# Online monitoring and control of the biogas process

**Boe, Kanokwan; Angelidaki, Irini**

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Kanokwan Boe

INSTITUTE OF ENVIRONMENT & RESOURCES

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Kanokwan Boe

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# Preface

This Ph.D. thesis is the result of a research project carried out at the Institute of Environment & Resources (E&R), Technical University of Denmark (DTU), during the period from October 2002 to February 2006. The project was financed by a Ph.D. scholarship from DTU. Throughout the period, Associate Professor Irini Angelidaki was the main supervisor and Dr. Damien John Batstone was the co-supervisor.

The thesis is organised in two parts. The first part is a dissertation providing backgrounds for understanding the important aspects of the biogas process, and also an updated knowledge about monitoring and control of the biogas process. Moreover, an application of a novel online VFA monitoring system is presented, as well as the methods for improving biogas production. The second part consists of the following papers.

Paper I Boe, K., Batstone, D.J. and Angelidaki, I. (2005) Online headspace chromatographic method for measuring VFA in biogas reactor. *Water Science and Technology*, **52,** 473-478.

Paper II Boe, K., Batstone, D.J. and Angelidaki, I. (2006) An innovative online VFA monitoring system for the anaerobic process, based on headspace gas chromatography. Submitted.

Paper III Boe, K., Batstone, D.J., Steyer, J.P. and Angelidaki, I. (2006) Comparison of process parameters for monitoring and control of the biogas process. Manuscript.

Paper IV Boe, K., Steyer, J.P. and Angelidaki, I. (2006) Monitoring and control of the biogas process using online VFA measurement. Manuscript.

Paper V Angelidaki, I., Boe, K. and Ellegaard, L. (2005) Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. *Water Science and Technology*, **52,** 189-194.

Paper VI Boe, K. and Angelidaki, I. (2006) Investigation of serial CSTR digester configuration for improving biogas production. Manuscript.

Paper VII Boe, K., Karakashev, D. and Angelidaki, I. (2006) Effect of post digestion temperature on serial CSTR biogas reactors. Manuscript.

Paper VIII Boe, K., Batstone, D.J. and Angelidaki, I. (2005) Optimisation of serial CSTR biogas reactors using modeling by ADM1. In: The First International Workshop on the IWA Anaerobic Digestion Model No.1 (ADM1), 2-4 September 2005, Lyngby, Denmark. Proceedings, pp. 219-221. International Water Association, London, UK.

The papers are not included in this www-version but may be obtained from the Library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Lyngby (library@er.dtu.dk)

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# Abstract

The demand for online monitoring and control of biogas process is increasing, since better monitoring and control system can improve process stability and enhance process performance for better economy of the biogas plants. A number of parameters in both the liquid and the gas phase have been suggested as process indicators. These include gas production, pH, alkalinity, volatile fatty acids (VFA) and hydrogen. Of these, VFA is the most widely recognised as a direct, relevant measure of stability. The individual, rather than collective VFA concentrations are recognised as providing significantly more information for diagnosis. However, classic on-line measurement is based on filtration, which suffers from fouling, especially in particulate or slurry wastes. In this project, a new online VFA monitoring system has been developed using gas-phase VFA extraction to avoid sample filtration. The liquid sample is pumped into a sampling chamber, acidified, added with salt and heated to extract VFA into the gas phase before analysis by GC-FID. This allows easy application to manure. Sample and analysis time of the system varies from 25-40 min. depending on the washing duration. The sampling frequency is fast enough for the dynamic of a manure digester, which is in the range of several hours. This system has been validated over more than 6 months and had shown good agreement with offline VFA measurement. Response from this sensor was compared with other process parameters such as biogas production, pH and dissolved hydrogen during overload situations in a laboratory-scale digester, to investigate the suitability of each measure as a process indicator. VFA was most reliable for indicating process imbalance, and propionate was most persistent. However, when coupling the online VFA monitoring with a simple control for automatic controlling propionate level in a digester, it was found that propionate decreased so slow that the biogas production fluctuated. Therefore, it is more proper to optimise biogas production, while using propionate (or VFA) as a warning indicator for process imbalance. Moreover, in this project, the investigations of serial CSTR configuration for improving biogas production were also carried out both in lab-scale experiments and by using the ADM1 computer model. It was shown that the serial CSTR configuration with long retention time in the first reactor and short retention time in the second reactor could improve biogas production from manure and could improve effluent quality in terms of VFA concentration, compared to a conventional single CSTR reactor. The temperature of the second reactor in the serial CSTR configuration also affected the amount of extra biogas yield. The serial CSTR configuration present in this study can be applied to the existing process in the Danish centralized biogas plants and requires only small process modification.

# Resumé

Der er et stigende behov for online overvågning og styring af biogasprocessen, da dette kan forbedre processtabiliteten og øge proceseffektiviteten, hvorved danske biogasanlægs økonomi kan forbedres. Et antal parametre i både væske- og gasfase har været foreslået som procesindikatorer. Disse inkluderer gasproduktionen, pH, alkalinitiet, samt koncentrationen af flygtige fede syrer (volatile fatty acids, VFA) og brint. Af disse er VFA de mest anerkendte som direkte indikator på processtabiliteten. I forhold til den totale VFA-koncentration giver de individuelle VFA en bedre information om processen samt en bedre diagnose af eventuelle procesproblemer. De klassiske online VFA-målinger er baseret på filtration, hvor filtrene ofte tilstoppes, specielt ved gylle med højt indhold af suspenderet materiale. I nærværende projekt, er der imidlertidig udviklet et nyt apparat til online VFA-måling, der er baseret på gasfase- ekstration af VFA fra prøven. Den flydende prøve pumpes ind i et prøvekammer, hvor der tilsættes syre og salt. Prøven opvarmes for, at ekstrakthere VFA, som herefter analyseres på gaschromatograf udstyret med flammeionisationsdetektor (GC-FID). Denne metode gør måling af VFA i gyllen meget nemmere. Prøveududtagningen og prøvebehandlingen tager fra 25–40 minutter afhængig af den programerede rensetid af prøvekammeret. Apparatet kan indstilles til en målefrekvens og derved kan man følge dynamiske ændringer i processen, som normalt ændrer sig over flere timer. Målesystemet er blevet valideret i laboratorieskala i over 6 måneder, og har vist god overensstemmelse sammenlignet med manuelle VFA målinger. Online VFA-parametre blev også sammenlignet med andre processparametre som foreksempel biogasproduktionen, pH og opløst brint under forskellige forstyrrelser af processen. Forsøgene blev foretaget i laboratorieskala, for at undersøge hvilke parametre der er bedst egnet til forudsigelse af procesforstyrrelser. VFA viste sig at være bedst til at indikere procesforstyrrelser og propionsyre var den sværest nedbrydelige. Ved at installere et simpelt kontrolprogram sammen med online VFA-målingen, til automatisk styring af propionsyre-niveauet i biogasreaktoren, blev det opdaget, at propionsyre- niveauet faldt så langsomt, at biogasproduktionen varierede meget. Derfor er det bedre at styre biogasproduktionen, ved at bruge propionsyre som en advarselsindikator på procesforstyrrelser. Derudover blev det undersøgt om en serieopbygning af to biogasreaktorer kunne optimere biogasproduktionen. Undersøgelserne foregik i laboratorieskala og ved brug af ADM1-computermodellen. Resultatet viste, at serieopbygningen med lang opholdstid i den første reaktor, og kort opholdstid i den anden reaktor, forøgede biogasproduktionen fra gylle. Endvidere kunne kvaliteten af den afgassede gylle forbedres med hensyn til VFA koncentrationen, sammenlignet med den konventionelle enkelt-reaktormodel. Temperaturen i den anden reaktor ved serieopbygning, har stor indflydelse på biogasproduktionen. Serieopbygningen kan direkte anvendes til de dansk biogasfællesanlæg og kun mindre procesændringer er nødvendige.

# Table of Content

# 1. Introduction and aim of the study

Anaerobic digestion for treatment of organic waste and biogas production is an environmentally attractive technology. It has environmental benefits with regard to waste treatment, pollution reduction, production of $CO_2$-neutral renewable energy and improvement of agricultural practices by recycling of plant nutrients (Tafdrup, 1995). A large number of biogas plants are currently found throughout the world. In Denmark the first centralized biogas plant was built in the 1980s. In 2001, Danish biogas plants treated approximately 1.2 million tonnes of manure and approximately 300,000 tonnes of organic industrial waste (Angelidaki and Ellegaard, 2002). Biogas production in Denmark has been increasing every year. It has been increasing 400% from 1990 to 2004 (Danish Energy Authority, 2005a), as illustrated in Figure 1. Currently, there are 20 large-scale centralised plants in operation, treating a mixture of approximately 75% manure and 25% organic waste. It has proven to be possible to build and operate large biogas installations which are economically sustainable with addition of organic waste. It is therefore expected that the use of biogas will rise (Danish Energy Authority, 2005b).

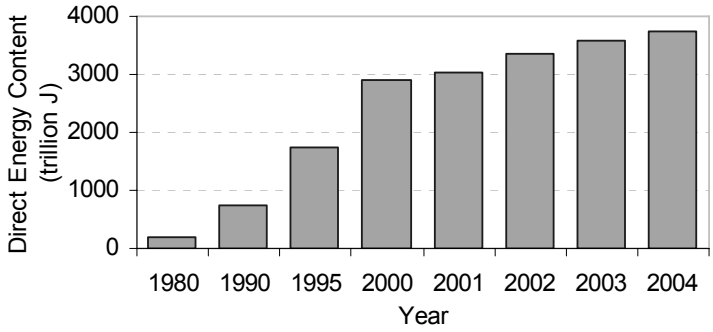



**Figure 1.** Energy production from biogas in Denmark
Data from: Danish Energy Authority (2005a)

Anaerobic digestion comprises several groups of microorganisms in a complex process. Some microbial groups are slow growing and sensitive to change in operating conditions. Therefore, special knowledge and process sensors are required for optimal operation. Under-loading enables stable operation, but this results in relatively low productivity, and consequently low economic profit. Additionally, very low loaded anaerobic digesters operate with low conversion rates. Increasing loading increases biogas production rate but risks overloading, which can lead to extended recovery times, consequent loss of production, and restart expenses. Often the process fails before the operators can take action due to lack of responsive sensors to provide an early warning. Better monitoring and control is an important tool to achieve process stability and optimised biogas production, without the risk of process failure. This contributes to a better economy of the biogas plants.

Easily measured indicators such as gas flow and pH are often too slow to arrest rapidly occurring overloads. In contrast volatile fatty acids are both an intermediate, and a core potential inhibitor, and the concentration of volatile fatty acids (VFA) is one of

the best indicators for process instability (Hill and Holmberg, 1988; Hickey and Switzenbaum, 1991c; Ahring *et al.*, 1995; Björnsson *et al.*, 2000; Mechichi and Sayadi, 2005). Off-line analysis of VFA can be slow, require specialist equipment, and may be expensive. Effective monitoring and control requires on-line analysis of control parameters. There have been many approaches to developing on-line VFA sensors. Total VFA has been measured by online titration systems (Powell and Archer, 1989; Bouvier *et al.*, 2002; Feitkenhauer *et al.*, 2002), respirometric techniques (Kong et al., 1996), a denitrifying biosensor (Rozzi *et al.*, 1997), and recently by a Fourier Transform Infra-Red (FT-IR) spectrometer (Steyer *et al.*, 2002a). However, individual VFA can provide more information of the process status. Several studies have highlighted the importance of individual VFA as an early warning indicator for process failure (Hill and Holmberg, 1988; Hill and Bolte, 1989; Cobb and Hill, 1991; Ahring *et al.*, 1995).

The online measurement of individual VFA has been mostly based on liquid sample filtration followed by gas chromatography (GC) (Slater *et al.*, 1990; Ryhiner *et al.*, 1993; Pind *et al.*, 2003a) or liquid chromatography (HPLC) (Zumbusch *et al.*, 1994). However, membrane fouling was the main problem in the application of these systems. It was clear that the efficiency of sample pre-treatment by membrane filtration decreased over time. The filter suffered from fouling and required extensive maintenance to obtain satisfactory results. In anaerobic digestion, the sampling system has to deal with complex, high-solid, or viscous samples, which makes membrane filtration even more susceptible to fouling. Especially for "difficult" substrates such as manure or solid waste, membrane fouling is extensive. So far there is no on-line monitoring system that can measure individual VFA with the acceptable level of simplicity and price. The systems existing on research level today are complicated, expensive, and require high levels of maintenance, which makes them impractical for implementation in biogas plants.

The aim of this Ph.D. project is to develop a new method for VFA on-line analysis, which can overcome the problems faced in other systems today and achieve a price and simplicity level that is economically attractive for the biogas sector and other anaerobic applications. In order to fulfill this objective, the following work-tasks were addressed:

1. Design and construction of a novel online measuring system, including optimisation and calibration.
2. Validation of the system by online monitoring of a lab-scale CSTR biogas reactor and comparison with other online parameters such as gas production, pH and dissolved hydrogen.
3. Application of the online measuring system for automatic control of the lab-scale reactor.

Apart from the main topic, several studies have also been carried out for improving the biogas production of the CSTR manure digester by optimising process configurations through both laboratory experiments and computer model. Through these studies, the effect of operating conditions and reactor configuration on the efficiency of biogas plants was also investigated.

