The Mesophilic and Thermophilic Anaerobic Digestion of Instant Coffee Waste Waters

Richard Mark Dinsdale BSc (Hons)

A submission presented in partial fulfilment of the requirements of the University of Glamorgan/Prifysgol Morgannwg for the degree of Doctor of Philosophy.

This research programme was carried out in collaboration with Nestlé, UK.

March 1998

Declaration

This is to certify that the thesis entitled "The Thermophilic And Mesophilic Anaerobic Digestion Of Instant Coffee Waste Waters" or any part has been presented or is currently submitted in candidature for any degree other than the degree of Doctor of Philosophy of the University of Glamorgan.

Candidate..... R Dinsdale .....

Richard Mark Dinsdale

Supervisor..... Freda R Hawkes .....

Freda R. Hawkes

March 19, 1998

TABLE OF CONTENTS

ACKNOWLEDEGEMENTS

I would like to thank my supervisors Freda and Dennis Hawkes for their invaluable guidance and support throughout this research project. My thanks also go to Alan Guwy for his advice and friendship without which the laboratory work would have been much more difficult. Thanks also go to Gary Burrows and Clive Reid at the EPSRC pilot plant for supplying numerous cups of coffee, 3000 litres of coffee waste and granules for this project. I would also like to acknowledge the support of Nestlé UK and their staff in this project. This work was conducted as part of a collaborative project between the University of Glamorgan, Loughborough University of Technology, University of Birmingham, Imperial College of Science and Technology and University of Newcastle upon Tyne and was funded by EPSRC Grant GR/H18494.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

An instant coffee factory's physical waste treatment systems and their resulting waste streams were identified and samples of wastewater were analysed to determine the physical and chemical characteristics relevant to anaerobic digestion. Two wastewater types were chosen for treatment by anaerobic digestion, a wastewater with a high level of suspended solids and a wastewater with the majority of the suspended solids removed.

The waste with a high level of coffee grounds was studied in mesophilic and thermophilic batch studies and mesophilic and thermophilic CSTR reactors fed daily. Samples were analysed to determine the degree of waste removal and changes in proximate composition before and after digestion. Continuous mesophilic digestion was achieved at a OLR of 1.3 kgCOD $m^{-3}day^{-1}$ (25 day HRT) with the addition of $Ca(OH)_2$, nitrogen, phosphorus and trace metals. A COD and VS removal of 60% was achieved. Continuous thermophilic digestion could not be established beyond 50 days without a increase in total VFA occurring.

The coffee waste low in suspended solids was treated in single stage mesophilic and thermophilic UASBs. Using the same UASBs the effect of thermophilic pre-acidification was also studied.

Stable anaerobic digestion of settled instant coffee wastewater was achieved for over 100 days in mesophilic and thermophilic UASB reactors. Thermophilic UASBs were seeded with mesophilic granules and converted to thermophilic operation by raising

the temperature to 55°C in one step. Both the mesophilic and thermophilic UASBs achieved stable anaerobic digestion at OLRs of up to 10 $kgCODm^{-3}d^{-1}$ and down to a HRT of 24 hours. The mesophilic UASB has superior performance than the thermophilic UASB but the thermophilic reactor achieved an higher OLR.

The thermophilic and mesophilic UASB reactors used in single stage operation were operated with thermophilic pre-acidification and studied over a period of more than 120 days. The thermophilic pre-acidification stage was operated with pH control or with $1.5gl^{-1}$ $NaHCO_3$ added to the feed with HRTs of 24, 18, 15 and 12 hours. Up to 38% of the total influent COD was converted to TVFA, the principal VFAs were *n*-butyric, acetic and propionic respectively. The thermophilic/mesophilic two stage system gave a consistent improvement in performance over the thermophilic/thermophilic two stage system especially at higher organic loading rates. The thermophilic/mesophilic system achieved greater COD removal than achieved by the thermophilic/thermophilic two stage system. Thermophilic pre-acidification gave an increase of 60% in the loading rate achievable by the mesophilic methanogenic stage and a 100% reduction in HRT compared with the single stage system.

ABBREVIATIONS

AAFEB	anaerobic attached film expanded bed reactor
AFB	anaerobic fluidized bed
Atm	atmospheres
BOD	biochemical oxygen demand
CASBER	carrier assisted sludge bed reactor
COD	chemical oxygen demand
CSTR	continously stirred tank reactor
EU	European Union
EPSRC	Engineering and Physical Sciences Research Council
HRT	hydraulic retention time
ITD	Inclined tubular digester
K_M	Michaelis constant (half saturation constant)
LCFA	long chain fatty acid
NAD	nicotinamide adenine di-nucleotide
OLR	organic loading rate
RBC	rotating biological contact reactor
RPI	retail price index
TVFA	total volatile fatty acid
TS	total solids
μ_{max}	maximum specific growth rate
UASB	upflow anaerobic sludge blanket
VFA	volatile fatty acid
VS	volatile solids

1. INTRODUCTION

Most food processing factories as a result of their activities produce large amounts of wastewater possessing significant polluting load. One such waste is instant coffee production wastewater. This waste has a high polluting load of up to 40,000mgO_2l^{-1}COD (chemical oxygen demand), significant levels of suspended solids (18 gl^{-1}), and a strong colour.

To prevent degradation of the surrounding environment measures will have to be taken to dispose of the wastewater. In UK with 96% of the population connected to sewer systems, this service is usually performed by the regional water service companies (Water Facts, 1995). Not only may the cost of this service be substantial but the water company may not be prepared to take certain types of waste due to infringement of consent limits. Significant rises in trade effluent charges may also be required to meet increased environmental standards such as the EEC Urban Waste Water Directive and future water quality legislation. When the regional water companies were privatised in 1989, a pricing regime of Retail Price Index (RPI)+K was legislated for 10 years to compensate for the increase investment required to meet increased water discharge standards (Kinnersley, 1994). This was in contrast to other utility privatisations where a price regime of RPI-X was imposed. Indeed the water companies have a further option to increase prices via the "pass through option" (Kinnersley, 1994). Therefore any company discharging to sewer could face the prospect of trade effluent charges rising faster than inflation on a yearly basis.

However by the intelligent selection of physical and biological treatment systems an effective and reliable on site waste treatment system could be installed. This would result in a waste with a reduced treatment cost and acceptable for discharge to the water company.

Increasing interest has been shown in anaerobic digestion as a biological treatment system as it has significant potential advantages over aerobic treatment. These advantages are lower electrical power usage, methane gas production, and lower microbial cell production. A comparison of the aerobic vs anaerobic treatment of a metric ton of COD removed is shown in Table 1.1.

Table 1.1. Comparison Of The Anaerobic And Aerobic Treatment Of A Metric Ton Of COD.

Factor	Anaerobic	Aerobic
Electrical Input	—	1100 kWh
Methane	1.16×10^7 MJ	—
Net cell production	20-150 kg	400-600 kg

(Data From Speece, 1983)

Anaerobic digestion does have some disadvantages. Anaerobic digestion is not an all in one solution, post-treatment will usually be necessary as BOD is not usually reduced sufficiently for discharge to open water. In particular nitrogen and phosphorus removal is limited. Industrial plants usually operate at 20-35°C although they can operate efficiently down to temperatures of 10-15°C (Mergaert *et al.*, 1992). This problem is usually easily overcome as most wastewaters are released at these temperatures and methane from the digestion process can be used to heat the reactor.

However it means for dilute low strength wastes (<500 mgCOD l^{-1}) discharged at less than 10-15°C anaerobic digestion may not be the most cost effective option.

Despite these disadvantages, the cost advantage for the anaerobic process can be substantial for wastes with a COD strength greater than 500 mgCOD l^{-1} and this cost advantage increases as the COD strength increases (see Fig.1.1) . For a waste of 2000 mgCOD l^{-1}, anaerobic treatment is a third of the cost of aerobic treatment alone (see Fig.1.1). If the COD influent strength rises to 12,000 mgCOD l^{-1}, anaerobic treatment is a tenth of the cost aerobic treatment (see Fig. 1.1).

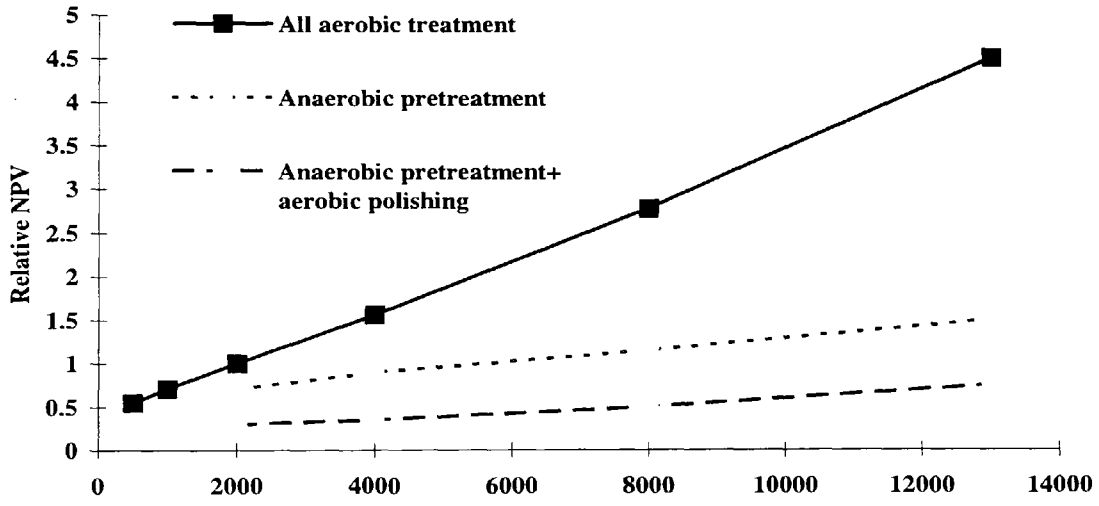

Figure.1.1. Comparison of Relative Net Present Values of Anaerobic Treatment, Aerobic Treatment and Anaerobic Treatment with Aerobic Polishing. Cost relative to all-aerobic treatment of 2000 mgl^{-1} COD effluent. (Data applies to 1996, courtesy of Eckenfelder, Black and Veatch)

An aerobic polishing stage would be required for the anaerobic stage, to meet the same effluent standard as a solely aerobic treatement. However the cost advantage of employing an anaerobic stage is still significant (see Fig.1.1).

One of the principal cost advantages of anaerobic digestion is the reduced need for the disposal of excess biomass production. The cost of this sludge disposal is likely to rise as implementation of the Urban Waste Water Treatment Directive will prevent disposal routes such as sea dumping (Council of the European Communities, 1991).

The anaerobic digestion process has not been implemented as extensively as the advantages over aerobic processes and its long history of waste treatment would suggest (Switzenbaum, 1994 and Speece, 1983 and 1996). One of the main reasons proposed for this, is a lack of confidence due to limited experience with the implementation and operation of anaerobic systems (Switzenbaum, 1994).

A way of overcoming this lack of confidence is to develop rational design procedures based on a clear understanding of the chemical, biological and physical phenomena occurring in anaerobic reactors. Laboratory-scale investigations are one way in which this information for rational design procedures can be determined.

Experiments can be performed to determine the level and rate of degradation of a particular waste. This will allow an estimation of reactor size and the degree of treatment which can be expected. Information from the analysis of the waste and operation of laboratory reactors can indicate what additions of nutrients, trace metals and alkalinity will be required. Compositional analysis will determine what fractions such as lipid, protein and lignin are present. The level of lignin can effect the degree of degradation which can be achieved. Fractions such as lipid and protein can cause problems in the operation of reactors if present at high levels (Lettinga and Hulshoff Pol, 1991). The use of waste pre-processing should be assessed to determine if pre-

treatments could result in a more efficient waste processing system. For example the removal of significant levels of suspended solids can allow the use of more cost effective digester type.

By following this procedure a waste treatment system can be implemented which may integrate waste pre-treatment, efficient reactor configuration and post treatment leading to a system which is cost effective and meets the expectations of the buyer.

Instant coffee wastewaters have a high organic strength and would seem to be ideal for treatment by anaerobic digestion. The anaerobic digestion of instant coffee waste waters has been attempted by several workers Lane (1983), Raetz (1990), Kida *et al.* (1992), Kostenberg and Marchaim (1993a and b, 1994), Lanting *et al.* (1989). Some success has been achieved although problems in maintaining long term stable digestion have been experienced.

There could be many reasons for the problems experienced in the anaerobic digestion of instant coffee wastes. There are several unique aspects about instant coffee wastes. The processing of the coffee beans consists of roasting the beans at temperature up to 540°C, grinding up the beans and extracting the soluble products with super heated steam (Clark and Macrae, 1985). Heating has been found to have significant effects on the composition and digestibility of animal feedstuffs (van Soest and Mason, 1991). Although plant materials have been widely treated by anaerobic digestion, the anaerobic digestion of heat roasted plant material has not been studied extensively.

Coffee is chemically complex containing up to 750 different chemical compounds (Clark and Macrae, 1985). These compounds may be inhibitory or toxic to the anaerobic digestion process, resistant to degradation or cause physical problems in the operation of a particular type of reactor. However by analysing the waste to determine composition, the nutrients and alkalinity supplementation required, pre-treatment options, assessing different reactor types and configurations a more stable anaerobic digestion process should be obtainable.

Instant coffee wastewaters are discharged at high temperatures (up to 70°C). The majority of full-scale anaerobic plants operate at mesophilic temperatures, so cooling of the waste would probably be required. However operation at thermophilic temperatures (55°C) could offer the prospect of higher loading rates and smaller plants resulting in reduced capital costs (Wiegant *et al.* 1986, Wiegant and Lettinga, 1985, Lanting *et al.* 1989, Souza *et al.* 1992). Along with the possible advantages of thermophilic digestion, problems have been experienced with the thermophilic anaerobic digestion being more sensitive to environmental factors than equivalent mesophilic systems (Shi and Forster, 1993, Macleod and Forster, 1988, Disley *et al.*, 1992). A tendency to run at relatively high levels of volatile fatty acids, in particular propionic acid, usually taken to be a sign of reactor failure, has also been reported (Wiegant *et al.* 1986, Souza *et al.* 1992).

As the waste is already heated to thermophilic temperatures and no energy is required to heat the waste, the opportunity for a more cost effective system should not be overlooked. Therefore the start-up and operation of thermophilic reactors will be investigated.

Two-phase anaerobic digestion is also a method of increasing the process efficiency of anaerobic digestion. In this configuration the treatment plant is divided into a pre-acidification stage and a methanogenic stage. Thermophilic pre-acidification has been relatively little studied, with the majority of the work been conducted at mesophilic temperatures. The effectiveness of combining with either a mesophilic or thermophilic methanogenic stage has also not been studied and both these aspects have been examined here.

This work was conducted as part of a EPSRC collaborative project between the University of Glamorgan and four other universities and a industrial partner, Nestlé UK. The EPSRC pilot plant, supervised by Loughborough University of Technology was also installed at Nestlé UK`s instant coffee factory at Hayes, Middlesex.

The EPSRC pilot plant consisted of four anaerobic reactors, a UASB, a anaerobic filter, a contact process and an expanded fluidised bed reactor. The first three had a nominal volume of $5m^3$ with the expanded bed reactor having a nominal volume of 0.5 m^3 (Caine *et al.*, 1991). All four reactors were supplied from a common balancing tank of 13 m^3 volume. The pilot plant had been previously used on a collaborative project to treat ice cream waste waters (Caine *et al.*, 1991 and Hawkes *et al.*, 1995).

The research conducted at the other universities was as follows :

- **University of Birmingham**: Biofilms, granules and microbial ecology.
- **Loughborough University of Technology**: Monitoring and control.
- **University of Newcastle**: Pre-acidification of complex organics in anaerobic digestion.

