Anaerobic Treatment of Agricultural Residues and Wastewater - Application of High-Rate Reactors

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Application of High-Rate Reactors

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ABSTRACT

The production of methane via anaerobic digestion of agricultural residues and industrial wastewater would benefit society by providing a clean fuel from renewable feedstocks. This would reduce the use of fossil-fuel-derived energy and reduce environmental impact, including global warming and pollution. Limitation of carbon dioxide and other emissions through emission regulations, carbon taxes, and subsidies on biomass energy is making anaerobic digestion a more attractive and competitive technology for waste(water) management.

This thesis is concerned with some important aspects of anaerobic digestion of solid potato waste, sugar beet leaves and opaque beer brewery wastewater. Studies were performed using batch, one-stage and two-stage processes using laboratory-, pilot- and full-scale anaerobic reactors. For improved understanding of the anaerobic digestion of solid potato waste, some of the aspects investigated in this work were the profiles of hydrolytic enzymes, the distribution of the major volatile fatty acids produced in the acidification stage, the organic matter degradation, methane yield and the effect of co-digestion.

During the hydrolysis of solid potato waste, both free and cell-bound hydrolytic enzyme activity was observed; amylase activity was found to be the highest followed by carboxylmethyl cellulase and filter paper cellulase. The fermentation products during batch anaerobic digestion of solid potato waste were chiefly acetic, butyric, propionic and lactic acid. The concentration and proportions of individual volatile fatty acids in the acidogenic stage are important in the overall performance of the anaerobic digestion system since acetic and butyric acids are the preferred precursors in methane formation.

The performance of two-stage anaerobic digestion systems under mesophilic and thermophilic conditions showed that the digestion period for solid potato waste was shorter under thermophilic conditions than under mesophilic conditions; the concentrations of volatile fatty acids in the effluent of the second-stage thermophilic upflow anaerobic sludge blanket (UASB) reactor were no different from those in the effluent from the mesophilic UASB reactor.

High rate reactors (packed-bed reactors with plastic and straw as biofilm carriers and a UASB reactor) were found to perform well during methanogenesis in two-stage anaerobic digestion of solid potato waste. The UASB performed better than the packed-bed reactor with plastic carriers. Straw, a common agricultural by-product, was confirmed to work well as a biofilm carrier.

Employing efficient but low-cost technology is important for increased utilisation of anaerobic digestion, and the possibility of doing this has been demonstrated by a simple pilot-scale, two-stage anaerobic digestion system for the treatment of solid potato waste and sugar beet leaves, alone and combined, with the recovery of biogas. Co-digestion of solid potato waste and sugar beet leaves improved the methane yield by 60% compared with that from digestion of the separate substrates in both batch and pilot-scale studies. Results from this work suggest that potato waste and sugar beet leaves are suitable substrates for anaerobic digestion giving high biogas yields, and could provide additional benefits to farmers.

The performance of a full-scale UASB reactor treating opaque beer brewery wastewater investigated over a period of two years enabled the brewery to meet the requirements for wastewater discharged into the municipal sewage system of Harare, Zimbabwe. The installation of a high-rate reactor by the brewery is an attractive economic and environmental alternative considering that an era of critical energy shortage, substantially higher energy prices and high demand on environmental protection lies ahead.

CONTENTS

THE PAPERS

This thesis consists of the present summary article and the following papers, referred to by their Roman numerals (**I-VII**) in the text. Paper **IV** was reprinted with permission from Elsevier and the others were published by kind permission of the journals concerned.

I Parawira W., Murto M., Read J.S. and Mattiasson B.
Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste.
Submitted for publication.

II Parawira W., Murto M., Read J.S. and Mattiasson B.
Volatile fatty acid production during anaerobic mesophilic digestion of solid potato waste.
Journal of Chemical Technology and Biotechnology (2004) 79: 673-677.

III Parawira W., Murto M., Read J.S. and Mattiasson B.
A study of two-stage anaerobic digestion of solid potato waste using reactors under mesophilic and thermophilic conditions.
Submitted for publication.

IV Parawira W., Murto M., Zvauya R. and Mattiasson B.
Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves.
Renewable Energy (2004) 29: 1811-1823.

V Parawira W., Read J.S., Mattiasson B. and Björnsson L.
Energy production from agricultural residues: high methane yields in pilot scale two-stage anaerobic digestion.
Submitted for publication.

VI Parawira W., Murto M., Zvauya R. and Mattiasson B.
Comparison of the performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate.
Submitted for publication.

VII Parawira W., Kudita I., Nyandoroh M.G. and Zvauya R.
A study of industrial anaerobic treatment of opaque beer brewery wastewater in tropical climate using a full scale UASB reactor seeded with activated sludge.
Process Biochemistry (2005) 40:593-599.

This thesis is concerned with some important aspects of anaerobic digestion based on research carried out using laboratory-, pilot- and full-scale anaerobic high-rate reactors. The maximum methane yield, the maximum organic loading rates for stable operation and other parameters of importance in the anaerobic process were investigated.

Papers I and **II** are concerned with the hydrolysis and acidification stage of anaerobic digestion giving the profile of hydrolytic enzyme activities during hydrolysis of solid potato waste and the volatile fatty acid production during the anaerobic digestion.

Papers III, V and **VI** report on comparisons of the performance of various types of high-rate reactors during two-stage anaerobic digestion of solid potato waste. In **Papers IV** and **V** the effect of co-digestion of solid potato waste and sugar beet leaves in batch and pilot-scale reactors, respectively, is described.

In **Paper VII**, industrial anaerobic treatment of opaque beer brewery wastewater using a full-scale upflow anaerobic reactor seeded with activated sludge in a tropical climate was investigated.

1 INTRODUCTION

Millions of tons of solid waste are generated each year from municipal, industrial and agricultural sources. Unmanaged organic waste fractions from farming, industry and municipalities decompose in the environment, resulting in large-scale contamination of land, water and air. These wastes not only represent a threat to environmental quality, but also possess a potential energy value that is not fully utilised despite the fact that they are cheap and abundant in most parts of the world. Methane (CH_4) and carbon dioxide (CO_2) emitted as a result of microbial activity under uncontrolled anaerobic conditions at dumping sites are released into the atmosphere and contribute to global warming (Baldasano and Soriano, 2000; Chynoweth *et al.,* 2001).

The Kyoto Protocol of 1997, signed by more than 60 countries (Morrissey and Justus 1999), calls for specific steps to be taken by the different parties involved. The developed nations (which contribute approximately 80% of global greenhouse gas emissions) that signed the protocol are committed to reducing CO_2 equivalents by an average of 5.2% by 2008-2012, compared with 1990 emissions. It is expected that changing the technology of waste treatment could lead to a substantial reduction in greenhouse gases with relatively low marginal costs and within a short time. Controlled anaerobic digestion of waste will also produce considerable amounts of methane that can replace fossil fuels, thereby reducing CO_2 emissions. Compared to other fuels, methane produces less atmospheric pollutants and CO_2 per unit energy and as a result it is being increasingly used for appliances, vehicles and power generation. Better waste management will also lead to other environmental benefits, such as reduction of surface water and groundwater contamination, transformation of organic waste into high-quality fertiliser, preventing waste of land and resources (Ayalon *et al.,* 2001; Francese *et al*., 2000; Kashyap *et al*., 2003).

Under modern environmental regulations, organic waste is becoming difficult to dispose of using traditional means. Recent legislation in the United States and European Union is forcing member countries to reduce the amount of biodegradable organic waste entering landfills (EU, 1996). Disposal of solid waste into domestic sewers is becoming less favourable because of increased sewer charges and the reluctance of municipal sewage treatment plants to accept these waste streams, which have high concentration of biodegradable organic matter. The greater part of crop residues on farms throughout the world is ploughed back into the soil after harvest, where microorganisms degrade them. As a result, nutrients are released which contribute to the eutrophication of lakes and watercourses. On the one hand, we have a problem caused by municipal, and agro-industrial waste, which is disposed of in the environment, and on the other hand, we have an energy crisis, which should be met by sustainable and cleaner technologies. Economy and technologies today largely depend upon energy resources that are not renewable. It is therefore necessary to identify and develop alternative sources of energy that are sustainable.

1.1 Biodegradable waste(water) treatment options

Waste can be treated by several means, such as controlled landfilling, composting, incineration, anaerobic degradation and recycling. Each method has its own advantages and disadvantages and areas of application.

Landfilling of solid waste represents the most widespread method of solid waste disposal in the world. Landfills are responsible for approximately 8% of anthropogenic methane emissions (Wang *et al*., 1997). Landfill gas contains roughly 50%-60% CH_4 and 40%-50% CO_2 and the decomposition of each metric ton of solid waste could potentially release 50-110 m^3 of carbon dioxide and 9-140 m^3 of CH_4 into the atmosphere (Ayalon *et al.,* 2001; Vieitez and Ghosh, 1999). The CO_2 released, although a greenhouse gas, does not have a net effect on global warming since the carbon in CO_2 is fixed by photosynthetic plants and returned to the carbon cycle. It is believed that 18%-20% of the global warming effect is due to CH_4 emission which traps 20-30 times more heat than CO_2 (Ghosh, 1997; Vieitez and Ghosh, 1999; Francese *et al.,* 2000).

In many areas, landfills are approaching the available capacity of land and hence, land is no longer available for the disposal of waste (Chynoweth *et al*., 2001). The practice of landfilling is also becoming less popular due to the generation of odour as communities expand into the proximity of treatment plants. Biodegradable organic waste decomposes slowly and takes many years to decompose completely (Chugh *et al.,* 1999). Leaching of soluble constituents (salts, soluble organics and heavy metals) into the soil and groundwater is also an important concern where the groundwater is used by communities or migrates into nearby streams (Fueyo *et al*., 2002). Furthermore, the valuable energy contained in the organic waste is lost if the methane is not collected from landfills (Tsukahara *et al*., 1999). Due to the considerable environmental impact of landfills, many of them are due to close in Europe as a result of EU legislation (Mata-Alvarez *et al*., 2000).

Waste incineration, like all combustion processes, releases CO, NO_X and volatile organic compounds, which cause environmental pollution, while a large amount of ash and residues from off-gas treatment requires further treatment (Ayalon *et al*., 2001). Municipal waste is incinerated to reduce volume, in order to reduce landfill costs and to recover energy, either for heating or electricity generation. Incineration can only be used for residues containing less than 50% water otherwise oil or gas must be added to fuel the combustion process (Chynoweth and Legrand, 1988). Increased awareness of the environmental hazards of raw waste incineration and landfilling is increasing the complexity of these operations and, consequently, their costs. The trend is therefore to minimise the amount of waste to be treated.

Alternative technologies to the above-mentioned methods are aerobic or anaerobic digestion of the waste under controlled conditions. Aerobic treatment or composting involves the use of oxygen as an electron acceptor by microorganisms during the degradation of organic matter into CO_2, water, nitrates and sulphates. Of all biological waste treatment methods, aerobic treatment is the most widespread process used throughout the world (more than 95% of biological treatment). The compost contains nutrients and is used as a soil conditioner in agriculture. Composting, although good at stabilising organic solid waste, can only be applied to structured solids with water contents between 50% and 60%. Anaerobic digestion with energy recovery is an attractive method for the treatment of solid waste and wastewater. Anaerobic digestion is a complex biochemical process carried out in a number of steps by several types of microorganisms in the absence of oxygen. Methane and carbon dioxide are the principal end products, with minor quantities of nitrogen, hydrogen, ammonia and hydrogen sulphide. This is what is called biogas. In nature this process occurs in environments such as hot springs, swamps, paddy fields, lakes and oceans and the intestinal tract of animals (Garcia *et al*., 2000).

In principle, all organic material can be anaerobically digested. Gunaseelan (1997) concluded in a review that a wide range of biomass, both terrestrial and aquatic, might provide potential sources of methane. Easily degradable substances are of course more suitable, as they can be degraded faster. Anaerobic digestion of the large quantities of municipal, industrial and agricultural solid waste can provide biogas as well as other benefits such as reduction in waste volume, the production of biofertiliser and valuable soil conditioners (Edelmann *et al.,* 2000a; Grommen and Verstraete, 2002; Lema and Omil, 2001; Lettinga, 2004). Anaerobic digestion can be used to treat high-, medium- and low-strength, hot, cold, complex and simple waste(water) (Lettinga, 1995; de Baere, 2000; Zeeman and Saunders, 2001).

1.2 Aerobic vs anaerobic degradation

Some of the advantages and disadvantages of aerobic and anaerobic technology for waste(water) treatment are summarised in Table 1. Anaerobic processes have many advantages over the corresponding aerobic processes, such as low consumption of energy and low sludge production, smaller space requirements and lower overall costs (Demirel and Yenigun, 2002; Ahn *et al*., 2001; Ligero *et al*., 2001; Lema and Omil, 2001; Lettinga and Hulshoff, 1991). On the other hand, aerobic digestion requires energy input to provide aeration. The anaerobic route has an obvious advantage in that it produces methane, a combustible gas with a high calorific value (24 MJ/m^3). Depending on the substrate and digestion process, the methane content of biogas is generally between 55% and 80%.

Methane may be used directly as a clean fuel in boilers to produce hot water and steam for sanitary washing, or in gas-fired absorption chillers for refrigeration. Alternatively, it can be used to power fuel cells or internal combustion generator systems to produce electric power to replace coal. Biogas can also be purified and upgraded and used as vehicle fuel. Over a million vehicles are now using biogas and fleet operators have reported savings of 40-50% in vehicle maintenance costs (Francese *et al.,* 2000). Methane burns very cleanly producing lower emissions and generating less CO_2 than other fuels per unit energy. This could mitigate atmospheric CO_2 levels through the replacement of fossil fuels (Chynoweth *et al.,* 2001). Biogas can be sold to electricity utilities at a price that is competitive with current prices of fossil fuels. Methane derived from anaerobic digestion is competitive in both energy efficiency and cost to other energy carriers such as biomass for combustion and ethanol (Stewart *et al*., 1984; Chynoweth *et al.,* 2001).

There are disadvantages associated with anaerobic treatment but with improved process knowledge the drawbacks are gradually being remedied (Lettinga, 1995). Regarding the susceptibility of methanogens and acetogens to xenobiotic substances, a great deal of information is now available on the extent of such toxicity and better insight is available into the countermeasures that can be taken. The slow initial start-up is a disadvantage quoted by many researchers (Lauwers *et al*., 1990; Hsu and Shieh, 1993), but this has been overcome by advanced reactor concepts, e.g., fixed-bed and fluidised-bed reactors (Schink, 2002). There is a great deal of information on the growth conditions for anaerobic organisms and gradually large quantities of highly active anaerobic sludge (ideal seed) are becoming available from existing full-scale installations, so the start-up of new reactors can be achieved within a few days or weeks (Seghezzo *et al*., 1998). The main drawback of anaerobic digestion is that it requires more stringent process control, odour generation from H_2S and other sulphur compounds, and it only reduces the organic pollution by 85-90%, which means that a second step, usually an aerobic stage, is needed to guarantee high effluent quality.

Table 1. Comparison of aerobic and anaerobic biological waste(water) treatment.

	Aerobic digestion	**Anaerobic digestion**
Start-up	• Short start-up period.	• Long start-up period.
Process	• Integrated nitrogen and phosphorus removal possible. • Production of high excess sludge quantities. • Large reactor volume necessary. • High nutrient requirements.	• No significant nitrogen or phosphorus removal, nutrients removal done via post treatment. • Production of very little excess sludge (5-20%). • Small reactor volume can be used. • Low nutrient requirements.
Carbon balance	• 50-60% incorporated into CO_2; 40-50% incorporated into biomass.	• 95% converted to biogas; 5% incorporated into microbial biomass.
Energy balance	• 60% of available energy is used in new biomass; 40% lost as process heat.	• 90% retained as CH_4, 3-5% is lost as heat, and 5-7% is used in new biomass formation.
Residuals	• Excess sludge production. • No need for post-treatment.	• Biogas, nitrogen mineralised to ammonia. • Post-treatment required for removal of remaining organic matter and malodorous compounds.
Costs	• Low investment costs. • High operating costs for aeration, additional nutrient and sludge removal, and maintenance.	• Often moderate investment costs. • Low operating costs due to low power consumption and additional nutrients hardly required.
State of development	• Established technology.	• Still under development for specific applications.

(Adapted from Lepisto and Rintala, 1997; Banerjee *et al.,* 1999; Zoutberg and Eker, 1999; Gijzen, 2001; Lettinga *et al.,* 1984; Lettinga, 2001).

2 BIOCHEMICAL AND MICROBIOLOGICAL ASPECTS OF ANAEROBIC DIGESTION

To ensure the correct design and application of anaerobic treatment systems it is essential to know and understand the process and technological aspects, and the biochemistry and microbiology of anaerobic digestion (Lema and Omil, 2001).