According to the work-task 1, the results from batch experiment for system design and the preliminary tests results of the system are presented in Paper I. In addition, the detailed description of the online VFA measuring system and the validation test results are shown in Paper II. The results from the work-task 2 and 3 are presented in Paper III and IV, respectively. Paper V reports the results from the investigation on effect of operating conditions and reactor configuration on the efficiency of full-scale biogas plants. In Paper VI, the studies for improving biogas production by applying a serial-CSTR configuration are presented, where the effects of temperature on the serial operation are reported in Paper VII. Moreover, the behavior of a serial-CSTR configuration has been simulated using the ADM1 computer model and the criteria for optimisation of serial operation are presented in Paper VIII.

# 2. The biogas process

Anaerobic digestion is a multi-step biological process where the organic carbon is converted to its most oxidized ($CO_2$) and most reduced ($CH_4$) state (Angelidaki *et al.*, 2003). The main product of the process is biogas which is a mixture of methane and carbon dioxide, as well as trace gases such as hydrogen sulfide and hydrogen. The process involves several groups of microorganisms which makes it complex and sensitive, and makes it a valid subject for control and optimisation.

The important processes in anaerobic digestion are hydrolysis, fermentation, acetogenesis, and methanogenesis, where hydrolysis is subject to the fermentation process, while acetogenesis and methanogenesis are linked. The hydrolysis step is an extra-cellular process where the hydrolytic and fermentative bacteria excrete enzymes to catalyse hydrolysis of complex organic materials into smaller units. The hydrolysed substrates are then utilised by fermentative bacteria. Fermentation products such as acetate, hydrogen and carbon dioxide can directly be used by methanogenic microorganisms producing methane and carbon dioxide, while other more reduced products such as alcohols and higher volatile fatty acids are further oxidised by acetogenic bacteria in syntrophic with the methanogens. Figure 2 shows carbon flow diagram of the biogas process, with individual processes described as follows.

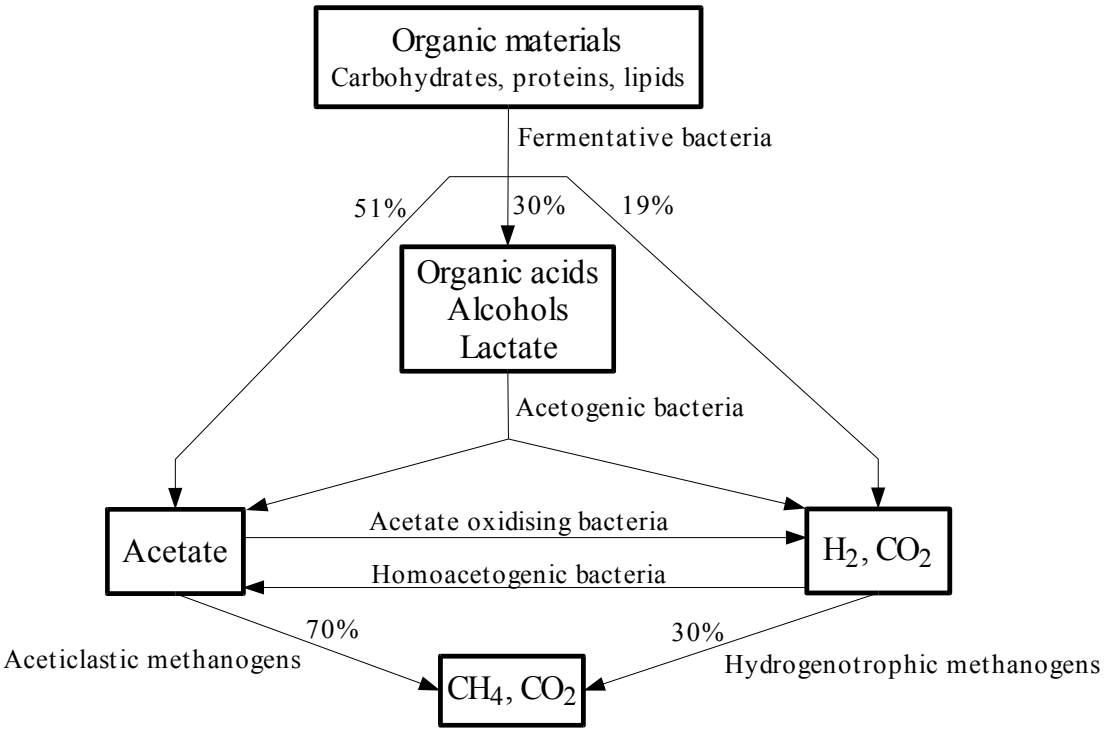



**Figure 2.** Carbon flow diagram of the biogas process
Adapted from Angelidaki *et al.* (2002)

## 2.1 Hydrolysis

Hydrolysis is an extra-cellular process in which organic particulates are broken down to soluble oligomers and monomers. It is an important step prior to fermentation process, as the fermentative bacteria cannot adsorb complex organic polymers directly into their cells. Hydrolytic enzymes include cellulase, cellobiase, xylanase and amylase for degrading carbohydrates into sugars, protease for degrading protein into amino acids, and lipase for degrading lipid into glycerol and long-chain fatty acids (LCFA) (Kaseng *et al.*, 1992; Parawira *et al.*, 2005). The hydrolysis process itself involves several steps, including enzyme production, diffusion, adsorption, reaction, and enzyme deactivation step (Batstone *et al.*, 2002a). The overall hydrolysis rate depends on organic material size, shape, surface area, biomass concentration, enzyme production and adsorption (Chyi and Dague, 1994; Batstone *et al.*, 2000). Moreover, competitive adsorption of enzyme on the inert substrate like lignin can also decrease hydrolysis efficiency (Converse and Optekar, 1993). This complicated process has been described by several kinetic models, for example, a surface-based kinetics model where the hydrolysis is related to the substrate concentration and particle surface area (Sanders *et al.*, 2000), or a two-phase model where the bacteria first attaches to particles, then releases enzyme to degrade material (Vavilin *et al.*, 1996). Hydrolysis is therefore a function of both biomass and substrate concentration. In contrast to low solids systems, for high solids systems, Batstone *et al.,* (2000) selected a surface area-dependent model to allow for a hydrolysis-limitations, when variation in particle size was expected. However, most authors explain hydrolysis as a lumped process using a first-order kinetic based on substrate (Pavlostathis and Giraldo-Gomez, 1991; Angelidaki *et al.*, 1993; Batstone *et al.*, 2002a). Hydrolysis was shown to be a rate-limiting step for digestion of high particulated substrate like swine waste, cattle manure and sewage sludge while methanogenesis is the rate-limiting step for readily degradable substrate (Vavilin *et al.*, 1997; Björnsson *et al.*, 2001b).

## 2.2 Fermentation

Fermentation is generally defined as a microbial process in which part of the organic molecule to be oxidized acts as terminal electron acceptor in the absence of exogenous electron acceptors such as nitrate or sulfate (Gujer and Zehnder, 1983; Zehnder and Stumm, 1988). The sugars obtained from the hydrolysis step can easily be degraded by fermentation process while LCFA is must be obligately oxidised by an external electron acceptor (Batstone *et al.*, 2002a). Amino acids can either be degraded through Stickland reactions, where one amino acid acts as an electron donor and another acts as an electron acceptor, or can be oxidised with an external electron acceptor (Ramsay and Pullammanappallil, 2001). Glucose fermentative microbes have branched metabolisms, which means they are able to metabolise the substrate in different pathways which give different amount of energy and produce different fermentation products (Dolfing, 1988). The fermentative bacteria can function at high concentrations of hydrogen and formate (Batstone *et al.*, 2002a). However, under this condition, the bacteria will use a metabolic pathway in which more reduced metabolites are produced, such as VFA,

lactate, and ethanol (Angelidaki *et al.*, 2002). Examples of different products from glucose fermentation are shown in Table 1.

**Table 1.** Examples of glucose fermentation products

| Products | Reaction |
|---|---|
| Acetate | $C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$ |
| Propionate + Acetate | $3C_6H_{12}O_6 \rightarrow 4CH_3CH_2COOH + 2CH_3COOH + 2CO_2 + 2H_2O$ |
| Butyrate | $C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 2H_2$ |
| Lactate | $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$ |
| Ethanol | $C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2CO_2$ |

(Thauer *et al.*, 1977; Schink, 1997; Batstone *et al.*, 2002a)

The dominant pathway depends on several factors such as substrate concentration, pH and dissolved hydrogen concentrations (Rodriguez *et al.*, 2006). Under very high organic load, lactic acid production becomes significant (Mattiasson, 2004). At low pH (< 5) the production of ethanol is increased, while at higher pH more VFA are produced (Ren *et al.*, 1997; Horiuchi *et al.*, 1999). At pH < 4, all fermentation processes may cease (Hwang *et al.*, 2004). However, hydrogen partial pressure has been reported to have most influence on the fermentation pathway. At low partial pressured of hydrogen the fermentation pathway to acetate and hydrogen is favoured (Thauer *et al.*, 1977; Klass, 1984; Schink, 1997).

Although the fermentative bacteria are not strictly dependent on syntrophic relationship, they still gain profit from the activities of the hydrogen-scavenging organisms, as the fermentative bacteria gain maximum energy yield when protons are used as electron acceptor with concurrent $H_2$ production (Dolfing, 1988; Schink, 1997). The reason is because the low hydrogen partial pressure (<10 Pa) allows the redox coenzyme NADH to release electrons as molecular hydrogen. Fermentation can produce more acetate, carbon dioxide, and hydrogen rather than ethanol or butyrate formation, thus allowing additional ATP synthesis from substrate level phosphorylation (Schink, 1997). One example is the fermentation of glucose by *Clostridium butyricum* which under normal condition the reaction is as following:

$$\text{Glucose} + 2H_2O \rightarrow 0.7\ \text{butyrate} + 0.6\ \text{acetate} + 1.3\ H^+ + 2CO_2 + 2.6H_2$$
$$(\Delta G^{\circ\prime} = -233\ \text{kJ/mol})$$

The reaction yields 3.3 ATP units per molecule of glucose. However, at $[H_2] < 10$ Pa, the overall reaction changes to:

$$\text{Glucose} + 2H_2O \rightarrow 2\ \text{acetate} + 2H^+ + 2CO_2 + 4H_2$$
$$(\Delta G^{\prime} = -280\ \text{kJ/mol})$$

,which yields 4 ATP units per molecule of glucose (Thauer *et al.*, 1977; Schink, 1997). Thus, in a system where the hydrogen-utilising organisms (such as methanogens or sulfate reducing bacteria) maintain low partial pressure of hydrogen, the carbon flow from carbohydrates will mainly go through acetate, hydrogen and carbon dioxide. However, higher VFA and alcohols are still produced from amino acid fermentation

(Schink, 1997). From Figure 2, the carbon flow diagram shows that under stable conditions, the fermentation pathway to acetate and hydrogen contributes the main carbon flow to methane formation. The products from fermentation step consist approximately 51% of acetate, 19% of $H_2$, and the rest are more reduced products such as higher VFA, alcohols or lactate (Angelidaki *et al.*, 2002).

## 2.3 Acetogenesis

Some fermentation products such as fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, and branched-chain and aromatic fatty acids, cannot directly be used in methanogenesis. In acetogenesis process, these products are oxidized to acetate and $H_2$ by obligated proton reducing bacteria in syntrophic relationship with methanogenic archaea as low $H_2$ partial pressure is essential for acetogenic reactions to be thermodynamically favorable ($\Delta G > 0$) (Schink, 1997; Stams *et al.*, 2005).

Among fermentation products, volatile fatty acids are the most common intermediates found in a mixed-culture anaerobic digester (Pind *et al.*, 2003b). During acetogenesis, propionate is mainly oxidized via the methyl-malonyl-CoA pathway yielding acetate, $H_2$ and $CO_2$ (De Bok *et al.*, 2004). N-butyrate and n-valerate degrade via β-oxidation to acetate and acetate + propionate, respectively (Batstone *et al.*, 2003). Iso-butyrate degrades via an isomerization to n-butyrate and further β-oxidation (Angelidaki and Ahring, 1995). The degradation of iso-valerate is complicated with $CO_2$ as co-substrate producing acetate and $H_2$. The pathway found in pure culture involves carboxylation, dehydrogenation of saturated fatty acid residue, and one substrate level phosphorylation step (Stieb and Schink, 1986). Volatile fatty acid oxidation reactions are shown in Table 2, showing that all the reactions become thermodynamically favorable at low $H_2$ partial pressure ($<10^{-4}$ atm).

**Table 2.** Fatty acids degradations

| **Substrate** | **Reaction** | $\Delta G°$ (kJ/mol) | $\Delta G°'$ (kJ/mol) |
|---|---|---|---|
| Propionate | $CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$ | +76.2 | -14.6 |
| *i*-butyrate | $CH_3(CHCH_3)COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ | +48.4 | -25.9 |
| Butyrate | $CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$ | +48.4 | -25.9 |
| *i*-valerate | $CH_3(CHCH_3)CH_2COOH + 2H_2O + CO_2 \rightarrow 3CH_3COOH + H_2$ | +20.2 | -36.8 |
| Valerate | $CH_3CH_2CH_2CH_2COOH + 2H_2O \rightarrow$ | +48.4 | -25.9 |

$\Delta G°'$ based on 25 ºC, pH 7, $P_{H2}$ $10^{-5}$ atm, $P_{CH4}$ 0.7 atm, organic acids 1 mM, and $HCO_3^-$ 0.1 M (Thauer *et al.*, 1977; Schink, 1997; Batstone *et al.*, 2002a; Batstone *et al.*, 2003)

Both $H_2$ and formate have been reported as important electron carriers in the acetogen/methanogen syntrophic relationship (Schink, 2002; De Bok *et al.*, 2004; Stams *et al.*, 2005). In normal methanogenic system the concentration of both $H_2$ and formate are very low, so it is difficult to determine which compound is the most important electron carrier. Moreover, many actogenic bacteria can release electron in the form of both $H_2$ and formate, and most of methanogenic organisms are also able to utilise both

(Stams *et al.*, 2005). Both electron carriers may be used simultaneously in one degradation process, or switching between each channel depending on the environmental conditions (Schink, 2002). The important factors affecting the interspecies transfer of $H_2$ and formate are solubility and diffusivity. $H_2$ diffuses approximately 30 times faster than formate, while formate has higher solubility which makes concentration gradient between the producer and consumer organisms can be up to a 1000 times higher than for $H_2$ (De Bok *et al.*, 2004). The flux of electron carrier transferred between organisms depends on the surface area of the producing bacterium, the diffusion constant, the concentration gradient, and the distance between organisms. Hence, formate transfer is important in suspended system with long distance between microorganisms, while $H_2$ transfer is more important for short-distance compact aggregates (De Bok *et al.*, 2004). However, it is obvious that the closely association of syntrophic partners is preferable for electron transfer as often observed in biofilm systems (Batstone *et al.*, 2004a).