- **Imperial College of Science and Technology**: Biodegradability and toxicity of recalcitrants.
- **University of Glamorgan:** Thermophilic and mesophilic anaerobic digestion.

1.1. Aims and objectives

The aims of the research are as follows:-

1. To determine the proximate composition and the chemical and physical factors of the coffee waste which would affect the anaerobic digestion process. To date there is little public information on instant coffee waste waters. Coffee waste would be analysed to determine its composition, e.g. lipid, ammonia and lignin content, components which would influence the anaerobic digestion process. Other factors such as the COD strength, suspended solids content and bicarbonate alkalinity of the waste, all having important influences on the anaerobic digestion process will be determined.

2. To date problems have been experienced in achieving long term anaerobic digestion of instant coffee wastes. Therefore continuous reactor studies will be conducted to determine if long term digestion of coffee waste is possible. The effect of additions such as nutrients, bicarbonate alkalinity will be investigated. The advantages of waste type selection on reactor operation and choice of reactor i.e. CSTR or UASB will also be investigated.

3. Thermophilic operation may offer certain advantages in the anaerobic digestion of coffee wastes. There is little information on the start-up of thermophilic reactors and few comparative studies on mesophilic or thermophilic digestion of industrial waste waters. Therefore the start up and operation of mesophilic and thermophilic methanogenic reactors on coffee waste will be investigated.

4. The pre-acidification of industrial waste waters can produce increases in methanogenic reactor efficiency, however most studies were conducted at mesophilic temperatures. As the thermophilic operation of instant coffee

wastewaters is a possibility, the efficiency of thermophilic acidification will be studied.

5. To date there are few or no comparative studies of mesophilic and thermophilic two-phase operation with industrial waste waters. Therefore the operation of mesophilic and thermophilic methanogenic reactors with thermophilic pre-acidification will be compared.

2. THE BIOTECHNOLOGY OF ANAEROBIC DIGESTION

2.1. Microbiology And Biochemistry Of Anaerobic Digestion

Most wastewaters will be composed of a complex mixture of organic molecules determined by the initial feedstock and the procedures used in the manufacturing process. The conversion of organic molecules to methane and carbon dioxide is dependent on the successful co-ordination of the metabolic activities of a diverse group of micro-organisms. Intensive research has been conducted into the microbiology and biochemistry to try and understand the fundamental mechanisms of the anaerobic digestion process. The anaerobic digestion process has six main metabolic activities (Gujer and Zehnder, 1983).

1. Hydrolysis of biopolymers

    1A. Hydrolysis of proteins
    1B. Hydrolysis of carbohydrates
    1C. Hydrolysis of lipids

2. Fermentation of amino acids and sugars

3. Anaerobic oxidation of long chain fatty acids and alcohols

4. Anaerobic oxidation of intermediary products such as volatile acids (except acetate)

5. Conversion of acetate to methane

6. Conversion of hydrogen and carbon dioxide to methane

This scheme can be simplified into four main metabolic activities as shown by Mosey (1983) in Figure 2.1. These activities are hydrolysis, acidogenesis, acetogenesis and methanogenesis.

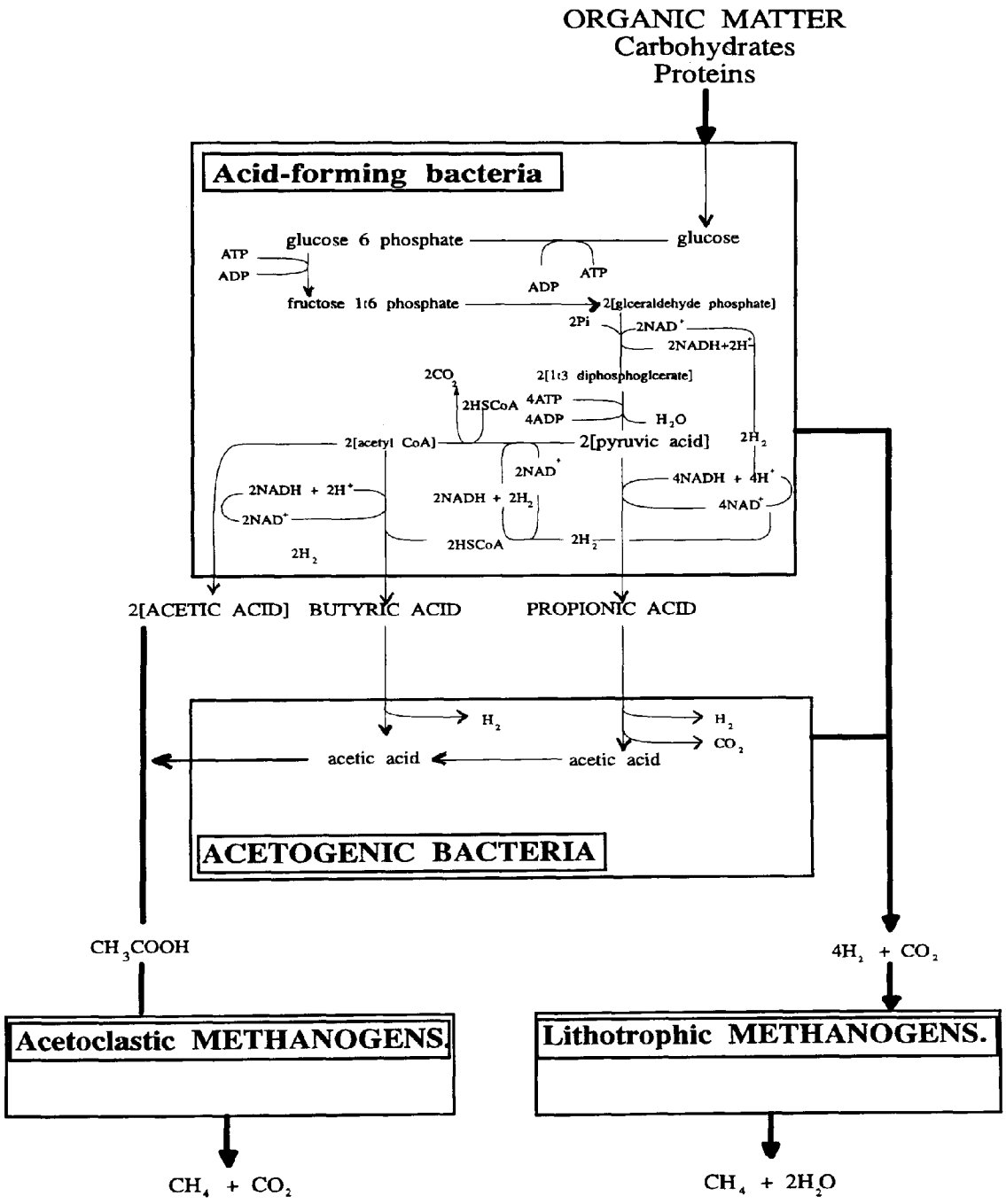

Figure 2.1. The Metabolic Pathways Of Anaerobic Digestion

(A.Guwy, 1995)

2.1.1. Hydrolysis and Acidogenesis

Bacteria can only take up small soluble organic molecules. The assimilation of larger or particulate organic material will require the breakdown or hydrolysis of this material. Hydrolytic bacteria from the geni *Clostridium, Bacillus, Staphylocccus* produce extra-cellular enzymes (lipases, proteases, cellulases etc.) which break down the larger proteins, lipids and polysaccharide molecules to sugars, amino acids, long chain fatty acids etc. (Stronach *et al.* 1986). The rate and degree of hydrolysis is affected by the waste type, pH and residence time in the reactor. Where the waste type is recalcitrant the hydrolysis stage is rate limiting for the anaerobic digestion process.

These substrates are then fermented to acetate, propionate, butyrate, valerate, ethanol, lactate, hydrogen, CO_2, ammonia and sulphide by the acidogenic bacteria. Succinate produced by many bacteria is decarboxylated by others to propionate and CO_2. The proportion of the organic products of the acidogenic bacteria is determined by the H_2 concentration and pH (Mosey, 1983, McCarty and Mosey, 1991). The end product which generates the most energy for the acidogenic bacteria is acetate. However this can only be achieved if the partial pressure of hydrogen is below 10^{-3} atm. As the partial pressure increases to 10^{-4} atm and above, the NADH is used to produce more reduced products such as propionic and butyric acids as the conversion to acetate becomes energetically unfavourable. In the elevated hydrogen levels the production of propionic acid predominates at neutral pH values but as the pH level becomes acidic then the production of butryic acid will begin to predominate (McCarty and

Mosey, 1991). In a stable digester the low H_2 partial pressure is usually maintained by the hydrogen utilising methanogens (Harper and Pohland, 1986).

2.1.2. Acetogenesis And The Role Of Interspecies Hydrogen Transfer.

The methanogens can only utilise a limited range of substrates (methanol, acetate and hydrogen). The acetogens (obligate hydrogen producing acetogenic bacteria) convert the fermentation products which the methanogens cannot use (alcohols, greater than two carbon VFAs, aromatic compounds) to the substrates which the methanogens can utilise. The Gibbs free energies (a measure of the energy used or released in a particular reaction) for the conversion of ethanol, propionic and butyric to acetate and hydrogen are energetically unfavourable i.e. positive (Eq. 1, 2, 3) at standard free biochemical energy levels (pH 7.0, 1 atm.).

$$CH_3CH_2OH + H_2O \longrightarrow CH_3COOH + H_2 \qquad (1)$$

$\Delta G'_0 = +6.2$ KJ mol^{-1}

(Sahm, 1984)

$$CH_3CH_2COOH + 2H_2O \longrightarrow CH_3COOH + CO_2 + 3H_2 \qquad (2)$$

$\Delta G'_0 = +76.1$ KJ mol^{-1}

(van Lier *et al.*, 1993)

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2\ CH_3COOH + 2\ H_2 \qquad (3)$$

$\Delta G'_0 = +48.1$ KJ mol^{-1}

(van Lier *et al.*, 1993)

However if the hydrogen partial pressure can be reduced then the $\Delta G'_0$ becomes progressively more negative (Figure 2.2).

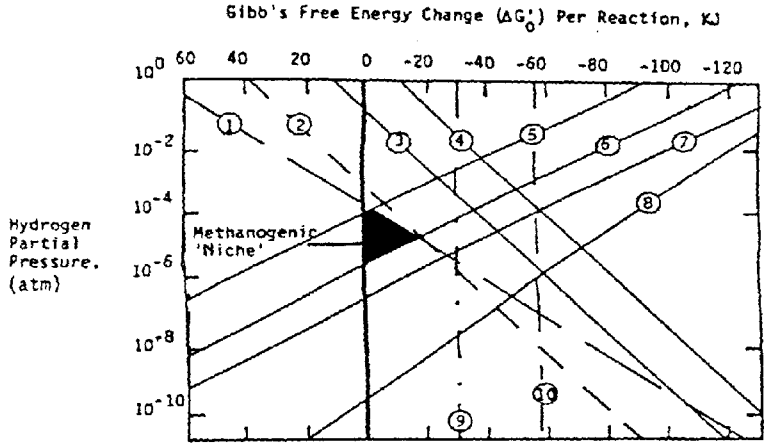

Figure 2.2. Graphical Representation Of The Hydrogen-Dependant Thermodynamic Favorability Of Acetogenic Oxidations And Inorganic Respirations Associated With The Anaerobic Degradation Of Waste Organics. (1) Propionic Oxidation To Acetic Acid, (2) Butyric Oxidation To Acetic, (3) Ethanol To Acetic, (4) Lactic To Acetic, (5) Acetogenic Respiration Of Bicarbonate, (6) Methanogenic Respiration Of Bicarbonate, (7) Respiration Of Sulphate To Sulphide, (8) Respiration Of Sulphite To Sulphide, (9) Methanogenic Cleavage Of Acetic Acid, (10) SRB-Mediated Cleavage Of Acetic Acid. (From Harper and Pohland, 1985).

The H_2 partial pressure must be maintained at between 10^{-6}-10^{-4} atmospheres for sufficient energy for growth to be obtained from propionic and butyric acid (Harper and Pohland, 1986). As H_2 is continually being generated it must continually be

removed for methanogenesis to occur. The removal of H_2 is achieved by conversion to methane by the hydrogen utilising methanogens. This symbiosis is known as interspecies hydrogen transfer and is crucial to the success of the anaerobic digestion process (Conrad *et al.*,1985). This low partial pressure is obtained from the close cell contact between the acetogens and the hydrogen utilising methanogens. It was initially thought hydrogen transfer occurred between the bacteria and a hydrogen pool. However from the hydrogen turn over rates and the hydrogen pool size it was calculated that up to 95% of the hydrogen was passed directly to the hydrogen utilising bacteria (Conrad *et al.*,1985). This transfer would require close contact and it would be an advantage for the anaerobic bacteria to adhere together in flocs, biofilms or granules for this to occur.

2.1.3. Methanogenesis

The final stage in the anaerobic digestion process is the production of methane. So far 19 methanogenic species have been identified in pure culture. These include *Methanobacterium., Methanobrevibacter, Methanococcus spps.* (Stronach *et al.* 1986). These bacteria have been difficult to culture *in vitro* as in nature they are dependent on other bacteria to provide a suitable environment for their growth. The fermentative bacteria provide a environment with a low redox potential -330 mV, extremely low in O_2 and suitable range of substrates (Sahm,1984).

The methanogens can only utilise a very narrow range of substrates i.e. H_2, acetate, formate, methanol and methylamine. The majority of species (lithotrophic methanogens) oxidise H_2 and reduce CO_2 to form methane (Eq.4).

$$4H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O \qquad (4)$$

$$\Delta G'_0 = -138.9 \text{ KJ mol}^{-1}$$

Formate is also utilised in a similar way (Eq.5)

$$4HCOOH \longrightarrow CH_4 + 3\ CO_2 + 2\ H_2O \qquad (5)$$

$\Delta G'_0 = -111$ KJ mol $^{-1}$

Only 3 species of methanogens which utilise acetate (aceticlastic methanogens) have been isolated in pure culture. From Eq. 6 it can be seen that the standard free energy change from the conversion of acetate into methane and carbon dioxide is very small.

$$CH_2COOH + H_2O \longrightarrow CH_4 + CO_2 \qquad (6)$$

$\Delta G'_0 = -32$ KJ mol^{-1}

(Sahm, 1984)

These methanogens grow slowly on this substrate. The growth rates (μ max) range from 0.21 to 0.43 day^{-1} (doubling times of 3 to 10 days) (Stronach *et al.* 1986). Although the acetoclastic methanogens gain little energy and grow slowly, radioactive labelling studies have shown that 70% of the methane produced in anaerobic digesters originates from acetate (Gujer and Zehnder, 1983).

Optimum methanogenesis is dependent on pH being maintained at between pH 6.5 to 7.5. (Sahm, 1984). Failure to do so results in reduction of gas production and no or little reduction in the polluting power of the effluent stream.

2.2. Reactor Types

The anaerobic biomass can be packaged in a variety of process configurations or reactor types. A review by Speece, (1996) identified twelve anaerobic reactor configurations, not including 2 stage types. This proliferation has occurred during the attempt to increase the effluent throughput and efficiency of anaerobic digestion. The main aim of these reactor variants is to maintain the highest concentration of anaerobic biomass in the reactor as possible. The higher the concentration of biomass the greater the amount of digestable organic waste that can be treated.