Anaerobic digestion consists of several interdependent, complex sequential and parallel biological reactions, during which the products from one group of microorganisms serve as the substrates for the next, resulting in transformation of organic matter mainly into a mixture of methane and carbon dioxide (Noykova *et al*., 2002; Pavlostathis and Giraldo-Gomez, 1991; Gujer and Zehnder, 1983). Anaerobic digestion takes place in four phases: hydrolysis/liquefaction, acidogenesis, acetogenesis and methanogenesis. A simplified model of anaerobic digestion, indicating the main metabolic stages, is shown in Figure 1 (adapted from Gujer and Zehnder, 1983). To ensure a balanced digestion process it is important that the various biological conversion processes remain sufficiently coupled during the process so as to avoid the accumulation of any intermediates in the system (Kaseng *et al*., 1992). Microorganisms from two biological kingdoms, the *Bacteria* and the *Archaea*, carry out the biochemical process under strict anaerobic conditions (Dugba and Zhang, 1999; Powell and Archer, 1989; Kalyuzhnyi *et al.,* 2000; Veeken and Hamelers, 2000).

It should be emphasised that the biochemistry and microbiology of anaerobic digestion of complex organic substrates is still not completely understood (van Lier *et al*., 2001; Lettinga and Hulshoff-Pol, 1991; Michaud *et al.,* 2002). Anaerobic microbial degradation as such represents interesting biochemical and microbiological challenges and is still an exciting field for investigation. Moreover, sound knowledge of the capacities, strategies and limitations of anaerobic digestion sets the stage for the design of successful reactor systems and concepts that promise new methods and ideas with every discovery.

2.1 Hydrolysis

The first stage is depolymerisation of the organic matter, during which the complex polymers viz., carbohydrates, proteins and lipids, are broken down into monomers by the extracellular enzymes produced by microorganisms (e.g., cellulase, amylase, protease and lipase). The hydrolases may be secreted (free) or anchored on the cell surface (cell-bound), and the enzymes may be endohydrolases or exohydrolases (Eastmann and Ferguson, 1981; Kaseng *et al.,* 1992).

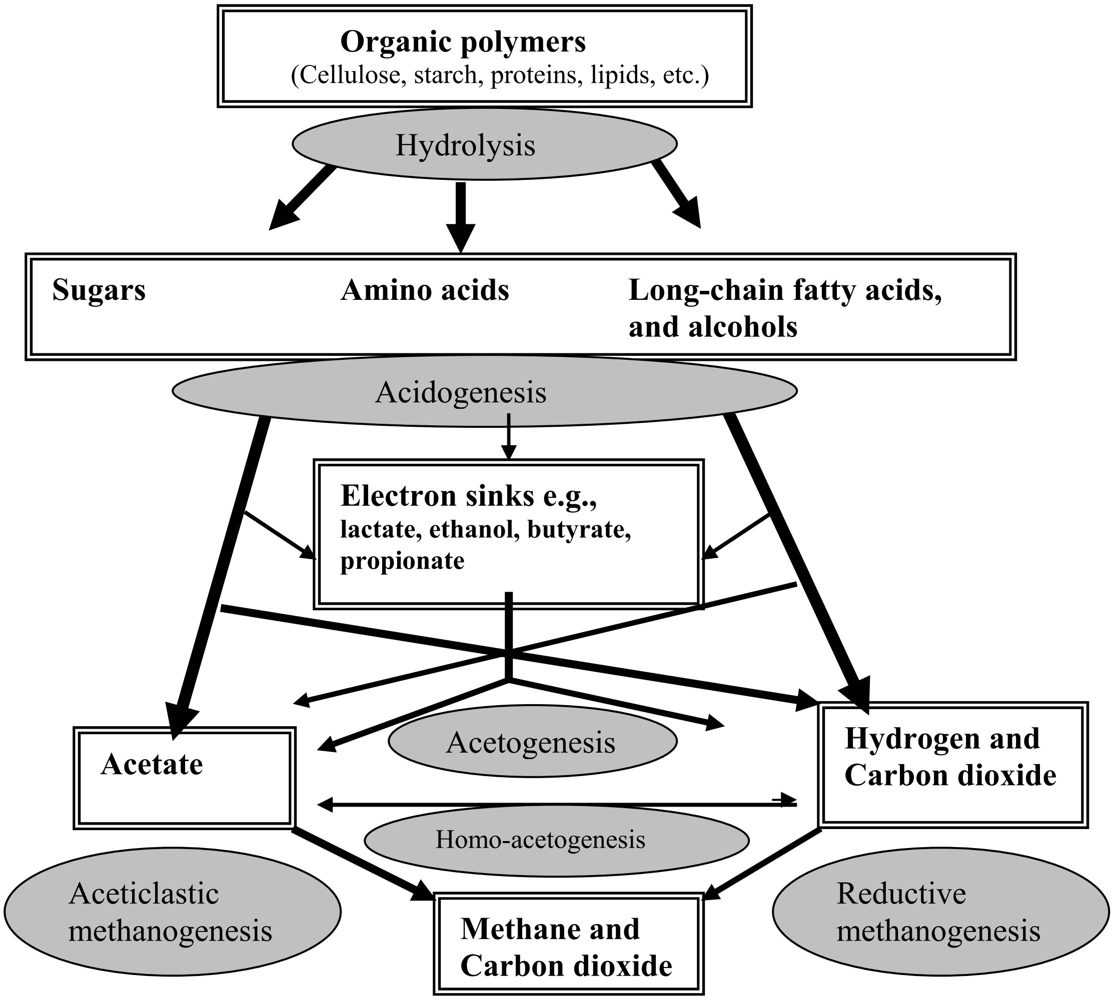

Figure 1. The main degradation pathways in anaerobic digestion

Proteins are broken down into amino acids, small peptides, ammonia and CO_2. In general, polysaccharides are converted into simple sugars, monomeric or dimeric. Starch is degraded into glucose units by a number of enzymes. Hydrolysis of cellulose by the cellulase enzyme complex yields glucose. Hemicellulose is biodegraded by special enzymes into a variety of monosaccharides such as glucose, galactose, xylose, arabinose and mannose (Elefsiniotis and Oldham, 1994a). Lipids are hydrolysed into long- and short-chain fatty acids and glycerol moieties by lipases and phospholipases. Lipases catalyse the stepwise hydrolysis of fatty acid ester bonds in triglycerides to release the corresponding fatty acids and eventually glycerol. Phospholipid metabolism by phospholipases results in the production of fatty acids and a variety of other organic compounds, depending on the substrate used (Pavlostathis and Giraldo-Gomez, 1991).

The hydrolytic and fermentative bacteria comprise both obligate and facultative anaerobes. This group of bacteria is also responsible for removing small amounts of oxygen introduced when feeding the digester. *Clostridia* and the *Micrococci* appear to be responsible for most of the extracellular lipase production. Proteins are generally degraded to amino acids by proteases secreted by *Bacteroides*, *Butyrivibrio*, *Clostridium*, *Fusobacterium*, *Selenomonas* and *Streptococcus* species (McInerney, 1988).

The profile of hydrolytic enzymes (both free and cell-bound enzyme activity) produced by microorganisms to hydrolyse solid potato waste during anaerobic digestion is presented in **Paper I**. The activity of the free enzyme was higher than that of the cell-bound for all the enzymes studied. The amylase activity was the highest, followed by carboxymethyl cellulase and filter paper cellulase, while the other hydrolytic enzymes were present at low activities. The point is that there is a varied repertoire of hydrolases with which microorganisms can attack organic particles and polymers to hydrolyse them into transportable molecules. Understanding of the hydrolytic capacity is crucial when working with solid waste, where hydrolysis is usually the rate-limiting step in the digestion.

2.2 Acidogenesis

During acidification, sugars, long-chain fatty acids and amino acids resulting from hydrolysis are used as substrates by fermentative microorganisms to produce organic acids, such as acetic, propionic, butyric and other short-chain fatty acids, alcohols, H_2 and CO_2, or by anaerobic oxidisers (Figure 1) (Kalyuzhnyi *et al.,* 2000; Gujer and Zehnder, 1983). Most of the products formed in the metabolism of glucose have, as an intermediate, pyruvic acid, which is produced via the glycolytic Embden-Meyerhof-Parnas (EMP) pathway. Depending on the anaerobic microbial species present, and reactor conditions, subsequent pyruvic acid fermentation can lead to the production of a number of C_1 to C_4 compounds such as volatile fatty acids (VFAs) e.g., acetic, propionic, and butyric acids, other organic acids (formic and lactic), alcohols, ketones, and aldehydes (Figure 2), (**Paper II**, Eastmann and Ferguson, 1981; Elefsiniotis and Oldham, 1994b; Pavlostathis and Giraldo-Gomez, 1991; Demirel and Yenigun, 2002). The production of VFAs by batch anaerobic digestion of solid potato waste is reported in **Paper II**. After 300 h digestion of potato waste on a small scale, the fermentation products were chiefly acetic acid, butyric acid, propionic acid and caproic acid, with insignificant amounts of iso-butyric, normal-valeric and iso-valeric acid. When the load of potato solids was increased, the VFA content was similar, but the amounts of acetic and lactic acids were higher.

Amino acids can also serve as energy and carbon sources for strict or facultative fermentative anaerobic bacteria. Short-chain VFAs (C_2 to C_5, straight-chain or branched) are generated via reductive deamination of aliphatic amino acids, specific fermentative pathways of individual amino acids, or an oxidation-reduction reaction between pairs of amino acids, known as the Stickland reaction (Elefsiniotis and Oldham, 1994b; Pavlostathis and Giraldo-Gomez, 1991).

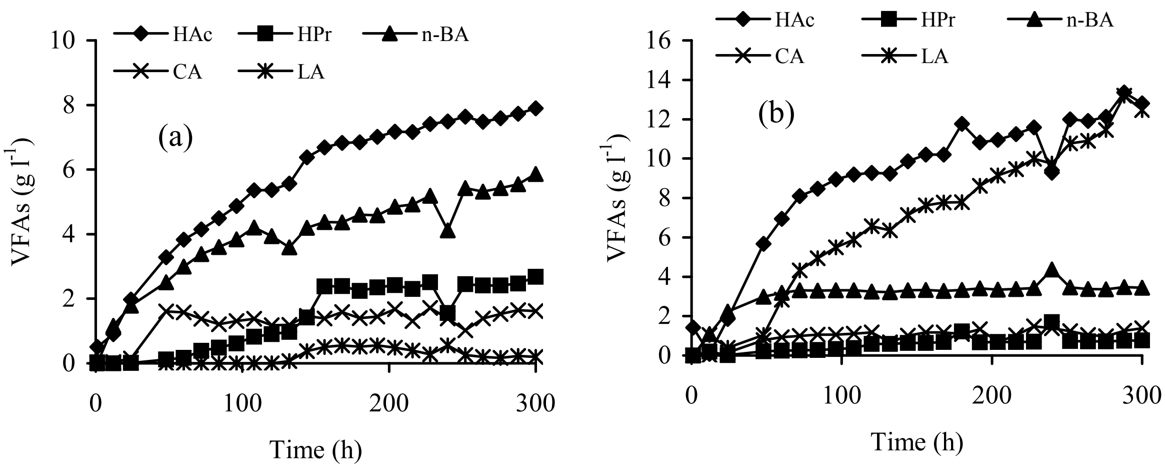

Figure 2. Time course for the production of VFAs and lactic acid during hydrolysis/acidification of potato solids at loads of 500 g (a) and 1, 000 g wet weight (b) (**Paper II**). (Note that the vertical scales are different).

Acidogenesis is usually the fastest reaction in the anaerobic conversion of complex organic matter in liquid phase digestion (Mosey and Fernandes, 1989). During steady state in anaerobic degradation, the main pathway is via acetate, carbon dioxide and hydrogen, and these reduced fermentation products can be used directly by the methanogens (Schink, 1997). The accumulation of electron sinks such as lactate, ethanol, propionate, butyrate and higher VFAs is the response of the bacteria to increased hydrogen concentration in the medium (**Paper II**, Schink, 1997). Many kinds of bacteria are involved in hydrolysis and acidogenesis and, therefore, several kinds of organic acids and alcohols are usually produced (Horiuchi *et al*., 2002). The concentration and proportion of individual VFAs produced in the acidogenic stage is important in the overall performance of the anaerobic digestion system since, acetic and butyric acids are the preferred precursors for methane formation (Hwang *et al*., 2001; **Paper II**).

2.3 Acetogenesis

The obligate hydrogen-producing acetogenic bacteria further degrade the electron sinks propionic, butyric and valeric acids, formed in acidogenesis to acetate, formate, carbon dioxide and hydrogen, (Figure 1; Table 2). This intermediate conversion is important for the successful production of biogas because the electron sinks are not utilised directly by the methanogens

(Mah, 1982). A clear distinction between acetogenesis and acidification reactions is not always present (Fox and Pohland, 1994).

The acetogens are very slow-growing, are sensitive to fluctuations in organic loads, are also sensitive to environmental changes, and long lag periods are likely to be required for these bacteria to adjust to new environmental conditions (Xing *et al*., 1997). They also depend on low partial pressure of hydrogen in order for the acetogenic degradation to proceed. Therefore, syntrophic associations with hydrogen-consuming methanogens are required (McCarthy, 1982; Fox and Pohland, 1994; Salminen *et al.,* 2000; Sekiguchi *et al* 2001). Syntrophy means, literally 'eating together' and refers to the interdependence of the hydrogen-producing and hydrogen-consuming methanogenic microorganisms. Such associations between acetogenic/acidogenic bacteria and methanogens are necessary when such reactions are thermodynamically unfavourable, as indicated by the conversion of simple substrate intermediates to acetate and hydrogen and their use to produce methane (Table 2). The acetogenic bacteria includes: (i) the valerate- and butyrate-degrading acetogenic bacteria, e.g. *Syntrophomonas wolfeii,* (ii) propionate-degrading acetogenic bacteria, e.g. *Syntrophobacter wolinii* and (iii) the homoacetogenic bacteria, which are responsible for converting the products of acidogenesis into acetic acid, hydrogen and carbon dioxide (Zinder, 1993).

Table 2. Acetogenic and methanogenic reactions: free energies (Adapted from Fox and Pohland, 1994; Garcia *et al*., 2000; Sekiguchi *et al*., 2001) pH 7, 1 atm plus all reactants and products at 1 M concentration respectively.

Acetogenic reactions			**Free energy per reaction ($\Delta G^{0,}$ kJ)**
Propionate		Acetate	
$CH_3CH_2COOH + 2H_2O$	$\longrightarrow$	$CH_3COOH + CO_2 + 3H_2$	+76.1
Butyrate		Acetate	
$CH_3CH_2CH_2COOH + 2H_2O$	$\longrightarrow$	$2CH_3COOH + 2H_2$	+48.1
Ethanol		Acetate	
$CH_3CH_2OH + H_2O$	$\longrightarrow$	$CH_3COOH + 2H_2$	+9.6
Lactate		Acetate	
$CH_3CHOHCOOH + 2H_2O$	$\longrightarrow$	$CH_3COOH + CO_2 + 2H_2 + H_2O$	-4.2
Methanogenic reactions			
Acetate	$\longrightarrow$	Methane	
$CH_3COOH + H_2O$		$CH_4 + CO_2 + H_2O$	-31.0
Carbon dioxide + hydrogen	$\longrightarrow$	Methane	
$CO_2 + 4H_2$		$CH_4 + 2H_2O$	-135.6
Syntrophic reaction:			
$2CH_3CH_2CH_2COOH + 4H_2O$	$\longrightarrow$	$4CH_3COOH + 4H_2$	+96.2
$CO_2 + 4H_2$	$\longrightarrow$	$CH_4 + 2H_2O$	-135.6
		Net reaction	-39.4

2.4 Methanogenesis

The methanogens utilise mainly H_2/CO_2 and acetic acid to form methane and carbon dioxide. The methanogens can also utilise a limited number of other substrates to form CH_4 such as methanol, methylamines, alcohols + CO_2 and formate (Kalyuzhnyi *et al.,* 2000; Hwang *et al*., 2001). H_2/CO_2-consuming methanogens reduce CO_2 (using it as an electron acceptor) via formyl, methenyl, and methyl, through association with specific coenzymes, to finally produce CH_4. About 70% of the methane is produced via the aceticlastic pathway (Archer, 1983; Klass, 1984; Lalman and Bagley, 2001; Solera *et al*., 2002; Zinder, 1984). Very few known species are capable of aceticlastic methane production, whereas nearly all known methanogenic species can produce methane from H_2/CO_2 (Hawkes and Hawkes, 1987). The hydrogen pathway is more energy yielding than the acetate pathway, it is normally not rate limiting, and it is important in keeping the hydrogen pressure low in the system.

In well-balanced anaerobic decomposition, all products of a previous metabolic stage are converted into the next, resulting in nearly complete conversion of the anaerobically biodegradable organic material into biogas without significant accumulation of intermediate products. If the process becomes unstable, e.g. when hydrogen partial pressure increases, this will lead to the accumulation of VFAs and a decrease in pH, inhibiting the pH-sensitive methanogens, finally leading to failure of the methanogensis stage and the whole anaerobic digestion process (**Papers III** and **VI**). Hydrogen is recognised as being the controlling parameter in the overall scheme of anaerobic waste digestion, but is rarely detected in well-functioning methanogenic digesters (Archer *et al.,* 1986).