Acetogenic bacteria not only profit from hydrogenotrophic methanogens, but also aceticlastic methanogens, as acetate removal has an influence on the energetics of VFA oxidizing reactions, especially in iso-valerate degradation, where three molecules of acetate and only one molecule of $H_2$ are formed (Schink, 2002). Moreover, acetate accumulation may have a biochemical inhibitory effect on acetogenesis (Kuninobu *et al.*, 1999).

Temperature also affects the thermodynamics of acetogenic reactions. The $H_2$ formation from organic acids oxidation becomes more energetic at higher temperatures, while $H_2$ consumption by the methanogens becomes less energetic. However, since diffusion coefficients become higher and diffusion gradients steeper, the organic acids degradation is expected to be faster under high temperature (De Bok *et al.*, 2004).

In a manure digester, the reactor is continuously inoculated from the feedstock. The microbial community in manure shows a close relationship to the animal gastrointestinal tract ecosystem, for example, the present of *Clostridium*, *Lactobacillus* and *Bacillus* in cow manure (Ouwerkerk and Klieve, 2001; Whitford *et al.*, 2001), and *Eubacterium*, *Clostridium*, *Bacillus*, *Lactobacillus*, *Streptococcus* and *Bacteroides* in pig manure (Snell-Castro *et al.*, 2005).

## 2.4 Methanogenesis

During methanogenesis, the fermentation products such as acetate and $H_2/CO_2$ are converted to $CH_4$ and $CO_2$ by methanogenic archaea. The methanogenic archaea are able to grow directly on $H_2/CO_2$, acetate and other one-carbon compound such as formate, methanol and methylamine (Schink, 1997; Stams *et al.*, 2005). Some methanogens can only utilise one substrate, e.g. *Methanosaeta* species use only acetate and *Methanobrevibacter arboriphilus* use only $H_2/CO_2$. Other methanogens are more versatile, e.g. *Methanospirillum hungatei* and *Methanobacterium formicicum* grow on both $H_2/CO_2$ and formate, and *Methanosarcina* species can use $H_2/CO_2$, acetate, methanol and a few other one-carbon compounds (Stams *et al.*, 2005).

Methanogenesis is affected by reactor operating condition such as temperature, hydraulic loading rate, organic loading rate, and feed composition (Fey and Conrad,

2000; McHugh *et al.*, 2003; Murto, 2003). Moreover, apart from methanogenic reactions, the inter-conversion between hydrogen and acetate catalysed by homoacetogenic bacteria also plays an important role in the methane formation pathway. Homoacetogens can either oxidize or synthesize acetate depending on the external hydrogen concentration (Schink, 2002; Kotsyurbenko, 2005). This makes it able to compete with several different microbes, including methanogens. The reactions related to acetate and hydrogen consumptions are shown in Table 3.

**Table 3.** Reactions related to methanogenesis

| | **Reaction** | $\Delta G°$ **(kJ/mol)** |
|---|---|---|
| Hydrogenotrophic methanogenesis | $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ | -135.0 |
| Aceticlastic methanogenesis | $CH_3COOH \rightarrow CH_4 + CO_2$ | -31.0 |
| Acetate oxidation | $CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$ | +104.0 |
| Homoacetogenesis | $4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$ | -104.0 |

(Thauer *et al.*, 1977; Schink, 1997; Batstone *et al.*, 2002a)

From Table 3, under standard temperatures, the hydrogen consumption by hydrogenotrophic methanogenesis is more favorable than homoacetogenesis. Acetate consumption by aceticlastic methanogeneis is also more favorable than acetate oxidation. Hydrogenotrophic methanogenesis functions better at high hydrogen partial pressure, while aceticlastic methanogenesis is independent from hydrogen partial pressure (Schink, 1997).

At higher temperatures (> 30 $^{o}$C) the acetate oxidation pathway becomes more favourable (Schink, 2002). It has been reported that methane formation through acetate oxidation can contribute up to 14% of total acetate conversion to methane under thermophilic conditions (60 $^{o}$C) (Petersen and Ahring, 1991). This corresponded to reports of a high abundance of hydrogen-utilising methanogens in a thermophilic digester (Griffin *et al.*, 1998). However, aceticlastic methanogens such as *Methanosarcina thermophila* also function at high temperature (Schink, 1997), thus, they will compete for acetate. At high acetate concentrations, aceticlastic methanogenesis is more favorable than syntrophic acetate oxidation (Zinder and Korch, 1984; Petersen and Ahring, 1991). However, in extreme thermophilic conditions (>65 $^{o}$C), which is beyond the optimum temperature (63 $^{o}$C) of aceticlastic methanogens, the syntrophic acetate oxidation pathway dominates despite high acetate concentrations (Lepistö and Rintala, 1999). In a test with 75 $^{o}$C sludge using $^{13}$C-labelled acetate as substrate, approximately 95% of the total acetate conversion was performed by syntrophic acetate oxidation (Van Lier, 1995).

Acetate oxidation has an effective lower temperature limit at 37 $^{o}$C (Schnurer *et al.*, 1996; Schink, 2002), since at lower temperatures, lower hydrogen partial pressure is needed to obtain energy for the oxidation step, as shown in Figure 3 (Schink, 1997). Under psychrophilic conditions (<15 $^{o}$C), the activity of hydrogen-utilising methanogens is very low. Homoacetogenesis takes over as the main hydrogen removal

function, and methane formation through aceticlastic methanogens becomes dominant (Kotsyurbenko *et al.*, 2001). Methane formation through homoacetogenesis under psychrophilic temperature can be up to 95% of the total methane production (Kotsyurbenko, 2005). However, aceticlastic methanogenesis is more sensitive to ammonia than hydrogen-utilising methanogenisis (Borja *et al.*, 1996; Sung and Liu, 2002). Acetate oxidation can dominate at moderate temperatures (mesophilic or below) if high ammonium concentrations inhibits the aceticlastic methanogens (Schnurer *et al.*, 1994; Schnurer *et al.*, 1999). This has also been reported in the digestion of swine waste at temperature 25 $^{o}$C, showing that at increased ammonia concentrations, the hydrogen-utilising *Methanomicrobiales* increased while the acetate-utilising *Methanosarcina* decreased (Angenent *et al.*, 2002). *Methanomicrobiales* was also found abundant in a mesophilic digester treating a mixture of pig and cattle manure together with industrial organic waste, whereas acetate concentration in the reactor was low (Hansen *et al.*, 1999a).

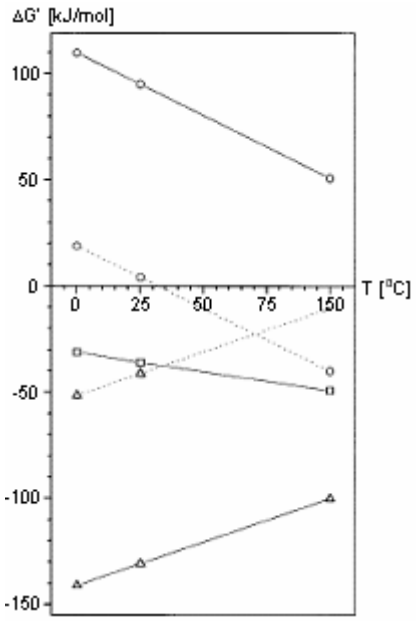



**Figure 3.** Temperature dependence of the free energy change in anaerobic hydrogen and acetate metabolism; solid lines, standard conditions (1 M concentrations, 1 atm pressure); dashed lines, the same for $10^{-4}$ atm of hydrogen; □: aceticlastic methanogenesis; O: acetate oxidation; Δ: hydrogenotrophic methanogenesis (from Schink, 1997)

As shown in Figure 2, in the normal anaerobic digesters, acetate is the precursor for up to 70% of total methane formation while the remainder originates from $H_2/CO_2$ (Klass, 1984). The two important aceticlastic methanogens are *Methanosaeta* and *Methanosarcina* (Murto, 2003; McMahon et al., 2004). In most anaerobic digesters treating a mixture of manure and organic wastes in Denmark, *Methanosarcina* are dominant, followed by hydrogen-utilisers *Methanobacteriales*, *Methanomicrobiales* and *Methanococcales* (Hansen *et al.*, 1999a; Karakashev *et al.*, 2005). The main factor that affects the populations of *Methanosaeta* and *Methanosarcina* is the level of VFA, especially acetate (Schmidt *et al.*, 2000; Karakashev *et al.*, 2005). *Methanosaeta* has

higher affinities to acetate but slower growth rate and it is found dominant at low acetate concentration (<1 mM), while *Methanosarcina* dominates at high acetate concentration (Schmidt *et al.*, 2000; Karakashev *et al.*, 2005). The high population of *Methanosaeta* imples that the methane pathway in such system goes mainly through aceticlastic methanogenesis since it can only use acetate (Murto, 2003). In psychrophilic condition, acetoclastic methanogenesis is known to be a key step for methane production (Chin *et al.*, 1999; McHugh *et al.*, 2003). Lay *et al.* (1996) reported that in the lake sediments under psychrophilic conditions the number of acetate-utilising methanogens were substantially higher than hydrogen utilisers. Chin *et al.* (1999) reported acetate as the main source for methanogenesis in anoxic rice field soils where *Methanosaeta* was dominant at 15 $^{o}$C, and *Methanosarcina* was dominant at 30 $^{o}$C. Moreover, *Methanosaeta* was found dominant in a psychrophilic digester treating low-strength synthetic sewage (McHugh *et al.*, 2003). These studies corresponds to the study from Lokshina and Vavilin (1999) which showed that acetate produced by homoacetogens from $H_2$ was the main precursor of methane in the psychrophilic digestion.

Methanogenesis removes hydrogen and acetate from the system and, thus, has strong influence on both acetogenesis and fermentation. Hydrogenotrophic methanogenesis occurs simultaneously with acetogenesis syntrophically. Hydrogeotrophic methanogenesis is a primary regulator in the anaerobic process and its failure in function will strongly affect the syntrophic acetogenic bacteria and the fermentation process as a whole (Schink, 1997). The accumulation of reduced fermentation products in anaerobic digester is mainly due to inadequate removal of hydrogen and acetate due to several reasons. For example, high organic load increases hydrogen and VFA production beyond the capacity of methanogens resulting in accumulation of VFA, or the decreasing in capacity of methanogens due to inhibition by toxic compounds or pH drop (<6) (Schink, 2002).

## 2.5 Reactor types and applications

Conventional anaerobic digesters are in batch, semi-continuous or continuous operations. Semi-continuous or continuous operations are preferable as maximum growth rate can be achieved constantly at steady-state by controlling the feed rate. In batch system, the steady-state cannot be achieved as the concentrations of components in the digester are changing with time (Klass, 1984).

Choice of reactor type is determined by waste characteristics, especially particulate solid contents. Solids and slurry waste are mainly treated in continuous flow stirred tank reactors (CSTRs), while soluble organic wastes are treated using high-rate biofilm systems such as anaerobic filters, fluidised bed reactors and upflow anaerobic sludge blanket (UASB) reactors (Angelidaki *et al.*, 2002).

In biofilm systems, the biomass is retained in the bioflim/granular aggregates such that sludge retention time (SRT) is much higher than hydraulic retention time (HRT), which has the advantage that the reactor can run at high flow rate and can tolerate higher toxic concentrations than CSTR systems. High-rate biofilm systems are normally run in continuous mode with HRT less than 5 days (often below 24 hours) (Pind *et al.*, 2003c). The systems can operate in a wide range of temperature from

psychropihilic conditions (3 $^{o}$C) (Lettinga *et al.*, 1999) to extreme-thermophilic conditions (80 $^{o}$C) (Lepistö and Rintala, 1997). Of the high-rate systems, the UASB is the most popular for anaerobic treatment of soluble organic wastes.

In CSTR systems, the biomass is suspended in the main liquid and will be removed together with the effluent such that the SRT is equal to the HRT. This makes it necessary to run at long HRTs, usually 10-20 days, to avoid washing out the slow-growing methanogens. In domestic sludge digestion, a sludge settler after the main reactor is often applied for recycling of the biomass, which makes it possible to run at shorter HRT (Pind *et al.*, 2003c). In anaerobic co-digestion based on manure slurry and organic wastes, which is the main focus in this thesis, the process is commonly operated with a CSTR in semi-continuous mode where the digester is intermittently supplied with the substrate after an equal amount of the digested material is withdrawn. The large-scale biogas systems normally have waste receiving tanks, which allow stable operation. The addition of organic waste to manure helps increase biogas production significantly, and is important for the economic viability of Danish biogas plants (Tafdrup, 1995). Continuous feeding is preferred, if optimising of heat-exchange is required. However, intermittent feeding saves pumping costs and ensures adequate times for pathogen reduction. Danish veterinarian research suggested the following heat treatment times for pathogen reduction; 70 $^{o}$C for 1 hour, 52 $^{o}$C for 10 hours, 53.5 $^{o}$C for 8 hours, or 55 $^{o}$C for 6 hours (Bendixen, 1994; Angelidaki *et al.*, 2003). Large-scale biogas plants in Denmark are normally operated under mesophilic conditions (36-38 $^{o}$C) with HRT 23 ± 4 days, or thermophilic conditions (51-53 $^{o}$C) with HRT 17 ± 4 days (Nielsen, 2006). Mesophilic plants normally have an extra sanitation installation, while themophilic plants use extra sanitation tanks or apply serial configuration to ensure the sanitation retention time.

