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Parawira, Wilson

2004

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

Wilson Parawira

Department of Biotechnology

Lund University


Academic thesis which, by due permission of the Faculty of Engineering at Lund University, will be publicly defended on Thursday 16 December, 2004, at 10:30 in lecture hall A, at the Center for Chemistry and Chemical Engineering, Sölvegatan 39, Lund, for the degree of Doctor of Philosophy in Engineering.

Faculty opponent: Professor Amelia K. Kivaisi, Applied Microbiology Unit, Department of Botany, University of Dar es Salaam, Dar es Salaam, Tanzania.
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 carboxymethyl 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.
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.

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.

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.
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 (CH₄) and carbon dioxide (CO₂) 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 CO₂ 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 CO₂ emissions. Compared to other fuels, methane produces less atmospheric pollutants and CO₂ 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 H₂S 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.

<table>
<thead>
<tr>
<th></th>
<th><strong>Aerobic digestion</strong></th>
<th><strong>Anaerobic digestion</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Start-up</strong></td>
<td>• Short start-up period.</td>
<td>• Long start-up period.</td>
</tr>
<tr>
<td><strong>Process</strong></td>
<td>• Integrated nitrogen and phosphorus removal possible.</td>
<td>• No significant nitrogen or phosphorus removal, nutrients removal done via post treatment.</td>
</tr>
<tr>
<td></td>
<td>• Production of high excess sludge quantities.</td>
<td>• Production of very little excess sludge (5-20%).</td>
</tr>
<tr>
<td></td>
<td>• Large reactor volume necessary.</td>
<td>• Small reactor volume can be used.</td>
</tr>
<tr>
<td></td>
<td>• High nutrient requirements.</td>
<td>• Low nutrient requirements.</td>
</tr>
<tr>
<td><strong>Carbon balance</strong></td>
<td>• 50-60% incorporated into CO₂; 40-50% incorporated into biomass.</td>
<td>• 95% converted to biogas; 5% incorporated into microbial biomass.</td>
</tr>
<tr>
<td><strong>Energy balance</strong></td>
<td>• 60% of available energy is used in new biomass; 40% lost as process heat.</td>
<td>• 90% retained as CH₄, 3-5% is lost as heat, and 5-7% is used in new biomass formation.</td>
</tr>
<tr>
<td><strong>Residuals</strong></td>
<td>• Excess sludge production.</td>
<td>• Biogas, nitrogen mineralised to ammonia.</td>
</tr>
<tr>
<td></td>
<td>• No need for post-treatment.</td>
<td>• Post-treatment required for removal of remaining organic matter and malodorous compounds.</td>
</tr>
<tr>
<td><strong>Costs</strong></td>
<td>• Low investment costs.</td>
<td>• Often moderate investment costs.</td>
</tr>
<tr>
<td></td>
<td>• High operating costs for aeration, additional nutrient and sludge removal, and maintenance.</td>
<td>• Low operating costs due to low power consumption and additional nutrients hardly required.</td>
</tr>
<tr>
<td><strong>State of development</strong></td>
<td>• Established technology.</td>
<td>• Still under development for specific applications.</td>
</tr>
</tbody>
</table>

(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).
Proteins are broken down into amino acids, small peptides, ammonia and CO₂. 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, H₂ and CO₂, 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 C₁ to C₄ 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 (C₂ to C₅, 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).](image)

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.
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.

<table>
<thead>
<tr>
<th>Acetogenic reactions</th>
<th>Free energy per reaction ($\Delta G^0$ kJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionate</td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_3\text{CH}_2\text{COOH} + 2\text{H}_2\text{O}$</td>
<td>$\text{CH}_3\text{COOH} + \text{CO}_2 + 3\text{H}_2$</td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 2\text{H}_2\text{O}$</td>
<td>$2\text{CH}_3\text{COOH} + 2\text{H}_2$</td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_3\text{CH}_2\text{OH} + \text{H}_2\text{O}$</td>
<td>$\text{CH}_3\text{COOH} + 2\text{H}_2$</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_3\text{CHOHCOOH} + 2\text{H}_2\text{O}$</td>
<td>$\text{CH}_3\text{COOH} + \text{CO}_2 + 2\text{H}_2 + \text{H}_2\text{O}$</td>
</tr>
</tbody>
</table>

| Methanogenic reactions                    |                                          |
| Acetate                                  |                                          |
| $\text{CH}_3\text{COOH} + \text{H}_2\text{O}$ | $\text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O}$ | -31.0 |
| Carbon dioxide + hydrogen                |                                          |
| $\text{CO}_2 + 4\text{H}_2$              | $\text{CH}_4 + 2\text{H}_2\text{O}$     | -135.6 |

| Syntrophic reaction                      |                                          |
| $2\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH} + 4\text{H}_2$ | $4\text{CH}_3\text{COOH} + 4\text{H}_2$ | +96.2 |
| $\text{CO}_2 + 4\text{H}_2$             | $\text{CH}_4 + 2\text{H}_2\text{O}$     | -135.6 |