Methanogenic microorganisms belong to the *Archaea*, a unique group phylogenetically different from the main group of prokaryotic microorganisms. Although they possess a prokaryotic cell structure and organisation, they share some common features with eukaryotes: homologous sequences in rRNA and tRNA, the presence of introns in their genomes, similar RNA polymerase subunit organisation, immunological homologues and translation systems (Garcia *et al*., 2000; Zinder, 1993). This group contains: (i) the acetotrophic methanogens, (ii) the hydrogenotrophic methanogens, and (iii) methylotrophs which convert methyl compounds such as methanol and methylamines. Of the many methanogenic genera, only two, *Methanosarcina* and *Methanosaeta*, are known to grow by the aceticlastic reaction (Zinder, 1984). Some of the acetate-utilising methanogens are *Methanosaeta soenhngenii* and *Methanosarcina barkeri* (Wiegant and Lettinga, 1985; Anderson *et al.,* 1994a). Species of *Methanosaeta* grow very slowly, with doubling times of 4 to 9 days (Lafitte-Trouqué and Forster, 2000; Zinder, 1984).

Methane-producing microorganisms are obligate anaerobes and very sensitive to environmental changes (Rozzi and DiPinto, 1994). The hydrogen-utilising methanogens have been found to be more resistant to environmental changes than aceticlastic methanogens and, therefore, methanogenesis from acetate has been shown to be rate limiting in several cases of anaerobic treatment of easily hydrolysable waste (Archer, 1983; Mosey and Fernandes, 1989).

3 IMPORTANT PROCESS PARAMETERS

The environmental factors that are important in the process of anaerobic digestion include temperature, pH and buffering systems, retention time, process configuration, and the solubility of gases, the availability of nutrients, and the presence of toxic components in the process (Björnsson *et al*., 2000; Demirel and Yenigun, 2002; Rajeshwari *et al*., 2000).

3.1 Temperature

The anaerobic degradation process is strongly influenced by temperature and the microorganisms can be divided into the following classifications: psychrophilic (0-20 °C), mesophilic (20-42 °C), and thermophilic (42-75 °C) (Hulshoff Pol, 1998). Anaerobic digestion reactors are normally operated within the mesophilic and thermophilic ranges (van Lier *et al*., 1996). Mesophilic processes require long hydraulic retention time and are not efficient in killing pathogenic microorganisms. To overcome these disadvantages, thermophilic conditions have been adopted for anaerobic digestion of some industrial organic waste, manure and domestic sewage (Archer, 1983; Song *et al*., 2004). Thermophilic anaerobic digestion may become an attractive alternative to mesophilic digestion because of the higher growth rates of the bacteria involved and, therefore, the high activities per unit biomass, and higher loading rate of organic materials that can be employed (Dugba and Zhang, 1999; Rintala and Lepisto, 1997; van Lier *et al.,* 2001; Wiegant and Lettinga, 1985). Whether it is economical to operate a digester at elevated temperatures is a matter of design, because the efficiency of operation must be offset against the heating costs.

Results of comprehensive studies suggest that thermophilic anaerobic digestion may be attractive for treating high-temperature industrial effluents and specific types of slurries (Lepisto and Rintala, 1997; Dinsdale *et al.,* 1997a; Lettinga, 1995; Zinder, 1984; Wiegant *et al*., 1985). Food industries, such as vegetable processing and canning factories, and alcohol distilleries, employ high-temperature unit operations, which generate hot effluents. Also, anaerobic treatment at high temperatures would mean elimination of cooling before treatment compared with the mesophilic process. The disadvantage of thermophilic digestion is the often-found high effluent VFA concentrations (Archer, 1983; Fang and Wai-Chung Chung, 1999; Ghosh 1998; van Lier *et al.,* 2001). Few high-rate thermophilic treatment systems are installed, despite the very promising results achieved in bench-scale investigations (Schraa and Jewell, 1984; Wiegant *et al.,* 1986; Van Lier *et al.,* 1994; Lettinga, 1995). Interpretation of the results published in the literature on the performance of reactors under thermophilic and mesophilic conditions show a degree of confusion, Table 3.

Table 3. Comparison of the performance of mesophilic and thermophilic anaerobic digestion (adapted from Ahn and Forster, 2000; Ahring, 1994; Ghosh, 1998).

Performance characteristics	Mesophilic digestion	Thermophilic digestion
Gas production rate	Contradictory reports	Contradictory reports
Pathogen reduction	Lower	Higher
Effluent VFAs	Lower	Higher (contradictory)
Dewaterability	Contradictory reports	Contradictory reports
Process stability	Higher	Lower (contradictory)
Methane content	Higher	Lower
Energy requirement	Lower	Higher
Odour	Lower	Higher
Product/substrate inhibition	Lower	Higher

In the present work, the performance of two-stage anaerobic digestion systems under mesophilic + mesophilic (37 °C), mesophilic (37 °C) + thermophilic (55 °C) and thermophilic + thermophilic (55 °C) conditions was investigated (**Paper III**). The digestion period was shorter in the mesophilic + thermophilic and thermophilic + thermophilic systems than in the mesophilic + mesophilic one. The concentrations of VFAs in the effluent from the second-stage thermophilic UASB reactor did not differ from those in the effluent from the mesophilic UASB reactor.

The hydrolysis and acidogenesis processes are not significantly affected by temperature, as among the mixed population there are always some bacteria that have their optimum within the temperature range in which the reactor is being operated. The acetogenesis and methanogenesis stages are carried out by fewer specialised species of microorganisms and are thus more likely to be sensitive to temperature.

Methanogenesis is also possible under psychrophilic conditions but occurs at lower rates (Nyns, 1986). Bacterial activity and growth decrease by one half for every 10 °C decrease in temperature below 35 °C (Hulshoff-Pol, 1998). Low temperatures or psychrophilic applications are of interest for wastewater produced in the bottling, malting and brewery industries, which produce cold effluents with organic concentrations below 1 kg COD /m^3 (Rebac *et al.,* 1997; van Lier *et al.,* 2001). However, some attempts to treat such dilute wastewater under psychrophilic conditions have not been successful (Lettinga *et al.,* 1999; Matsushige *et al.,* 1990; van der Last *et al.,* 1992). However, Rebac e*t al.,* (1997) reported successful application of anaerobic treatment to low-strength malting wastewater in a pilot-scale, expanded granular-sludge-bed system under psychrophilic conditions.

3.2 pH and alkalinity

The anaerobic degradation process is highly pH dependent because each of the microbial groups involved in the reactions has a specific pH range for optimal growth. The aspects influenced by pH include utilisation of carbon and energy sources, efficiency of substrate dissimilation, synthesis of proteins and various types of storage material, and the release of metabolic products from the cell (Elefsiniotis and Oldham, 1994b).

The optimal pH for methane-producing microbes is 6.8-7.2, while for acid-forming bacteria it is around 6 (Moosbrugger *et al.,* 1993; Zoetemeyer *et al*., 1982). The growth rate of methanogenic microbes decreases sharply below pH 6.6 (Mosey and Fernandes, 1989). The aceticlastic methanogens have been found to be more sensitive to low pH values than the hydrogenotrophic methanogens (Brummeler, 1993). The sensitivity of aceticlastic methanogens to low pH values may be the result of deterioration of the energy-generating process of the organisms. Variations in pH levels from 6.0 to 8.0 have been reported to affect the dominant microbial populations in the acidic phase (Demirel and Yenigun, 2002). In a one-step anaerobic treatment process, the pH is typically maintained at conditions more optimal for methanogens to prevent the predominance of acid-forming bacteria, which may cause the accumulation of VFAs. Acidogenesis can occur at pH values approaching neutrality. Efficient methanogenesis from a digester operating in a steady state should not require pH control, but at other times, for example, during start-up or with unusually high feed loads, pH control may be necessary. pH can only be used as a process indicator when treating waste with low buffering capacity, such as carbohydrate-rich waste (**Paper VI**).

An important parameter in anaerobic digestion systems is alkalinity, which is a measure of the chemical buffering capacity of the aqueous solution. It is essential that the reactor contents provide enough buffering capacity to neutralise any possible VFA accumulation in the reactor and to maintain pH (6.7 to 7.4) for stable operation (**Paper VI**, Callander and Barford, 1983). Carbonic acid (bicarbonate), hydrogen sulphide, dihydrogen phosphate and ammonia are the compounds that provide a significant buffering capacity in the useful region around pH 7. However other compounds such as VFAs and ammonia may be present and also contribute to the alkalinity. The predominant VFAs in anaerobic systems are acetic and propionic acids, and they buffer in pH intervals of 3.7-5.7 and 3.9-5.9, respectively, (Jenkins *et al*., 1991). The procedure of measuring alkalinity described in Standard Methods (1998) is by titration of a sample to pH 4.3. At this pH more than 99% of the bicarbonate system is converted to carbonic acid and thus is measured in the procedure. If VFAs are present, more than 80% of the total VFAs will be measured. This leads to overestimation of the alkalinity, as the VFAs do not provide any useful buffering capacity. Hill and Jenkins (1989) proposed a new endpoint measurement, titration to pH 5.75. At this pH, 80% of the bicarbonate will have been titrated but less than 20% of the VFAs will have contributed to the alkalinity. This alkalinity is referred to as partial alkalinity (PA) as opposed to total alkalinity (TA) at pH 4.3. In the present work, PA measurement was found to be suitable for process monitoring during anaerobic digestion of potato leachate (**Paper VI)**.

The presence and concentration of a buffering compound depends on the composition of the substrate and the total organic load. If the pH in an anaerobic bioreactor decreases, it is recommended that feeding be stopped and the buffering capacity increased. In this work, the methane yield increased from UASB reactors after recovery from overloading conditions without addition of buffering agents such as calcium carbonate, sodium bicarbonate or lime (**Paper III**).

3.3 Volatile fatty acids

The concentration of VFAs is one of the most important parameters in the monitoring of the anaerobic digestion process. It is commonly agreed that VFA build-up is the result of unbalanced digestion conditions (Björnsson *et al*., 1997). The decrease in pH accompanying accumulation of VFAs is the main cause of toxicity and reactor failure in the anaerobic digestion process (Ahring *et al*., 1995). This is because the toxicity of VFAs is pH dependent since only the nonionized forms are toxic to microorganisms. VFAs are toxic at pH values where they exist in protonated forms, as they then can penetrate the cell membrane. When they are inside the cell, where the pH is around 7, they are ionized and the hydrogen ion released will cause a decrease in the intracellular pH (Björnsson, 2000). The pH gradient across the membrane is essential for ATP formation and therefore bacterial growth.

The concentrations of acetic, propionic and butyric acids are considered the best indicators of the metabolic state of the most sensitive microbial groups in the anaerobic system (Rozzi, 1991). The studies presented in **Papers III-VI** all confirmed the suitability of monitoring VFAs to detect imbalances in the anaerobic digestion processes investigated.

3.4 Nutrients

All organisms need essential ingredients for their growth, viz. macronutrients and trace elements, and a lack of these nutrients will negatively affect their growth (Lettinga, 1995). The study of the effects of micronutrients on anaerobic digestion is now a promising and exciting field of research (Gonzalez-Gil *et al.,* 1999; Lettinga, 2001). Nutrients such as nitrogen and phosphorus, and trace elements (sulphur, potassium, calcium, magnesium, iron, nickel, cobalt, zinc, manganese and copper) are required for efficient anaerobic degradation and these are usually present in sufficient amounts in most wastes that are treated in anaerobic digesters (Rajeshwari *et al.,* 2000).

Inhibitory and toxic effects of heavy metals on the acidogenic stage of anaerobic digestion have been reported in various studies (e.g. Demirel and Yenigun, 2002). According to Lin (1993) copper and zinc were the most toxic, while lead was the least toxic heavy metal to acidogens. Yenigun *et al.* (1996) reported inhibitory effects of copper and zinc in batch digesters in the range of 1-10 mg/dm^3 for copper and 5-40 mg/dm^3 for zinc, showing that copper was more toxic than zinc to acidogens. Heavy metals have also been reported to inhibit the degradation of VFAs to methane in anaerobic digestion (Lin, 1992).

The most important nutrients are nitrogen and phosphorus, and the optimum C:N:P ratio for high methane yield is reported to be 100:3:1 (Rajeshwari *et al.,* 2000). If the C/N ratio is high there is a risk of nutrient deficiency and a low buffering capacity will result in a more sensitive process (Nyns, 1986). If the nitrogen content is high the problem of ammonia inhibition may arise because the degradation of nitrogenous compounds will release ammonium.

The digestibility of carbohydrate-rich wastes can be improved by mixing them with those containing high amounts of nitrogen to improve the C:N ratio. The amount of one type of organic waste generated at a particular site at a certain time may not be sufficient to make anaerobic digestion cost-effective all year round. Co-digestion then becomes a suitable alternative as it is a well-established concept (Ahring *et al*., 1992; Kaparaju *et al*., 2002; Misi and Forster, 2001) and it has many advantages (Mata-Alvarez *et al*., 2000; Callaghan *et al*., 2002). Co-digestion as a process has been examined for a wide range of waste combinations (e.g. Edelmann *et al*., 2000b; Rushbrook, 1990; Tafdrup, 1994). In the present work, co-digestion of solid potato waste and sugar beet leaves was investigated in laboratory-scale, anaerobic batch digestion (**Paper IV**) and in pilot-scale, two-stage anaerobic digestion (**Paper V**). Co-digestion improved the methane yield by 31-62% compared with digestion of potato waste alone in the batch experiments. Co-digestion gave a 60% higher methane yield than the digestion of the individual substrates in the pilot-scale, two-stage anaerobic digestion process (Figure 3). The marked increase in methane yield could be attributed to positive synergism established in the digestion liquor and the supply of additional nutrients by the co-substrates resulting in an improved C:N ratio.

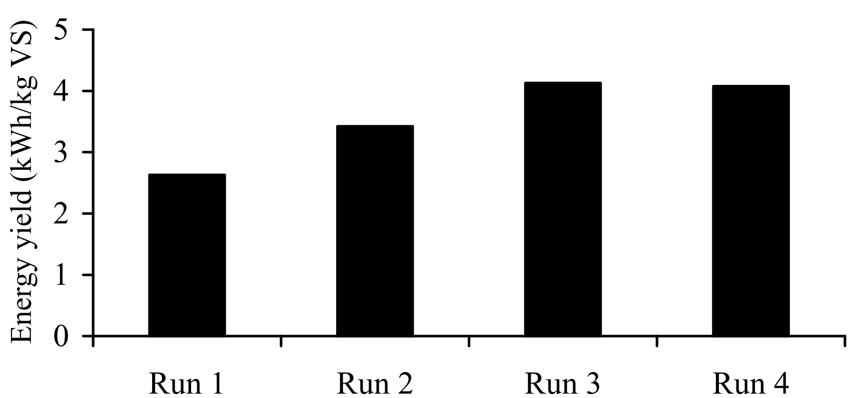

Figure 3. Energy yield per kg of organic dry matter (volatile solids (VS)) during pilot-scale, two-stage anaerobic digestion (with straw packed-bed reactor for the methanogenic stage) of agricultural residues. Four experimental runs were performed: 1. unpeeled potatoes, 2. peeled potatoes, 3. co-digestion, beet leaves: potatoes 1:2, 4. co-digestion, beet leaves: potatoes 1:3.

3.5 Organic loading rate and hydraulic retention time

The OLR is the quantity of organic matter fed per unit volume of the digester per unit time, (e.g., g VS/l/day). OLR plays an important role in anaerobic wastewater treatment in continuous systems and is a useful criterion for assessing performance of the reactors (Rajeshwari *et al.,* 2000; Lissens *et al*., 2001). High OLRs and low sludge production are among the many advantages of anaerobic processes over other biological processes (Batstone *et al.,* 2002). A comparison of the OLRs for stable operation of different reactors during anaerobic digestion investigated in this work is given in Table 4. The results show that OLR depends on several factors, such as reactor type and substrate. The performance of a UASB reactor and an anaerobic packed-bed reactor with plastic carriers when treating potato waste leachate at different OLRs was investigated (**Paper VI**). Two-stage, anaerobic digestion of solid potato waste using UASB methanogenic reactor under mesophilic and thermophilic conditions is reported in **Paper III**. The maximum OLR for stable operation was 36 g COD/l/day for the thermophilic UASB reactor compared with 11 g COD/l/day for the mesophilic one. In the pilot-scale study (**Paper V**) the processes were not optimized, so the highest OLRs in this study should not be taken as maximum values. The results obtained in this study are comparable to those in the literature. There are correlations between the type of carriers used for biomass immobilisation and the performance of the anaerobic filters (Anderson *et al*., 1994a; Picanco *et al.,* 2001).