## 2.6 Factors affecting the stability of the biogas process

The factors affecting the biogas production are mainly based on operating conditions and the feedstock. Operating conditions such as pH and temperature influence directly on the microorganisms. Disturbances from the feed include waste composition and concentration, and toxic and inhibitory compounds. Sometimes, toxic compounds are not initially present in the feed but are produced inside the reactor from substrate degradation (e.g., VFA and ammonia).

### *2.6.1 Substrate and nutrients*

Substrate type and compositions directly determines the biogas yield. Anaerobic substrate input is often measured in term of total chemical oxygen demand (COD) or total volatile solid (VS). It is important to distinguish between available degradable fraction and inert fraction, as a considerable fraction of the input COD or VS is inert (Gossett and Belser, 1982; Batstone *et al.*, 2002b; Møller *et al.*, 2004). Manure, which contains high water and recalcitrant fractions has lower methane yield per VS or COD than easily degradable substrate such as organic waste. An example of the different carbon fractions in manure from different sources is shown in Figure 4. Lignin is considered as non-degradable anaerobically. Carbohydrate is divided into easily

degradable (E) and slowly degradable (S) fractions, while lipid, protein and VFA are considered as easily degradable (Møller *et al.*, 2004). The methane yield of manure is in the range of 100-400 L $CH_4$ / kg VS, where pig manure has higher methane yield than cow manure due to more protein and lipid content, less lignin, and less slowly degradable carbohydrate. This factor strongly depends on the animal feedstock (Møller *et al.*, 2004).

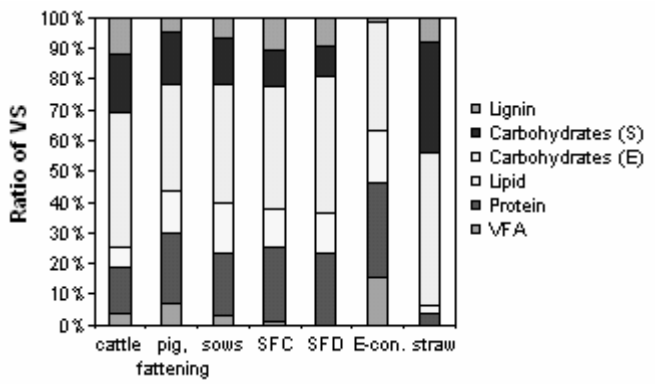



**Figure 4.** The average composition of VS in freshly excreted cattle manure, fattening pig manure, sow manure, solid fraction from centrifugation of pig manure (SFD), solid fraction from chemical precipitation of manure (SFC), liquid fraction pretreated with a decanting centrifuge (E-conc.) and wheat straw (from Møller *et al.*, 2004)

In contrast, most industrial organic wastes contain a high fraction of easily degradable substrate, which gives high methane yield and VFA production. It is therefore important to control organic and hydraulic load according to the capacity of the reactor. Underloading the process (low substrate input rate) results in low biogas production rate. Although this can prevent process failure, it is uneconomical because the capacity of the process is not fully utilised. Moreover, the process is running in sub-optimal level and the microbial populations are present in a slow and undynamic state (Ahring and Angelidaki, 1997). It has been reported that the system with a history of very stable operation was more difficult to recover from imbalance, since the organic acid degrading organisms were present in low concentration due to low organic acids supply at steady state (McCarty and Mosey, 1991; McMahon *et al.*, 2004). Increasing the load gives more biogas production but risks overloading, which results in VFA accumulation. High concentration of VFA decreases pH and makes VFA become more toxic to the methanogens, which can lead to process breakdown.

Sufficient nutrients are also important to microbial cell growth. Macro- nutrients such as carbon, hydrogen, nitrogen and oxygen are the main components in biomass cells. Sulphur, phosphorus, potassium, calcium, magnesium and iron are required for specific proteins (Kayhanian and Rich, 1995). These macro-nutrients should be present in the cell around $10^{-4}$ M, while the micro-nutrients such as nickel, cobolt and copper are required in smaller amount (Angelidaki *et al.*, 2002). Most nutrients can be inhibitory if present in high concentrations. Sulfide and phosphate can decrease the metal ion bioavailability by precipitating. Normally, all the nutrients are present in sufficient quantities in swine and cow manure.

*2.6.2 Operating conditions*

Temperature

Anaerobic digestion can be applied in a wide range of temperature from psychrophilic (<20 $^{o}$C) (Kashyap *et al.*, 2003), mesophilic (25-40 $^{o}$C), thermophilic (45-60 $^{o}$C) (Pfeffer, 1974), and even extreme-thermophilic conditions (>60 $^{o}$C) (Lepistö and Rintala, 1999). Temperature has direct effect on physical-chemical properties of all components in the digester and also affects thermodynamic and kinetic of the biological processes. Temperature determines if specific reaction is favourable and thus, also influences methenogenesis as discussed in item 2.3. Increasing temperature has several advantages (Van Lier, 1995), e.g.

- Increase solubility of organic compounds which makes them more accessible to the microorganisms.
- Increase chemical and biological reaction rates, thus, accelerates the conversion process so the reactor can be smaller and can run with shorter HRT.
- Improve several physical-chemical properties such as improve diffusivity of soluble substrate, increase liquid-to-gas transfer rate due to lower gas solubility, decrease liquid viscosity which makes less energy required for mixing and also improve liquid-solid biomass separation.
- Increase death rate of pathogenic bacteria especially under thermophilic conditon, which decreases retention time required for pathogen reduction (Bendixen, 1994; Smith *et al.*, 2005).

Moreover, the organic acid oxidations reactions become more energetic at higher temperature which is advantage for degradation of LCFA, VFA and other intermediates (Van Lier, 1995).

Nonetheless, high temperature can have some negative effect. Increasing temperature decreases pKa of ammonia, thus, increases the fraction of free-ammonia ($NH_3$) which is inhibitory to microorganisms. In addition, increasing temperature increases pKa of VFA, which increase its undissociated fraction, especially at low pH (4-5) such as in the acidogenic reactor (Van Lier, 1995). This makes the thermophilic process more sensitive to inhibition. However, due to many advantages of high temperature, thermophilic operation is popular in anaerobic applications where ammonia inhibition is not a major consideration. In Danish full-scale biogas plants with co-digestion of manure and organic wastes, the practical operating temperature for mesophilic condition is 35-38 $^{o}$C and thermophilic condition is 52-56 $^{o}$C (Ahring, 1994).

pH and buffering capacity

pH level has an effect on enzyme activity in microorganisms, since each enzyme is active only in a specific pH range and has maximum activity at its optimal pH (Lay *et al.*, 1997). Each group of microorganisms has different optimal pH ranges. Methanogenic archea can function in quite narrow pH interval from 5.5-8.5 with an optimal range of 6.5-8.0 (Nielsen, 2006). Fermentative bacteria can function in wider pH range from 8.5 down to pH 4 (Hwang *et al.*, 2004). A study of glucose fermentation where methanogens was washed out by short HRT showed that at pH 5-7, the main

products were acetic and butyric acid, while at pH 8.0, the main products were acetic and propionic acids. This phenomenon was reversible, showing that fermentative bacteria have different optimal pH in respect to the fermentation products (Horiuchi *et al.*, 2003). The pH level also affects acid-base equilibrium of different compounds in the digester. At low pH, free VFA can cause weak acid, while at high pH, free ammonia can likewise cause weak base inhibition. In a mixed-culture anaerobic digester the optimal pH range is 6.6-7.4 (Moosbrugger *et al.*, 1993).

Buffering capacity, or the solution resistance to pH change is also important for process stability. The main buffer in anaerobic digesters is bicarbonate ($HCO_3^-$), with a pKa of 6.3, while the main generated acids are VFA, with an aggregate pKa of approximately 4.8 (Moosbrugger *et al.*, 1993). Other compounds normally found in the digester also influence the pH balance if present at high concentration, for example, ammonia ($NH_4^+/NH_3$, pKa 9.3), hydrogen sulfide ($H_2S/HS^-/S^{2-}$, pKa 7.1 and 13.3) and hydrogen phosphate ($H_3PO_4/H_2PO_4^-/HPO_4^{2-}/PO_4^{3-}$, pKa 2.1, 7.2 and 12.3). Manure digesters (mainly a mixture of cow and pig manure) normally have high feed bicarbonate buffering capacity and a high ammonia content, which makes the pH stable around 7.5-8.0 (Pind *et al.*, 2003c), and the system can tolerate rather high concentration of VFA before pH drop.

Mixing intensity

Several studies showed that the mixing intensity in a CSTR reactor has an effect on process inhibition and recovery from organic overload (McMahon *et al.*, 2001; Stroot *et al.*, 2001; Vavilin and Angelidaki, 2005). Stroot *et al.* (2001) studied acetate and proprionate accumulation in CSTR digesters treating municipal solid wastes and biosolids with aggressive startup and organic overloading. They found that while acetate was eventually consumed, propionate persisted throughout system operation and began to decrease only after mixing level was reduced. They also found that a reactor with minimised mixing can tolerate higher organic load than the reactor with intensive mixing. Vavilin and Angelidaki (2005) tested CSTR digesters treating municipal solid wastes and manure, and found that when organic loading was high, intensive mixing resulted in acidification and process failure, while low mixing intensity was crucial for successful digestion. They hypothesized that mixing was preventing establishment of methanogenic zones in the reactor space and evaluated this in a mathematical model. However, McMahon *et al.* (2001) suggested that the inhibition due to strong mixing inhibited the syntrophic oxidation of VFA by disrupting the spatial juxtaposition of syntrophic bacteria and their methanogenic partners.

### *2.6.3 Toxic/inhibiting compounds*

Inhibitory compounds are either present already in the substrate or generated during the degradation. The most common inhibitors are formed during degradation of the substrate, such as VFA, LCFA, ammonia and sulfide. Some inhibitors are present already in the substrate, such as LCFA, heavy metals and antibiotics.

VFA is the main intermediate in anaerobic digestion, and accumulates under process imbalance. At lower pH the VFA becomes more toxic due to increase its

undissociated fraction. The undissociated VFA can freely cross the cell membrane, then dissociate which lowers internal pH and disrupts homeostasis (Switzenbaum *et al.*, 1990). The concentration threshold for VFA inhibition depends on reactor buffering capacity.

Ammonia comes mainly from degradation of protein waste. A study on 18 full-scale centralized biogas plants in Denmark showed that ammonia was a significant factor affecting the process stability (Paper V). Ammonia toxicity increases at high pH and high temperature due to higher concentration of free ammonia which is known to be inhibitory. However, the methanogens can be acclimated to ammonia. The study from Sung and Liu (2002) showed that the acclimated aceticlastic methanogens could tolerate total ammonia under thermophilic condition up to 2 g-N/L without any inhibition. However, the methanogenic activity decreased when further increasing ammonia concentration, and the total inhibition occurred at 10 g-N/L. Moreover, they also found that pH had influence on the inhibitory effect of ammonia. Under high ammonia concentration, the inhibitory effect on methanogens was lowest at pH 7.0-7.5.

Sulfate and sulfur compounds are also present in protein waste. In anaerobic conditions, the oxidised sulfur compounds are used as electron acceptors by sulfate-reducing bacteria and reduced to $S^{2-}$. Both acetogenic and methanogenic organisms are affected by the presence of sulfate since sulfate-reducing bacteria are metabolically versatile and many of them can oxidise all fermentation products to carbon dioxide with reducing sulfate as electron acceptor (Schink, 1997). At low concentrations of sulfate, sulfate-reducing bacteria compete with methanogenic archaea for hydrogen and acetate, and at high concentration the sulfate-reducing bacteria also compete with acetogenic bacteria for propionate and butyrate (Stams *et al.*, 2005). Sulfate-reducing bacteria can easily outcompete hydrogenotrophic methanogens for hydrogen. The percent electron flow to the sulfate-reducing pathway increased with the increasing influent sulfate, while flow toward the methanogenesis was correspondingly reduced (Khanal and Huang, 2005). It has been reported that the hydrogenotrophic methanogens were completely washed-out due to competition with hydrogen-utilising sulfate reducers in an anaerobic UASB reactor treating sulfate-rich wastewater (Yamaguchi *et al.*, 1999). However, aceticlastic methanogens can compete reasonably well with acetate-degrading sulfate reducers. Sulfate-reducing bacteria grow much faster on propionate and butyrate than acetogenic bacteria (Stams *et al.*, 2005).

Sulfide produced from sulfate reduction also has inhibitory effect at even low concentration as 0.003-0.006 M total S or 0.002-0.003 M $H_2S$ (Speece, 1996). The toxicity of sulfide is related to the undissociated species, $H_2S$, since the neutral molecule can pass unopposed through the cell membrane (Speece, 1983; McCartney and Oleskiewiez, 1991). However, others claimed that the toxicity should rather be related to total sulfide concentration at pH higher than 7.2 (O'Flaherty *et al.*, 1998).