New reactor designs attempt to increase the ratio of solids retention time (SRT) to hydraulic retention time (HRT). The SRT is the major consideration in anaerobic technology. The key class of micro-organisms (the methanogens) have a low synthesis ratio and specific growth rates between 0.21 to 0.43 day^{-1} (Sahm, 1984). This can be an advantage as little excess sludge is produced. However if biomass is required to replace continually washed out biomass, start up reactors from a small inoculum or adapt to changes in feed concentration or composition then low biomass production is a disadvantage. Because of the low specific growth rate, a minimum SRT of 4 days at 35°C will be required to prevent "washout". "Washout" occurs when insufficient biomass growth occurs to maintain the bacterial population within the reactor ie. the HRT is too short. By retaining the biomass solids (increasing the SRT), shorter HRTs can be employed without washout occurring. If a large SRT is used, it will result in a system which is easier to start up, more stable and having reduced sludge production. The smaller the HRT, the smaller the reactor volume and

the smaller the capital cost. Therefore the main aim of reactor design development has been directed at increasing the SRT while reducing the HRT.

The simplest way of doing this is to settle and recycle the biomass leaving the digester in an external settler (anaerobic contact process). The baffled reactor is an extension of this principle, has plates in the reactor to settle and prevent the biomass leaving the reactor. In the upflow anaerobic sludge blanket (UASB) the bacteria form a "blanket" of bacteria. The majority of the "blanket" is maintained in the bottom of the digester due to its mass and baffles forming the settler unit at the top of the reactor. The success of the UASB reactor is dependent on two factors. To maintain a high concentration of biomass, most of the biomass should be formed into large grains or granules (3-4 mm in diameter). At the bottom of the bed are the larger granules, further up the bed the granules decrease in size and flocculated biomass appears. The design of the settler must prevent the granules and floculated biomass escaping in the effluent outflow.

In attached biomass systems the bacteria are encouraged to form biomass films on inert material which is incapable of leaving the reactor. In expanded bed and fluidised bed reactors the bacteria form biofilms on particles of activated carbon or sand. The increased density of these biomass clumps combined with appropriate reactor design allow the biomass to be trapped in the reactor. Anaerobic filters employ packing material physically fixed in the reactor. This packing material is larger than that used in fluidised bed reactors and can be porous or non-porous in nature. The bacteria either form films on the packing material or are trapped as flocs between packing material pieces.

Further details of reactor types can be found in general anaerobic digestion texts such as Stronach, (1986) or reviews such as Hickey *et al.* (1991). More detailed reviews of UASBs and anaerobic filters can be found in Lettinga and Hulshoff Pol, (1991) and Young (1991) respectively.

Table 2.1. Feedstock Characteristics For Various Reactor Systems.

Reactor Type	Feedstock Characteristic
ITD (Inclined tubular digester)	High SS, low to high strength
CSTR (Continuously Stirred Tank Reactor)	High SS, low to high strength
Contact	Small but significant SS, low to moderate strength
UASB (up-ward flow anaerobic sludge blanket)	Low SS, low to high strength
Anaerobic Filter	Significant biodegradable SS, low to high strength
RBC (rotating biological contact reactor)	Low SS, low to moderate strength
CASBER (Carrier assisted sludge bed reactor)	Low SS, moderate strength
AAFEB (Anaerobic attached-film expanded bed)	Low SS, low to high strength
AFB (Anaerobic fluidised bed)	Low SS, moderate to very high strength

Taken from Stronach, *et al.*,(1986).

The successful and cost effective operation of the anaerobic process is dependent on the choice of the most appropriate configuration for the waste type. The characteristic of the waste, which usually determines the type of reactor used, is the level of suspended solids in the waste.

Unfortunately from Table 2.1 it can be seen that if the wastewater has a high concentration of suspended solids then there is only a limited choice of reactors, either the CSTR or ITD. Predominately the CSTR is used for high SS waste. A low level of suspended solids is taken to be where less than 10% of the COD of the waste is formed by suspended solids (Mergaert *et al.*, 1992). If the wastewater has a low level of suspended solids, then a wide variety of anaerobic digesters can be used. As yet there is little concurrence which is the most efficient reactor type to use. Studies have been attempted to compare the effectiveness of a variety of reactor types in concurrent experiments (Hawkes *et al.*, 1995). However such studies have been performed on a limited number of waste waters. Any conclusions reached so far may have to be mitigated by the limited number of waste waters used, different operational strategies in start-up and steady state operation etc. (Hickey *et al.* 1991).

However the large numbers of UASBs employed would suggest that the UASB type has significant commercial advantages (Lettinga and Hulshoff Pol, 1991). For the anaerobic treatment of industrial waste waters, UASBs made up 67% of the anaerobic systems installed world wide (see Fig. 2.3).

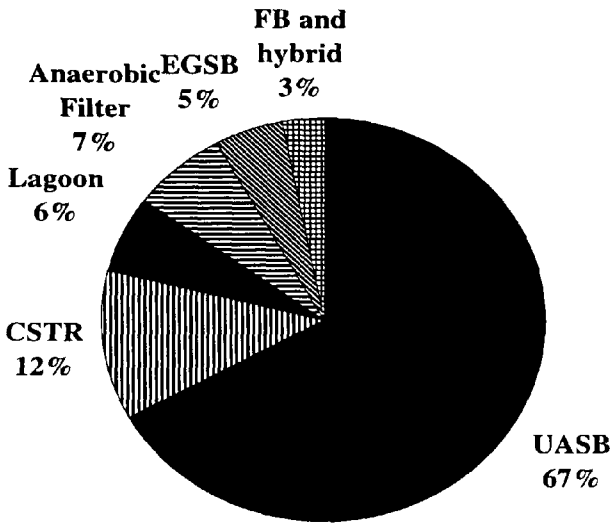

Figure 2.3. Distribution of Industrial Anaerobic Waste Treatment Plants in 1996. (Data supplied by Habets, L., Paques Water Systems BV)

The dominant position of the UASB is been further consolidated by developments in the UASB concept such as the expanded granular bed reactor (EGBR), a cross between a fluidised bed and UASB. This reactor type is commercialised as the Biothane Biobed or the Paques Biopac-IC reactor.

2.3 Monitoring The Effectiveness Of The Anaerobic Digestion Process

The effectiveness of the anaerobic digestion process is measured by determining the operational status of the reactor under a controlled set of operating conditions. The principal controlled reactor conditions are temperature, hydraulic retention time (HRT) and organic loading rate (OLR or B_V). The HRT is defined in Eq. 7.

$$\text{HRT (Days)} = \frac{\text{reactor volume}}{\text{feed flow rate in 24 hours}} \qquad (7)$$

The OLR or B_V is the amount of organic material applied per reactor volume per day Eq.8. The units of OLR include kg COD $m^{-3}day^{-1}$ or kg VS $m^{-3}day^{-1}$.

$$\text{OLR} = \frac{\text{organic concentration of feed} * \text{daily flow rate of feed}}{\text{reactor volume}} \qquad (8)$$

The operational status of the anaerobic digestion process can be monitored by analysing manually samples taken at discreet time intervals (off-line analysis). Increasing interest has been shown in on-line monitoring where automatic analysers continually monitor a sample stream from the reactor. On-line analysers allow measurements to be taken without operators and computer monitoring and control strategies to be implemented. Efforts are being directed at developing new robust sensors and using artificial intelligence techniques in computer monitoring and control strategies (Hawkes and Hawkes, 1994). Using these techniques it is hoped

that more efficient systems result and compliance with increasingly stringent legislation can be achieved (van Vooren, *et al.* 1996).

Either off-line or on-line analysis is directed at measuring parameters either in the liquid phase or gas phase. Measurements in the gas phase usually include the volume of biogas produced and composition. The two main components of the biogas are methane and carbon dioxide. The production of biogas with a high content of methane (50-80%) indicates that the digester is functioning well. Increasing carbon dioxide content is an indication of an upset to the digester. Other trace components measured in the biogas are hydrogen, carbon monoxide and hydrogen sulphide. Hydrogen and carbon monoxide can be monitored to determine the health of the digester (Mosey and Fernandes, 1989, Switzenbaum *et al.*, 1990). Hydrogen sulphide concentration is measured due its toxic and corrosive nature. A high concentration can also reduce the cost effectiveness of biogas utilisation.

The most important liquid phase measurements are pH and bicarbonate alkalinity. The methanogens are pH sensitive, if the pH is not within the range of pH 6.5 -7.5 then reactor performance can decline. The bicarbonate alkalinity measurement has been shown to be a more sensitive monitoring parameter than pH (Guwy, 1995). The bicarbonate alkalinity is a measure of the buffering capacity of the reactor over the range of pH 5.3-7.3.

Another important parameter is the level of volatile fatty acids (VFA) in the reactor liquid. Anaerobic digesters can operate at a wide range of levels of TVFA. Operation above 300-500 mgl^{-1} may indicate that the methanogenic activity is insufficient to remove the available substrate, although reactors can operate successfully at higher levels of TVFA. This not only results in a lower removal of the pollution but

destruction of the buffering capacity of the reactor. A rise in VFA is usually a sign of system stress.

The usual purpose of the anaerobic digester is to reduce the polluting ability of a wastewater effluent. The efficiency of removal of polluting power can be determined by measuring the level of biochemical oxygen demand (BOD), chemical oxygen demand (COD) or total organic carbon (TOC) removal. When the waste is highly particulate, analysis of total and volatile solids are the easiest measurements to perform.

The determination of active biomass in anaerobic digesters is difficult and time consuming to perform. Efforts have been directed at monitoring DNA, co-enzymes, bacterial lipids, ATP, protein, cell number and micro-calorimetry (Hickey *et al.* 1991). Accurate and easy measurement of active biomass would aid greatly in comparing anaerobic digestion studies and developing models of the process.

3. ANAEROBIC DIGESTION OF COFFEE PRODUCTION WASTE WATERS

The production of coffee results in the production of large quantities of waste. The initial processing generates 33 million tons of liquid and solid waste (82% w/w of fruit harvested). In the production of instant coffee only 33% of the raw material forms the final product, resulting in the production of approximately 2 million tons of waste per annum (Kostenberg and Marchaim, 1993b). The presence of such large quantities of organic waste constitutes a significant waste disposal problem. The anaerobic digestion process has been investigated as a possible solution.

Previous anaerobic digestion research into coffee industry wastes falls into two areas. These are the anaerobic digestion of waste from the initial processing of the coffee berry, and the waste from the production of instant coffee from the coffee bean. This research is concerned with the latter. There is little composition data to determine the similarities and differences between the wastes, however treatment by anaerobic digestion has met with variable success with both wastes.

3.1. Treatment Of Primary Processing Waste Waters

After picking, the coffee cherries are processed either via the dry method or the wet method. In the dry method, the cherry is dried and the skin, mesocarp and internal membrane (the husk) is then removed to leave the coffee bean. In countries bordering the Caribbean the wet method is employed resulting in a higher value product but greater amounts of liquid waste. The cherry is first washed and pulped to remove the mesocarp. The mesocarp (pulp) is then pressed to form pulp juice and a solid bagasse. Then the bean is fermented to remove the membrane, washed and dried (Calzada *et al.*, 1989).

The raw coffee pulp has been found difficult to digest, requiring 40-60 days to digest (Calzada *et al.,* 1981). Problems were also experienced using coffee pulp juice. In single-stage mesophilic CSTR digesters, reactor failure was experienced when the loading rate was increased above 1.39 kg VS $m^{-3}day^{-1}$ (10 day HRT). Two-stage operation was found to be more stable, resulting in a 70% VS destruction at an OLR of 1.8 kg VS $m^{-3}day^{-1}$(HRT of 8 days).

Using high rate anaerobic digesters to treat liquid effluent higher OLRs and lower HRTs were achieved (Calzada *et al.*, 1984). In a single-stage filter stable digestion was attained at 3.6 kg VS $m^{-3}day^{-1}$(5 day HRT). By using a two-stage anaerobic filter stable anaerobic digestion was possible at an OLR of 8.8 kg VS $m^{-3}day^{-1}$ (2 Day HRT) with a 60-71% reduction in COD being seen.

Although there is little compositional information to compare the digestion of primary waste waters with secondary waste waters some indications for the successful anaerobic digestion of secondary waste waters may be gained. More successful anaerobic digestion seems to occur when high rate digesters are used and the two stage concept is applied.

3.2. Treatment Of Secondary (Instant Coffee) Processing Waste Waters

3.2.1. Studies on Waste Containing Coffee Grounds

Laboratory-scale anaerobic digestion of spent coffee grounds has been attempted at mesophilic temperatures by Lane (1983), Oi *et al.*(1980) and Raetz (1990) and at thermophilic temperatures by Kida *et al.* (1992), Raetz (1990) and Kostenberg and Marchaim (1993a, 1993b, 1994).

Raetz (1990) analysed the composition of the coffee grounds and performed batch studies on the grounds at mesophilic (35°C) and thermophilic temperatures (60°C). Up to 60% reduction of volatile solids was thought possible. The degree of degradation had to be calculated from the partial breakdown products as the biogas contained no methane at mesophilic temperatures and only 10% methane at thermophilic temperatures. This lack of biogas production was probably due to problems in controlling pH. Repeated additions were made to maintain the pH at 6.8-7.2 but the pH rapidly fell to 4.0-4.5. The fall in pH was due to the production of volatile fatty acids, mainly acetic, propionic and butyric.

The extent of the biodegradation was mainly calculated from the level of volatile fatty acids. At mesophilic conditions 30 % of the organic content had been degraded over 19 days, 35 % after 26 days. Under thermophilic conditions 22 % of the organic content had been degraded. No enhancement in biodegradation was seen when the batch studies were supplemented with nitrogen and phosphate.

The compositional analyses showed that the grounds were principally lignocellulosic in nature (67% of total solids) but 27% of total solids was lipid. The slow rate of biodegradation and limited biogas production from the spent coffee grounds was thought to be due to its lignocellulosic nature and a possible inhibitor of methanogenesis being present in the waste. When greater than 15% of spent coffee grounds were added to the batch studies a slower rate of hydrolysis occurred. An inhibitory compound was thought to be present in the waste as a decrease in biogas production per unit weight of substrate was seen as more solids were added.

In an attempt to increase the biodegradability of coffee grounds Oi *et al.* (1980) investigated treatment of coffee grounds with dioxane (a lignin extractor), ammonia, cellulolytic enzymes or sulphuric acid. After pre-treatment the grounds were digested anaerobically at 37°C, either with or without additions of nitrogen and a easily biodegradable carbon source (inositol). The most effective treatment (dioxane treatment, incubation with cellulolytic enzymes and addition of nitrogen source and inositol) increased gas production from 67 ml per g of coffee grounds to 335 ml per g of coffee grounds in a 14 day batch study. This corresponds to an increase in biodegradation from 10% to 41% destruction of total solids. Although increasing the digestibility of coffee waste would be advantageous, the increase in digestibility has to be offset by the increased costs for the processes used to increase digestibility.

Lane (1983) used small-scale batch digesters and a larger 10 litre CSTR to study the anaerobic digestion of coffee grounds at 35°C and 40°C. The feedstock consisted of $80gl^{-1}$ total solids and was fed at a loading rate of $4kgTSm^{-3}$ day^{-1}. The coffee grounds were found to be 87% and 95% convertable to biogas in the short term studies at 36°C and 40°C respectively. In the longer term continuous study the coffee grounds were found to be initially 70% biodegradable at 20 day HRT with solids recycle. However gas production gradually declined and failed over a 80 day period. Nutrient addition of nitrogen and phosphorus and trace elements had no effect. Long term

acclimatisation experiments and conversion to thermophilic temperatures were tried but with no success. Lane concluded that inhibition was due to some unidentified component in coffee waste. Attempts were made to identify the inhibitory component. The waste was analysed and found to contain necessary levels of Ca, Mg, Mn, Fe, Cu and Zn. Heavy metal analysis found no toxic levels of Cd, Pb, Ni, Co, As or Hg. Caffeine levels in the waste were found to be between 1.4-6.9 mgl^{-1}. In tests caffeine levels of 670 mgl^{-1} did not inhibit the anaerobic digestion of glucose. Brown pigments were extracted and dried from the coffee waste and roasted cereal grains and chicory but none of these components was found to be inhibitory. The only compound found to be inhibitory was the addition of instant coffee.