The hydraulic retention time (HRT) is one of the most important design parameters influencing the economics of digestion (Maharaj and Elefsiniotis, 2001; Elefsiniotis and Oldham, 1994d). For a given volume of wastewater, a shorter HRT translates into a smaller digester, and therefore, more favourable economics (Dugba and Zhang, 1999). In the case of continuously stirred reactors, where the HRT is long, overloading results in biomass washout and this leads to process failure. The solids retention time will be long if particles are retained in the digester, for example, in the high-rate reactors described in the next section. Upflow anaerobic sludge blanket (UASB), fixed-film, expanded- and fluidised-bed reactors can withstand higher OLRs. Even if there is a shock load resulting in failure, the system rapidly recovers. Moreover, high COD removal is achieved even with a short HRT (Rajeshwari *et al.,* 2000). Simple waste may pass through high-rate digesters, e.g., the UASB reactor, with retention times of merely hours. Complex waste, such as animal manure, must be digested at HRTs of 10 days or more because of the high fraction of recalcitrant organic matter present in cattle manure (Sung and Santha, 2003).

Table 4. Comparison of OLR for stable operation of second-stage methanogenic reactors during mesophilic anaerobic digestion.

Substrate	Reactor Type	Scale	Max OLR (g COD/l/d)	HRT (days)	Reference
Hydrolysed solid potato waste	UASB	Lab	11.0	2.4	**Paper III**
Hydrolysed solid potato & sugar beet waste	Packed-bed, straw carrier Packed-bed, plastic carrier	Pilot Pilot	20 18	1.0 1.4	**Paper V**
Potato waste leachate	UASB Packed-bed, plastic carrier	Lab Lab	6.1 4.7	3.3 4.3	**Paper VI**
Potato-maize wastewater	UASB	Lab	14	1.3	Kalyuzhnyi *et al.* 1996
Synthetic wastewater	Packed-bed, straw carrier	Lab	4	0.5	Guitonas *et al.* 1994
Dairy wastewater	Sintered glass carrier Packed-bed, PVC rings	Lab Lab	21 4	0.5	Anderson *et al*. 1994a

4 ANAEROBIC BIOREACTOR CONFIGURATIONS

4.1 High-rate wastewater bioreactors

The successful application of anaerobic digestion technology to the treatment of industrial wastewater is critically dependent on the development and use of high-rate anaerobic bioreactors (Barber and Stuckey, 1999; Chynoweth *et al*., 2001; van Lier *et al*., 2001). This is because large volumes of effluent have to be treated and optimally designed bioreactors can decrease the treatment time and increase the treatment efficiency, leading to an overall lowering of the treatment cost. The application of high-rate reactors has enhanced the recognition of anaerobic digestion as a cost-effective and efficient technology for environmental protection. High-rate reactors meet the following two conditions: (a) high retention of viable sludge under high organic loading conditions, and (b) good contact between biomass and incoming wastewater, resulting in reduced reactor size and low process energy requirements (Lettinga *et al*., 1997; Rajeshwari *et al*., 2000).

The average growth rate of methanogens is much lower than that of acidogens; the overall rate of the biomethanation process is controlled by the methanogenic step if the wastewater does not contain particulate matter. It has been observed that the rate of biomethanation can be accelerated by enhancement of the rate of conversion of VFAs to methane by increasing the concentration of the methanogens in the reactor. This can be achieved by two methods, viz.: making the individual cells agglomerate to form 'sludge granules' so that they have better settling properties and are not washed out of the reactor, or by making the cells grow while attached to an inert 'carrier' material which has a higher specific gravity than cells (Björnsson *et al*., 1997; Lettinga *et al*., 1997; **Papers III, V, VI**).

High-rate bioreactors include the UASB, packed-bed and fluidised-bed reactors, based on the mechanism used to achieve biomass retention within the bioreactors (Figure 4). These bioreactors provide a high reaction rate per unit reactor volume thus reducing reactor volume and ultimately allowing the application of high volumetric loading rates (Borja *et al.,* 1994; Barber and Stuckey, 1999; Rebac *et al.,* 1997).

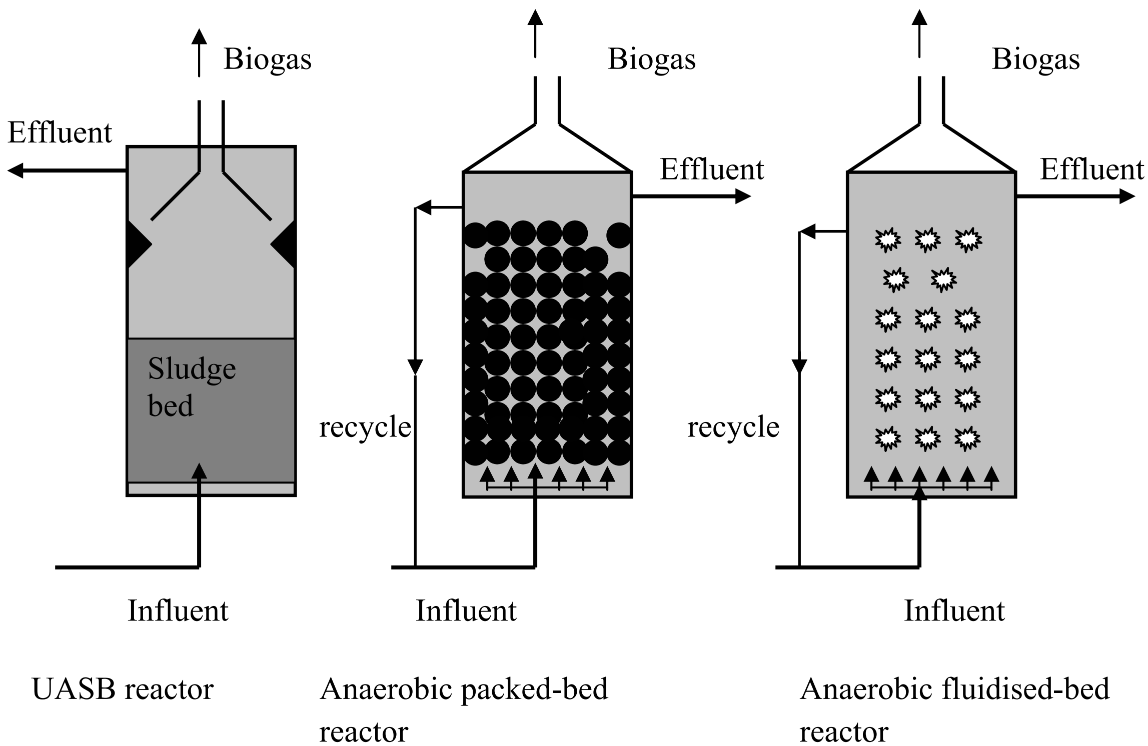

Figure 4. Anaerobic reactor configurations

4.1.1 Upflow anaerobic sludge blanket reactors

The UASB reactor is a high-capacity methane bioreactor with a sludge bed, or blanket of settled microorganisms through which the wastewater flows upwards (Yan *et al.,* 1990). The main advantage of the UASB process is that no support material is required for retention of the high-density anaerobic sludge (Lettinga *et al*., 1980; Elias *et al.,* 1999; Zoutberg and Eker, 1999). However, the absence of carriers necessitates the availability and maintenance of highly settleable biomass, either as flocs or as dense granules (0.5-2.5 mm in size) (Callander and Barford, 1983; Lettinga, 1995). The simple design of UASB reactors ensures a uniform distribution of incoming wastewater around the base of the digester, sufficient cross section to prevent excessive biomass entrapment, and effective separation of gas, biomass and liquid (Callander and Barford, 1983; Fox and Pohland, 1994). A three-phase separator, (biogas, liquid and biomass) serves to separate the biogas on the one hand, and the bacterial mass, which is returned into the active lower zone of the reactor, on the other hand (Lettinga *et al.,* 1984; Yan *et al.,* 1990; Lettinga, 1995). The UASB does not require the expense and energy consumption of pumps for recirculation of effluent (Lettinga and Hulshoff 1991; Rajeshwari *et al.,* 2000; Wentzel *et al*., 1995). In practice, the UASB reactor distinguishes itself by being highly reliable under constantly varying conditions. The dense structure and high settleability of the sludge (60-80 m/h), allow upflow anaerobic reactors to be operated at very high upflow liquid velocities, without loss of granules (Lettinga and Hulshoff, 1991; Wentzel *et al.,* 1995; Zoutberg and Eker, 1999). Bench- and pilot-scale studies indicate that it is possible to operate this type of reactor at an OLR of 40 kg COD/m^3/day at HRTs of 4-24 h with a COD reduction of more than 80% (Bowker, 1983). In UASB reactors channelling problems occur only at low loading rates and when the distribution by the feed inlet is poor.

There are certain disadvantages of the UASB design. The bed can be disrupted if the influent flow rate is too fast, or if gas production is too vigorous. The bioreactor may not treat particulate wastes effectively since particles appear to interfere with flocculation and may also accumulate in the bed, thus reducing its effectiveness per unit volume. Another disadvantage is that the reactor requires granular seed sludge for faster start-up.

The food industry throughout the developed world has become an active user of this anaerobic treatment technology, for example, the sugar processing industry, breweries and potato processing plants. In this work, UASB reactors have been applied successfully for treatment of potato hydrolysate and at maximum OLR ranging from 6 to 36 g COD/l/day (**Papers III** and **VI**). A study of industrial anaerobic treatment of opaque beer brewery wastewater in a tropical climate using a full-scale UASB reactor was carried out for two years (**Paper VII**). The UASB reactor enabled the brewery to meet the permissible levels of COD in the wastewater for discharge into the municipal sewage system of Harare in Zimbabwe.

4.1.2 Expanded granular sludge bed system

The latest generation of high-rate anaerobic treatment systems is the expanded granular sludge blanket (EGSB) process, which has become increasingly popular, mainly because of their very high loading potential in comparison with conventional UASB reactors (Lettinga, 2001). The EGSB reactor (modified form of the UASB reactor) makes use of higher superficial liquid velocity (5-10 m/h), and therefore contact between the wastewater and sludge is further improved (Lettinga, 1995). Both the UASB and the EGSB processes use granular anaerobic biomass, have the same operation principles, but differ in terms of geometry and process parameters (Zoutberg and Eker, 1999). In the EGSB process, granular biomass is expanded by the high gas and liquid upflow velocities, hence mainly granular sludge will be retained in an EGSB system, with a significant proportion of the sludge being in the expanded or fluidised state in higher regions of the bed.

The advantages of this system are found in its small footprint and higher loading rates compared with conventional UASB systems. The loading rates may reach values up to 20-40 kg COD/m^3/day, depending on the type of system and wastewater to be treated (van Lier *et al*., 2001). One of the most serious problems associated with expanded-bed digesters is the instability of the granular conglomerates during continuous operation. This also applies, though to a much lesser extent, to UASB reactors and loss of biomass might occur due to: (i) granule disintegration, (ii) wash-out of hollow granules, (iii) occurrence of fluffy granules, and (iv) scaling due to inorganic precipitates.

4.1.3 Anaerobic biofilm reactors

In these reactors microorganisms grow as a biofilm on the surface of inert carriers. Anaerobic biofilm reactors can be subdivided into: (a) anaerobic packed-bed reactors with a stationary support medium for microorganism attachment, and (b) fluidized- or expanded-bed reactors where the support itself is in motion in the liquid stream.

Anaerobic packed-bed reactors

The components of an anaerobic packed-bed reactor are a wastewater distributor and a medium-support structure, a headspace to allow for accumulation and capture of methane gas, and effluent recycling equipment (Figure 4). In packed-bed reactors (sometimes called fixed-bed reactors) wastewater is passed in either upflow or downflow mode over a population of microorganisms attached to an inert solid support carrier e.g., gravel, plastic carriers, ceramic rings, glass beads or baked clay, (Lettinga *et al.,* 1984; Rajeshwari *et al*., 2000). Low biodegradable biomass such as straw and wood chips has also been found to be suitable as microbial carrier (Andersson and Björnsson, 2002, **Papers I** and **V**). Microorganisms exist not only in the spaces within the carriers, but also attached to its surface, hence, a high-density microbial population is retained within the reactor, thus allowing a biomass retention time longer than the HRT (di Berardino *et al.,* 2000).

Packed-bed reactors have an advantage over UASB reactors in that they are not susceptible to biomass washout by hydraulic shock loads. However, they can be subject to clogging due to an increase in biofilm thickness or a high concentration of suspended solids in the wastewater. Channelling might also occur, and microbial attachment during start-up may be slow. The main limitation of this reactor design is that the reactor volume is relatively high in comparison to other high-rate processes because of the volume occupied by the carriers (Hawkes and Hawkes, 1987). In this work, packed-bed reactors were applied in the methanogensis of hydrolysate from crop residues at OLRs ranging from 4.7 to 20 g COD/l/day (**Papers V** and **VI**).

Anaerobic fluidised- or expanded-bed reactors

Expanded-bed or fluidised-bed reactors consist of a reactor filled with granular material to which biomass adheres in a thin film, a wastewater distributor, head space for the collection of methane gas, and effluent-recycling systems. Wastewater flowing up through these reactors fluidises, or at least expands the bed of particles to which the microorganisms are attached by 20-50%, so good contact between wastewater and biomass is ensured (Chen *et al.,* 1985; Lettinga *et al.,* 1984). Due to the need to maintain fluidised conditions in the medium, upflow velocities are generally an order of magnitude greater than in the upflow anaerobic fixed-bed reactor. The main difference between the expanded-bed and fluidised-bed reactors is that in an expanded bed the sludge bed is located in the lower part of the reactor, whereas in a fluidised bed the sludge is distributed throughout almost the entire reactor volume. The suspended particles are in constant motion, and the bed appears to be boiling, thus channelling or clogging is prevented and very efficient substrate transfer is achieved. In the fluidised state, each carrier provides a large surface area for biofilm formation and growth. This enables the attainment of higher reactor biomass hold-up, and better mass transfer to the biofilm (Lauwers *et al*., 1990; Switzenbaum and Jewell, 1980). The support particles should be of a low density to minimise energy requirements for fluidisation. Support media used are small sized particles (0.5 mm) of sand, basalt, pumace, PVC particles or carbon granules. These reactors can be used to treat particulate waste and the resulting conversion has been comparable or even superior to those obtained with UASB reactors (Cho *et al*., 1996; Rajeshwari *et al*., 2000; Barber and Stuckey, 1999).

Fluidised- or expanded-bed biofilm reactors have the disadvantage of requiring power input for bed expansion. There is also a problem of excessive growth on the carrier under mild shear conditions (upper part of the reactor) and no growth on the carrier under high shear conditions (lower part of the reactor) required to fluidise the carrier. Fluidised-bed systems require a sophisticated feed-inlet distribution system to ensure fluidisation.

At present, it is not possible to single out any specific process as all-round and optimally suited under all circumstances. Each of the high-rate processes has its merits and limitations. Many variables have to be taken into consideration in the treatment of each waste(water). To choose the most appropriate type of reactor for a particular application, it is essential to conduct a systematic evaluation of different reactor configurations with the waste(water).

4.2 Processes for solid waste degradation

Biodegradable solid waste management is a major challenge worldwide (Grommen and Verstraete, 2002). This kind of waste is generated by agriculture, industry and municipalities in appreciable quantities. The generation of agro-industrial, agricultural and municipal solid waste worldwide is shown in Table 5. Uncontrolled decomposition of each metric ton of solid waste could potentially release 50-110 m^3 CO_2 and 90-140 m^3 CH_4 into the atmosphere, contributing to global warming (Vièitez and Ghosh, 1999). It is estimated that global warming may be reduced by up to 20% by using discarded biomass and waste for the production of biofuel, as well as other benefits to society and the environment (Bouallagui *et al*., 2003; Vièitez and Ghosh, 1999). Anaerobic digestion of biodegradable organic waste is in many situations an environmentally attractive way of treating solid biodegradable waste, while at the same time producing energy in the form of biogas (de Baere, 2000). Biodegradable waste has considerable potential as a source of energy in both developed and developing countries.

Table 5. Total production of municipal solid waste (MSW), industrial and agricultural waste in million tons/year. (Source: Kashyap *et al*., 2003).

Waste	**India**	**Brazil**	**Sudan**	**USA**	**Sweden**
MSW	135.5	44.0	2.3	148.0	5.3
Sewage	44.9	8.02	1.4	16.0	0.6
Manure	653.0	470.0	68.0	306.0	13.2
Agricultural residues	200.0	47.0	8.1	573.0	12.6
Biomass	140.0	496.8	192.3	427.0	14.0

The greatest advances in anaerobic digestion processes have been made using essentially liquid waste, and slurries (Brummeler, 1993; de Baere and Verstraete, 1984). The main limiting factor in the conversion of particulate materials is the relatively low rate of hydrolysis compared with the rate of acid formation from soluble materials (Ghosh, 1987; Mata-Alvarez *et al.,* 2000; Shin *et al.,* 2001; Vavilin *et al.,* 2001; Andersson and Björnsson, 2002). The strategies to treat solid waste include anaerobic batch and two-stage systems.