LCFA is found at high concentration in hydrolysed vegetable oil and is also formed during degradation of fat and lipids. LCFA adsorbs on the bacteria cell wall, which inhibits the transport of essential nutrients (Henderson, 1973). 18-C LCFA such as oleic and linoleic acid are inhibitory even at concentrations as low as 1.5 g/L (Angelidaki and Ahring, 1992; Templer *et al.*, 2006). A study from Templer *et al.*

(2006) showed that the inhibitory effect of LCFA on hydrogenotrophic methanogens was progressively worse from linoleic acid (18:2) > oleic acid (18:1) > stearic acid (18:0). No adaptation on LCFA has been observed but the microorganisms can recover after dilution of LCFA by adding new substrate that does not contain LCFA (Rinzema *et al.*, 1994; Pereira *et al.*, 2003).

Heavy metals are found in industrial and domestic wastewater. In co-digestion application, domestic sludge is the main source of heavy metal in anaerobic digester. Heavy metals are bio-available and toxic when present in ionic form as they can bind to the ion-exchange site on the cell membrane and form a matrix with extracellular enzymes (Hickey *et al.*, 1989). Some heavy metals such as nickel and copper are necessary as nutrients at low concentrations (below $10^{-4}$ M) but toxic at higher concentrations (Angelidaki *et al.*, 2002). Codina *et al.* (1998) reported the relative toxicity of metals from an inhibition test on methanogenic activities as Zn<Cr<Cu<Cd<Ni<Pb. Normally heavy metals do not cause substantial problems in anaerobic digesters, since the ionic concentration is kept low due to sulfide and carbonate precipitation. A digester with high solids can tolerate higher amount of heavy metals (Hickey *et al.*, 1989).

Antibiotics are normally found in wastewater from pharmaceutical industries, domestic wastewater, and in manure from livestock feed additives. Normally, in manure based digestion, the antibiotics from feed additives do not cause inhibition if used at recommended levels in the animals (Nielsen, 2006).

Oxygen and nitrate are also inhibitory to methanogens. However, when present at existing concentrations, all oxygen and nitrate are quickly depleted by oxidation of readily available substrate or sulfide.

# 3. Monitoring parameters of the biogas process

Monitoring of anaerobic digesters is necessary to ensure successful operation. Since anaerobic digestion is a complex process involving several groups of microorganisms which are sensitive to many operating factors as discussed in Chapter 2, it is important to be able to detect process imbalance in the early stage so the action can be taken in time to prevent process failure. As with other biological processes, anaerobic digestion can be monitored by measuring substrate conversion (COD or VS removal), intermediates accumulation (VFA, pH, alkalinity, $H_2$, CO), product formation (gas production rate, $CH_4$, $CO_2$), microbial communities (populations, diversity), or microbial activities, as shown in Figure 5.

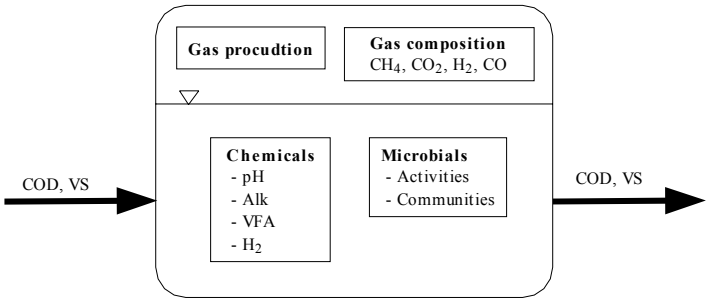



**Figure 5.** Monitoring parameters of the biogas process from liquid and gas phases

The ideal indicators should reflect the current process status and be easy to measure, preferably on-line (Switzenbaum *et al.*, 1990). Moreover, the responses of parameters to process imbalances should be significant compared to the background fluctuations (Ahring and Angelidaki, 1997). During the last 20 years, a number of parameters have been studied and suggested for anaerobic process indicators. The common indicators for monitoring of biogas process are gas production, gas composition, pH, alkalinity and volatile fatty acids (VFA) (Hawkes *et al.*, 1993; Ahring and Angelidaki, 1997). However, alternative parameters such as measurement of microbial activities and microbial communities are also gaining interest. These measures are as follows.

## 3.1 Biogas production

Biogas production is the most common parameter to be measured. It can be expressed in terms of rate (volume gas produced per unit time, e.g. $m^3$-gas/day), or yield (volume gas produced per unit feed, e.g. $m^3$-gas/ton-feed). It is an important parameter as it indicates overall performance of the process. However, it cannot be used for indicating process imbalance since the change in biogas production rate depends on hydraulic and organic loading rate, and the biogas yield also depends on feed composition, as discussed in Chapter 2.6.1. Moreover, it has low sensitivity to overloading compared to other process indicators, a decrease in biogas production is often occurred after the process is

severely inhibited or already broken down, thus, it is not an effective early warning indicator (Switzenbaum *et al.*, 1990; Moletta *et al.*, 1994).

## 3.2 Methane and carbon dioxide

The biogas is mainly consisted of $CH_4$ and $CO_2$. The ratio of $CH_4$ to $CO_2$ is normally stable in the reactor and a change of the ratio can be due to process imbalance. However, the methane ratio also depends on substrate composition, temperature, pH and pressure (Liu, 2003). Since the dissolution of $CO_2$ is strongly dependent on pH, fluctuation of pH can also change gas composition. A better indicator is therefore methane production, rather than gas-methane composition (Hansson *et al.*, 2002; Liu, 2003).

Methane production combines biogas production to the measurement of methane percent. The parameter can be expressed similarly to the biogas production, as rate or yield. Methane production rate (L-$CH_4$/day) has been successfully used as an online indicator for controlling a glucose-fed CSTR digester (Chynoweth *et al.*, 1994) and also recommended elsewhere (Hansson *et al.*, 2002; Liu, 2003). However, Ahring *et al.* (1995) has tested the use of methane production rate and methane yield (mL-$CH_4$/gVS) as a process indicator and showed that methane production rate also depended on reactor loading, not only the status of the process. The methane yield could reflect process imbalance but the change was relatively small. Moreover, they suggested that the use of methane yield alone was doubtful since it could recover again even though the accumulation of VFA continued. Similar to the biogas production, the methane response was significant only after the process imbalance was well developed (Switzenbaum *et al.*, 1990).

## 3.3 pH

pH is normally relatively easy to measure, and often the only liquid-phase parameter that is measured online. The change in pH can be both an indicator, and the cause of process imbalance, since the microorganisms can function only in a specific range of pH as discussed in Chapter 2.6.3. The pH of the feed can also affect pH in the digester. The use of pH as a process indicator is normally based on the fact that a pH drop corresponds to VFA accumulation. Some anaerobic systems apply pH monitoring and control where acid or base are added to ensure suitable pH for microbial growth. In a reactor with low buffering capacity and no pH control, VFA accumulation can decrease pH quickly, and pH is an effective process indicator. However, it is not recommended to use pH for indicating process imbalance in a well buffered system where the change of pH from VFA accumulation is often slow and too small (Björnsson *et al.*, 2000; Paper III). The high buffering capacity will resist pH change and the pH drop will often occur after the process is severely imbalanced (Mechichi and Sayadi, 2005; Nielsen, 2006). In manure digester the VFA could accumulate up to 100 mM while pH varied only 0.5 units. Accumulation of VFA caused by ammonia inhibition did not significantly change pH due to the buffering capacity offered by the high content of ammonia (Angelidaki and Ahring, 1994).

## 3.4 Alkalinity or buffering capacity

Alkalinity or buffering capacity is a better alternative than pH for indicating VFA accumulation, since the increased VFA will directly consume alkalinity before large pH changes. However, total alkalinity (TA) measured by titration of the sample to pH 4.3 was proved to be insensitive since the combination of VFA and bicarbonate results in a stable TA level (Hill and Jenkins, 1989; Hill, 1990; Björnsson *et al.*, 2001b). The partial alkalinity (PA) or bicarbonate alkalinity measured by titration the sample to pH 5.75 has empirical correlation to VFA accumulation (Hawkes *et al.*, 1994). However, this relationship is not observed during VFA accumulation in response to ammonia overload, as the ammonia adds alkalinity to the system (Björnsson *et al.*, 2001b). Other authors suggested the ratio of VFA/TA as an indicator where the healthy digester should have the ratio in the range of 0.1-0.35 (Switzenbaum *et al.*, 1990).

## 3.5 Volatile fatty acids

Volatile fatty acids (VFA) accumulation during process imbalance directly reflects a kinetic uncoupling between acid producers and consumers (Switzenbaum *et al.*, 1990). The VFA concentration has been most suggested for monitoring of anaerobic digester (Hill and Holmberg, 1988; Hickey and Switzenbaum, 1991c; Anderson and Yang, 1992; Moosbrugger *et al.*, 1993; Ahring *et al.*, 1995; Björnsson *et al.*, 2000; Lahav *et al.*, 2002; Feitkenhauer *et al.*, 2002; Mechichi and Sayadi, 2005; Paper III). In a low buffered system, pH, partial alkalinity and VFA measurements are useful for process monitoring whereas in highly buffered system only VFA is reliable for indicating process imbalance (Murto *et al.*, 2004). VFA is commonly measured by gas chromatograph (GC) with flame ionization detection (FID), for individual VFA, or titration to give total VFA, which is cheaper and widely used in commercial biogas plants. Several titration methods for determination of total VFA have been proposed, e.g. a simple titration (Anderson and Yang, 1992), a 5-point titration (Moosbrugger *et al.*, 1993), and an 8-point titration (Lahav *et al.*, 2002).

However, several studies have pointed out that individual VFA can give more important information as an early warning before process failure (Hill and Holmberg, 1988; Hill and Bolte, 1989; Cobb and Hill, 1991; Ahring *et al.*, 1995). The level of iso-butyric and iso-valeric acid had been suggested as an indicator of stress level in advance of process failure (Hill and Holmberg, 1988; Hill and Bolte, 1989; Cobb and Hill, 1991), while Ahring *et al.* (1995) suggested the concentration of n-butyric and iso-butyric as a better indicator. Propionic acid is known to be the most thermodynamically unfavourable. During process overload or stress conditions, increases in hydrogen partial pressure can thermodynamically affect the degradation of propionate before other VFA (Pauss and Guiot, 1993). Some authors used propionic acid as a sole process indicator (Renard *et al.*, 1991; Hansson *et al.*, 2002), while others suggested to use the variation in propionic:acitic acid ratio as an indicator for impending failure (Hill *et al.*, 1987; Marchaim and Carsten, 1993).

Although VFA is excellent for indicating organic overload and the toxic condition where acid consumers and methanogens are inhibited, the VFA response is

unclear under toxic stress where acid producers are also inhibited, for example, under high concentration of LCFA (Paper III).

## 3.6 Hydrogen

Hydrogen is important as both an intermediate and electron carrier in the digestion process. Hydrogen concentration affects thermodynamics and degradation pathway of the anaerobic degradation process as described in Chapter 2. High hydrogen concentration can inhibit volatile acids degradation, resulting in VFA accumulation. Thus, hydrogen accumulation has been suggested as an early stage indicator for process imbalance (Archer, 1986; McCarty and Smith, 1986; Collins and Paskins, 1987; Hickey and Switzenbaum, 1991b; Mathiot *et al.*, 1992; Steyer *et al.*, 2002b). It has also been reported as an effective parameter to indicate toxic inhibition from heavy metals during batch anaerobic sludge digestion (Hickey *et al.*, 1989).

However, since hydrogen is formed in the liquid phase, the sensitivity of hydrogen in biogas is also limited by the liquid-to-gas mass transfer rate (Pauss *et al.*, 1990a; Frigon and Guiot, 1995; Björnsson *et al.*, 2001a). Guwy *et al.* (1997) measured online hydrogen in the headspace of a fluidised bed digester treating baker's yeast wastewater and found that hydrogen concentration in biogas was not correlated to the propionic acid concentration. In contrast, Pauss and Guiot (1993) found that the concentration of dissolved hydrogen had good correlation with propionic acid. However, they found that dissolved hydrogen could not be predicted from gas measurements due to supersaturation of the liquid (Björnsson *et al.*, 2000). Dissolved hydrogen has been widely used as an indicator (Whitmore and Lloyd, 1986; Smolenski and Robinson, 1988; Pauss *et al.*, 1990b; Kuroda *et al.*, 1991; Krämer and Conrad, 1993; Strong and Cord-Ruwisch, 1995; Cord-Ruwisch *et al.*, 1997; Björnsson *et al.*, 2001a)

The sensitivity of hydrogen depends on several factors. Whitmore and Lloyd (1986) used dissolved hydrogen concentration as a control parameter to regulate glucose supply for a thermophilic digester and found that the accumulation of hydrogen depended on the input step size. The steady state level of hydrogen also depends on the type of reactors and wastes. Collins and Paskins (1987) found hydrogen partial pressure in the reactor headspace varied between 15-199 ppm in 20 mesophilic sewage sludge digesters under normal operation. Hickey *et al.* (1987b; 1989) found that when hydrogen partial pressure increased, the degradation pathway will shift to more reduced intermediate such as VFA, which decreases hydrogen production and thus limits the sensitivity of hydrogen. Voolapalli and Stuckey (2001) found that the predominance of formate production as an alternative electron acceptor under neutral pH (pH>7) could result in a strong dampening of the hydrogen response in the early stage of step shock load. Several authors concluded that hydrogen could not give early warning for process inhibition from gradual overload (Strong and Cord-Ruwisch, 1995; Cord-Ruwisch *et al.*, 1997; Guwy *et al.*, 1997; Voolapalli and Stuckey, 2001; Björnsson *et al.*, 2001b).