Kostenberg and Marchaim (1993a, 1993b, 1994) operated thermophilic (55°C) fed batch CSTRs on coffee grounds. The feedstock had $192gl^{-1}$ total solids ($191gl^{-1}$ volatile solids). In one set of the experiments used grounds pre-treated with a soil grinder.

Stable anaerobic digestion was achieved at OLRs up to 8.6 kg VS m^{-3} day^{-1} at a HRT of 20 days. When the OLR was increased to 10 kg VS m^{-3} day^{-1}, overload occured with the VFA levels rising to 3000 mgl^{-1}, with a high percentage of propionic acid (Kostenberg and Marchaim, 1993b). From the biogas production a VS destruction of between 48-72% was calculated (Kostenberg and Marchaim, 1993b). To ensure stable anaerobic digestion the addition of alkali was needed. In experiments the addition of 0.3 g g VS^{-1} CaO was found to be superior to the addition of 0.3 g gVS^{-1} ammonium bicarbonate or 0.3 g gVS^{-1} sodium bicarbonate (Kostenberg and Marchaim, 1993b). The addition of CaO was found to give the highest biogas production and lowest level of VFA. When the anaerobically digested coffee waste was added as a supplement to peat moss superior growth characteristics resulted. This was thought to be due the digested coffee waste containing a plant growth promoting or a fungicidal agent (Kostenberg *et al.* 1994).

Kida *et al.* (1992) treated coffee grounds in a thermophilic (53°C) batch study and in a two-stage thermophilic (53°C) system. The feedstock was 200 gl^{-1} total solids (197gl^{-1} volatile solids) coffee grounds. In the single-stage system difficulty was experienced in maintaining a neutral pH and gas production was not reproducible. The two-stage system consisted of a first stage which retained the solids and produced a liquor which was fed to a methanogenic stage fluidised bed. This system was more stable than the single stage system. However the degree of solids reduction was not stated.

From the review of work treating coffee grounds, the long term continuous mesophilic anaerobic digestion of waste containing coffee grounds was not achieved. In the study by Lane, (1983) the mesophilic digester became unstable after 80 days of operation at a loading rate of 4kgTS$m^{-3}day^{-1}$ due to some inhibitory component in the waste. Thermophilic operation seems to offer the prospect of more stable operation. Kida *et al.* (1992) operated a thermophilic reactor for 50 days, however in effect coffee ground leachate was used in a retained biomass system. Using CSTRs, Kostenberg and Marchaim (1993a, 1993b, 1994) did achieve stable digestion with periods of up to 150 days of operation being studied and at organic loading rates of up to 8.6 kg VS m^{-3} day^{-1}.

3.2.2. Studies on Coffee Ground Free Waste

The removal of grounds from the coffee waste results in strong organic liquid waste which allows the implementation of retained biomass systems. There are several reports of these systems particularly at industrial scale.

Lanting *et al.* (1989) operated a pilot-plant UASB, treating coffee waste which had COD strength of 2649-5244 mgO_2CODl^{-1} and 227-993mgl^{-1} suspended solids. In two mesophilic experiments OLRs of 12-13 kgCOD m^{-3} day^{-1} were achieved but after 50

days of operation the reactors failed due to increasing levels of TVFA. Thermophilic operation was attempted and stable operation was achieved at OLRs of 10-11 $kgCODm^{-3}$ day^{-1} for a period of 120 days. A total COD removal of 69% was achieved in the thermophilic studies, 13% higher than that achieved in mesophilic operation. It was therefore recommended that thermophilic operation be used to treat instant coffee waste waters. The addition of metal trace elements was also thought to aid stability.

Full scale operation has been reported by Hajipakkos (1992) and Lettinga and Hulshoff Pol (1991) and Gornall (1991). The latter two references present little information except that a mesophilic UASB reactor is used in both cases. In contrast to Lanting *et al.* (1989) all these reactors operate at mesophilic temperatures. However the OLRs were lower than that reported by Lanting *et al.* (1989) the highest being 6kgCOD m^{-3} day^{-1}(Hajipakkos, 1992).

In the 880 m^3 plant described by Hajipakkos (1992) who presents more information, a mesophilic UASB was used to treat coffee waste after treatment in a settler and pre-acidification stage. This UASB was installed at General Foods, Banbury, Oxford in 1989. The waste initially had a strength of 4000mgO_2CODl^{-1} and 1500 mgl^{-1} suspended solids. The waste fed to the digester after pre-treatment had a strength of 3275mgO_2CODl^{-1} and 500mgl^{-1} suspended solids. A 55% reduction in COD and a 52% reduction in suspended solids was achieved by the UASB. Problems were experienced with H_2S levels in the waste leaving the UASB. A submerged aerobic filter was used to reduce the sulphate levels from 40 mgl^{-1} to 1 mgl^{-1}. Gornall (1991) reports a 3% level of H_2S in the biogas from a UASB treating coffee waste. This caused problems in the utilisation of biogas due to the corrosive nature of H_2S.

Treating coffee waste with reduced levels of suspended solids in a UASB seems to be the preferred commercial option. However some reports suggest that the reactors

can become unstable at relatively low OLRs and the biogas may contain high levels of H_2S. Improvements in the operation of UASBs treating coffee waste may be improved by employing thermophilic digestion and the effect of a pre-acidification stage may also be worth investigating.

3.2.3. Studies on Synthetic Coffee Wastes

To further understand the anaerobic digestion of instant coffee waste, laboratory-scale experiments have been performed using various synthetic feed stocks to mimic instant coffee waste waters. These include instant coffee itself (McDougall *et al.* (1993), Fernandez and Forster, (1993a, 1994), and coffee bean extract (Fernandez and Forster, 1993b). McDougall *et al.* (1993) and Fernandez and Forster, (1993a and b, 1994) used anaerobic filters while Shi and Forster (1993) operated a UASB. McDougall *et al.* (1993) also utilised an anaerobic filter in a two-phase system with an acidogenic CSTR.

McDougall *et al.* (1993) and Fernandez and Forster, (1993a and b) could achieve stable mesophilic digestion at organic loading rates up to 4 kgCOD $m^{-3}d^{-1}$. The level of COD removal ranged from 60-80% with coffee extract and 65 % with instant coffee. By utilising two-phase digestion, the COD removal was increased to 78%, a 13% improvement (McDougall *et al.*, 1993).

When thermophilic operation was attempted problems were experienced in establishing satisfactory thermophilic digestion. When converting a single stage filter to thermophilic operation, McDougall *et al.* (1993) were still experiencing TVFA levels of 1000 mgl^{-1} at the end of 41 days of thermophilic operation. Problems with

thermophilic filters were also experienced by Fernandez and Forster, (1993a and b). Reactor failure was experienced with gas production falling from 1.2 l l^{-1} day^{-1} to 0.1 l l^{-1} day^{-1} and a corresponding fall in COD removal from 50% to 5%. Shi and Forster (1993) achieved COD removals of greater than 75% in a thermophilic UASB at OLRs of 4 kgCOD $m^{-3}d^{-1}$ (HRT 24 hours). This level of performance could only be achieved when calcium was added to counteract the inhibitory effects of potassium present in the waste (Shi and Forster, 1993). Optimum performance was achieved when calcium : potassium ratio was 8:12 (Shi and Forster, 1994). A similar effect was found in thermophilic filters, where optimum performance was achieved when no additional potassium (a side effect of the phosphorus source) was added and calcium phosphate added instead. When these changes were introduced stable thermophilic performance could be achieved with COD removals reaching 70% (Fernandez and Forster, 1993b). A performance improvement from 60 % COD removal to 80% COD removal was also seen in the mesophilic filters (Fernandez and Forster, 1993b).

As with real coffee ground free waste problems were experienced in establishing stable anaerobic digestion with synthetic coffee ground free wastes. However more problems were experienced with thermophilic digestion of synthetic wastes than mesophilic digestion. This was in contrast to Lanting *et al.* (1989) who found the reverse when using actual coffee waste. However the identification of inhibitory concentrations of potassium being present in the synthetic feedstocks and that improved digestion could be achieved by adding calcium to the feedstock should be borne in mind if problems with real coffee waste were experienced. Also the OLRs

were modest in the synthetic studies, being no more than 4 kgCOD $m^{-3}d^{-1}$. It would be hoped higher OLR rates could be achieved in any further studies.

3.3. Improving The Anaerobic Digestion Of Instant Coffee Waste Waters

Increasing the throughput or treatment efficiency of the anaerobic digestion process would be of obvious commercial advantage. A variety of strategies have been investigated to achieve this. Efforts have been directed at improving reactor design to increase treatment efficiency, reduce HRT and increase loading rates. By employing appropriate monitoring and control strategies reactors can be operated at greater levels of throughput while reducing the chance of reactor instability (Hawkes and Hawkes, 1994). Two strategies which may be particularly suited to the anaerobic digestion of instant coffee wastewaters are thermophilic digestion and two-phase digestion.

3.3.1. Thermophilic Anaerobic Digestion

Three temperature optima have been reported for the anaerobic digestion process psychrophilic (around 15°C), mesophilic (around 35°C) and thermophilic (around 55°C) temperatures (Romero *et al.*,1988). Methanogenesis has been found to occur up to 75°C but the optimum temperature is thought to be 55-60°C (van Lier, 1996). The advantage of the higher temperature ranges is that the process will proceed at a faster rate than the lower temperature ranges as stated by the Arrhenius equation (Pavolostathis and Giraldo-Gomez, 1991). The implications for waste treatment are that a set amount of waste could be treated more quickly and in a smaller plant,

hopefully resulting in a more cost effective system. For this reason interest in thermophilic operation has been shown for many years, although thermophilic operation also has disadvantages.

The advantages and disadvantages of thermophilic operation over mesophilic anaerobic digestion have been reviewed by Buhr and Andrews, (1977). These include:

(1) Increased conversion rate of organic matter.

(2) Increased efficiency of the % fraction of organic solids converted.

(3) Increased reduction of pathogenic organisms.

(4) Improved solid-liquid separation.

The disadvantages noted were:

(1) High energy requirement for heating.

(2) Poor supernatant quality.

(3) Poor process stability.

Other disadvantages have also been suggested :

(4) Increased sensitivity to inhibitory compounds.

(5) Difficulties in generating thermophilic biomass.

The main proposed advantage for thermophilic operation is that due to the increased reaction rates at higher temperatures a greater fraction of the waste is converted to biogas in a shorter time period. This would result in a waste stream been treated to the same treatment efficiency in smaller reactor and reduced capital cost. In studies on cow manure, thermophilic operation (50-60°C) resulted in greater VS conversion

at identical HRT as mesophilic operation (Varel *et al.*, 1980). Operation at a shorter HRT was also possible at thermophilic temperatures than at mesophilic temperatures. Using thermophilic filters treating non-fat milk powder, twice the COD loading rate could be treated than in the equivalent mesophilic system (Harris and Dague, 1993). This corresponded to a OLR of 49.5 kgCOD $m^{-3}d^{-1}$in the thermophilic reactors. High loading rates of 120-150 kgCOD $m^{-3}d^{-1}$ have also been achieved in thermophilic UASBs on VFA substrates (Wiegant and De Mann, 1986). Stable operation at even higher organic loading rates of 160 kgCOD $m^{-3}d^{-1}$ with 94-98% soluble COD removal has been achieved in mesophilic UASBs with no pre-acidification (Fang and Chiu, 1993). In the treatment of coffee wastes Lanting *et al.* (1989) found thermophilic operation to be superior to mesophilic operation.

In certain waste types e.g. waste activated sludge, the waste is dewatered to reduce transportation cost after digestion. Thermophilically digested sludge has been reported to produce greater filtration yields and reduced consumption of coagulant than mesophilically treated sludge (Buhr and Andrews, 1977).

Digestion at thermophilic temperatures also results in a greater destruction of pathenogenic organisms than operation at mesophilic temperatures. Deactivation of helminth eggs (Buhr and Andrews, 1977) and fecal *Streptococci* (Mathrani *et al.* 1994) have been achieved by using thermophilic digestion. The use of thermophilic digestion can negate the need for a pasteurisation step for disposal of anerobic digester sludges to agricultural land (Danish Energy Agency, 1992). A pasteurisation step is a recommended requirement in certain countires to minimise the disease risk in land disposal (Buhr and Andrews, 1977, Danish Energy Agency, 1992).

As well as advantages the thermophilic digestion process also has a number of disadvantages. The usual temperature chosen for thermophilic digesters is 55°C

which is substantially higher than the ambient temperature. This means that extra energy input and hence cost is required to heat the feedstock. However some wastes such as vinasses and instant coffee waste are discharged at temperatures which require no extra energy input (Souza *et al.*, 1992). Also the advantages of thermophilic operation may outweigh the extra cost, for example where a pasteurisation step is a mandatory requirement to minimise disease risk (Buhr and Andrews, 1977, Danish Energy Agency, 1992).

A greater sensitivity to inhibitory compounds by thermophilic organisms compared to mesophilic organisms has been reported, for example increased thermophilic sensitivity to potassium (Shi and Forster, 1993), heavy metals (Macleod and Forster, 1988) and organic compounds (Disley *et al.* 1992). However this inhibition could be overcome in some cases by additions of other compounds (Shi and Forster, 1994) and by the selection of a more appropriate reactor design (van Lier, 1996).

A related problem is the tendency of thermophilic reactors to operate at relatively high levels of TVFA compared to the equivalent mesophilic system (van Lier, 1996, Souza *et al.*, 1992, Wiegant and DeMann, 1986). This could be due to inhibition by toxic waste components or some fundamental disadvantage in the thermophilic biochemistry. Comparisons of the substrate affinity of thermophiles and mesophiles has found that the predominant methanogen in thermophilic UASBs *Methanothrix* does not have a lower substrate affinity than its mesophilic counterpart (van Lier, 1996). However *Methanosarcina* sp,. does have lower substrate affinity than its mesophilic equivalent leading to higher effluent VFA levels where conditions lead to this organism predominating (van Lier, 1996). It has also been found that

immobilised thermophilic biomass can have a high apparent K_M leading to high effluent VFA levels at high loading rates (van Lier *et al.*, 1996).

Any thermophilic anaerobic digester will have to be seeded with an inoculum containing thermophilic anaerobes, the higher the concentration of thermophiles the quicker the start up will be. Mesophilic laboratory-scale and full-scale reactors can be quickly started up with a large inoculum from currently operating mesophilic digesters. In particular UASBs can be more quickly started up with obvious commercial advantage when granules from other reactors are used (Goodwin *et al*, 1992). Unfortunately there are few full-scale thermophilic anaerobic digesters operational from which to provide a thermophilic inoculum (Fang and Lau, 1996). Therefore in the study of any thermophilic wastewater treatment, attention will have to be given to the start up of the anaerobic digestion process.

It does seem that thermophilic organisms are present in many mesophilic inocula and are successfully used to start up thermophilic digesters (Zinder, 1986). Mesophilic seed has been used successfully to start up CSTRs (Romero *et al*, 1988) and UASBs (van Lier, 1996, Wiegant and DeMann, 1986). However this can take up to between 75 and 110 days for UASBs (Fang and Lau, 1996) and over a year was required to start up a particular thermophilic cow manure digester (Danish Energy Agency, 1992).

Thermophilic anaerobic digestion could offer a commercial advantage in achieving greater waste throughput in the treatment of instant coffee wastewaters. As the waste is released at thermophilic temperatures there is no requirement to heat it. Attention

will have to be given to the start-up of the reactors and possible sensitivity to components in the waste.