4.2.1 Batch system

In normal batch systems, bioreactors are filled once with fresh waste, with or without the addition of seed material, and allowed to go through all the degradation steps sequentially, leading to the formation of biogas. A minimum amount of water is added and the leachate can be recirculated to the top of the fermenting waste (Figure 5). This is the principle of e.g., the Biocel process (Brummeler, 2000).

In sequential batch design, the leachate of a freshly filled reactor, containing high levels of soluble organic compounds, is recirculated to another, more mature, reactor where methanogenesis takes place. The leachate of the latter reactor, containing little acids and loaded with pH-buffering bicarbonates, is pumped back into the initial reactor. This configuration ensures cross-inoculation between new and mature reactors, which eliminates the need to mix the fresh waste with seed material (Brummeler, 2000; Lissens *et al.,* 2001).

Anaerobic batch digestion is useful because it can be performed with simple, inexpensive equipment and with waste with total solids concentration as high as 90%, e.g., straw. It is also useful in laboratory-scale studies in assessing the rate at which a material can be digested and determining the yields of biogas obtainable (Badger *et al.,* 1979; Lettinga, 2001; Lastella *et al*., 2002; Lissens *et al*., 2001; Stewart *et al*., 1984). The major disadvantages of batch systems are their large footprint, a possible need for a bulking agent and a lower biogas yield caused by impairment of the percolation process due to channelling or clogging due to compaction (Ouedraogo, 1999).

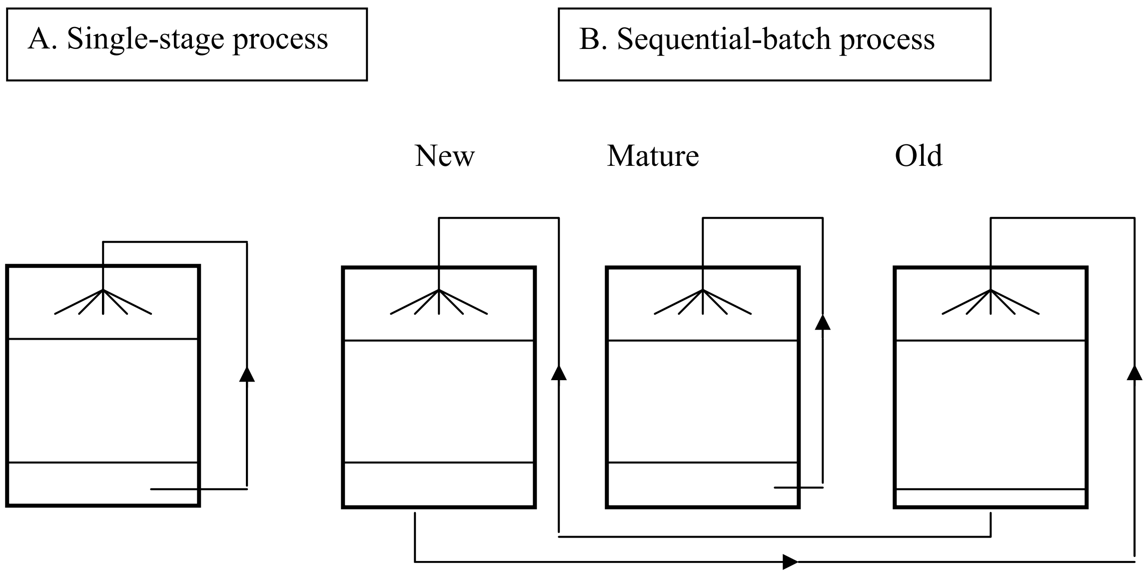

Figure 5. Configuration of leachate recycling patterns in different batch systems (adapted from Lissens *et al.,* 2001).

The specific features of batch processes, such as a simple design and process control, low water consumption and robustness together with the low investment cost, make them particularly attractive for applications in both developed and developing countries (Brummeler, 2000; Lissens *et al.,* 2001; Lastella *et al.,* 2002). One of the successful developments is the dry anaerobic composting (DRANCO) process (de Baere and Verstraete, 1984; de Baere 2000). Other anaerobic batch digestion systems are Valorga and Vagron (Gijzen, 2002).

In this work, anaerobic batch digestion of solid potato waste, alone and in combination with sugar beet leaves, was investigated (**Paper IV**). The effects of increasing the concentration of potato waste, expressed as a percentage of total solids, and the initial inoculum-to-substrate ratio on methane yield were investigated. A maximum methane yield of 0.32 l CH_4/g $VS_{degraded}$ was obtained at 40% of total solids and an inoculum-to-substrate ratio of 1.5 (Figure 6). Co-digestion of potato waste and sugar beet leaves improved the methane yield by 31-62% compared with digestion of potato waste alone.

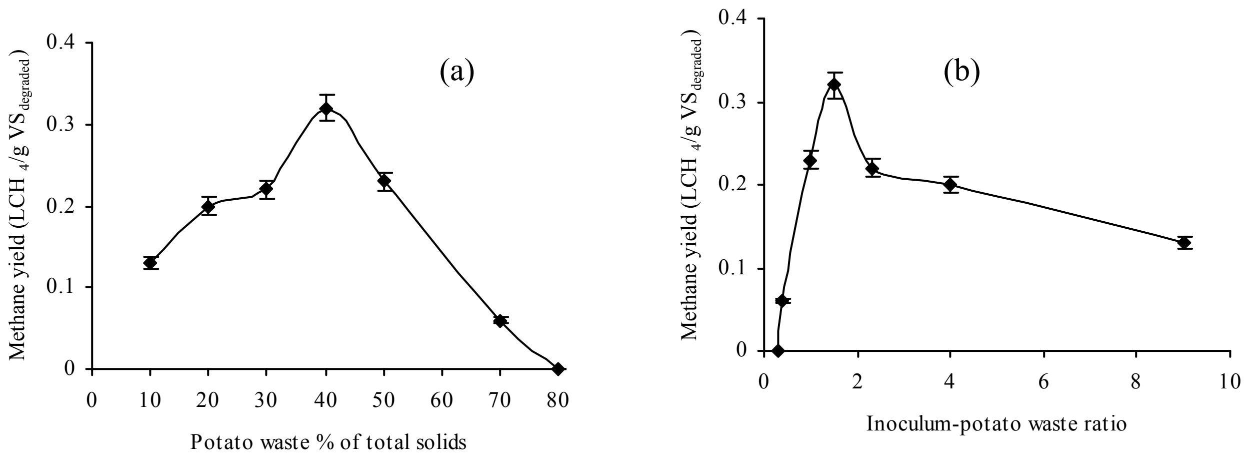

Figure 6. Methane yields (L CH_4/ g $VS_{degraded}$) during batch anaerobic digestion of potato solids at different potato waste concentrations (a) and inoculum-to-substrate ratios (b).

4.2.2 Continuously stirred tank reactor

The CSTR is the most common type of reactor applied to sewage and manure treatment with low solids content (2-10% total solids). Many solid waste fractions are also treated in CSTRs after slurrying with liquid. In the continuously stirred tank reactor, the rate of feeding should be continuous for maximum efficiency, but for practical reasons the reactor is fed intermittently; the most common frequency being once a day (Gunaseelan, 1997). These reactors are continuously mixed, and mixing may be achieved by mechanical means or by gas recirculation, where digester gas is bubbled back through the digester contents (Hawkes and Hawkes, 1987). The main characteristic of a CSTR system is that its SRT (sludge retention time) is equal to its HRT, and thus no biomass retention occurs (Zeeman and Saunders, 2001). The CSTR is normally operated at an HRT of 20-30 days and a loading rate of 1.7 kg VS/m^3/day. A long HRT has to be employed to prevent biomass washout. The disadvantages of the CSTR are that the effluent will contain some fraction of freshly added, undigested feed material, together with some of the still active microbial population, and the methane yield is usually low. The addition of large amounts of water requires large reactor volumes and leads to high costs for the post-treatment of the digester residue (Gunaseelan, 1997). Due to these limitations, there have been developments in the design of new reactors suitable for thick slurries and semi-solid waste.

The CSTR is used for conventional, single-stage anaerobic digestion. In one-stage systems (the conventional application in the CSTR), hydrolysis, acidogenesis and methanogenesis are combined in the same vessel under the same process conditions (Yu *et al.*, 2002). In such systems the production of VFAs from easily degradable solids can lead to the accumulation of VFAs resulting in a fall in pH and subsequent inhibition of methanogenesis (Yu *et al.*, 2002; Ghosh *et al.*, 2000). Conditions that are favourable for the growth of acid-producing bacteria, such as short HRT and low pH, are inhibitory to the methanogens (Ghosh, 1998; Ince, 1998; Anderson *et al.,* 1994b). As a result, one-stage systems are operated at solid retention times longer than 15 days, at low feed solids concentrations and low loading rates, in large reactors (CSTRs) but with low net energy production (Ghosh, 1987).

4.2.3 Two-stage anaerobic digestion

There has long been interest in separating the acid- and methane-forming populations in two or more serial reactors to reduce problems associated with stability and control in one-stage digestion (Callander and Barford, 1983; Cohen *et al.,* 1982; Breure *et al.,* 1985; Ghosh *et al.,* 1975; Pohland and Ghosh, 1971). Two-stage anaerobic digestion is a process configuration using two separate reactors, one for liquefaction (hydrolysis) and acidification, and the other for biomethanation, connected in series (Ghosh and Poland 1974; Vavilin *et al.,* 2001). This process configuration is also referred to in the literature as two-step or two-phase digestion (Figure 7). All the high-rate reactors discussed in Section 4.1.1 can be used as methanogenic reactors in the two-stage process.

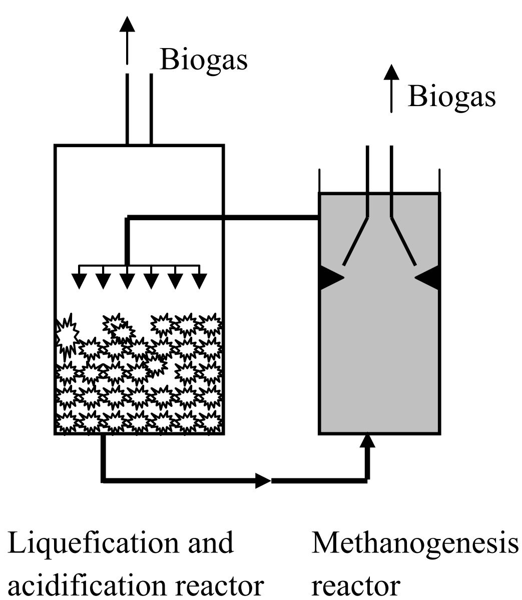

Figure 7. Two-stage set-up for the anaerobic digestion of solid waste.

The two-stage system allows for optimisation of both microbial processes with respect to nutritional requirements, physiology, physical requirements and growth rates (Anderson *et al.,* 1994b; Ghosh *et al.,* 2000; Elefsiniotis and Oldham, 1994c). In two-stage anaerobic digestion, the acidogenic-stage reactor is maintained at a low alkalinity and develops a high CO_2 and low CH_4 content in the gaseous environment. Acidifying organisms dominate in the first reactor and the major biochemical reaction is enzymatic hydrolysis and fermentation. The methanogenic-stage bioreactor is maintained at pH above 7 and high alkalinity resulting in high specific methanogenic activity (Fox and Pohland, 1994; Ghosh, 1990). The conditions that favour methanogenic activity also retard the growth of fermentative acidogens. Some opinions on the advantages of the two-stage system over the one-stage system when treating the same waste(water) given in the literature are listed in Table 6.

Table 6. The advantages of the two-stage system over the one-stage system when treating the same waste(water).

- Two-stage systems can treat three times the organic loading of a one-stage process, and therefore have shorter hydraulic retention time for rapidly degradable waste. The volumetric capacity of the two-stage system is theoretically higher than that of a single-stage system.
- Significantly higher biomass conversion efficiency and higher COD removal efficiency.
- Higher methane concentration (80-85%) in the biogas produced because the specific activity of methanogens is increased.
- Better process reliability, resilience and stability, especially with variable waste conditions and readily degradable waste, which causes unstable performance in one-stage systems.
- Physical separation of the acidogenic and methanogenic phases allows maintenance of appropriate densities of the acid- and methane-producing microbes enabling maximisation of their rates.
- The acid phase and methane phase can be started much more easily and quickly than in conventional, single-stage digesters.
- The acidification reactor can serve as a buffer system when the composition of the wastewater is variable and can help in the removal of compounds toxic to the methanogens.
- Based on information from full-scale operating systems, two-stage systems produce less and better quality Class A biosolids. This is the main reason for using the two-stage process.
- Foaming is limited to, at worst, the start-up in all two-phase systems. Foaming problems can be controlled by keeping the feed solids above 5% TS, which is an advantage in itself.

(Adapted from: Yu *et a*l., 2002; van Lier *et al.,* 2001; Lissens *et al.,* 2001; Ghosh *et al.,* 2000; Dinsdale *et al*., 2000; Ince and Ince 2000; Wilson, 2000; Solera *et al.,* 2002; Elias *et al.,* 1999; EL-Gohary *et al*., 1999; Ince, 1998; Dinsdale *et al.,* 1997a; Anderson *et al.,* 1994b; Fox and Pohland, 1994).

The disadvantages of two-stage anaerobic digestion are possible disruption of syntrophic interspecies hydrogen transfer and the loss of methane potential by H_2 and CO_2 production in the acidogenic phase (Gunaseelan, 1997). They are also more difficult to engineer, implement and operate, and there is lack of process experience and applicability to some kind of waste. The extra investment cost and operating complexity of two-stage systems have caused two-stage systems to be limited to a small market (Lettinga, 1995; Elias *et al.,* 1999; de Baere, 2000).

About 90% of the full-scale plants currently in use in Europe for anaerobic digestion of waste(water) rely on one-stage systems (de Baere, 2000). Industrialists prefer one-stage systems because simpler designs suffer less frequently from technical failure and have smaller investment costs. Industrial applications often showed that complete pre-acidification had adverse effects on the stability of anaerobic sludge systems (Lettinga, 1995; Ahn *et al.,* 2001; van Lier *et al.,* 2001). The required level of pre-acidification depends on the actual wastewater characteristics, such as COD concentration and buffering capacity. Comparisons are further complicated by the different cultures that tend to dominate in single-stage and two-stage systems (Fox and Pohland, 1994). The two-stage process can be recommended for all waste with unbalanced ratios of C:N:P, such as agro-industrial residues with exceptionally high protein levels or waste that acidifies quickly, like potato waste and wastewater. Well-balanced waste with average C:N:P ratios of about 100:5:1 can be treated with the cheaper one-stage process, which will be preferred in most situations. Two-stage anaerobic digestion is more widely applied in the degradation of the organic fraction of municipal solid waste, representing 10% of the anaerobic treatment capacity in Europe (de Baere, 2000; Pavan *et al.,* 2000). The two-stage treatment of solid agricultural residues has been less well investigated (Andersson and Björnsson, 2002). In this work, some features of the two-stage process were investigated such as the profiles of hydrolytic enzymes in the process the degree of acidification, the distribution of the major VFAs produced in the acidification reactor, the COD removal, methane yield and the effect of co-digestion (**Papers I, III, V**) for improved understanding of the two-stage process. The performance of two-stage anaerobic digestion for agricultural residues carried out in this work using various high-rate reactors gave good methane yields and these are compared with literature data in Table 7.

Table 7. Energy yield in this study compared with two-stage anaerobic digestion literature data.

Feed	**Reactor type**	**Temperature** (°C)	**Energy yield** (kWh / kg VS)	**Reference**
Solid potato waste	Two-stage, lab-scale	37	3.9	**Paper I**
Solid potato waste	Two-stage, lab-scale	37	4.9	**Paper III**
Solid potato waste	Two-stage, pilot-scale	37	3.4	**Paper V**
Co-digested beet tops and potato	Two-stage, pilot-scale	37	3.9	**Paper V**
Sugar beet pulp	Two-stage, pilot-scale	35	3.5	Weiland, (1993)
Fruit and vegetables	Two-stage, lab-scale	35-38	5.0	Virtua *et al*. (1989)
Food waste	Two-stage, pilot-scale	35-38	4.3	Lee *et al*. (1999)

Two-stage anaerobic digestion -Temperature options

Different temperature conditions can be applied to two-stage reactors, such as mesophilic + mesophilic, mesophilic + thermophilic (meso + thermo), thermophilic + mesophilic (thermo + meso) and thermophilic + thermophilic (thermo + thermo) conditions. The thermophilic + mesophilic anaerobic digestion process was developed as a means of taking advantage of thermophilic digestion, while mitigating its undesirable qualities, especially odour and foaming tendencies. The system offers the advantages of a high digestion rate and pathogen destruction capability with the thermophilic process, and lower energy requirement and good effluent quality with the mesophilic process.