Hydrogen responds rapidly to an overload of readily degradable organics but it is insensitive to slowly degradable material since its response is generally dampened by solid hydrolysis (Hickey and Switzenbaum, 1991a; Kidby and Nedwell, 1991). Kidby

and Nedwell (1991) measured online hydrogen in the headspace of a CSTR digester treating sewage sludge. They found that hydraulic overload led to process failure where biogas production and methane yield were markedly decreased without significant change in hydrogen concentration. The increase of hydrogen was detected long after the failure had occurred. They concluded that it was due to high content of cellulose material in the feed where hydrolysis was the rate-limiting step and suggested that the use of hydrogen as an indicator was more suitable for reactors treating easily biodegradable wastes. The study in Paper III (Figure 6a) also found that the hydrogen response was low when the feed was added with particulate carbohydrate (fibre) and rapeseed oil, which are known to be slowly degradable (from point A), and the response was much higher when the feed was also added with glucose (from point B). This agreed with the results from Mosey and Fernandes (1989) where hydrogen accumulated significantly in a CSTR sewage digester after a glucose pulse.

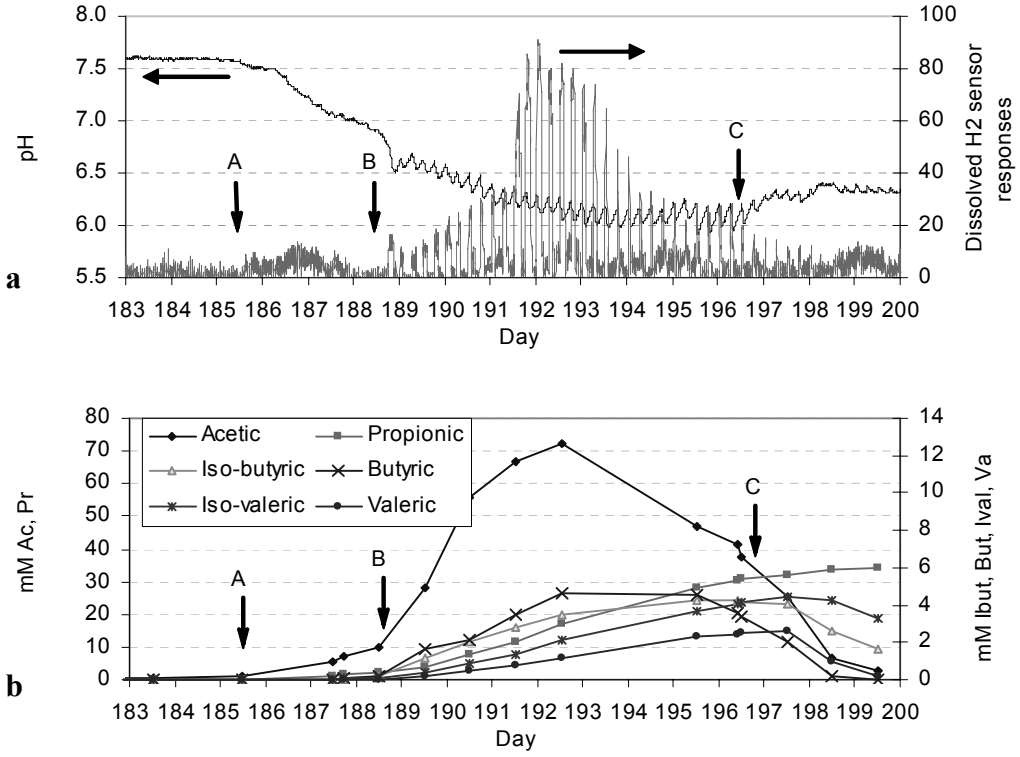



**Figure 6.** Dynamic response of dissolved $H_2$ and pH (6a) and VFA (6b) during an organic overload; left axis (6a): pH, right axis (6a): dissolved $H_2$ sensor response; day 183-185: normal operation, from point A: start adding fibre and rapeseed oil in each feed, from point B: start adding glucose in each feed, from point C: back to normal feed again (Paper III)

From Figure 6a hydrogen increased suddenly after each feed and reached the peak level after 30 min., corresponding to a small pH drop. The hydrogen level decreased again within a few hours while pH increased. The same result was found in the study of Kidby and Nedwell (1991), as shown in Figure 5. They explained that temporal increase in hydrogen concentration immediately after feeding was due to the utilisation of soluble, hydrolysed organic molecules initially present in the feed.

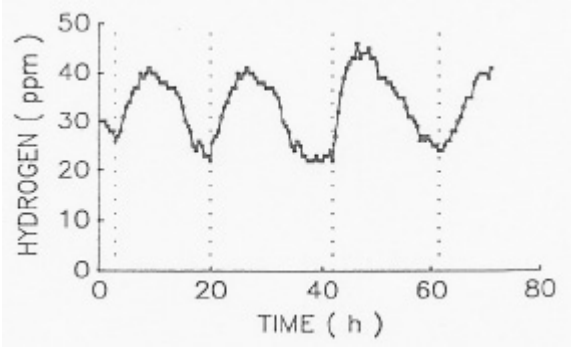



**Figure 7.** Variation of hydrogen concentration in the headspace biogas of the CSTR sewage sludge digester under normal condition at HRT 20 days with daily feeding. Dotted vertical lines indicate time of feeding (from Kidby and Nedwell, 1991).

Hydrogen is very sensitive to organic overload, but not retentive compared to VFA. As seen from Figure 6a and 6b, between point B and C, though the organic overload was still present, the hydrogen decreased rapidly, while acetate and butyrate decreased slowly. Propionate was the best indicator, as it persisted and increased even after the extra organic load was removed (point C).

In fact, rapid hydrogen variations can be a natural reponse to normal microbial activity, rather than an indicator of problems. Archer, (1986) found that hydrogen responded very fast to organic overload but also rapidly returned to normal concentrations without any VFA accumulation. The study in Paper III showed that even short exposure to air could increase dissolved hydrogen without any change of VFA or biogas. Switzenbaum *et al.* (1990) and Guwy *et al.* (1997) concluded that the variation in hydrogen concentration was a short-term event, with no correlation to other indicators, or reactor performance. Thus hydrogen was not recommended as a stand alone indicator, but rather in combination with other parameters, e.g. gaseous $H_2$ and alkalinity (Kaspar and Wuhrmann, 1978a; Kaspar and Wuhrmann, 1978b; Archer, 1989), gaseous $H_2$ and CO (Hickey and Switzenbaum, 1991a), gaseous $H_2$, pH and gas production (Moletta *et al.*, 1994), or dissolved $H_2$ as a complement to VFA monitoring (Björnsson *et al.*, 2001b).

## 3.7 Carbon monoxide

Carbon monoxide is a possible intermediate in the metabolic pathway of both acetogens and methanogens (Hickey *et al.*, 1989; Switzenbaum *et al.*, 1990) and it has been reported to be evolved during methanogenis from acetate (Hickey *et al.*, 1987a). Carbon monoxide was found in significant levels during toxic inhibition by heavy metals (Hickey *et al.*, 1989). It also showed good potential for indicating organic and hydraulic overloads in a sewage sludge digester. The level of gaseous carbon monoxide has been reported to be directly related to acetate concentration, and inversely related to methane concentration (Hickey and Switzenbaum, 1991a). However, the response of carbon monoxide could also be dampened by solids hydrolysis, in a similar manner to hydrogen (Hickey and Switzenbaum, 1991a), and further applications of carbon monoxide as a process indicator have not been found.

## 3.8 Organic matter reduction

There are many industrial applications in which the main purpose of anaerobic digestion is focused on treatment of organic waste instead of gas production. For this purpose, the organic removal, which is the different between the organic content before and after treatment, is an important parameter to be monitored. The organic removal in anaerobic digestion has been reported to be measured in terms of TS, VS, TOC, COD or BOD (Pind, 2001; Steyer *et al.*, 2002a; Liu *et al.*, 2003; Wang *et al.*, 2005). These parameters are suitable for monitoring of anaerobic digestion applied to the waste with soluble organic matter, such as high-rate systems. For solid and slurry wastes treated by suspended system where biomass are washed out with the effluent, it is difficult to distinguish between residue undigested particulate organics and the biomass from the reactor.

## 3.9 Microbial communities and activities

The monitoring of microbial community is based on the number of population and identification of organisms, especially archaea, relating to environmental conditions and performance (Dearman *et al.*, 2006), where activity measurement is focused on the status of microbial metabolisms (Sørensen and Ahring, 1993). The number of methanogenic populations can be directly counted and has shown to be related to methane production (Solera *et al.*, 2001). The attempt to measure biomass density using near-infrared spectroscopy (NIR) has also been reported (Nordberg *et al.*, 2000; Zhang *et al.*, 2002). Microbial identification can be done using fluorescence *in-situ* hybridisation (FISH) (Schmidt *et al.*, 2000). Measurement of microbial diversity and community structure can be done by the genetic fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE) and terminal restriction fragment length polymorphism (T-RFLP) (Muyzer and Smalla, 1998; Collins *et al.*, 2003). Microbial activities can be directly measured in batch tests such as specific methanogenic activity (SMA) (Sørensen and Ahring, 1993). Moreover, the level of specific co-enzymes relating to cell metabolism such as $F_{420}$ and NADH have also been used to correlate with microbial activities or the number of active organisms in the reactor (Peck and Chynoweth, 1992).

The monitoring of microbial communities and their specific activities are normally done for describing process behaviour, rather than for control purpose, since most of the methods are only available off-line, and require specialist equipment and expertise.

# 4. Online monitoring and control applications

One of the most focused topics in anaerobic digestion currently is online monitoring and control. The increase in number of large-scale biogas plants also increases the demand for proper monitoring and control of these systems (Ahring and Angelidaki, 1997). Monitoring and control systems are applied differently depending on the applications (Batstone *et al.*, 2004b). With online monitoring and control, process optimisation is possible through maximising the utilization of process capacity and minimising the lost from process failure. According to Switzenbaum *et al.* (1990), "while much progress has been made in anaerobic treatment technology, only through the development of better monitoring and control strategies will the anaerobic treatment process reach its full potential for waste management".

## 4.1 Online monitoring applications

Although gas composition is limited in sensitivity to overloads, gas phase monitoring still has the advantage that the sample is very clean compared to a liquid sample, which makes a gas sensor much simpler (Lantz and Leupold, 1998; Steyer *et al.*, 2002b). Biogas production is a standard online measurement in most biogas plants. Gas composition can be measured online using a GC coupled with an automatic gas sampling loop (Lantz and Leupold, 1998). Methane can also be measured by infrared analyser (Moletta *et al.*, 1994), and hydrogen can be measured by an electrochemical sensor (Exhaled Hydrogen Monitor GMI) (Moletta *et al.*, 1994).

In the liquid phase, pH is a very common on-line sensor (Batstone *et al.*, 2004b). Alkalinity and total VFA can be measured using online titration (Bouvier *et al.*, 2002; Feitkenhauer *et al.*, 2002). Another method for online measurement of alkalinity has also been reported by continuous measurement of the carbon dioxide flow rate evolved from a stream of sample solution after saturation with gaseous $CO_2$ and subsequent acidification with excess acid (Hawkes *et al.*, 1993; 1994).

The online measurement of dissolved hydrogen has been widely studied. Kuroda *et al.* (1991), and Strong and Cord-Ruwisch (1995) used a platinum black electrode for direct measurement of dissolved $H_2$ concentration. Others used membrane diffusion techniques to extract dissolved hydrogen and measurement with different sensors, such as mass spectrometry (Whitmore and Lloyd, 1986; Whitmore *et al.*, 1987), hydrogen/air fuel cell detectors (Pauss *et al.*, 1990b; Pauss and Guiot, 1993), hydrogen electrodes, gas chromatography (Smolenski and Robinson, 1988; Krämer and Conrad, 1993; Cord-Ruwisch *et al.*, 1997) and a pallidium-metal oxide semiconductor sensor (Björnsson *et al.*, 2001a)

Individual VFA has been measured online mainly based on sample preparation by filtration, and subsequent conventional analysis. Zumbusch *et al.* (1994) used an ultra-filtratioin module to purify the sample before analysis by HPLC. Slater *et al.* (1990) and Ryhiner *et al.* (1993) used membrane filtration followed by GC analysis. Both applied the system on the soluble substrate. Only Pind *et al.* (2003a) has attempted to measure VFA online in particulate sample such as manure. The system contained three step filtration; pre-filtration by a rotating filter inside the reactor, ultra filtration by

a membrane cartridge, and a mini-filter for final purification, followed by GC analysis through automatic injection. Moreover, there have also been reports for online measurement of acetate and propionate by near-infrared spectroscopy (NIR) (Nordberg *et al.*, 2000; Hansson *et al.*, 2002). In this research work, a new online VFA monitoring system was also been developed using ex-situ VFA extraction from the liquid sample followed by automatic gas injection into the GC (Paper I, II).

Total VFA has also been estimated from online measurement of other parameters such "readily biodegradable COD" or biological oxygen demand (BOD), measured by biosensor based on microbial respiration (Liu *et al.*, 2004b), a denitrifying biosensor (Rozzi *et al.*, 1997), or the use of an empirical model to estimate VFA from online-pH measurement (Münch and Greenfield, 1998). TOC has been measured online using oxidation by ultraviolet light coupled with an infrared detector (Moletta *et al.*, 1994). Infra-red spectrometry (IR) has been reported for simultaneous online measurement of TOC, COD and total VFA (Steyer *et al.*, 2002a).