3.3.2 Multi-Stage Anaerobic Digestion

In an ideal situation, any waste treatment system would consist of a single stage or unit operation. However in the real world to achieve satisfactory performance or produce improved performance in waste treatment systems, the system will consist of a series of stages or unit operations. These unit operations can be a series of physical, chemical or biological operations organised to produce the most effective waste treatment system. Anaerobic digestion as a unit process has been improved by employing physical pre-treatment e.g. settling to remove suspended solids or chemical e.g. Fenton`s reagent to improve biodegradability or another biological process either as a pre-treatment or post-treatment e.g. aerobic polishing.

The anaerobic digestion process itself has been separated into a number of stages to improve efficiency. A variety of staged anaerobic digestion strategies has been proposed. Two or more anaerobic digesters have been arranged in series to improve the treatment efficiency. Stronach *et al.*, (1986) reported improved COD removal from 80% to 94% by using two fluidised beds in series. Using a thermophilic filter in series with a mesophilic filter, COD removals were increased to 96% compared to the 68% achieved in a single stage mesophilic filter (Kaiser and Dague, 1994). A staged process need not necessarily result in an increased number of reactors. Van Lier *et al.* (1996) designed a compartmentalised upflow (baffled) reactor which effectively contained 5 reactors in one reactor. Acidification bacteria were found in

the lower compartments and other bacterial types in the upper compartments e.g. propionate oxidisers. Using this, reactor more stable and effective thermophilic operation was achieved at higher loading rates than a single stage system.

The most widely studied anaerobic staged system is the so called two-phase system. In this system the anaerobic process is divided into two reactors, with the first reactor optimised for hydrolysis/acidification and the second for acetogenesis/ methanogenesis (Cohen *et al.*, 1980, Ghosh *et al.*, 1984). The two-stage concept as been promoted has offering the following advantages over a single-stage system in treating wastewaters: increased COD removal (McDougall *et al.* 1993), increased stability to shock loads (Bull *et al.*, 1984, Cohen *et al.*, 1982), increased turn over rate (Cohen *et al.*, 1980, Ghosh *et al.*, 1984) and greater resistance to inhibitory compounds e.g. lipid (Komatsu *et al.* 1991) or hydrogen sulphide (Nanninga and Gottschall, 1986).

By increasing the maximum turn over rate of the methanogenic system, a smaller methanogenic stage will be required, resulting in lower capital cost. Using a two-stage system the maximum loading rate achievable by a mesophilic UASB system was over three times higher whilst still maintaining the same COD removal than the same methanogenic system fed unacidified feed (Cohen *et al.*, 1980). Using a soft drink wastewater between 3 to 6 times higher OLRs and up to 50% shorter HRTs were achieved by employing two-phase anaerobic digestion over a single-stage system (Ghosh *et al.*, 1984).

This advantage in reactor throughput would have to be offset against the capital cost of building a pre-acidification tank for a two-stage system. However in practice most industrial scale plants require buffer tanks to manage the factory flowrate to the anaerobic digester (Hajipakkos, 1992, Nanninga and Gottschall, 1986). These tanks could be converted to pre-acidification tanks at little extra cost although in fact uncontrolled pre-acidification does occur in these tanks in the majority of cases (Nanninga and Gottschall, 1986).

An unexpected increase in wastewater strength or flow may lead to reactor overload and a reduction in reactor efficiency or in some cases reactor failure. Using a pre-acidification reactor, the methanogenic stage avoided reactor failure or maintained its efficiency under shock loading conditions. If the performance did decline the recovery was quicker in the two stage system (Bull *et al.*, 1984, Cohen *et al.*, 1982). The two-phase system was able to maintain stability during a 6-8 fold increase in COD load compared to the maximum load achievable in the single-stage system (Cohen *et al.*, 1982). In the same two-phase system after the overload was finished, normal efficiency was regained in 20% of the time required by the single stage system. A two-phase fluidised bed system maintained superior COD removal compared to a single-stage system under a 10°C decrease in temperature or 100% flow rate increase or a 100% feed COD increase (Bull *et al.*, 1984).

Some wastes contain compounds which are toxic, inhibitory or cause problems such as foaming in the methanogenic system. Using a two-stage system these problems have been reduced. In the treatment of potato-processing wastewater, toxicity due to hydrogen sulphide was reduced by using a two-stage system. This system provided

conditions which the sulphate reducing bacteria were less able to grow (Nanninga and Gottschall, 1986). Lipid toxicity was reduced by the more toxic unsaturated long fatty acids being converted to less toxic saturated fatty acids in acidogenic stage before being fed to the methanogenic stage (Komatsu *et al.* 1991).

Other advantages of pre-acidification which have been noted are reduced suspended solids levels and COD levels in the effluent and increased methane content of the methanogenic stage. In a two-phase fluidised bed 50% lower levels of suspended solids were found in the effluent compared to the single-stage system effluent (Bull *et al.*, 1984). Little methane production occurs in the pre-acidification stage, the main gas produced being carbon dioxide. This results in the methane content of the methanogenic phase of the two-phase system being 10% higher than in the single-stage system (Ghosh *et al.*, 1984).

Pre-acidification may offer significant advantages in the operation of anaerobic digesters treating instant coffee wastewaters. Such wastewaters have been found to contain up to 12% w/w of lipid (described in this thesis) so the two-stage system could offer a significantly more efficient treatment process (Komatsu *et al.* 1991). McDougall *et al.*, (1993) using a synthetic coffee waste in a mesophilic two-phase filter achieved a 13% greater COD removal than the equivalent single stage system. The main work in pre-acidification has been directed at mesophilic temperatures (Bull *et al.*, 1984, Cohen *et al.*, 1982, Cohen *et al.*, 1980), thermophilic pre-acidification has been relatively little studied. Phased temperature operation i.e. thermophilic reactor followed by a mesophilic reactor may offer advantages such as increased COD removal (Kaiser and Dague, 1994).

4. MATERIALS AND METHODS

4.1. Analytical Procedures

Where standard analytical methods were used a short description is contained in this section with a full description of the procedure in Appendix 1.

4.1.1. Collection and Preservation of Samples

Samples were collected and preserved according to the guidelines specified by Standard Methods, (APHA, 1989). Where possible any analysis was performed as soon as the sample was taken. Otherwise samples were stored at 4^{0}C in a domestic refrigerator for short periods or frozen at -20^{0}C. Where possible samples were acidified to prevent biological action.

4.1.2. Total Solids

Total solids (TS) were determined by drying to constant weight a known volume of sample at 105 ± 2^{0}C (APHA, 1989).

4.1.3. Volatile Solids

The level of volatile solids was determined by incinerating at 500 ± 50^{0}C to constant weight a sample which had undergone total solids analysis as described in **4.1.2.** (APHA, 1989).

4.1.4. Suspended Solids

Suspended solids were determined as described in Standard Methods (APHA, 1989).

4.1.5. Volatile Suspended Solids

A sample which had undergone suspended solids analysis as described in **4.1.4.** was incinerated at 500±50^{0}C to constant weight (APHA, 1989).

4.1.6. Settleable and Non-settleable Solids

Settleable and non-settleable solids were determined according to Standard Methods, APHA, (1989).

4.1.7. Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) was determined using the sealed tube method and mercury free reagents as described in Standard Methods (HMSO, 1986).

A number of different COD parameters were determined depending on the sample pre-treatment.

Total COD = analysed from well mixed sample.

Settled COD = analysed from sample left to stand in a 250 ml beaker at room temperature for one hour. Sample taken from 100 ml mark on beaker.

Filtered COD = analysed from filtrate after filtering with GF/C filter.

4.1.8. pH

The pH (hydrogen ion activity) was determined by electrometeric measurement Standard Methods (APHA, 1989).

4.1.9. Bicarbonate Alkalinity

Bicarbonate alkalinity was determined by titration as in Jenkins *et al.* (1983). This method differs from the Standard Methods (APHA, 1989) determination of bicarbonate alkalinity as a titration endpoint of pH 5.75 is used instead of a endpoint of pH 4.3. A compensation factor is required to compensate for the higher pH endpoint. However the higher pH endpoint ensures there is no or little interference from the buffering capacity of volatile fatty acids (Jenkins *et al.*, 1983).

4.1.10. Temperature

The temperature of waste and reactor samples was determined using a mercury filled thermometer (Standard Methods, APHA, 1989). The thermometer was calibrated in 1°C intervals from -20 to +110°C and corresponded to British Standard (BS) 1704.

4.1.11. Gas Composition

The methane and carbon dioxide content of the biogas was determined by GC (gas chromatography) as described in Peck *et al.* (1986).

4.1.12. Hydrogen Sulphide Content of Biogas

The hydrogen sulphide content of the biogas was determined by the use of Draeger type gas indicating tubes. A 100 ml sample of biogas was drawn up through a Kitagawa 1000 ppm or 2000 ppm hydrogen sulphide colour indicating tube with a Kitagawa Toxic Gas detector syringe (Alltech Associates, Carnforth, England). The hydrogen sulphide level was indicated by the extent of colour change along the indicating tube and was corrected for the atmospheric temperature and pressure.

4.1.13. Volatile Fatty Acid Analysis

Individual volatile fatty acid levels (C_2-C_5) were determined by GC fitted with FID after extracting the VFA with diethyl ether from an acidified sample as described by Peck *et al.* (1986). The advantage of the diethyl ether extract method is that column fouling from components in the waste is reduced.

4.1.14. GC Mass Spectroscopy

GC mass spectroscopy analysis was performed on the diethyl ether fraction of an acidified sample extracted as in section **4.1.13.**. Analysis was performed on a Hewlett Packard 5890 GC fitted with a Hewlett Packard 5971 Mass Selective Detector. The resulting mass spectra was analysed with MS Chemstation software running on a Hewlett Packard Vectra IBM compatible.

4.1.15. Total Lipid

Total lipid was determined gravimetrically after chloroform/methanol extraction (Bligh and Dyer, 1959). To prevent oxidation of the extract an antioxidant, BHQ (Butyrated Hydroxy Quinoline) was used as well as evaporation of the chloroform with N_2 was employed.

4.1.16. Holocellulose (Hemicellulose and α-Cellulose) Determination

The amount of hemicellulose and α-cellulose was determined as in a method described by Allen (1974). The first step in the analysis was the delignification stage achieved by boiling with acidified sodium chlorite. The residual mass after washing with deionised water and diethyl ether is termed holocellulose. To determine the level of α-cellulose in the holocellulose, a sample which had undergone the analysis for holocellulose was treated with potassium hydroxide to break down hemicellulose leaving the α-cellulose (Allen, 1974). The hemicellulose was derived by subtracting the α-cellulose value from the holocellulose value.

4.1.17. Lignin Determination

The lignin content of a sample was determined gravimetrically after removing all other organic compounds by solvent extraction and digestion with sulphuric acid (Allen, 1974).

4.1.18. Soluble Tannin and Lignin Determination

The level of soluble tannin and lignin was analysed according to the method described by the APHA, (1989). This is a spectrophotometric method based on the Folin-Ciocalteau reaction and registers all hydroxylated aromatic compounds including tannin, lignin, phenol and cresol. The reagents used were those supplied in the Hach Tannin and Lignin Test kit (Camlab Ltd., Cambridge).

4.1.19. Ammonia Nitrogen Determination

Ammonia nitrogen was determined by distillation and titration as in Standard Methods (APHA, 1989).

4.1.20. Total Kjeldahl Nitrogen Determination

Kjeldahl nitrogen was determined using a mercury free method with a copper catalyst, (HMSO, 1986), the subsequent ammonia produced was determined by distillation and titration, (APHA, 1989).

4.1.21. Crude Protein Determination

The level of crude protein was estimated by multiplying the organic nitrogen concentration by a factor of 6.25 (Allen, 1974). The organic nitrogen concentration was calculated by subtracting the ammonia concentration from the total Kjeldahl nitrogen concentration.

4.1.22. Sodium, Calcium and Potassium Determination

The sodium, calcium and potassium levels were determined by using a single beam Corning 400 Photometer. The machine was calibrated using a three point calibration for each individual ion made from a 1000 mgl^{-1} standard (Fison Scientific Ltd., Loughborough). Between each sample a sample of deionised water was analysed. A sample was filtered using a Whatman GF/C filter to remove any particulate matter. The filtrate was then analysed.

4.2. Experimental Apparatus

Procedures 4.2.1 and 4.2.2 were used in the anaerobic digestion studies on coffee waste containing coffee grounds as described in Chapter 6.

4.2.1. Batch Anaerobic Digestion Studies

The batch study reactor consisted of a 5 litre Quickfit vessel (Bibby, Stone, UK) fitted with a gas tight lid which had sufficient ports for gas output to gas meter, head space gas analysis and liquid sample port. In the mesophilic study the reactors were incubated at 35°C and at 55°C in the thermophilic studies, by placing in a water bath maintained at the set temperature by a Grant Heater (Grant Instruments Ltd., Cambridge, UK). Gas produced was passed through a Dreschel bottle containing acidified water (5% H_2SO_4).

In the mesophilic study gas volume was determined by wet type gas meter (Alexander Wright Ltd., London, UK). In the thermophilic study gas volume biogas volume was measured continuously by an electronic low flow gas meter (Guwy *et al.* 1995). The gas meter was monitored continuously using the digital inputs of a RTI 815 interface board (Analog Devices Inc., USA) connected to a Viglen III 386SX IBM compatible computer (Middlesex, UK). The datalogging software was custom written in Turbo C++ as described in Guwy *et al.*, (1995). The data were saved as a text file and can be manipulated using the Microsoft Excel spreadsheet. The gas meter was calibrated by passing a range of known gas flows generated by a Watson and Marlow pump (Falmouth, UK.). The gas flow was measured by a factory

calibrated bubble flow meter (SAGA4000, Ion Science Ltd. Cambridge, UK). The calibration graph was generated using Technicurve for Windows.

Six 5 litre vessels were used. The digesters were incubated at mesophilic (35°C) or thermophilic (55°C) temperatures. Four vessels were filled with 2 litres of waste containing coffee grounds, defined as waste stream C in this thesis (see chapter 5, Figure 5.1), 3 litres of deionised water and sufficient homogenised UASB granules to provide inoculum of 3 gl^{-1} as volatile solids. The inoculum source used for the batch studies were UASB granules from a mesophilic UASB treating paper mill effluent at Davidsons paper mill, Aberdeen.

For the mesophilic study a control digester was set up containing 2 litres of deionised water instead of coffee waste but with all other additions. In the thermophilic study the control digester was supplemented with 7.3 gl^{-1} cellulose (Avicel) and 5.4 gl^{-1} D-glucose to determine if an active thermophilic culture was achieved. These values of cellulose and glucose were chosen to provide suspended solids and soluble COD similar to the coffee waste. All reactors had nutrient supplementation. Nitrogen (urea) and phosphorus (ammonium phosphate) were added to give a COD:N:P ratio of 400:7:1. Trace elements and metals were supplied in the amounts as described in Standard Methods (HMSO, 1988). Sodium bicarbonate was added at concentration of 9 gl^{-1} to maintain the digestion at the optimum pH for anaerobic digestion.

The progress of the digestion was determined by monitoring gas production and composition, pH and VFA levels. When gas production had ceased to be measurable and VFA levels had fallen to less than 300 mgl^{-1}, the digestion was left for a further 2

weeks and then deemed to have been completed. One litre of well mixed digester contents was then removed for analysis along with a sample taken prior to digestion to determine the compositional changes after digestion. The analyses performed were for volatile solids, lipid, α-cellulose, hemicellulose and lignin. The volatile solids reduction calculation was adjusted for the initial inoculum which was assumed to be not degraded over the experiment. The lipid values were adjusted for lipid found in the inoculum which was assumed to remain constant over the duration of the experiment. Lignin, α-cellulose and hemicellulose were assumed not to be present in the inoculum.