Meso + thermo anaerobic digestion was originally pioneered to meet the time-temperature requirement for pathogen reduction, and it was based on the premise that exposure of mesophilically digested sludge to the enzyme systems of thermophilic anaerobes could result in further volatile solids reduction (Ghosh, 1998). The increased volatile solids reduction was achievable because the solid retention time of meso-thermo digestion process was longer than that of conventional mesophilic digestion process. Since mesophilic digestion overcomes some of the limitations of thermophilic digestion, the application of temperature-phased, two-stage anaerobic digestion could provide the 'best of both worlds' regarding mesophilic and thermophilic fermentation, together with the advantages of reactor staging. The performance of the two-stage anaerobic digestion process in treatment of some industrial waste(water)s is listed in Table 8. There are few reports on the application of the two-stage system to treat solid potato waste.

Table 8. Performance of some two-stage anaerobic digestion processes (adapted from Demirel and Yenigun, 2002). All results were based on bench-scale reactors.

Reactor Configuration	**Waste**	**OLR** (kg CODm^3/day)	**Temperature regime**	**Removal** (%)	**Reference**
Solid-bed + UASB	Solid potato waste	11	Meso+Meso	90 (COD)	**Paper III**
Solid-bed + UASB	Solid potato waste	36	Thermo+Thermo	85 (COD)	**Paper III**
CSTR+AF	Soft drink		Meso+Meso	96 (COD)	Ghosh *et al.* (1985)
CSTR+ UASB	Instant coffee	16	Thermo+Meso	77 (COD)	Dinsdale *et al.* (1997b)
CSTR +AF	Dairy	5	Meso+Meso	90 (COD)	Ince, (1998)
Two-stage	Instant coffee	2.8	Meso+Meso	97 (COD)	Strydom *et al.* (1997)

Two-stage anaerobic digestion options - High-rate reactors

The high-rate reactors (Section 4.1.1) can be applied in the second step, methanogenesis, of the two-stage anaerobic digestion process. In this work, a UASB methanogenic reactor and a methanogenic reactor packed with wheat straw biofilm carriers were compared (**Paper I**). The performance of UASB reactors in two-stage configurations under mesophilic and thermophilic conditions for the first and second stage when treating solid potato waste was also investigated (**Paper III**). In the pilot-scale study, two configurations of the second methanogenic stage were investigated, a straw packed-bed reactor and a reactor containing plastic carriers (**Paper V**). The performance of the methanogenic reactors is listed in Table 4,7 and 8. In all these studies, the hydrolysis and acidification stage and the methanogenic stage were reasonably well separated, as indicated by the high CO_2 production, high VFA concentration and low pH in the acidogenic reactors compared with high methane production, low VFA concentration and above-neutral pH in the effluent of the methanogenic reactors.

5 APPLICATIONS OF ANAEROBIC DIGESTION TECHNOLOGY

5.1 An Outlook

Developments over the past 15 years have shown that the anaerobic treatment process is an attractive and viable alternative for the stabilisation of a wide range of industrial waste and wastewater (Gijzen, 2001). Interest in many industrial sectors in applying anaerobic digestion is increasing rapidly (Lettinga, 2001; Lema and Omil, 2001; Lettinga, 2004). The application of the anaerobic treatment process in waste management includes septic tanks (for on-site systems), sludge digesters, industrial wastewater treatment, municipal wastewater treatment, hazardous waste management (aromatic compounds and halogenated compounds), municipal solid waste management, agricultural waste management, and biogas generation (Switzenbaum, 1995; Verstraete and Vandevivere, 1997). These applications are in various stages of development, from proven technologies (e.g. septic tanks and sludge stabilisation), to developing technologies (e.g. use in hazardous waste remediation). While anaerobic technology has been applied historically to non-complex, agro-industrial wastewater at mesophilic conditions, the application of anaerobic treatment is expanding to include extremely complex wastewater and thermophilic and psychrophilic conditions (van Lier *et al.,* 2001; Lettinga 2001).

Anaerobic digestion started to gain a niche among treatment technologies for organic solid waste arising from municipal solid waste, market waste or other industrial organics in Europe in around 1995. In 1995, anaerobic capacity represented less than 5% of the total biological treatment capacity in Europe but this has steadily increased throughout the continent (de Baere, 2000). In Sweden in 2000, approximately 50% of solid biodegradable waste was being incinerated, 45% was deposited in landfills, and 5% biologically treated (mainly composted but anaerobic digestion was gaining interest) (Sonesson *et al*., 2000). In Sweden there have been increased demands for a reduction in emissions from incinerators and landfills, as well as for improved treatment efficiency in sewage plants. During 2001, 1.4 TWh of biogas was produced in Sweden and the potential annual contribution from biogas has been estimated to be up to 25 TWh, of which 14 TWh could be derived from the agricultural sector (Nordberg and Edström, 2003). Germany, the Netherlands and Denmark are leaders in modern anaerobic digestion technology. The country with greatest experience of using centralised, large-scale digestion facilities is Denmark, where 18 large, centralised plants are in operation (Angelidaki *et al*., 2004). In many cases, these facilities co-digest manure, organic industrial waste, and source-sorted municipal solid waste. MSW is attracting renewed interest as a feedstock for anaerobic digestion. There is widespread of use of anaerobic digestion in Germany, with approximately 850 biogas plants (with reactor volumes between 100 and 4, 000 m^3) on farm scale as well as on large industrial scale with good performance in 2000 (Weiland, 2000). In 2000, there were also 14 centralised large-scale plants with treatment capacities between 16,000 and 126,000 tons per year in Germany. In the Netherlands and Belgium, the market shares were 15.6% and 11.9%, respectively, of the biological treatment capacity provided by anaerobic technologies (de Baere, 2000). The two-stage anaerobic digestion process is relatively new compared with other anaerobic digestion configurations. It has been applied for treatment of high-strength industrial waste and municipal sludge in Europe, the United States, Malaysia, India and other countries (Ghosh, 1998).

5.2 Perspectives of anaerobic treatment in developing countries

Although reliable regional energy statistics are not readily available, existing estimates of energy use in eastern and southern Africa indicate a significant and persistent dependence on traditional biomass energy technologies and limited use of modern, sustainable energy technologies. The use of biogas is widespread in countries such as China and India for cooking, lighting and operation of small engines, but this is not the case in most African countries (Reddy *et al*., 2000). In most developing countries, for example, Bangladesh, Burundi, Bolivia, Ivory Coast, Tanzania and Thailand, biogas is produced through anaerobic digestion of human and animal excreta using the Chinese fixed-dome digester and the Indian floating-cover biogas digester, which are not reliable and have poor performance in most cases (Omer and Fadalla, 2003). These plants were built for schools and small-scale farmers, in most cases by non-governmental organisations. Most of the plants have only operated for a short period due to poor technical quality. There is thus a need to introduce more efficient reactors to improve both the biogas yields and the reputation of the technology. The development of large-scale anaerobic digestion technology in eastern and southern Africa is still embryonic, although the potential is there. The development of anaerobic digestion technology for effective and feasible waste and wastewater management constitutes an ideal solution to the increasing waste(water) problems in developing regions (Gijzen, 2001). The main limitations to the adoption of large-scale biogas technology are both institutional and economic. Establishing a self-sustaining institutional system that can collect and process urban waste and effectively market the generated biogas fuel is a complex activity that calls for sophisticated organisational capability and initiative.

The combination of methane production with waste treatment processes is very attractive, and this bodes well for the improved management of biodegradable organic waste in Zimbabwe and other developing countries (Ayalon *et al*., 2001; Lema and Omil, 2001). Energy is an essential factor in the development of a country since it stimulates and supports economic growth and development. The power generated by hydroelectric and thermal power stations in these countries is below the demand. For example, Zimbabwe has not been able to increase its electricity generation capacity since 1985 and imports 40% of her electrical energy need from neighbouring countries (Mbohwa and Fukuda, 2003). Although the population in urban areas is rising, around 80% of the population still lives in rural areas. Wood is the most important domestic fuel and about 80% of the population mainly in rural and peri-urban areas depends on it for cooking, lighting and heating. Biogas technology can be used to improve rural infrastructure and to substitute imports and fossil energy sources. Biogas would be important in rural communities allowing local energy generation from local resources, thus making the communities independent of centrally distributed energy, and oscillations and uncertainties of the world energy market (Kashyap *et al*., 2003). Agriculture is the backbone of economic and social development in developing countries, and this generates large amounts of biomass waste, which could be used to produce biogas (Omer and Fadalla, 2003). Developing countries are located in regions where the climate is warm most of the time, and this is the main factor that makes the use of anaerobic technology appropriate and less expensive, even for the treatment of low-strength industrial waste and wastewater (Foresti, 2001).

The application of anaerobic digestion to industrial wastewater treatment in Zimbabwe would be a welcome development. The biogas from the plant studied in this work is currently vented to the atmosphere, whereas further benefits of the plant could be realised by tapping the energy generated by the anaerobic process in the form of methane (**Paper VII**). Some of the difficulties encountered in the introduction of anaerobic wastewater treatment for industrial waste in developing countries are listed below (Lettinga, 1995; 2001; Switzenbum, 1995; Tafdrup, 1995; Iza *et al*., 1991):

- Inexperienced contractors and consultants, resulting in poor-quality plants, and poor choice of materials.
- Lack of reliable information on the potential of the technology.
- Complete absence of academic, bureaucratic, legislation and commercial infrastructure in the region/country.
- Lack of knowledge on the system in practice, sometimes even in research institutes and universities.
- Complete absence of pilot studies, and no full-scale experience.
- No properly educated operators, lack of credibility, lack of technical knowledge on maintenance and repair.
- Uninformed or poorly informed authorities and policy makers.
- Research at universities is frequently considered to be too academic in nature, even when it is quite applied.
- In some cases, where land is available and inexpensive, waste stabilisation ponds provide a more economical solution than anaerobic treatment.

In order to promote the implementation and proper use of anaerobic digestion technology, it is important to initiate long-term anaerobic digestion and other renewable energy training and capacity-building programmes, and to perform scientific work in this field (through appropriate research). It is important to establish contacts between research and university groups and experienced contractors, and to initiate collaboration with polluting industries, i.e., to interest them in the system, either for use as an environmental protection method, or for energy production. In addition, experts should provide reliable and pertinent information about the method and its potential to local authorities, politicians, and the public in general. There is also need and to obtain grants from the government or international organisations, and industry for pilot-plant and/or demonstration-scale projects (Foresti, 2001; Karekezi, 1994).

6 CONCLUDING REMARKS

The anaerobic treatment process is increasingly being recognized as the core method for an advanced technology for environmental protection and resource preservation and, combined with other suitable methods; it represents a sustainable and appropriate method of treating waste and wastewater in both developed and developing countries. The successful application of anaerobic digestion to the treatment of biodegradable solid waste and wastewater is critically dependent on the development and use of high-rate bioreactors among other factors. There is a considerable amount of biodegradable waste that is suitable for biogas production, and in the present study, solid potato waste is used as a representative of many kinds of carbohydrate-rich waste. The purpose of the present work was to investigate the performance of different high-rate bioreactors when treating agricultural residues and industrial wastewater. The following conclusions have been drawn from the work described in this thesis.

One important aspect in promoting anaerobic processes is to demonstrate the appropriate anaerobic technology for waste(water), where it is not the common practice today. In this work it has been demonstrated how anaerobic digestion in high-rate reactors (**Papers I, III, V, VI**) may provide an alternative to other uses of solid potato waste such as ethanol production and cattle feed, and to sugar beet leaves which are currently not used for biogas production. In **Paper VII**, the application of a full-scale UASB bioreactor for treating opaque beer brewery wastewater is described, enabling the brewery to meet the permissible levels (in terms of COD) for effluent discharge into the municipal sewage treatment system in a developing country. The organic matter reduction is transmitted to municipal treatment plants and subsequently to the environment. Further benefits of the plant could be realised by tapping the energy generated by the anaerobic process in the form of methane gas. It can be concluded that the installation of an anaerobic wastewater treatment plant by the brewery is an extremely attractive economic and environmental alternative considering that an era of critical energy shortages, substantially higher energy prices, and higher demand on environmental protection lies ahead.

Hydrolysis is the first step during the anaerobic digestion of solid waste and is the rate-limiting step of the degradation. To overcome this limitation, more knowledge about the enzymatic degradation is needed. The profiles of hydrolytic enzymes (both free and cell-bound enzyme activities) produced by microorganisms to hydrolyse solid potato waste during anaerobic digestion are presented in **Paper I**. There is a varied repertoire of hydrolases with which microorganisms can attack organic particles and polymers to hydrolyse them into transportable molecules. Anaerobic digestion of solid potato waste was also shown to produce valuable intermediates, such as acetic, propionic, butyric and lactic acids (**Paper II**).

The successful application of high-rate reactors as the second-stage in two-stage anaerobic digestion of solid agricultural waste was demonstrated (**Papers I, III, V, VII**). The results confirmed that straw, a common agricultural by-product, works well as a biofilm carrier in the methanogenic stage. The UASB reactor was found to perform better than anaerobic packed bed reactors.

Both mesophilic and thermophilic anaerobic digestion of solid potato waste can be applied depending on the aim of the anaerobic process; in some processes the volumetric reduction of the waste has priority, while for others the biogas yield is more important. The acceptable costs and loading rates for each process vary with demands (**Paper III**).

Employing efficient but low cost technology is important for increased utilisation of anaerobic digestion, and the possibility of doing this has been demonstrated by a simple pilot-scale, two-stage anaerobic digestion system for the treatment of solid potato waste and sugar beet leaves alone and combined with the recovery of biogas (**Paper V**). Encouraging results obtained in the study indicate the feasibility of large-scale application of the process**.**

The benefit of co-digestion of solid potato waste and sugar beet leaves was shown, the methane yield improved by 60% compared with the methane yield from digestion of the separate substrates (**Papers IV, V**). The high methane yield favours the application of the two-stage process to co-digestion of solid potato with sugar beet leaves. Results from this study suggest that potato waste and sugar beet leaves are suitable substrates for anaerobic digestion for the production of biogas, and could provide additional benefits to farmers. The general conclusion is that starch-rich substrates may be mixed with others rich in nitrogen and then co-digested.

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REFERENCES

Andersson, J. and Björnsson, L. 2002 Evaluation of straw as a biofilm carrier in the methanogenic stage of two-stage anaerobic digestion of crop residues. *Bioresource Technology*, **85**, 51-56.

Anderson, G.K., Kasapgil, B. and Ince, O. 1994a Comparison of porous and non-porous media in upflow anaerobic filters when treating dairy wastewater. *Water Research*, **28**, 1619-1624.

Anderson, G.K., Kasapgil, B. and Ince, O. 1994b Microbiological study of two-stage anaerobic digestion during start-up. *Water Research*, **28**, 2383-2392.

Ahn, Young-Ho, Kyung-Sok, Min, and Speece R.E. 2001 Pre-acidification in anaerobic sludge bed process treating brewery wastewater. *Water Research*, **35**, 4267- 4276.

Ahn, J-H., and Forster, C.F. 2000 A comparison of mesophilic and thermophilic anaerobic upflow filters. *Bioresource Technology*, **73**, 201-205.

Ahring, B.K., Angelidaki, I. and Johansen, K. 1992 Anaerobic treatment of manure together with industrial waste. *Water Science and Technology*, **30**, 241-249.

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

Ahring, B.K. 1994 Status on science and application of thermophilic anaerobic digestion. *Water Science and Technology*, **28**, 165-176

Angelidaki, I., Boe, K. and Ellegaard L. 2004 Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. Proceedings of the 10th World Congress in Anaerobic Digestion, Montreal Canada, 29th August-2nd September.

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

Archer, D.B. 1983 The microbial basis of process control in methanogenic fermentation of soluble wastes. *Enzyme Microbial Technology*, **5**, 162-169.

Archer, D.B., Hilton, M.G., Adams, P. and Wiecko, H. 1986 Hydrogen as a process control index in pilot scale anaerobic digester. *Biotechnology Letters*, **8**, 197-202.

Ayalon, O., Avnimelech, Y., and Shechter, M. 2001 Solid waste treatment as a high-priority and low cost alternative for greenhouse gas mitigation. *Environmental Management*, **27**, 697-704.

Baldasano, J.M. and Soriano, C. 2000 Emission of greenhouse gases from anaerobic digestion processes: comparison with other municipal solid waste treatments. *Water Science and Technology*, **41**, 275-282.

Banerjee, A., Elefsiniotis, P., and Tuhtar, D. 1999 The effect of addition of potato-processing wastewater on the acidogenesis of primary sludge under varied hydraulic retention time and temperature. *Journal of Biotechnology*, **72**, 203-212.

Barber, W.P. and Stuckey, D.C. 1999 The use of anaerobic baffled reactor (ABR) for wastewater treatment: A Review. *Water Research*, **33**, 1559 -1578.