Microbial communities or activity has been correlated to co-enzymes such as $F_{420}$ and NADH which can be online measured by fluorescent analysis (Peck and Chynoweth, 1992). Nordberg *et al.* (2000) used near-infrared spectroscopy (NIR) for measuring phospholipid fatty acids correlating to the biomass density.

## 4.2 A new online VFA monitoring system

Most of the online VFA monitoring systems available are based on filtration for sample preparation, which suffers from fouling, and require extensive maintenance. The objective of this Ph.D. project was to develop a new technique for online measurement of VFA by gas extraction (Paper I, II). Since gas extraction avoids membrane fouling, thus it has a great potential to deal with high solids environments such as manure slurries. In this work, an online VFA monitoring system has been developed based on headspace gas chromatography (HSGC) technique. HSGC has been widely used for analysis of volatile compounds in liquid and solid samples (Seto, 1994). There have also been reports on applications of HSGC method for offline VFA analysis, including static HSGC (Cruwys *et al.*, 2002) and dynamic HSGC (Ábalos *et al.*, 2000). Static HSGC involves the equilibration of liquid or solid sample in a closed vial at high temperature to extract VFA into gas phase, and injection of headspace gas with a gas tight syringe into the GC. Dynamic HSGC is done by continuous purging of the liquid or solid sample with an inert gas through the headspace or sparging into the liquid. The volatiles are then trapped in an adsorbent and released under high temperature for GC measurement. In this thesis, an online VFA system was developed from static HSGC. However, my method has a unique characteristic that a variable headspace volume is used for gas extraction under atmospheric pressure, rather than constant headspace volume as in the static HSGC.

The main principle of operation is extraction of the VFA in the liquid sample by *ex-situ* stripping and subsequent analysis by GC-FID (gas chromatography-flame ionization detection). The method consists of the following; (a) 40 mL of liquid sample from a recirculation loop to a sampling cell, (b) acidification with 4 mL of 34% phosphoric acid ($H_3PO_4$) to obtain final pH<2, (c) adding 2 mL of sodium hydrogen

sulfate salt ($NaHSO_4$), (d) heating to 75 $^oC$, (e) gas sampling with a frictionless glass syringe using a stepper motor for gas injection and 5-mL gas sampling loop for volume control, (f) gas injection and analysis in GC-FID, and (g) sample ejection, cleaning and flushing of sampling system. The details of system construction and implementation are described in Paper II. The GC responses from online system were calibrated against the VFA concentration directly measured from the liquid by GC and have shown linear correlation in the application range of VFA in manure digester (Figure 8).

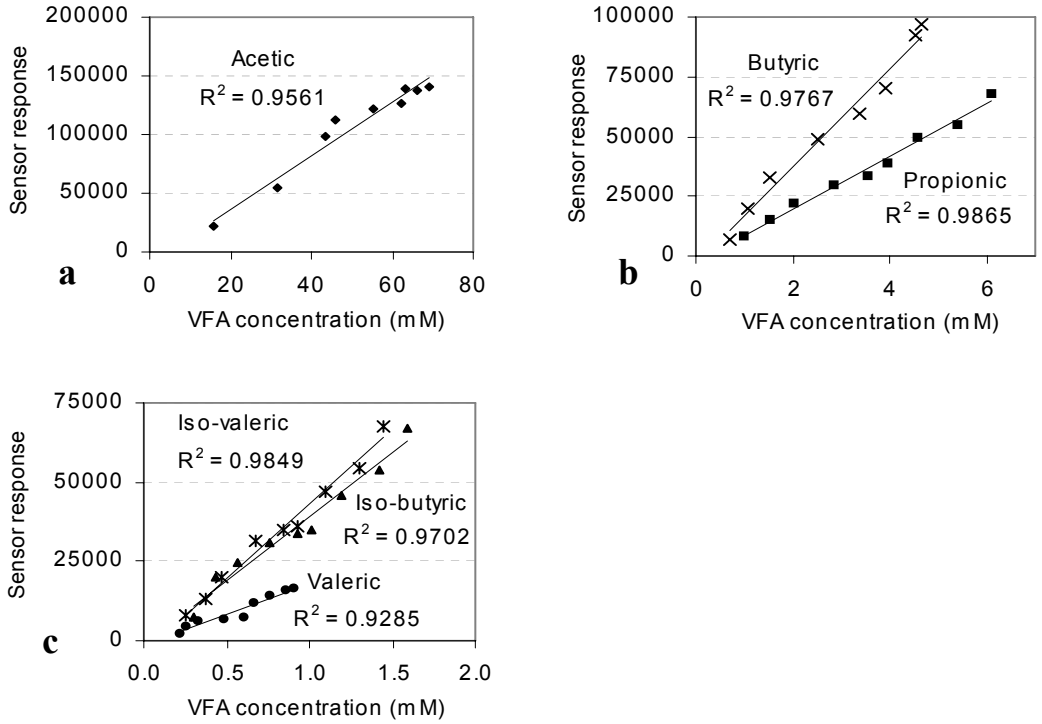



**Figure 8.** Correlation of the sensor response from online measurement and the VFA concentration from offline analysis; (a) Acetic acid, (b) Propionic and Butyric acid, (c) Isobutyric, Isovaleric and Valeric acid

The system takes the reactor sample from the liquid circulation loop, which makes it able to work with any kinds of reactor configurations. One sensor can be connected to several reactors by using the liquid circulation loop to transfer the fresh sample to the system. In the present sensor design, all parts, except for GC machine, can be assembled and maintained using simple techniques. The sample cell can be automatically cleaned by caustic solution during a wash cycle. The gas tubes can be operated for many months before any cleaning requirements. No equipment parts in the system are consumable except for chemicals and gases for the GC. The GC maintenance is also minimised because gas samples are much cleaner than liquid sample. The sample volume of 40 mL can easily be sourced from a full-scale reactor. The sample and analysis time is from 25-40 min. depending on the washing duration, which is suitable for full-scale biogas reactors since the VFA dynamic is in the range of several hours.

## 4.3. Process control and optimisation

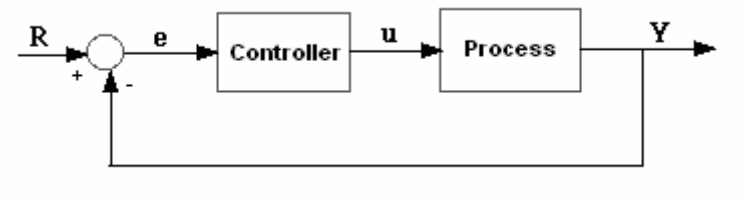
Process control has been mainly applied in high-rate digesters treating industrial wastes with mostly soluble organics, and has generally not been applied in municipal sludge, or solids/manure digesters (Batstone *et al.*, 2004b). However, solids and slurry digesters have potential to be improved since significant methane potential is lost with the effluent in the form of VFA and other undigested organics.

The main components in a control system are process output variables, manipulated variables, disturbances, and control approach (Pind *et al.*, 2003c). The process output variables are monitoring parameters that can indicate process status resulting from process control. Process variables could be monitored online (Puñal *et al.*, 2003), or offline if slower control action is applied (Renard *et al.*, 1991). However, the parameters should be available online if automatic control will be applied. Most of the monitoring parameters suggested for the biogas process have been described in Chapter 3 with the online monitoring applications in Chapter 4.2 and 4.3. The manipulated variables are process parameters that can be regulated, and have effect on the process evolution. The manipulated variable can be adjusted manually by the operator, e.g. feed rate and mixing intensity, or automatically by another control, e.g. pH and temperature (Pind *et al.*, 2003c). The manipulated variables should have a range of operation to facilitate refined control, rather than on-off control. The disturbances are process parameters or external parameters that cannot be regulated, but also have effect on the process evolution, e.g. COD in the feed and inhibiting compounds concentration. (Pind *et al.*, 2003c).

The aim of process control in anaerobic digestion is not only to ensure digester stability, but also to enhance process performance (Pind *et al.*, 2003c). The control type can be divided into open-loop and closed-loop control. Open-loop control is when the process is controlled after a control program without taking the monitoring parameters into account, for example, using timer program. Close-loop control is when the process is regulated after the control program where the monitoring parameters are taken into account (Liu, 2003). Since all advanced control applications are closed-loop control, thus it will be focused here. The closed-loop control can further divided into feedback and feedforward system. In feedback control, the process output variables are used as an input for control compilation and the manipulated variable are regulated to achieve the desired value of output variables. In feedforward control, the disturbances are measured as control-function input and the manipulated variables were then regulated to suppress the effect of disturbance before entering the process.

There are many control approaches applied in anaerobic digestion. The complexity of process control increases with the diversity of the control objectives, ranging from simple controller (e.g. on/off, PID), to the complicated adaptive control or artificial intelligence control schemes (e.g. fuzzy, knowledge based, and neural network controllers) (Pind *et al.*, 2003c). The on/off control is straightforward. A manipulated variable is turned on or off if a process output variable exceeds a set point. This control approach is simple but the results may be very fluctuating, since it does not take into account the offset between process output and the setpoint (Liu, 2003). The proportional-integral-derivative (PID) controller is a combination of three terms with

one parameter for every term, i.e. proportional gain ($K_P$), integral gain ($K_I$), and derivative gain ($K_D$), as shown in Figure 8. The proportional (P) action is a linear proportion of control signal (u) and the tracking error (e), which is the difference between process output (Y) and setpoint (R). It can reach steady state in the absence of disturbances but can result in offset. The integral (I) action summarizes the errors and compensates for offset but can increase overshoot signal on startup. In derivative (D) action the control signal is proportional to the derivative (or slope) of the error which will speed up the control action when rapid load changes take place, thus minimize overshooting (Liu, 2003).




$$u = K_P e + K_I \int e dt + K_D \frac{de}{dt}$$

**Figure 9.** PID control diagram

One example of on/off control was used during startup and operation of a UASB reactor treating petrochemical wastes containing short chain fatty acids where the feed pump was turned on or off according to the pH setpoint (Pretorius, 1994). Another empirical control application was used by Denac *et al.* (1990), where the effluent quality expressed in total acids concentration was successfully controlled by regulating feed rate and using alkaline consumption (for maintain pH at 7) as the controlled variable. The PID controller has been used by Marsili-Libelli and Beni (1996) to maintain the bicarbonate alkalinity in an anaerobic filter by manipulating the bicarbonate dosing ($NaHCO_3$). In Paper IV, a simple logic control has also been applied to control the level of propionate in a CSTR manure digester.

However, the bioprocess is nonlinear, highly dynamic, and the process characteristic itself can change over time. The parameters in the PID control are dependent on the desired state of the process and may need to be tuned after setpoint changes. Alternatively, a more advanced controller such as adaptive control, which is non dependent on a linear assumption, or can change parameters, may be preferable. This control approach is able to estimate the model parameters from the process output parameters based on the given process model and the control objective. The model is then used to determine the control signal. The success of an adaptive controller is dependent on the ability to predict the response of the process. However, it does not necessarily require a complex model. The advantage of using a model based control is that it can give better understanding of the process behavior (Pind *et al.*, 2003c). An example of adaptive control application is seen in Renard *et al.* (1991). By steering the dilution rate, the propionate concentration was kept constant during startup and steady-state running of a pilot-scale completely mixed digester treating spent liquor from citric acid fermentation. Bernard *et al.* (2001) successfully used a model-based adaptive linearising controller to control the ratio of intermediate alkalinity to total alkalinity,

and the level of total alkalinity, by regulating feed flow in a pilot-scale anaerobic filter treating winery wastewater.

Fuzzy logic is generally a ruled-base logic controller where the rules are based on simple logic with interval value (0-1), rather than just on (1) or off (0). The complexity is increasing with increasing number of rules and number of variables (Pind *et al.*, 2003c). Bernard *et al.* (2001) had also used fuzzy control with two control variables (the ratio of intermediate alkalinity to total alkalinity and the level of total alkalinity), to regulate feed flow in a pilot-scale anaerobic filter treating winery wastewater. Puñal *et al.* (2003) successfully used fuzzy control to control the VFA concentration by manipulate feed flow in the same system as Bernard *et al.* (2001).

Neural networks have an advantage that they can map the non-linear relationship between the input-output pairs without requiring the knowledge process kinetics model (Pind *et al.*, 2003c). The processing units are normally arranged in layers where the first layer receives a number of input signals, summarizes, and send the produced outputs further to the next layer and so on. The final result is then produced in the output layer through an output function. This approach has been successfully used for control methane production in a lab-scale sewage sludge digester where several parameters were measured for model calibration, i.e. feed rate, pH, total VFA, acetate, and propionate concentration (Holubar *et al.*, 2002). More advanced control can also be done with combination of different control approaches, for example, Steyer *et al.* (1997) combined the fuzzy logic and artificial neural networks for online diagnosis of reactor where several process parameters (i.e. pH, temperature, recirculation flow, feed flow and gas flow) are measured.

Application of control has been used for optimising biogas production. Liu *et al.* (2004a) used a cascade PID controller embedded into a rule-based supervisory system to control biogas production using pH as an overload alarm. The gas flow setpoint was automatically reset according to the defined rules for optimising the process performance where biogas is maximized without overloading. The concept of this method was similar control approach proposed by Steyer *et al.* (1999) with slightly different control functions. The concept was to add disturbance on purpose in the feed flow, then analyse the response compared to the expected one to decide if the feed flow should be increased or not. By this way, the disturbances from substrate concentration will be rejected.

# 5. Improving biogas production

In large-scale biogas plants, maximisation of energy yield in relation to treatment cost is being prioritised (Angelidaki *et al.*, 2005). Process optimisation through better monitoring and control is one of the ways to improve process efficiency, as described in Chapter 4. Other ways can be through improving process design, pre-treatment of the substrate or the removal of toxic components, which will be reviewed as following.