4.2.2. Semi-Continuously Stirred Tank Reactors (SCSTR) And Operation

Four 5 litre stirred digesters were constructed. An example is shown in Figure.4.1. The main vessel was a modified 5l Quickfit vessel (Bibby, Stone, UK) with a outflow port fitted by a glassblower. A gas tight lid was fitted with sufficient ports for stirrer guide, feed/liquid sample port, gas sample port, and gas output. The digesters were maintained at either mesophilic temperature (35°C) or thermophilic temperature (55°C) by a water jacket consisting of Neoprene tubing coiled around the digester. Water was passed through the tubing on a closed loop system with a reservoir by a thermostatically controlled Grant Flow heater (Grant Instruments Ltd., Cambridge, UK). Each digester was stirred at 300 rpm for 5 minutes every 6 hours by a Heidolph motor (Heidolph GmbH, Kelheim, Germany).

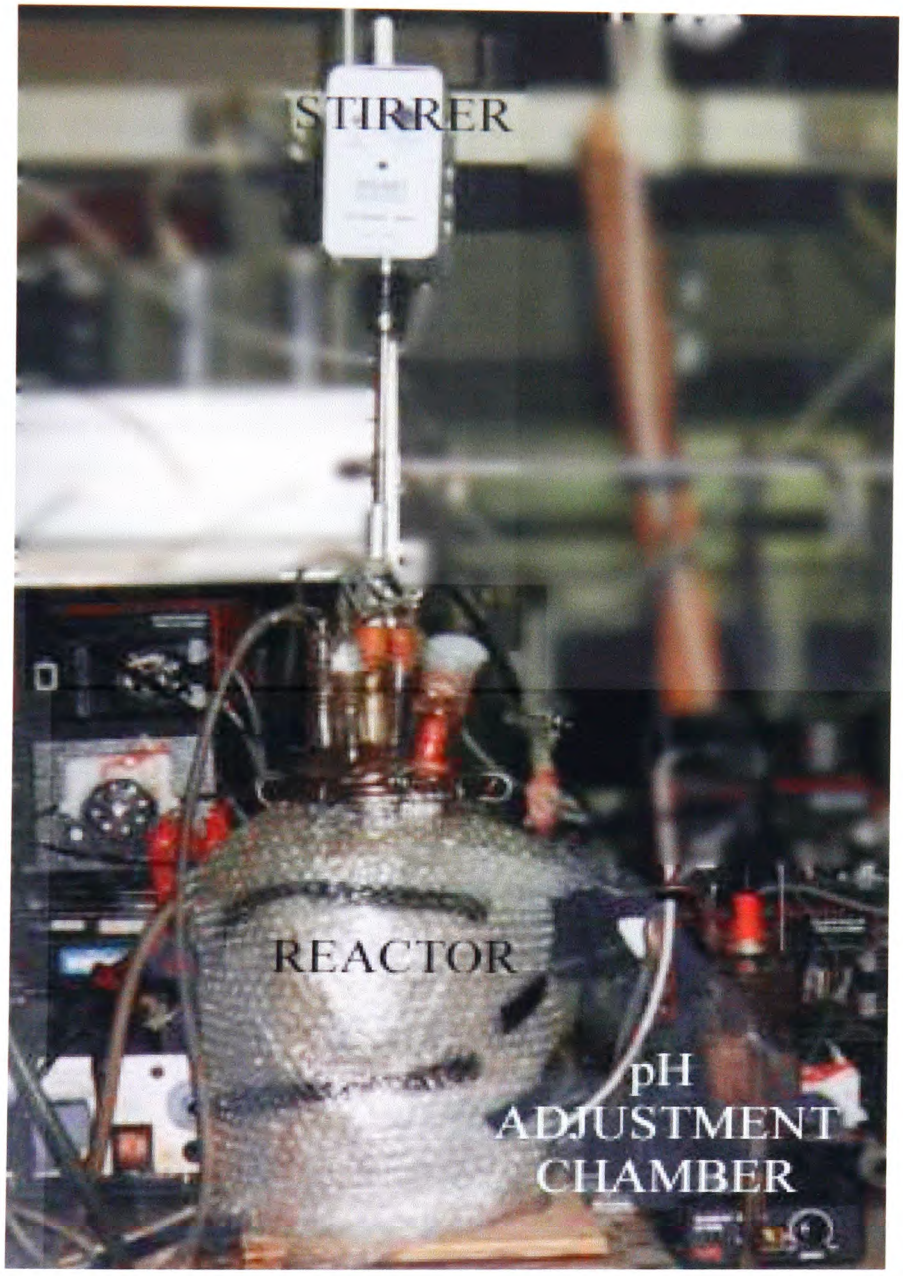

Figure 4.1. The CSTR Reactors Used In The Studies On The Waste Containing Coffee Grounds And As The Thermophilic Pre-Acidification Reactors.

Biogas produced was passed through a Dreschel bottle containing acidified water. Biogas volume was measured by a 4 channel water displacement gas meter similar to the design of Moletta and Albagnac (1982). This gas meter was calibrated as described in section 4.2 and Guwy *et al.*, (1995). The gas meter was monitored continuously with the computer hardware and software as described Guwy *et al.*, (1995).

The vessels were seeded by adding to each digester sufficient homogenized UASB reactor granules to give a volatile solids level due to the inoculum of 5 gl^{-1} in the final volume. To each digester was added 1.3 litres of coffee waste containing grounds (waste stream C, see chapter 5, Figure 5.1), 9 gl^{-1} sodium bicarbonate, and the volume was made up to 5 litres with deionised water. The digesters were left to acclimatise for 20 days, after which feeding was commenced at day zero. To provide as stable conditions as possible and because of the particulate and oily nature of the waste the digester was fed daily by hand (seven days a week). Initial retention times were chosen which were typical for the start-up of thermophilic and mesophilic CSTRs (Romero *et al.*, 1988). The mesophilic digesters were fed at a loading rate of 1.3 kg COD $m^{-3}day^{-1}$ (25 day HRT) and the thermophilic digesters at a 1.6 kg COD $m^{-3}day^{-1}$ (20 day HRT).

Two studies were performed described as follows :

In the first continuous study inoculum was taken from a UASB reactor treating paper mill effluent and was from the same batch used to seed the EPSRC Anaerobic

Facility pilot plant. The feed had 9 gl^{-1} sodium bicarbonate added and was not supplemented in any other way.

To investigate the importance of nutrient and Ca^{2+} addition, a second study was initiated using the same apparatus.

In the second continuous study the feed was neutralised by the addition of $1gl^{-1}$ $Ca(OH)_2$ and nutrient supplemented. The nutrients added were as follows: nitrogen (urea) and phosphorus (ammonium phosphate) were added to give a COD:N:P ratio of 400:7:1. Trace metals were supplied by the addition of 2 ml per litre of trace metal solution as described in Standard Methods (HMSO, 1988). The inoculum was taken from a UASB reactor of the EPSRC Anaerobic Facility pilot plant, located at Nestlé, Hayes, Middlesex, UK, which had been fed on settled coffee waste. The pilot plant reactor operates with TVFA levels of 700-1500 mgl^{-1}.

In the first study two mesophilic reactors were operated with daily feeding by hand for 35 days and monitored in total for 120 days. Also two thermophilic reactors were fed daily by hand until day 47 and monitored until day 120.

For the second study one mesophilic reactor was fed daily by hand and monitored for a period of 99 days and the other mesophilic reactor fed daily by hand and monitored for 80 days until operation was halted due to a public holiday. Also one thermophilic reactor was fed daily by hand for 48 days and monitored for 99 days. The second thermophilic reactor suffered repeated heater malfunction after 10 days of feeding and operation was discontinued.

Gas production, biogas composition, bicarbonate alkalinity, pH, TVFA and individual VFA levels were monitored on a regular basis. In the second study volatile solids destruction and COD destruction was determined for both mesophilic reactors. Samples were analysed in triplicate on six separate occasions. Samples were taken on days 50, 65, 71, 78, 85, 92 and 99 for the 99 day mesophilic digester and on days 30, 45, 51, 58, 65, 72 and 79 for the 80 day mesophilic digester. COD reduction was determined on samples taken on eight separate occasions which were analysed in triplicate. The COD samples were taken on days 45, 51, 73, 78, 79, 80, 84 and 92 for the 99 day digestion and on days 25, 31, 53, 58, 59, 60, 64 and 72 for the 80 day digestion.

4.2.3. Single Stage UASB Reactors And Operation

The apparatus and procedures described in this section were used in the study of unacidified coffee ground free waste in mesophilic and thermophilic UASB reactors as described in Chapter 6.

Four 5 litre perspex UASB reactors were constructed (see Figure 4.2 and Figure 4.3). The main body consisted of a 640 mm long perspex tube of 100 mm i.d., 110 mm o.d. Feed was pumped through a T piece situated at the bottom of the reactor. Effluent exited at the top of the reactor via a U-bend, which was arranged such that the reactor had 4.8 litre liquid volume. The sample port position used for pH and bicarbonate alkalinity was 60 mm below the liquid level in the reactor. The gas separator consisted of a perspex baffle and a polypropylene funnel. The funnel

resulted in a baffle at 50° from the horizontal as suggested by Lettinga and Hulshoff Pol (1991). Reactor temperature was maintained using a thermostatically controlled Grant Flow heater (Grant Instruments Ltd., Cambridge, UK) connected to the water jacket. Two UASB reactors were maintained at the mesophilic temperature of 35°C, and the other two at the thermophilic temperature of 55°C.

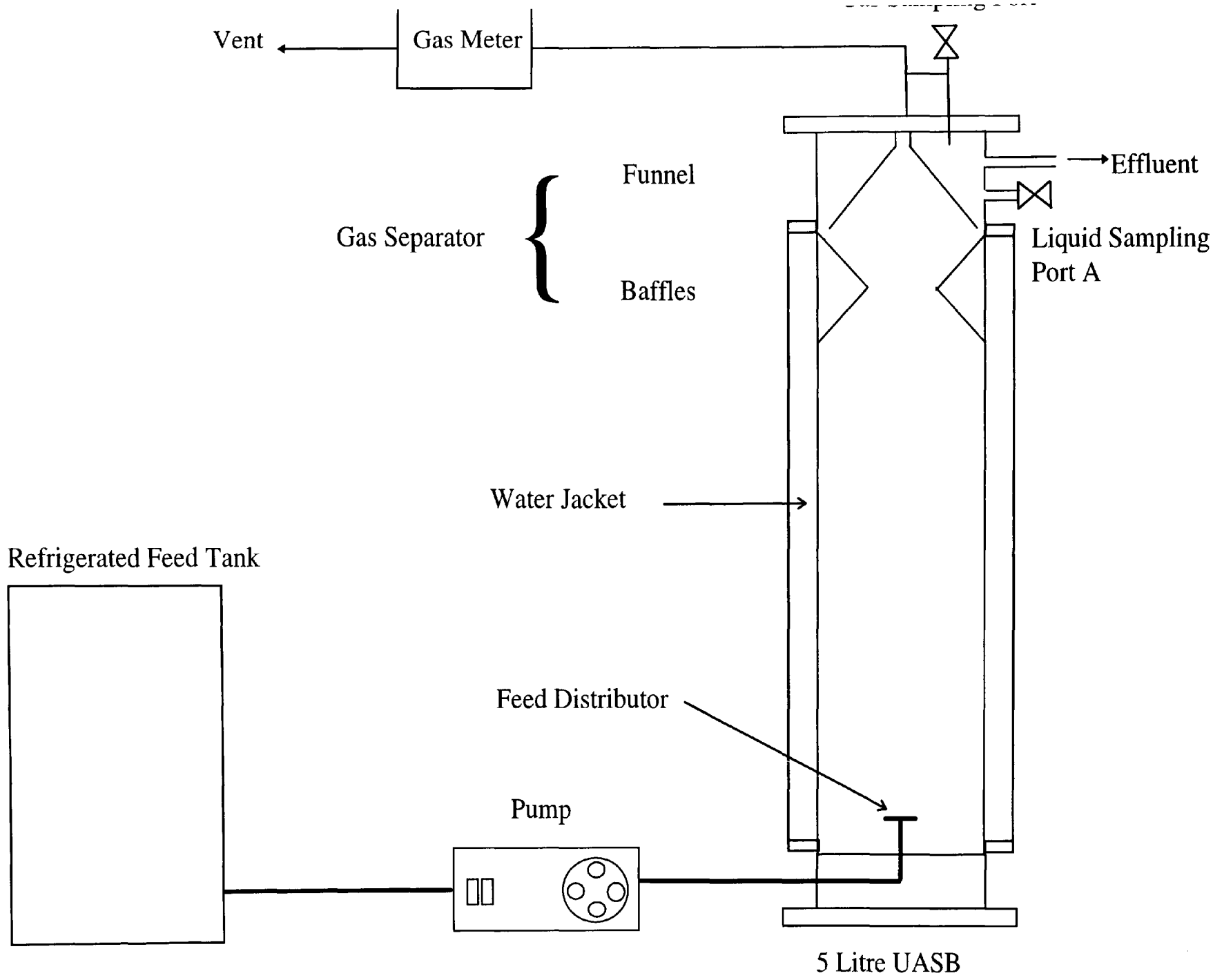

Figure.4.2 A Schematic Diagram of Single Stage UASB

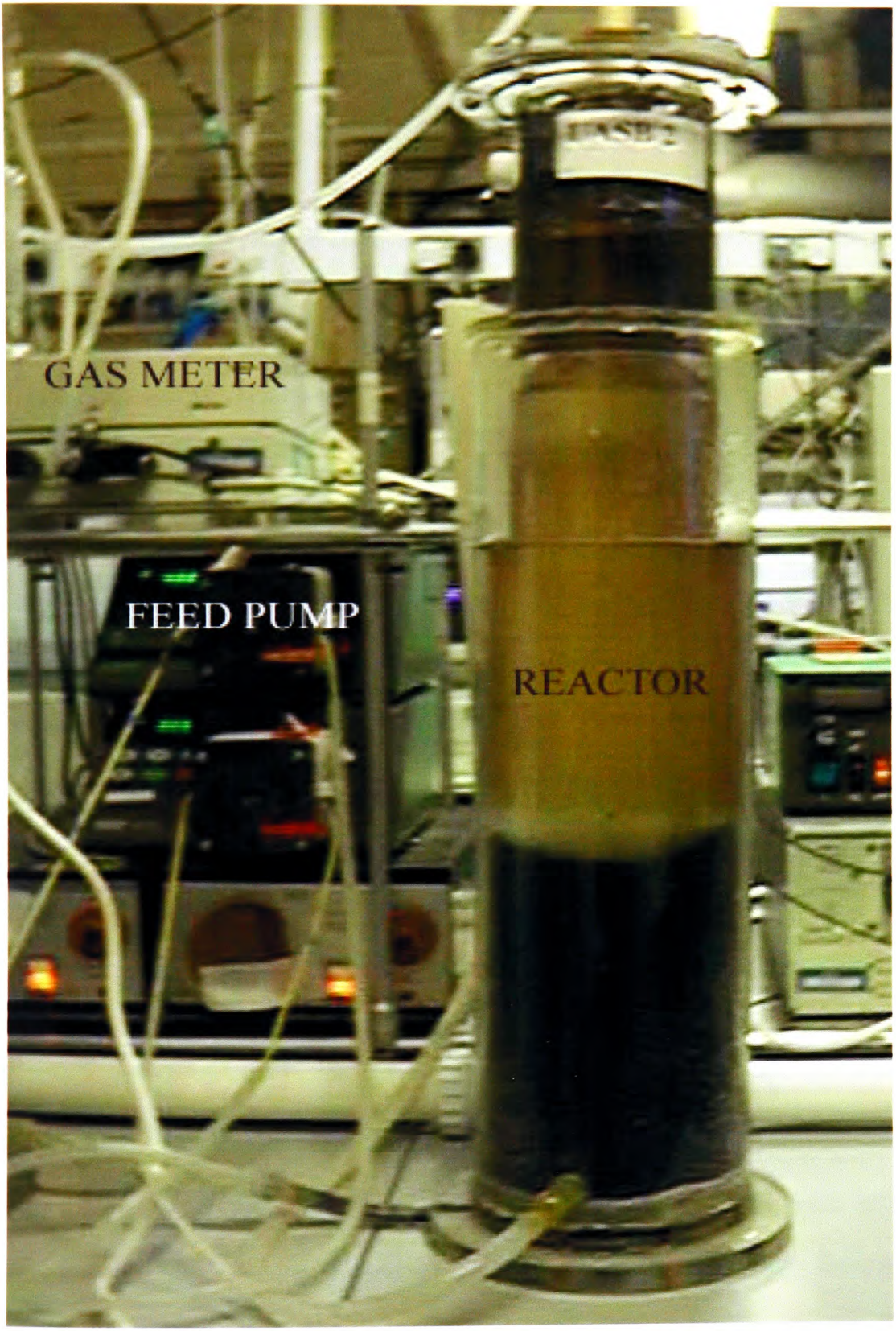

Figure 4.3. The UASB Reactors Used In Single And Two-Phase Operation.