Batstone, D.J., Keller, J., Angelidaki, I., Kalyuhnyi, S.V., Pavlostathis, S.G., Rozzi, A., Sanders, W.T.M., Siegrist, H. and Vavilin, V.A. 2002 The IWA Anaerobic Digestion Model. *Water Science and Technology*, **45**, 65-73.

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

Björnsson, L., Murto, M. and Mattiasson, B. 2000 Evaluation of parameters for monitoring an anaerobic co-digestion process. *Applied Microbiology and Biotechnology*, **54**, 844-849.

Borja, R., Martin, A., Luque, M., and Alonso, V. 1994 Kinetics of methane production from wine distillery wastewater in an immobilized cell bioreactor using sepiolite as support medium. *Resources Conservation and Recycling*, **10**, 317-327.

Bouallagui, H., Cheikh, R.B., Marouuani, L. and Hamdi M. 2003 Mesophilic biogas production from fruit and vegetable waste in tubular digester. *Bioresource Technology*, **86**, 85-89.

Bowker, R.P.G. 1983 New wastewater treatment for industrial applications. *Environmental Progress*, **2**, 235-242.
Breure, A.M. and van Andel, J.G. 1984 Hydrolysis and acidogenic fermentation of a protein, gelatin, in an anaerobic continuous culture. *Applied Microbiology and Biotechnology*, **20**, 40-45.
Breure, A.M., van Andel, J.G., Burger-Wiersma, T., Guijt, J. and Verkuijlen, J. 1985 Hydrolysis and acidogenic fermentation under anaerobic conditions in a laboratory scale upflow reactor. *Applied Microbiology and Biotechnology*, **21,** 50-54.
Brummeler, E.T. 1993 Dry anaerobic digestion of the organic fraction of municipal solid waste. *PhD Thesis*, Wageningen Agricultural University, Wageningen, The Netherlands.
Brummeler, E.T. 2000 Full scale experience with the BIOCEL process. *Water Science and Technology*, **41**, 299-304.
Callaghan, F.J. Wase, D.A.J., Thayanithy, K. and Forster C.F. 2002 Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. *Biomass and Bioenergy*, **22**, 71-77.
Callander, I.J. and Barford, J.P. 1983 Recent Advances in Anaerobic Digestion Technology. *Process Biochemistry*, **18**, 24 - 30.
Chen, S.J., Li, C.T. and Shieh, W.K. 1985 Performance evaluation of the anaerobic fluidised bed system: 1. Substrate utilisation and gas production. *Journal Chemical Technology and Biotechnology*, **35**B, 101-109.
Cho, J.K., Kim, B.S., Park, S.C., Choi, Y.S. and Chang, H.N. 1996 Effect of stepwise seeding on the performance of four anaerobic biofilters treating a synthetic stiilage waste. *Biomass and Bioenergy*, **10**, 25-35.
Chugh, S., Chynoweth, D.P., Clarke, W., Pullammanappallil, P., and Rudolp, V. 1999 Degradation of unsorted municipal solid waste by leach-bed process. *Bioresource Technology*, **69**, 103-115.
Chynoweth, D.P. and Legrand, R. 1988 Anaerobic digestion as an integral part of municipal waste management. In: Proceedings of the Conference on Landfill, Gas and Anaerobic Digestion of Solid Waste, Chester, England, 4-7 October. Thomas, H., Alston, Y.R. and Richards, G.E. eds. Pp. 467-480.
Chynoweth, D.P., Owens, J.M. and Legrand R. 2001 Renewable methane from anaerobic digestion of biomass. *Renewable energy*, **22**, 1-8.
Cohen, A., Breure, A.M., van Andel, J.G. and van Deursen, A. 1982 Influence of phase separation on the anaerobic digestion of glucose. II. Stability and kinetic responses to shock loadings. *Water Research*, **16**, 449 - 455.
de Baere, L. and Verstraete, W. 1984 Anaerobic digestion of solid and semi-solid substrates, In: Ferrero G.L., Ferranti M.P., and Naveau H. (Eds) Anaerobic digestion and carbohydrate hydrolysis of waste, Elsevier Applied Science Publishers, London, pp.195-208.
de Baere, L. 2000 Anaerobic digestion of solid waste: state of the art. *Water Science and Technology*, **41**, 283-290.
Demirel, B. and Yenigun, O. 2002 Two-phase anaerobic digestion processes: A Review. *Journal of Chemical Technology and Biotechnology*, **77**, 743-755.
di Berardino, S., Costa, S., and Converti, A. 2000 Semi-continuous anaerobic digestion of a food industry wastewater in an anaerobic filter. *Bioresource Technology*, **71**, 261-266.
Dinsdale, R.M., Hawkes, F.R and Hawkes, D.L. 1997a Mesophilic and thermophilic anaerobic digestion with thermophilic pre-acidification of instant-coffee-production wastewater. *Water Research*, **31**,1931 -1938.
Dinsdale, R.M., Hawkes, F.R and Hawkes, D.L. 1997b Comparison of mesophilic and thermophilic upflow anaerobic sludge blanket reactors treating instant coffee production wastewater. *Water Research*, **31**, 163 -169.
Dinsdale, R.M., Premier, G.C., Hawkes, F.R. and Hawkes, D.L. 2000 Two-stage anaerobic co-digestion of waste activated sludge and fruit/vegetable waste using inclined tubular digesters. *Bioresource Technology*, **72**, 159-168.

Dugba, P.N. and Zhang, R. 1999 Treatment of dairy wastewater with two-stage anaerobic sequencing batch reactor systems – thermophilic versus mesophilic operations. *Bioresource Technology*, **68**, 225-233.
Eastmann, J.A. and Ferguson, J.F. 1981 Solubility of particulate organic during the acid phase of anaerobic digestion. *Journal Water Pollution Control Federation*, **53**, 352-366.
Edelmann, W., Schleiss, K. and Joss, A. 2000 Ecological, energetic and economic comparison of anaerobic digestion with different competing technologies to treat biogenic wastes. *Water Science and Technology*, **41**, 263-273.
Edelmann, W., Engeli, H. and Gradenecker, M. 2000b Co-digestion of organic solid waste and sludge from sewage treatment. *Water Science and Technology*, **41**, 213-221.
Elefsiniotis, P. and Oldham, W.K. 1994a Substrate degradation patterns in acid-phase anaerobic digestion of municipal primary sludge. *Environmental Technology*, **15**, 741-751.
Elefsiniotis, P. and Oldham, W.K. 1994b Influence of pH on the acid-phase anaerobic digestion of primary sludge. *Journal of Chemical Technology and Biotechnology*, **60**, 89-96.
Elefsiniotis, P. and Oldham, W.K. 1994c Anaerobic acidogenesis of primary sludge: The role of solids retention time. *Biotechnology and Bioengineering*, **44**, 7-13.
Elefsiniotis, P. and Oldham, W.K. 1994d Effect of HRT on acidogenic digestion of primary sludge. *Journal of Environmental Engineering*, **120**, 645-660.
El-Gohary, F.A. and Nasr, F.A. 1999 Cost-effective pre-treatment of wastewater. *Water Science and Technology*, **39**, 97-103.
Elias, A., Barona, A., Ormazaba, J., Ibarra, G. and Caamano, J. 1999 Anaerobic treatment of acidified and non-acidified substrata in UASB reactors. *Journal of Chemical Technology and Biotechnology*, **74**, 949-956.
EU. 1996 Strategy paper for reducing methane emissions. Communication from the Commission to the Council and the European Parliament COM (96) 557 *http://europa.eu.int/comm7dg11/docum/96557en.pdf*
Fang, H.H.P. and Wai-Chung Chung, D. 1999 Anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. *Water Science and Technology*, **40**, 77-84.
Foresti, E. 2001 Perspectives on anaerobic treatment in developing countries. *Water Science and Technology*, **44**, 141-148.
Fox, P. and Pohland, F.G. 1994 Anaerobic treatment applications and fundamentals: substrate specificity during phase separation. *Water Environment Research*, **66**, 716-724.
Francese, A.P., Aboagye-Mathiesen, G., Olesen, T., Co'rdoba, P.R. and Sineriz, F. 2000 Feeding approaches for biogas production from animal wastes and industrial effluents. *World Journal of Biotechnology*, **16**, 147-150.
Fueyo, G., Gutierrez, A. and Berrueta, J. 2002 Anaerobic degradation: the effect of the combined treatment of substrates on the refractory fraction. *Journal of Chemical Technology and Biotechnology*, **77**, 910-916.
Garcia, J.L., Patel, B.K.C. and Ollivier, B. 2000 Taxonomic, phylogenetic, and ecological diversity of methanogenic *Archaea, Anaerobe*, **6**, 205-226.
Ghosh, S. and Pohland, F.G. 1974 Kinetics of substrate assimilation and product formation in anaerobic digestion. *Journal of Water Pollution Control and Federation*, **40**, 748-759.
Ghosh, S., Conrad, J.R. and Klass, D.L. 1975 Anaerobic acidogenesis of wastewater sludge. *Journal of Water Pollution Control Federation*, **47**, 30-45.
Ghosh, S. and Klass, D.L. 1978 Two-Phase Anaerobic Digestion. *Process Biochemistry*, **13**, 15-24.
Ghosh, S., Ombregt, J.P. and Pipyn, O. 1985 Methane production from industrial wastes by two-phase anaerobic digestion. *Water Research*, **19**, 1083-1088.
Ghosh, S. 1987 Improved sludge gasification by two-phase anaerobic digestion. *Journal of Environmental Engineering*, **113**, 1265-1284.
Ghosh, S. 1990 Principles and potentials of biphasic fermentation. Biogas Technologies and Implementation Strategies International Conference, 473-497.

Ghosh, S. 1997 Anaerobic digestion for renewable energy and environmental restoration, Pp. 9-16, In: Proceedings of the 8th International Conference on Anaerobic digestion, Sendai International Center, Sendai, Japan, May 25-29.
Ghosh, S., Henry M.P, Sajjad A., Mensinger M.C. and Arora J.L. 2000 Pilot-scale gasification of municipal solid wastes by high-rate and two-phase anaerobic digestion (TPAD). *Water Science and Technology*, **41**, 101-110.
Ghosh, S. 1998 Temperature-Phased Two-Phase Anaerobic Digestion. Proceedings of WEFTC Water Environment Federation 71st Annual Conference, **2**, 189-199.
Gijzen, H.J. 2002 Anaerobic digestion for sustainable development: a natural approach. *Water Science and Technology*, **45**, 321-328.
Gijzen, H.J. 2001 Anaerobes, aerobes and phototrophs, a winning team for wastewater management. *Water Science and Technology*, **44**, 123-132.
Gonzalez-Gil, G., Kleerebezem, R. and Lettinga, G. 1999 Effects of nickel and cobalt on kinetics of methanol conversion by methanogenic sludge as assessed by on-line CH_4 monitoring. *Applied and Environmental Microbiology*, **65**, 1789-1793.
Grommen, R. and W. Verstraete 2002 Environmental biotechnology: the ongoing quest. *Journal of Biotechnology*, **98**, 113-123.
Guitonas, A., Paschalidis, G. and Zouboulis, A. 1994 Treatment of wastewaters by fixed bed anaerobic reactors with organic support. *Water Science and Technology*, **29**, 257-263.
Gunaseelan, V.N. 1997 Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy*, **13**, 83-114.
Gujer, W. and Zehnder, A.J.B. 1983 Conversion processes in anaerobic digestion. *Water Science and Technology*, **15**, 127-167.
Hawkes, F.R. and Hawkes, D.L. 1987 Anaerobic Digestion, In Bu'lock, J. and B. Kristiansen (eds.), Basic Biotechnology. Academic press, London, 337-358.
Hill, D.T. and Jenkins, S.R. 1989 Measuring alkalinity accurately in aqueous systems containing high organic acid concentrations. *Trans. ASAE*, **32**, 2175-2178.
Horiuchi, J.I., Shimizu, T., Tada, K., Kanno, T. and Kobayashi, M. 2002 Selective production of organic acids in anaerobic acid reactor by pH control. *Bioresource Technology*, **82**, 209-213.
Hsu, Y. and Shieh, W.K. 1993 Start-up of anaerobic fluidised bed reactors with acetic acid as the substrate. *Biotechnology and Bioengineering*, **41**, 347-353.
Hulshoff-Pol, L.W. 1998 Waste characteristics and factors affecting reactor performance. Lecture Notes by Hulshoff Pol in International Course on Anaerobic Wastewater Treatment, Wageningen Agricultural University, The Delft, Netherlands.
Hwang, S., Lee, Y. and Yang, K. 2001 Maximisation of acetic acid production in partial acidogenesis of swine wastewater. *Biotechnology and Bioengineering*, **75**, 521-529.
Ince, O. 1998 Performance of a two-phase anaerobic digestion system when treating dairy wastewater. *Water Research*, **32**, 2707-2713.
Ince, B.K. and Ince, O. 2000 Changes to bacterial community make-up in a two-phase anaerobic digestion system. *Journal of Chemical Technology and Biotechnology*, **75**, 500-508.
Ince, B.K. Ince, O., Anderson, G.K. and Arayici, S. 2001 Assessment of biogas as an energy source from anaerobic digestion of brewery wastewater. *Water, Air and Soil Pollution*, **126**, 239-251.
Iza, J., Colleeran, E., Paris, J.M. and Wu, W.M. 1991 International workshop on anaerobic treatment technology for municipal and industrial wastewaters: summary paper. *Water Science and Technology*, **24**, 1-7.
Jenkins, R.S., Morgan, J.M. and Zhang, X. 1991 Measuring the usable carbonate alkalinity of operating anaerobic digesters. *Journal of Water Pollution Control and Federation*, **63**, 28-34.
Jennett, J.C. and Dennis, N.D. Jr. 1975 Anaerobic filter treatment of pharmaceutical waste. *Journal of Water Pollution Control and Federation*, **47**, 104-121.
Kalyuzhnyi, S., Veeken, A. and Hamelers, B. 2000 Two-particle model of anaerobic solid-state fermentation. *Water Science Technology*, **41**, 43 - 50.

Kalyuzhnyi, S., Sklyar, V.I., Davlyatshina, M.A., Parshina, S.N., Simankova, M.V., Kostrikina, N.A. and Nozhevnikova, A.N. 1996 Organic removal and microbiological features of UASB reactor under various organic loading rates. *Bioresource Technology*, **55**, 47-54.

Kaparaju, P., Luostarinen, S., Kalmari, J. and Rintala, J. 2002 Co-digestion of energy crops and industrial confectionery by-products with cow manure: batch-scale and farm-scale evaluation. *Water Science Technology*, **45**, 275 - 280.

Kaseng, K., Ibrahim, K., Paneerselvam, S.V. and Hassan, R.S. 1992 Extracellular enzymes and acidogen profiles of a laboratory-scale two-phase anaerobic digestion system. *Process Biochemistry*, **27**, 43-47.

Kashyap, D.R., Dadhich, K.S. and Sharma, S.K. 2003 Biomethanation under psychrophilic conditions: a review. *Bioresource Technology*, **87**, 147-153.

Karekezi, S. 1994 Disseminating renewable energy technologies in sub-Saharan Africa. Annual Review of Energy and Environment, **19,** 387-421.

Klass, D.L. 1984 Methane from Anaerobic Fermentation. *Science*, **223**, 1021-1028.

Lafitte-Trouqué, S. and Forster C.F. 2000 Dual anaerobic co-digestion of sewage sludge and confectionery waste. *Bioresource Technology*, **71**, 77-82.

Lalman, J.A. and Bagley, D.M. 2001 Anaerobic degradation and methanogenic inhibitory effects of oleic and stearic acids. *Water Research*, **35**, 2975-2983.

Lastella, G., Testa, C., Cornacchia, G., Notornicola, M., Voltasio, F. And Sharma, V.K. 2002 Anaerobic digestion of semi-solid organic waste: biogas production and its purification. *Energy Conservation and Management*, **43**, 63-75.

Lauwers, A.M., Heinen, W., Gorris, L.G.M., and van der Drift, C. 1990 Early stages in biofilm development in methanogenic fluidised-bed reactors. *Applied Microbiology and Biotechnology*, **33**, 352-358.

Lee, J.P., Lee, J.S. and Park, S.C. 1999 Two-phase methanisation of food wastes in pilot scale. *Applied Biochemistry and Biotechnology*, **77-79**, 585-593.

Lema, J.M. and Omil, F. 2001 Anaerobic treatment: a key technology for sustainable management of wastes in Europe. *Water Science and Technology*, **44**, 133-140.

Lettinga, G., Van Velsen, A.F.M., Hobma, S.W., De Zeeuw, W. and Klapwjik, A. 1980 Use of the upflow sludge blanket reactor concept for biological waste water treatment, especially for anaerobic treatment. *Biotechnology and Bioengineering*, **22**, 699-734.

Lettinga, G., Hulshoff Pol, L.W., Koster, I.W., Wiegant, W.M., De Zeeuw, W.J., Rinzema, A., Grin, P.C., Roersma, R.E. and Homba, S.W. 1984 High-rate anaerobic wastewater treatment using UASB reactor under a wide range of temperature conditions. *Biotechnology and Genetic Engineering Reviews*, **2**, 253-284.