## 5.1 Improving process design

### *5.1.1 Increase biomass retention*

Increasing biomass retention helps improve process efficiency and stability and is the key aspect of high-rate anaerobic systems (Björnsson, 2000). A dense packing of microorganisms is essential for an efficient interspecies hydrogen transfer, a process that plays a key role in methanogenesis (Stams *et al.*, 2005). In CSTR systems, the traditional method for biomass retention is to have a sludge sedimentation unit and re-circulate the biomass back into the reactor. Other methods include increasing hydraulic retention time or improving mixing pattern, for example, by switching off the stirrer half an hour before and after substrate addition to increase biomass retention due to enhanced sedimentation (Hansen *et al.*, 1999b).

### *5.1.2 Improve reactor configuration and operation*

Increasing temperature can allow the most significant improvement to biogas production due to several effects as described in Chapter 2.6.2. In Denmark, there is an increasing number of large-scale biogas plants that are switching to thermophilic condition (Nielsen, 2006).

For slowly degradable substrates, application of two-phase CSTR systems where thermophilic acidogenesis step with short HRT is followed by the mesophilic methanogenesis step with long HRT can increase biogas production due to improving hydrolysis and also improving pathogen reduction (Oles *et al.*, 1997; Huyard *et al.*, 2000). The two-phase configuration of extreme thermophilic ($68^{o}$C) followed by thermophilic ($55^{o}$C) has also been shown to improve biogas production compared to a single thermophilic CSTR for digestion of cattle manure (Nielsen *et al.*, 2004). However, Mtz.-Viturtia *et al.* (1995) reported that a single CSTR could also achieve nearly the same yield as the two-phase system for the easily degradable substrate.

Another configuration that has been studied in this research is the serial-CSTR configuration with a large main digester followed by a small post digester. This configuration makes it possible to run the main reactor with high loading rate and still ensure low VFA concentration in the effluent from the second reactor (Paper VI). Increasing the load in the main reactor also increases microbial activities according to Monod kinetics. This was demonstrated in Paper VIII using computer model which showed that increasing substrate concentration resulting in improving biogas production rate. Moreover, in the full-scale biogas operation with effluent storage, the extra biogas yield can also be recovered from the effluent storage (Tafdrup, 1994), and the study in

Paper VII showed that increasing the temperature of the effluent storage to 55 $^{o}$C can recover extra biogas more than 10%.

## 5.2 Pre-treatment of substrate

Another way to increase biogas production is to increase the biodegradability by pre-treatment of the substrate such as domestic sludge, solid waste, and most of the agricultural with high content of cellulose and hemi-cellulose (Ahring and Angelidaki, 2000). Since decreasing particle size of cellulose material helps increase hydrolysis and fermentation rate (Hu *et al.*, 2005), pre-treatment of domestic sludge is widely applied, such as mechanical destruction, heat treatment, or chemical treatment by $H_2O_2$ or $O_3$ (Dohányos *et al.*, 1997; Neyens and Baeyens, 2003). For solid organic wastes, the common pre-treatment method is to use hydrolysis reactor to liquefy the substrate before feeding into methanogenic reactor (Scherer *et al.*, 2000; Pavan *et al.*, 2000). Pre-treatment of the manure by thermophilic aerobic condition has also been reported (Pagilla *et al.*, 2000). The separation of lignin and cellulose out from manure waste can also obtain higher biogas yield per volume feed inlet (Møller *et al.*, 2004), or the use of anaerobic acidogenic stage in the two-phase system as described above can also apply as a pre-treatment stage.

## 5.3 Removal of toxic component

In many cases where toxic compounds are present in the feed, the process can operate in a so-called inhibited steady-state, which means stable operation, but with low biogas yield. Biogas production can be improved by removing the toxic/inhibitory compounds. The level of sulphate/sulphide can be decreased by ferric chloride ($Fe_2Cl_3$) or ferrous chloride (FeCl) to precipitate sulphate/sulphide in the form of ferrous sulphide (Tafdrup, 1994; Hansen *et al.*, 1999b). Yamaguchi *et al.* (1999) reported the use of a sulfide stripping device incorporated to a UASB reactor to alleviate sulfide inhibition in treating sulphate-rich wastewater. Ammonia level can be decreased by free-ammonia stripping (Siegrist *et al.*, 2005). The effect from ammonia inhibition can be counteracted by decreasing pH or by co-digestion with other compounds. Addition of bentonite which contains $Ca^{2+}$ and $Na^{+}$, or bentonite-bound oil was reported to help stabilisation of the process with high ammonia concentration (Angelidaki and Ahring, 1993). The adsorption of LCFA compound on the bentonite or activated carbon has also been reported to decrease the problem of LCFA inhibition (Angelidaki *et al.*, 1990). Antibiotics can be removed by flocculation before entering a UASB reactor (Deng *et al.*, 1998).

## 5.4 Co-digestion

Co-digestion of manure and organic wastes has proved to be very successful way for improving biogas production according to the Danish experience (Ahring *et al.*, 1992; Tafdrup, 1994). Since manure has quite low methane yield, typically from 10-20 $m^3$ $CH_4$/ton of manure treated, co-digestion of manure with easily degradable organic waste will significantly improve biogas production. Economic analysis of the Danish biogas plants revealed that the operation will be economically when the average biogas yield is

higher than approx. 30 $m^3$ biogas per $m^3$ biomass treated, which normally requires a 25% organic waste ratio. However, the lower ratio may be sufficient if concentrated wastes are available (Gregersen, 2003). Another advantage of co-digestion is that the high water content in manure helps dilute the concentrated organic waste which would be inhibitory and difficult to treat separately. Moreover, a high buffering capacity in manure makes the process more resistant to the effect from VFA accumulation (Angelidaki and Ellegaard, 2002).

# 6. Concluding remarks

Anaerobic digestion is a complex process and sensitive to changes in environmental conditions. The understanding in process behavior is important for proper reactor operation, as well as to be able to diagnose and solve process problems. Online monitoring has received increasing attention, as it is a necessary requirement for process control and consequently improved process performance and better economy of the biogas plants. A good online monitoring should be based on reliable indicators. The system should be robust, with low maintenance requirement, and low cost compared to the total investment and operating costs of the plant. The online information can also be an excellent aid to support decision making and problem solving for the plant operators.

As conclusion, the main outputs of this project can be summarized as follows.

- A novel online VFA monitoring system has been developed. It is based on extraction of VFA from a liquid sample by *ex-situ* stripping and subsequent analysis by GC-FID. To design the system prototype, a set of batch experiments were carried out and the optimal conditions for VFA extraction were determined (Paper I). The system was constructed and was validated successfully for a period of longer than 6 months. Good agreement with the offline VFA measurements was shown (Paper II). The system is based on a simple concept, which allows easy application to solids and slurry samples such as manure. Sample and analysis time of the system varies from 25-40 min. depending on the washing duration. The sampling frequency was proven to be suitable for studying process dynamics of manure digesters, which are usually operated with retention times of several days and have dynamic behaviour in the range of several hours.
- Response from the online VFA sensor was compared with other online process parameters such as biogas production, pH and dissolved hydrogen during overload situations in a lab-scale manure digester, to evaluate the suitability of these parameters as process indicators (Paper III). VFA was shown to be most reliable for indicating process imbalance, and propionate was most persistent. Biogas production could well indicate total process performance, but could not indicate stress conditions of the process. Hydrogen was sensitive to overload of easily degradable organics, but the response was not retentive compared to VFA. pH was not suitable as process indicator due to the high buffer capacity of manure.
- The online VFA sensor coupled with a simple control was tested for automatic control of propionate level in a lab-scale manure digester by regulating feed rate (Paper IV). It was found that decreasing feed rate resulted in decreased biogas production before propionate decreased, as propionate was very persistent. This caused high fluctuations in the biogas production. Thus, it was recommended to optimise the process using biogas production as the main control parameter and combine the use of VFA (or propionate) as a warning indicator in control algorithm.
- Effect of operating conditions and reactor configuration on efficiency of Danish centralised biogas plants was also investigated (Paper V). It was found that

ammonia affected the methane yield of the process. High ammonia concentrations in the reactor resulted in lost methane production. The lost methane potential could be retrieved from the digester effluent by post digestion. The amount of methane recovered in post digestion was found dependent on temperature.

- Serial CSTR digestion for improving biogas production was investigated both in lab-scale experiments (Paper VI), and by using the ADM1 computer model (Paper VIII). It was found that the serial CSTR configuration with long retention time in the first reactor and short retention time in the second reactor could improve biogas production from manure and could improve effluent quality in terms of VFA concentration, compared to a conventional single CSTR reactor. Moreover, the effect of temperature on the second reactor of the serial CSTR configuration was also investigated (Paper VII). It was found that the biogas recovery at low temperature (15 $^{o}$C) was very inefficient. The second reactor that operated at the same temperature as the first reactor (55 $^{o}$C), was running very stable with high biogas recovery which constituted up to 16% of total biogas production. Moreover, an enrichment of methanogens in the second reactor could be observed.

Further improvement of the online VFA monitoring system can still be done. For the application on samples with low VFA, the sensitivity of the system could be improved by increasing sample volume and optimised mixing for better gas transfer. Additionally, the sensor response can be increased by increasing gas sampling loop volume. The concept of the system is general and thus the method could be applied to different applications with different optimised VFA extraction conditions.

Improving monitoring and control, together with improving process design and operation can help the anaerobic digestion process become more reliable and economical. Hopefully, this study can contribute to an increase of interest in application of anaerobic digestion for waste treatment and bioenergy production as a sustainable and profitable technology.

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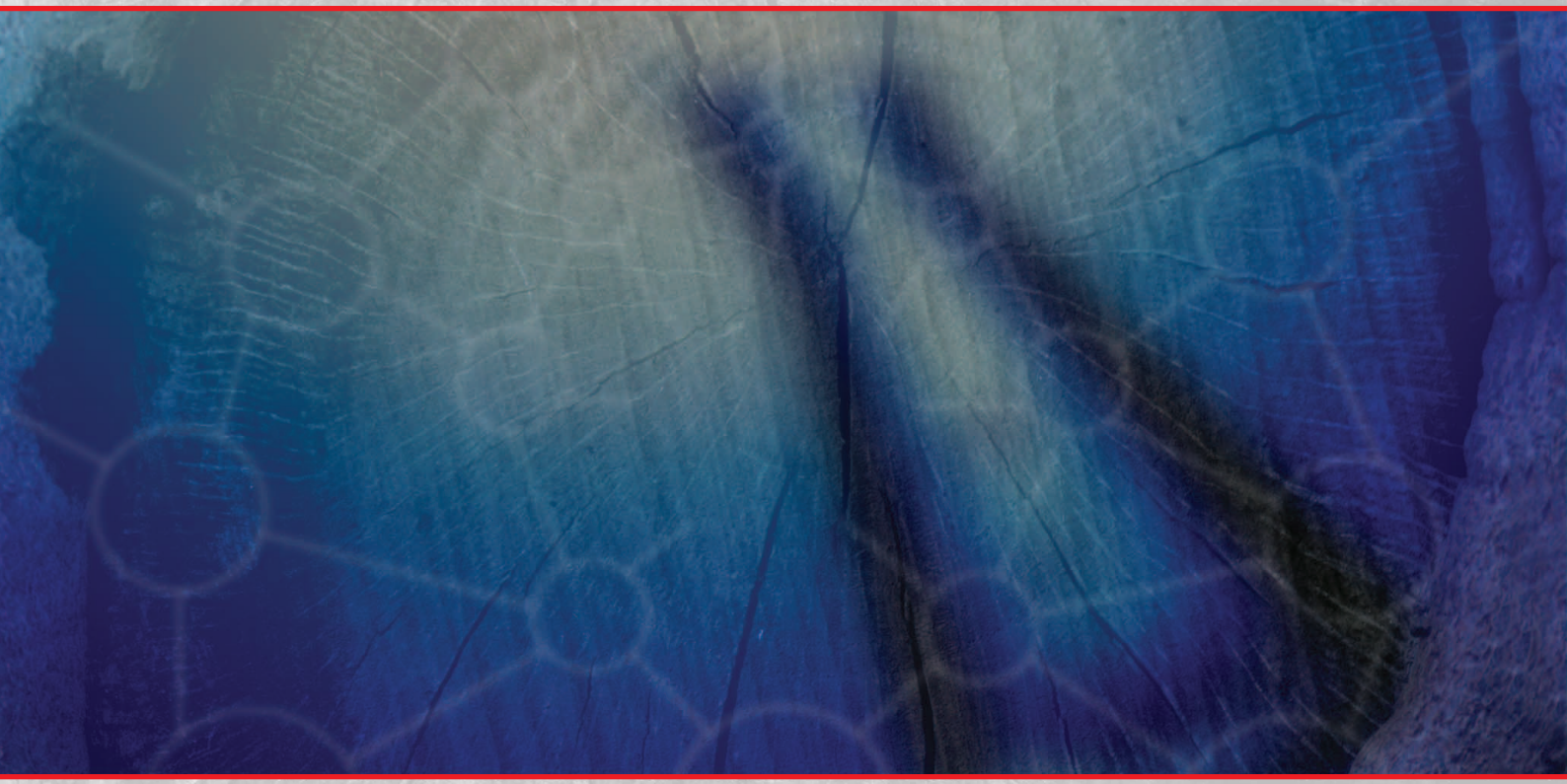

**Institute of Environment & Resources**
Technical University of Denmark
Bygningstorvet, Building 115
DK-2800 Kgs. Lyngby

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@er.dtu.dk

Please visit our website www.er.dtu.dk

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