Biogas volume was measured continuously by an electronic low flow gas meter described by Guwy *et al.* (1995). The gas meter was calibrated and monitored using the procedure and data logging apparatus described by Guwy *et al.* (1995). The feed was supplied by a Watson and Marlow 300U peristaltic pump (Falmouth, England) from two continuously mixed 20 litre containers. After day 31 the feed was maintained at 5 °C by placing the feed container in a domestic larder refrigerator. This reduced the amount of biomass growth in the peristaltic tubing and prevented any significant pre-acidification.

Coffee waste containing coffee grounds was obtained from waste stream C (see Chapter 5, Figure 5.1) at the Nestlé instant coffee factory, Hayes, London, UK. The waste was frozen until required, after which the coffee grounds were removed by settling at room temperature for 1 hour and siphoning off the top layer. This procedure resulted in coffee waste free of coffee grounds with a total COD ranging from 7,400 to 18,000 mgO_2l^{-1}. From day 30 of the experiment the coffee waste was blended or diluted with deionised water to produce a feed with a COD of 10,000 mgO_2l^{-1}. Nitrogen (urea) and phosphorus (ammonium phosphate) were added from day 1 to give a COD:N:P ratio of 400:7:1. Trace elements were supplied by the addition of 2 ml per litre of trace element solution as described in Standard Methods (HMSO, 1988). Sodium bicarbonate was also added at 1.5 - 2.0 gl^{-1}.

All reactors were seeded with 1.6 litres of mesophilic granules from the EPSRC pilot plant Anaerobic Facility operating at the Nestlé instant coffee factory, Hayes, London, UK, treating instant coffee waste water (Quarmby and Forster 1995). The granules had been washed and sieved (2 mm sieve) to remove any non-granular material. The inoculum gave a volatile solids concentration of $14.5gl^{-1}$ and a sludge

bed height of 210 mm. The reactors were warmed up rapidly to the required temperature (35°C or 55°C) and four days later feeding was started (day 0) at a 2 day HRT and an OLR of 3.6-7 $kgCODm^{-3}d^{-1}$. During start up to minimise the addition of sodium ions, levels of bicarbonate alkalinity were monitored and extra sodium bicarbonate was added if the bicarbonate alkalinity level dropped below 1000 $mgCaCO_3l^{-1}$. The reactors were operated and monitored for a period of 110 days. After start-up, changes in OLR were brought about by changes in feed flow rate (i.e. in HRT) introduced over a 2 day period. For each change in OLR the reactor was maintained at those conditions until steady state was reached and for sufficient time so that at least 3 HRTs had been achieved. Steady state operation was defined as achieving VFA levels and COD removal similar to that achieved at the lowest OLR.

The mesophilic UASB and thermophilic UASB were operated as described in Table 4.1.

Table. 4.1. Operating Conditions of the Single Stage Mesophilic and Thermophilic UASBS

HRT	OLR
48 hour HRT	OLR of 3.6-7 $kgCODm^{-3}d^{-1}$
48 hour HRT	OLR of 5 $kgCODm^{-3}d^{-1}$
36 hour HRT	OLR 6.6 $kgCODm^{-3}d^{-1}$
24 hour HRT	OLR 10 $kgCODm^{-3}d^{-1}$
21 hour HRT	OLR 11.4 $kgCODm^{-3}d^{-1}$
18 hour HRT	OLR 13.3 $kgCODm^{-3}d^{-1}$

The reactors were analysed regularly to determine COD removal, VFA levels, pH , bicarbonate alkalinity, biogas production and composition (CO_2, methane and hydrogen sulphide).

4.2.4. Pre-acidification Study Reactors And Operation

A pre-acidification stage was used to study the effect of pre-acidification on the operation of UASBs treating coffee ground free waste as reported in Chapter 7.

The two stage reactor apparatus consisted of a continuously stirred thermophilic pre-acidification reactor which then supplied acidified feed to a methanogenic UASB reactor. Pre-acidification reactors 1 and 2 (AC1 and AC2) were connected to a mesophilic and a thermophilic methanogenic UASB reactor respectively and operated simultaneously on the same feed (See Figure 4.4).

The two pre-acidification reactors each consisting of a 5 litre Quickfit vessel (Bibby, Stone, UK) (total volume 6.2 litres, liquid volume 5.6 litres) with a side arm (see Figure 4.1). A water jacket consisting of Neoprene tubing was coiled around the reactor. Water was passed through the tubing on a closed loop system with a reservoir by a thermostatically controlled Grant Flow heater (Grant Instruments Ltd., Cambridge, UK). The reactors were maintained at 55°C and were continuously stirred at 100 rpm with a Heidolph motor (Heidolph GmbH Kelheim, Germany).

Both reactors were fitted with an Ingold xerolyt gel filled electrode, type HA405-DXK-S/120, (Mettler Toledo, Leicester, UK) and a Kent EIL9142 pH meter/controller (ABB Kent Industries Ltd, Stonehouse, Gloucestershire, UK). The

reactor pH was maintained at pH 6 by the action of the pH controller activating a Watson and Marlow 300U peristaltic pump (Falmouth, England) which pumped in the required amount of 50 gl^{-1} NaOH.

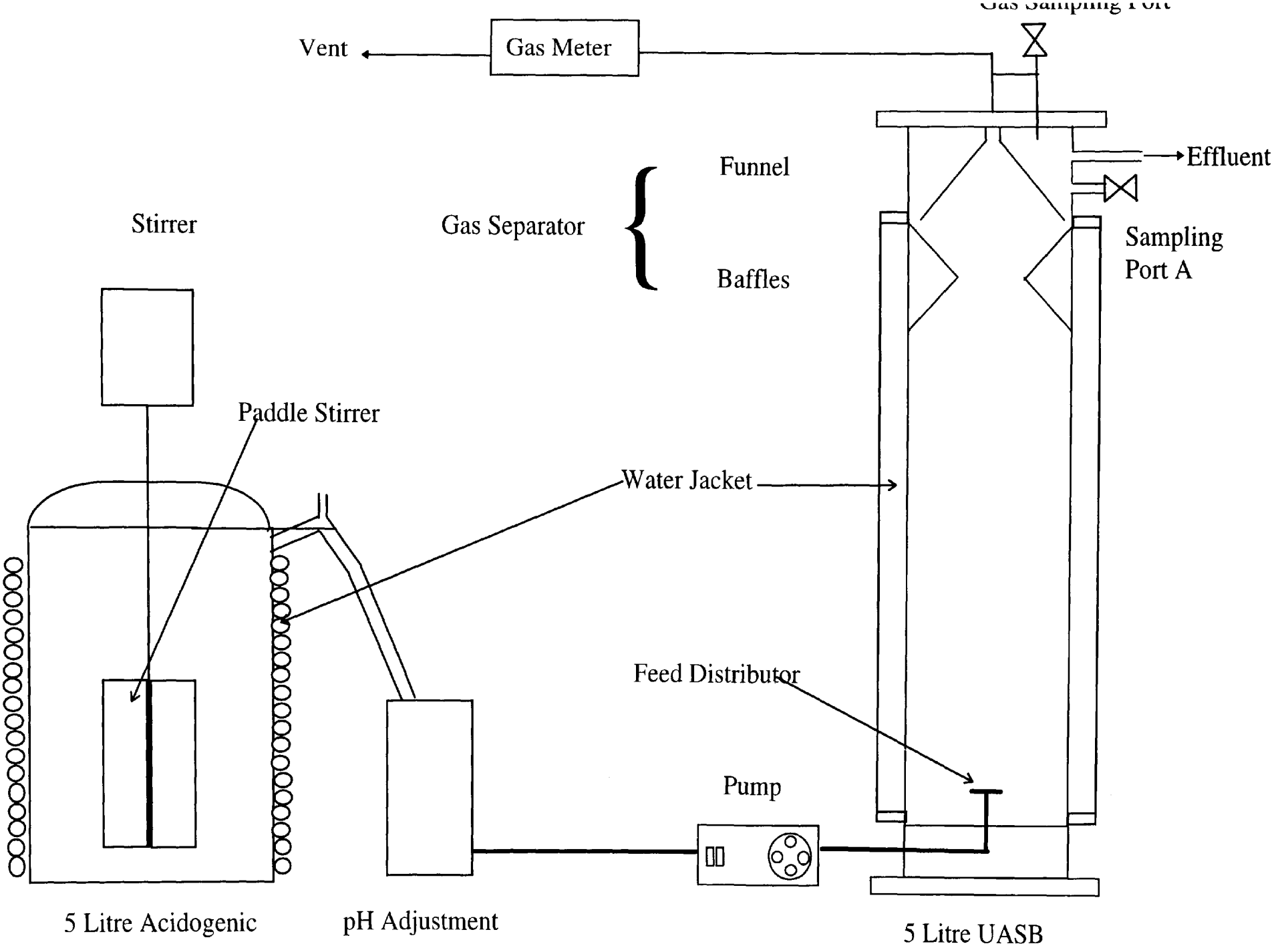

Figure.4.4 A Schematic Diagram of the Two Stage Reactor

Coffee waste was supplied as in Section 4.2.3, except for when the pH controllers were used no sodium bicarbonate was added to the feed. The unacidified feed was supplied to the acidification reactors by a Watson and Marlow 300U peristaltic pump (Falmouth, England) from two continuously mixed 20 litre containers. The feed was maintained at 5°C by placing the feed container in a domestic larder refrigerator. This reduced the amount of biomass growth in the peristaltic tubing and prevented any significant pre-acidification.

The acidified effluent exited by gravity to a 150 ml pH adjustment chamber, continually mixed with a magnetic stirrer and fitted with an Ingold xerolyt gel filled electrode, type HA405-DXK-S/120, (Mettler Toledo, Leicester, UK) and pH controller unit. The pH feed was then supplied to the UASB reactors. The acidified feed was adjusted to pH 6.7 by the action of the pH controller activating a Watson and Marlow 300U peristaltic pump (Falmouth, England) which pumped in the required amount of 50 gl^{-1} NaOH.

The acidified feed was then pumped by a Watson and Marlow 300U peristaltic pump (Falmouth, England) either to a mesophilic UASB or a thermophilic UASB as described in Section 4.2.3 and previously operated as described in Chapter 7. Prior to the start of the experimental work reported in Chapter 8 the reactors were maintained at either 35°C or 55°C but not fed for 10 days.

The two pre-acidification reactors and two UASBs were operated over a period of 125 days as described below.

The pre-acidification reactors were inoculated with 310 ml of homogenised UASB granules from a UASB digester treating paper mill effluent (Davidsons paper mill, Aberdeen). This gave an initial inoculum concentration of 2.7 g VS l^{-1}. Feeding was started (day 0) at a 24 hour HRT to the acidogenic stage and changes in OLR were

effected by changes in feed flow rate introduced over a 2 day period. In AC1 from day 0 to day 69 and in AC2 from day 0 to day 58, the pH controller activated a pump to add 50 gl^{-1} NaOH when the pH dropped below pH 6.0. For AC1 after day 69 and for AC2 from day 58 no pH adjustment was made but the instead the feed to the system contained 1.5gl^{-1} of sodium bicarbonate.

Table. 4.2. Operating Conditions of the Thermophilic Pre-acidification Reactors

HRT	OLR
24 hour with with pH control	10 $kgCODm^{-3}d^{-1}$
24 hour HRT without pH control	10 $kgCODm^{-3}d^{-1}$
18 hour HRT without pH control	13.3 $kgCODm^{-3}d^{-1}$
15 hour HRT * without pH control	16 $kgCODm^{-3}d^{-1}$
12 hour HRT without pH control	16 $kgCODm^{-3}d^{-1}$

* Thermophilic UASB not operated with the pre-acidification reactor under these conditions

Thermophilic pre-acidification was studied at the HRTs and OLRs as stated in Table. 4.2.

The reactors were monitored on a regular basis to determine pH, VFA level and composition and biogas composition (carbon dioxide and methane).

The mesophilic and thermophilic UASBs used in the single stage operation as described in Chapter 7 were used as the methanogenic stage in this pre-acidification study. The biogas monitoring and other analytical procedures were as described previously.

A portion of the effluent from the pre-acidification reactors AC1 and AC2 was passed to the UASB reactors such that the HRT of both the acidogenic and methanogenic stages was the same despite the differences in reactor volume. Feeding was started on day 0 at a 24 hour HRT to the methanogenic stage, giving a 48 hour HRT in the whole system. Since there was minimal COD removal in the acidogenic stage, the OLR to both stages at this HRT was 10 $kgCODm^{-3}d^{-1}$.

From day 0 to day 69 for the mesophilic UASB and from day 0 to day 58 for the thermophilic UASB, the pH of the acidified feed was adjusted to pH 6.7 in the intermediate pH adjustment chamber by the addition of 50 gl^{-1} NaOH. After these time periods, no pH adjustment was made. The two-phase thermophilic and mesophilic UASBs were operated at the HRTs and OLRs described in Table 4.3. The reactors were analysed regularly to determine COD removal, VFA levels, pH, bicarbonate alkalinity, biogas production and composition (CO_2 and methane).

Table. 4.3. Operating Conditions of the Two-phase Mesophilic and Thermophilic UASBS

HRT	OLR
24 hour HRT	OLR 10 $kgCODm^{-3}d^{-1}$
21 hour HRT	OLR 11.3 $kgCODm^{-3}d^{-1}$
18 hour HRT	OLR 13.3 $kgCODm^{-3}d^{-1}$
15 hours HRT*	OLR 16 $kgCODm^{-3}d^{-1}$
12 hours HRT	OLR 16 $kgCODm^{-3}d^{-1}$Yes

* Thermophilic UASB not operated under these conditions.

5. IDENTIFICATION AND ANALYSIS OF THE COFFEE FACTORY WASTE STREAMS

The factory's waste streams were surveyed to identify and characterise waste streams to be used in anaerobic digestion studies. The factory's waste treatment systems and their resulting waste streams are shown in Figure 5.1. Spent coffee grounds are removed from the extraction process to be initially dewatered by the extractor screws. The liquid extracted forms stream A and the solids are removed to the hydraulic screw systems. This system presses out liquid from the grounds to produce stream B. The solids are ground up and then incinerated. Waste streams A and B are pooled together to form waste stream C. This stream is currently discharged to sewer to be treated by the local water company after settling in a underground weir system. Due to the positioning of this tank it was not possible to collect samples from this stream for safety reasons.

During the study a new physical treatment system, a hydraulic filter press (plate and frame press) was installed for assessment purposes. This filters waste stream C, the liquid effluent forms stream D and is largely discharged to sewer and the solids are landfilled. Some of waste stream C and D has been used to supply the EPSRC anaerobic pilot plant facility after settling.