Net reaction -39.4
2.4 Methanogenesis

The methanogens utilise mainly \( \text{H}_2/\text{CO}_2 \) and acetic acid to form methane and carbon dioxide. The methanogens can also utilise a limited number of other substrates to form \( \text{CH}_4 \) such as methanol, methylamines, alcohols + \( \text{CO}_2 \) and formate (Kalyuzhnyi et al., 2000; Hwang et al., 2001). \( \text{H}_2/\text{CO}_2 \)-consuming methanogens reduce \( \text{CO}_2 \) (using it as an electron acceptor) via formyl, methenyl, and methyl, through association with specific coenzymes, to finally produce \( \text{CH}_4 \). 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 \( \text{H}_2/\text{CO}_2 \) (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 Methanoaeta soenngenii 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).

<table>
<thead>
<tr>
<th>Performance characteristics</th>
<th>Mesophilic digestion</th>
<th>Thermophilic digestion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas production rate</td>
<td>Contradictory reports</td>
<td>Contradictory reports</td>
</tr>
<tr>
<td>Pathogen reduction</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Effluent VFAs</td>
<td>Lower</td>
<td>Higher (contradictory)</td>
</tr>
<tr>
<td>Dewaterability</td>
<td>Contradictory reports</td>
<td>Contradictory reports</td>
</tr>
<tr>
<td>Process stability</td>
<td>Higher</td>
<td>Lower (contradictory)</td>
</tr>
<tr>
<td>Methane content</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Energy requirement</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Odour</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Product/substrate inhibition</td>
<td>Lower</td>
<td>Higher</td>
</tr>
</tbody>
</table>

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/m³ (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 et 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 non-ionized 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.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reactor Type</th>
<th>Scale</th>
<th>Max OLR (g COD/l/d)</th>
<th>HRT (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysed solid potato waste</td>
<td>UASB</td>
<td>Lab</td>
<td>11.0</td>
<td>2.4</td>
<td>Paper III</td>
</tr>
<tr>
<td>Hydrolysed solid potato &amp; sugar beet waste</td>
<td>Packed-bed, straw carrier</td>
<td>Pilot</td>
<td>20</td>
<td>1.0</td>
<td>Paper V</td>
</tr>
<tr>
<td>Hydrolysed solid potato &amp; sugar beet waste</td>
<td>Packed-bed, plastic carrier</td>
<td>Pilot</td>
<td>18</td>
<td>1.4</td>
<td>Paper V</td>
</tr>
<tr>
<td>Potato waste leachate</td>
<td>UASB</td>
<td>Lab</td>
<td>6.1</td>
<td>3.3</td>
<td>Paper VI</td>
</tr>
<tr>
<td>Potato waste leachate</td>
<td>Packed-bed, plastic carrier</td>
<td>Lab</td>
<td>4.7</td>
<td>4.3</td>
<td>Paper VI</td>
</tr>
<tr>
<td>Potato-maize wastewater</td>
<td>UASB</td>
<td>Lab</td>
<td>14</td>
<td>1.3</td>
<td>Kalyuzhnyi et al. 1996</td>
</tr>
<tr>
<td>Synthetic wastewater</td>
<td>Packed-bed, straw carrier</td>
<td>Lab</td>
<td>4</td>
<td>0.5</td>
<td>Guitonas et al. 1994</td>
</tr>
<tr>
<td>Dairy wastewater</td>
<td>Sintered glass carrier</td>
<td>Lab</td>
<td>21</td>
<td>0.5</td>
<td>Anderson et al. 1994a</td>
</tr>
<tr>
<td></td>
<td>Packed-bed, PVC rings</td>
<td>Lab</td>
<td>4</td>
<td>0.5</td>
<td>Anderson et al. 1994a</td>
</tr>
</tbody>
</table>
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).
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 methanogenesis 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 m³ CO₂ and 90-140 m³ CH₄ 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).