Lettinga, G., Hulshoff Pol, L.W., Zeeman, G., Field, J., van Lier, J.B., van Buuren, J.C.L. 1997 Anaerobic treatment in sustainable environmental production concepts. In: *8th International Conference on Anaerobic Digestion. Sendai*, pp. 32-39.

Lettinga, G. and Hulshoff-Pol, L.W. 1991 UASB process designs for various types of wastewaters. *Water Science and Technology*, **24**, 87-107.

Lettinga, G. 1995 Anaerobic digestion and wastewater treatment systems. *Antonie van Leeuwenhoek*, **67**, 3-28.

Lettinga, G., Rebac, S., Parshina, S., Nozhevnikova, A., van Lier, J.B. and Stams, A.J.M. 1999 High rate anaerobic treatment of wastewater at low temperatures. *Applied and Environmental Microbiology*, **65**, 1696-1702.

Lettinga, G. 2001 Digestion and degradation, air for life. *Water Science and Technology*, **44**(8), 157-176.

Lettinga, G. 2004 With anaerobic treatment approach towards a more sustainable and robust environmental protection. In: *10th International Conference on Anaerobic Digestion. Montreal, Canada, 29th August-3rd September*, Vol 1 pp. 2-12.

Lepisto, S.S. and Rintala, J.A. 1997 Start-up and operation of laboratory-scale thermophilic upflow anaerobic sludge blanket reactors treating vegetable processing wastewaters. *Journal of Chemical Technology and Biotechnology*, **68**, 331-339.

Ligero, P., de Vega, A. and Soto, M. 2001 Influence of HRT (hydraulic retention time) and SRT (solid retention time) on the hydrolytic pre-treatment of urban wastewater. *Water Science and Technology*, **44**, 7-14.

Lissens, G., Vandevivere, P., de Baere, L., Biey, E.M. and Verstraete, W. 2001 Solid waste digesters: process performance and practice for municipal solid waste digestion. *Water Science and Technology*, **44**, 91-102.

Lin, C.Y. 1993 Effect of heavy metals on acidogenesis in anaerobic digestion. *Water Research*, **27**, 147-152.

Lin, C.Y. 1992 Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. *Water Research*, **26**, 177-183.

Maharaj, I and Elefsiniotis, P. 2001 The role of HRT and low temperature on the acid-phase anaerobic digestion of municipal and industrial wastewaters. *Bioresource Technology*, **7**, 191-197.

Mah, R.A. 1982 Methanogenesis and methanogenic partnerships. *Philosophical Transactions Royal Society London*, **B297**, 599-616.

Mata-Alvarez, J., Macé, S. and Llabre`s, P. 2000 Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Review paper. *Bioresource Technology*, **74**, 3-16.

Matsushige, K., Inamori, Y., Mizuochi, M., Hosomi, M. and Sudo, R. 1990 The effects of temperature on anaerobic filter treatment for low – strength organic wastewater. *Environmental Technology*, **11**, 899-910.

Mbohwa, C and Fukuda, S. 2003 Electricity from bagasse in Zimbabwe. *Biomass and Bioenergy,* **25**, 197-207.

McCarthy, P.L. 2001 The development of anaerobic treatment and its future. *Water Science and Technology*, **44**, 149-156.

McCarthy, P.L. 1982 One hundred years of anaerobic treatment. In: Hughes et al., (eds) *Anaerobic Digestion* 1981, Elsevier Biomedical, Amsterdam, p. 3-22.

McInerney, M.J. 1988 Anaerobic hydrolysis and fermentation of fats and proteins. In: *Biology of Anaerobic Microorganisms*, A.J.B. Zehnder (ed), John Wiley and Sons p. 373-416.

Michaud, S., Bernet, N., Buffie're, P., Roustan, M. and Moletta, R. 2002 Methane yield as a monitoring parameter for the start-up of anaerobic fixed film reactors. *Water Research*, **36**, 1385-1391.

Misi, S.N. and Forster C.F. 2001 Batch co-digestion of multi-component agro-wastes. *Bioresource Technology*, **80**, 19-28.

Moosbrugger, R.E., Wentezel, M.C., Ekama, G.A., and Marais, G.V. 1993 Weak acid/bases and pH control in anaerobic systems – A review. *Water SA*, **19**, 1-10.

Mosey, F.E. and Fernandes, X.A. 1989 Patterns of hydrogen in biogas from the anaerobic digestion of milk sugars. *Water Science and Technology*, **21**, 187-196.

Morrissey, W.A., and Justus, J.R. 1999 Global climate change. Committee for the National Institute for the Environment. http://www.cnie.org/nle/clim-2.html

Nordberg, Å., and Edström, M. 2003 Treatment of animal waste in co-digestion biogas plants in Sweden. In: *The Future of Biogas in Europe II.* Al Seidi, T., and Holm-Nielsen, J.B. (eds). European Biogas Workshop. 2-4 October, University of southern Denmark, Esbjerg, Denmark.

Noykova, N., Muller, T.G., Gyllenberg, M., and Timmer, J. 2002 Quantitative analysis of anaerobic wastewater treatment processes: Identifiability and Parameter Estimation. *Biotechnology and Bioengineering*, **78**, 89-103.

Nyns, E.J. 1986 Biomethanation processes. In: Schonborn W. (ed), *Microbial Degradations*, **18**, 207-267.

Omer, A.M. and Fadalla, Y. 2003 Biogas technology in Sudan, Technical note. *Renewable Energy*, **28**, 499-507.

Ouedraogo, A. 1999 Pilot-scale two-phase anaerobic digestion of the biodegradable organic fraction of Bamako district municipal solid waste. In: *II International Symposium*

Anaerobic Digestion of Solid Waste, Barcelona, June 15-17. (eds J. Mata-Alvarez, A. Tilche and F. Cecchi), Vol. 2, pp.73-76. Int. Assoc. Wat. Qual.
Pavan, P., Battistoni, P., Cecchi, F. and Mata-Alvarez, J. 2000 Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): performance and kinetic study. *Water Science and Technology*, **41**, 111-118.
Pavlostathis, S.G. and Giraldo-Gomez, E. 1991 Kinetics of anaerobic treatment. *Water Science Technology*, **24**, 35 - 59.
Picanco, A.P., Vallero, M.V.G., Gianotti, E.P., Zaiat, M and Blundi, C.E. 2001 Influence of porosity and composition of supports on the methanogenic biofilm characteristics developed in a fixed bed anaerobic reactor. *Water Science Technology*, **44**, 197 - 204.
Pohland, F.G. and Ghosh, S.1971 Developments in anaerobic treatment process. *Biotechnology and Bioengineering*, **2**, 85-106.
Powell, G.E. and Archer, D.B. 1989 On-line titration method for monitoring buffer capacity and total volatile fatty acid levels in anaerobic digesters. *Biotechnology and Bioengineering*, **33**, 570 –577.
Rajeshwari, K.V., Balakrishnan, M., Kansal, A., Kusum Lata, and Kishore, V.V.N. 2000 State-of-the-art of anaerobic digestion technology for industrial wastewater treatment. *Renewable and Sustainable Energy Reviews*, **4**, 135-156.
Rebac, S., van Lier, J.B., Janssen, M.G.J., Dekkers, F., Swinkels, K.T.M., and Lettinga, G.1997 High rate anaerobic treatment of malting waste water in a pilot-scale EGSB system under psychrophilic conditions. *Journal of Chemical Technology and Biotechnology*, **68**, 135-146.
Reddy, A.K.N., Smith, K.R. and Williams, R.H. 2000 Rural energy in developing countries. In: UNDP World Energy Assessment: Energy and the Challenge of Sustainability edited by J. Goldemberg. Pp 367-384.
Rintala, J.A. and Lepisto, S.S. 1997 Pilot-scale thermophilic anaerobic treatment of wastewaters from seasonal vegetable processing industry. *Water Science and Technology*, **36**, 279-285.
Rozzi, A. 1991 Alkalinity considerations with respect to anaerobic digesters. *Med. Fac. Landbouww. Rijksuniv. Gent.,* **56**, 1499-1514.
Rozzi, A. and DiPinto, A.C. 1994 Start up and automation of anaerobic digesters with automatic bicarbonate control. *Bioresource Technology*, **48**, 215-219.
Rushbrook, P.E. 1990 Co-disposal of industrial wastes with municipal solid wastes. *Resources Conservation and Recycling*, **4**, 33-49.
Salminen, E., Rintala, J., Lokshima, L.Y. and Vavilin, V.A. 2000 Anaerobic batch degradation of solid poultry slaughterhouse waste. *Water Science and Technology*, **41**, 33-41.
Schraa, G. and Jewell, W.J. 1984 High rate conversions of soluble organics with the thermophilic anaerobic attached film expanded bed. *Journal of Water Pollution Control and Federation*, **56**, 226-232.
Schink, B. 2002 Anaerobic digestion: concepts, limits and perspectives. *Water Science and Technology*, **45**, 1-8.
Schink, B. 1997 Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, **61**, 262-280.
Seghezzo, L., Zeeman, G., van Lier J.B., Hamelers, H.V.M. and Lettinga G. 1998 A review: The anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology*, **65**,175-190.
Sekiguchi, Y., Kamagata, Y. and Harada, H. 2001 Recent advances in methane fermentation technology. *Current Opinion in Biotechnology*, **12**, 277-282.
Shin, H.S., Han, S.K., Song, Y.C., and Hwan, E.J. 2000 Biogasification of food residuals. *Biocycle International*, **August**, 82-86.
Solera, R., Romero, L.I. and Sales, D. 2002 The evolution of biomass in a two-phase anaerobic treatment process during start-up. *Chemical and Biochemical Engineering Quarterly*, **16**, 25-29.

Sonesson, U., Björklund, A., Carlsson M. and Dalemo M. 2000 Environmental and economic analysis of management systems for biodegradable waste. *Resources, Conservation and Recyling*, **28**, 29-53.

Song, Y.C., Kwon, S.J. and Woo, J.H. 2004 Mesophilic and thermophilic co-phase anaerobic digestion compared with single-stage mesophilic and thermophilic digestion of sewage sludge. *WaterResearch*, **35**, 3441-3447.

Sung S. and Santha, 2003 Performance of temperature-phased anaerobic digestion (TPAD) system treating dairy cattle wastes. *Water Research*, **37**, 1628-1636.

Stewart, D.J., Bogue, M.J. and Badger, D.M. 1984 Biogas production from crops and organic wastes 2. Results of continuous digestion tests. *New Zealand Journal of Science*, **27**, 285-294.

Strydom, J.P., Britz, T.J. and Mostert, J.F. 1997 Two-phase anaerobic digestion of three different dairy effluents using a hybrid bioreactor. *Water SA*, **23**, 151-156.

Switzenbaum, M.S. and Jewell, W.J. 1980 Anaerobic attached-film expanded-bed reactor treatment. *Journal of Water Pollution Control and Federation*, **52**, 1953-1965.

Switzenbaum, M.S. 1995 Obstacles in the implementation of anaerobic treatment technology. *Bioresource Technology*, **53**, 255-262.

Tafdrup, S. 1994 Centralised biogas plants combine agricultural and environmental benefits with energy production. *Water Science and Technology*, **30**, 133-141.

Tafdrup, S. 1995 Viable energy production and waste recycling from anaerobic digestion of manure and other biomass materials. *Biomass and Bioenergy*, **9**, 303-314.

Tsukahara, K., Yagishita, T., Ogi, T. and Sawayama, S. 1999 Treatment of liquid fraction separate from liquidised food waste in an Upflow Sludge Bed Blanket. *Journal of Bioscience and Engineering*, **87**, 554 - 556.

van der Last, A.R.M., and Lettinga, G. 1992 Anaerobic treatment of domestic sewage under moderate climatic (Dutch) conditions using upflow reactors at increased superficial velocities. *Water Science and Technology*, **25**, 167-178.

van Lier, J.B., Van der Zee, F.P., Tan, N.C.G., Rebac, S. and Kleerebezem, R. 2001 Advances in high rate anaerobic treatment: staging of reactor systems. *Water Science and Technology*, **44**, 15-25.

van Lier, J.B., Boersma, F., Debets, M.M.W.H. and Lettinga, G. 1994 'High rate' thermophilic anaerobic wastewater treatment in compartmentalised upflow reactors. In: Paper Pre-prints of the Seventh International Symposium on Anaerobic Digestion Cape Town, January 23-27, South Africa, 338-347.

van Lier, J.B., Rebac, S. and Lettinga, G. 1996 High rate anaerobic wastewater treatment under psychrophilic and thermophilic conditions. In: Proceedings of the IAWQ-NVA International Conference on advanced wastewater treatment, Amsterdam, 23-25 September.

Vavilin, V.A., Rytov, S.V., Lokshina, L.Y., Rintala, J. A., and Lyberatos, G. 2001 Simplified hydrolysis models for the optimal design of two-stage anaerobic digestion. *Water Research*, **35**, 4247- 4251.

Veeken, A.H.M. and Hamelers, B.V.M. 2000 Effect of substrate-seed mixing and leachate recirculation on solid state digestion of biowaste. *Water Science and Technology*, **41**, 255-262.

Viéitez, E.R. and Ghosh, S. 1999 Biogasification of solid wastes by two-phase anaerobic fermentation. *Biomass and Bioenergy*, **16**, 299-309.

Verstraete, W. and Vandevivere, P. 1997 Broader and newer applications of anaerobic digestion. Proceedings of the 8th International conference on Anaerobic Digestion Sendai, Japan.

Viswanath, P., Devi, S.S., and Nand K. 1992 Anaerobic digestion of fruit and vegetable processing wastes for biogas production. *Bioresource Technology*, **40**, 43- 48.

Viturtia, A. Mtz., Mata-Alvarez, J., Cecchi, F. and Fazzini G. 1989 Two-phase anaerobic digestion of a mixture of fruit and vegetable wastes. *Biological Wastes*, **29**, 189-199.

Wang, Y.S., Odle, W.S., Eleazer, W.E., and Barlaz, M. A. 1997 Methane potential of food waste

anaerobic toxicity of leachate produced during food waste decomposition. *Waste Management and Research*, **15**, 149-167.
Wentzel, M.C., Moosbrugger, R.E., Sam-Soon, P.A.L.N.S., Ekama, G.A. and Marais, G.V.R. 1995 Tentative guidelines for waste selection, process design, operation and control of upflow anaerobic sludge bed reactors. *Water Science and Technology*, **31**, 31-42.
Wiegant, W.M. and Lettinga, G. 1985 Thermophilic anaerobic digestion of sugars in upflow anaerobic sludge blanket reactors. *Biotechnology and Bioengineering*, **27**, 1603-1607.
Wiegant, W.M., Claassen, J.A., and Lettinga, G. 1985 Thermophilic anaerobic digestion of high strength wastewaters. *Biotechnology and Bioengineering*, **XXVII**, 1374-1381.
Weiland, P. 1993 One- and two-step anaerobic digestion of solid agroindustrial residues. *Water Science and Technology*, **27**, 145-151.
Weiland, P. 2000 Anaerobic waste digestion in Germany – Status and recent developments. *Biodegradation*, **11**, 415-421.
Wilson, T.E. 2000 Two phased anaerobic digestion options. *Proceedings of Biosolids Management in the 21st Century*. April 10-11th, Mary land, 136-148.
Xing, J., Criddle, C. and Hickey, R. 1997 Effects of a long-term periodic substrate perturbation on an anaerobic community. *Water Research*, **31**, 2195-2204.
Yan, J.Q., Lo, K.V. and Liao, P.H. 1990 Anaerobic digestion of cheese whey using an upflow anaerobic sludge blanket reactor: III. Sludge and substrate profiles. *Biomass*, **21**, 257-271.
Yenigun, O., Kizilgun, F. and Yilmazer, G. 1996 Inhibition effects of zinc and copper on volatile fatty acid production during anaerobic digestion. *Environmental Technology*, **17**, 1269-1274.
Yu, H.W., Samani, Z., Hanson, A. and Smith, G. 2002 Energy recovery from grass using two-phase anaerobic digestion. *Wastewater Management*, **22**, 1-5.
Zeeman, G. and Saunders, W. 2001 Potential of anaerobic digestion of complex waste(water)s. *Water Science and Technology*, **44**, 115-122.
Zinder, S.H. 1984 Microbiology of anaerobic conversion of organic wastes to methane: recent developments. *American Society for Microbiology*, **50**, 294-298.
Zinder, S.H. 1993 Physiological ecology of methanogens. In: Ferry J.G. (ed.), *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*. Chapman and Hall, New York. p. 128-206.
Zoetmeijer, R.J., v.d. Heuvel, J.C. and Cohen, A. 1982 pH influence on acidogenic dissimilation of glucose in an anaerobic digester. *Water Research*, **16**, 303-311.
Zoutberg, G.R. and Eker, Z. 1999 Anaerobic treatment of potato processing wastewater. *Water Science and Technology*, **40**, 297-304.