<table>
<thead>
<tr>
<th>Waste</th>
<th>India</th>
<th>Brazil</th>
<th>Sudan</th>
<th>USA</th>
<th>Sweden</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSW</td>
<td>135.5</td>
<td>44.0</td>
<td>2.3</td>
<td>148.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Sewage</td>
<td>44.9</td>
<td>8.02</td>
<td>1.4</td>
<td>16.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Manure</td>
<td>653.0</td>
<td>470.0</td>
<td>68.0</td>
<td>306.0</td>
<td>13.2</td>
</tr>
<tr>
<td>Agricultural residues</td>
<td>200.0</td>
<td>47.0</td>
<td>8.1</td>
<td>573.0</td>
<td>12.6</td>
</tr>
<tr>
<td>Biomass</td>
<td>140.0</td>
<td>496.8</td>
<td>192.3</td>
<td>427.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>

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 CH₄/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\textsubscript{4}/ g VS\textsubscript{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\textsuperscript{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.

![Two-stage set-up for the anaerobic digestion of solid waste.](image)

**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 CO2 and low CH4 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 al., 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 H₂ and CO₂ 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.

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

<table>
<thead>
<tr>
<th>Reactor Configuration</th>
<th>Waste</th>
<th>OLR (kg CODm$^{-3}$/day)</th>
<th>Temperature regime</th>
<th>Removal (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-bed + UASB</td>
<td>Solid potato waste</td>
<td>11</td>
<td>Meso + Meso</td>
<td>90 (COD)</td>
<td>Paper III</td>
</tr>
<tr>
<td>Solid-bed + UASB</td>
<td>Solid potato waste</td>
<td>36</td>
<td>Thermo + Thermo</td>
<td>85 (COD)</td>
<td>Paper III</td>
</tr>
<tr>
<td>CSTR + AF</td>
<td>Soft drink</td>
<td>16</td>
<td>Meso + Meso</td>
<td>96 (COD)</td>
<td>Ghosh et al. (1985)</td>
</tr>
<tr>
<td>CSTR + UASB</td>
<td>Instant coffee</td>
<td>16</td>
<td>Thermo + Meso</td>
<td>77 (COD)</td>
<td>Dinsdale et al. (1997b)</td>
</tr>
<tr>
<td>CSTR + AF</td>
<td>Dairy</td>
<td>5</td>
<td>Meso + Meso</td>
<td>90 (COD)</td>
<td>Ince, (1998)</td>
</tr>
<tr>
<td>Two-stage Instant coffee</td>
<td>2.8</td>
<td>Meso + Meso</td>
<td>97 (COD)</td>
<td></td>
<td>Strydom et al. (1997)</td>
</tr>
</tbody>
</table>
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 CO₂ 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 use of anaerobic digestion in Germany, with approximately 850 biogas plants (with reactor volumes between 100 and 4,000 m³) 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.
ACKNOWLEDGEMENTS

My dream has come true, thanks to a massive amount of hard work, support and dedication – partly by me – but also by a lot of other people.

I am deeply grateful to my initial supervisor Professor R. Zvauya, University of Zimbabwe, for encouraging me to consider studying for a PhD and for devoting his time and knowledge to help me to complete part of this study, and to Professor John S. Read, who took over the task after Professor Zvauya left University of Zimbabwe.

In particular, I would like to thank Professor Bo Mattiasson, with whom much of the work in this thesis was carried out, for giving me the opportunity to work in this dynamic, cosmopolitan, and stimulating Department of Biotechnology. I am very grateful for all the expert advice, guidance and support you gave me. More importantly, thank you for allowing me to carry out independent research work, which is important in my life.

Dr. Marika Murto and Dr. Lovisa Björnsson, with whom I had the pleasure and honour to work in these projects. Your ideas were most constructive and timely revision of the manuscripts is greatly appreciated.

Kersti and Siv, for their friendliness, professionalism and success in creating a good atmosphere at the department.

Dores Cirne, Irene Bohn, Anthony Mshandete, Jing Liu, Raul Munoz Torre, Ana Soares, Hamid Zilouei, Enrique Terrazas, Mattias Svensson, Kjell Christensson and Teresa Alvarez Aliaga and many others in the Environment group, other present and all former members of the department from all over the world, for creating a pleasant academic atmosphere.

My employer, the University of Zimbabwe and the Institute of Food, Nutrition and Family Sciences, for granting me study leave to carry out this work, and my workmates, for their encouragement and support throughout the project.

Chibuku Breweries’ Technical Department for their cooperation and allowing part of this research to be carried out at their premises using their facilities.

The Swedish International Development Cooperation Agency (SIDA/SAREC), the Swedish National Energy administration (STEM) and the University of Zimbabwe Research Board, for funding this work.

My wife Charity, for her support and encouragement. Tawanda, Tatenda and Tanaka for enduring my absence when you needed me most, and the whole of my big family for the encouragement and support.

Mum and Dad, I wish you were still around to witness this dream unfolding into reality. Mum you were simply a godsend, I miss you so much. I love you both and your love to me was priceless.

Thank you all, and many others too numerous to name here.
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