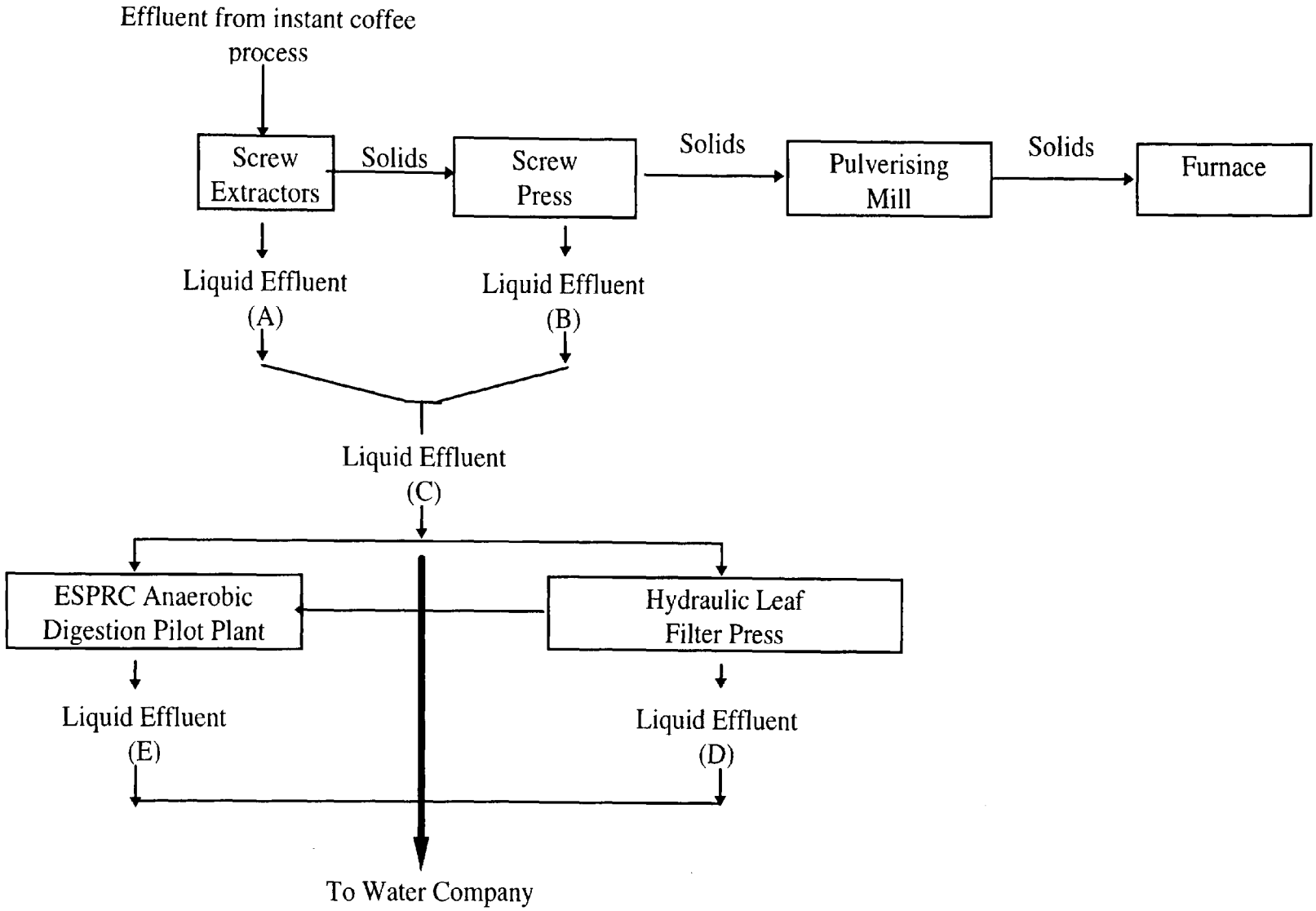

Figure. 5.1 The Instant Coffee Waste Treatment Process

Samples of waste stream A, B, and C were taken on two separate occasions and analysed. The waste stream physical characteristics are shown in Table 5.1, chemical characteristics in Table 5.2 and the proximate analysis is shown in Table 5.3. Some analysis was performed on the hydraulic filter press effluent. However due to operational problems with this system it was not possible to take further samples in the time period.

Table 5.1 Physical Characteristics of Waste Streams A,B,C, and D.

Analysis	Stream A (Dewatering Screws)	Stream B (Hydraulic Screw Press)	Stream C (Combined Stream)	Stream D (Hydraulic Leaf Press)
Total Solids gl^{-1}	16.2-16.8 SD.=0.3/0.7	31.6-59.1 SD.=0.4/0.9	23.9-27.5 SD.=0.6/0.8	10.6-16.8 SD.=0.2/0.1
Volatile Solids gl^{-1}	15.8-16.4 SD.=0.3/0.7	31.2-57.3 SD.=0.4/0.9	23.1-27.1 SD.=0.6/0.8	10.3-16.3 SD.=0.2/0.1
Suspended Solids gl^{-1}	9.3-9.8 SD.=0.3/0.2	19.9-52.4 SD.=0.6/0.7	14.1-18.3 SD.=0.5/0.7	3.1-5.7 SD.=0.1/0.2
Settleable Solids gl^{-1}	8.6-8.4 SD.=0.04/0.04	17.6-51.3 SD.=0.09/0.09	12.5-16.4 SD.=0.04/0.12	NA
Non-settleable suspended solids gl^{-1}	0.7-1.4 SD.=0.04/0.04	2.3-1.1 SD.=0.09/0.09	1.6-1.7 SD.=0.04/0.12	NA
Temperature °C	77	64	68-67	NA

NA = Not available
SD.= Standard Deviation of the two sets of analysis.eg. analysis set a/ analysis set b

The physical characteristics of the four waste streams are shown in Table 5.1. It can be seen that all four waste streams have a high level of volatile solids and total solids.

A high level of volatile solids (>10 gl^{-1}) would indicate that anaerobic digestion should be a cost effective treatment for the waste (Mergaert *et al.*, 1992). The percentage volatile solids in all cases was between 97 and 99 % of total solids. This would indicate that a large organic fraction would be available for biodegradation.

High percentage volatile solids is typical of instant coffee waste being reported by a number of authors. The values reported were 99.7% (Kostenberg and Marchaim, 1993a), 99% (Raetz, 1990), 98.5% (Kida *et al.*, 1992). If biological treatment was not suitable, disposal by incineration would be an alternative as there is relatively little ash to dispose of.

The suspended solids of waste streams A, B, and C is high, being between 9 and 52 gl^{-1}, forming at least 60% of total solids. The level of suspended solids is important as it affects the rate of biodegradation and the type of digester which can be used to treat the waste. At this level of suspended solids a CSTR reactor would be commonly be used with correspondingly modest loading rates (Wheatley *et al.*, 1997, Stronach *et al.*, 1986). Modern designs of anaerobic reactors such as UASBs require a low level of suspended solids, less than 10% of the waste strength (Mergaert *et al.*, 1992).

The settleable solids, however show that large amounts of the suspended solids, 90% can be removed by settling. This may produce waste which will allow the use of high rate digesters to treat this waste such as filters and UASBs. The settled grounds could then be incinerated or treated in a high solids biological system such as aerobic composting or the anaerobic Dranco process.

The temperature of the waste when it is discharged is high at 61-77°C. This high temperature would offer the cost effective use of a thermophilic digestion system as the waste would not require an energy input to heat the waste to thermophilic

temperatures. Thermophilic systems may offer the prospect of higher loading rates then the equivalent mesophilic systems (Lanting *et al.*, 1989 and Varel *et al.*, 1980), leading to smaller reactors and reduced capital cost. Therefore the study of thermophilic anaerobic digestion would be a logical option.

Table 5.2 shows the chemical characteristics of the waste streams. The analysis was performed in at least triplicate.

Table 5.2 Chemical Characteristics Of The Waste Streams A, B, C And D

Analysis	Stream A (Dewatering Screws)	Stream B (Hydraulic Screw Press)	Stream C (Combined Stream)	Stream D (Hydraulic Leaf Press)
Total COD (mgO_2l^{-1})	19,800-19,700 SD=400/700	45,100-92,000 SD=965/7054	35,900-40,000 SD=1,400/1,400	22,000-28,000 SD=/3445
Soluble COD (mgO_2l^{-1})	NA	NA	9,600-6,400 SD=400/200	11,900/13,000 SD=800/300
Nonsettleable COD (mgO_2l^{-1})	11,600-11,500 SD=700/200	12,200-11,100 SD=400/200	13,000-11,500 SD=900/300	15,800/ NA SD=600/ NA
pH	4.2	4.3	4.6-4.3	3.9
Bicarbonate Alkalinity ($mgCaCO_3l^{-1}$)	0	0	0	0

NA = Not available

SD= Standard Deviation of the two sets of analysis. eg. analysis set a/ analysis set b

All four waste streams have a high total COD ranging from 20,000 to 90,000mgO_2l^{-1}. The higher the COD level, the more economically viable the anaerobic digestion

system is (Mergaert *et al.*, 1992). However it must be readily biodegradable or a long retention time would be required and a large digester would be the result. Soluble COD tends to be more biodegradable than particulate COD, therefore the characteristics of the waste must be taken in to account in order to select the most efficient waste treatment option. The high strength of the waste however means there is a large scope for cost savings in effluent disposal.

In streams A, B and C the total COD can be greatly reduced by settling. Using settling to remove the particulate fraction may offer a cost effective way of reducing the polluting power of the waste. Incineration could then be used to dispose of the settled material.

The waste streams have an acid pH (pH 3.9 to pH 4.6) and no bicarbonate alkalinity. For stable anaerobic digestion the bicarbonate alkalinity (buffering capacity) of the digester must be maintained at, at least $1000mgCaCO_3l^{-1}$ (Graef and Andrews, 1974). Therefore without the addition of buffering capacity, anaerobic digestion of this waste could be difficult to control. For this waste the provision of alkalinity could be a major cost, however the utilisation of appropriate control strategies (Hawkes and Hawkes, 1994) or using two-stage operation could result in significant cost saving in alkalinity supply (Wheatley *et al.*, 1997).

Table 5.3 shows the proximate composition of the waste streams. The largest identified component is lipid comprising 21% to 37% of total solids. A lipid level of 18% to 31% of total solids was found by Raetz (1990). Although lipid is biodegradable, a high proportion of lipid can lead to a number of problems. The high level of lipid can lead to problems in sampling, handling and digester operation. The lipid tends to coat everything it comes in to contact with and is not evenly dispersed in aqueous environments due to its hydrophobic nature. Lipid accumulation on surfaces has led to the blocking of effluent pipes and anaerobic filter packing

material (Hawkes *et al.*, 1995). Prevention or hindrance of the granulation process in UASBs has also been reported with wastes containing lipid (Hawkes *et al.*, 1995, Lettinga and Hulshoff Pol, 1991).

Table 5.3 Proximate Composition of Waste Streams A, B, and C.

Analysis	Stream A (Dewatering Screws)	Stream B (Hydraulic Screw Press)	Stream C (Combined Stream)
Ash gl^{-1}	0.4-0.4	0.4-1.8	0.8-0.4
(%)	(2.5-2.4)	(1.3-3.0)	(3.3-1.4)
	SD=0.02/0.01	SD=0.02/0.02	SD=0.04/0.03
Holocellulose	2.5-2.2	9.6-22.1	3.5-5.8
gl^{-1}	(15.4-13.1)	(30.3-37.4)	(14.6-19.1)
(%)	SD=0.2/0.2	SD=0.5/1.46	SD=0.2/0.4
α cellulose gl^{-1}	1.3-1.9	2.6-8.9	2.7-3.8
(%)	(8.0-11.3)	(8.2-15.0)	(11.1-13.8)
	SD=0.1/0.2	SD=0.1/0.3	SD=0.2/0.3
Lipid gl^{-1}	3.4-5.6	11.6-19.1	7.1-7.3
(%)	(21.0-33.3)	(36.7-32.3)	(29.7-26.5)
	SD=0.3/0.8	SD=0.8/0.7	SD=1.2/1.1
Protein gl^{-1}	0.3-0.3	0.6-0.2	0.4-0.3
(%)	(1.8-1.8)	(1.9-0.3)	(1.7-1.1)
	SD=0.01/0.01	SD=0.01/0.01	SD=0.01/0.01
Unkown	9.6-8.3	9.4-15.9	12.1-12.3
Fraction gl^{-1}	(59.3-49.4)	(29.8-27.0)	(50.6-55.2)
(%)			

SD.= Standard Deviation of the two sets of analysis.eg. analysis set a/ analysis set b

Lipid toxicity to anaerobic digestion has also been reported by several authors including Angelidaki *et al*, (1990) and Hanaki *et al.*, (1981). The problem of toxicity was not due to the lipid itself but the immediate breakdown products of the lipid,

long chain fatty acids (LCFA) (Koster and Cramer, 1987 and Hanaki *et al.*, (1981). The levels of lipid which initiated inhibition were lower than the levels found in the coffee waste. Ahring, *et al.*, (1991) found neutral lipid levels of 2.5 gl^{-1} or free LCFA levels of 0.1-0.5 gl^{-1} to be inhibitory to methanogenic bacteria, while Rinzema *et al.* (1989) found inhibition occurred at levels of 100 mg l^{-1} free LCFA in UASB reactors.

The level of cellulose was 13-37% of total solids, the value found by Raetz (1990) was 35% to 48%. Raetz also identified 0.65% ash and 5.7% protein. This protein level is higher than the amount found in this study which was at most 1.9%. The level of protein is important on two counts: supply of nutrients (nitrogen) for bacterial cell growth and supplying alkalinity in the form of ammonium carbonate (Rozzi, 1983). To supply sufficient nitrogen for growth, available nitrogen must be found at a ratio of at least 400 mg COD to 7 mg of N (Stronach *et al.*, 1986).

There remains a large percentage (50%) of the total solids unaccounted for. This is not surprising as the coffee bean is chemically complex and is further complicated by degradation products formed in the roasting process, (Clarke and Macrae 1985). A Tannin and Lignin (Folin-Ciocalteau reaction) analysis of soluble fraction showed it to consist of 80% Tannin and Lignin but this test reacts with a wide variety of reducing compounds, (Box, 1983).

This unidentified fraction does not cause any serious problems as the effectiveness of the process would be determined by the COD and volatile solids removal. The procedure to determine the unknown fraction would be time consuming requiring the

application and development of techniques of molecular size determination, derivatisation studies and GC mass spectrometry.

One of the aims of the waste stream survey was to select wastes for study in anaerobic digestion studies. It was decided to use real waste, although synthetic wastes are often used, as they allow more reproducible and convenient feed stock to be used. However it was felt in this case that a synthetic waste could not replicate the complex nature of this waste. Also with the history of problems with this type of waste any work with real waste would be a more significant addition to knowledge. So despite the significantly greater difficulties in using real waste in particular with handling problems, it was thought using real waste would produce the more useful results.

The waste stream chosen for further work in the anaerobic digestion studies was the combined stream (waste stream C). This is mainly because it is the waste stream currently discharged into the sewer. The effluent from the filter press was not used although it would offer the prospect of lower suspended solids than the other waste streams. It was not selected as Nestlé were operating the machinery on a trial basis and a secure supply of waste could not be guaranteed for the length of the project.
Waste stream C was used to produce two waste types: waste containing significant levels of coffee grounds and a waste stream with lower levels of grounds with the grounds removed by settling. The removal of 90% of the suspended solids in this way would allow digesters such as UASBs to be used with this waste. The settled solids could then be disposed of by incineration which may be more economic than anaerobic digestion.

5.1. Conclusions From The Survey Of Waste Streams

From the survey of the waste streams several conclusions were reached.

1. Two distinct liquid wastes based on waste stream C would be used in the anaerobic digestion studies, waste stream C containing significant levels of suspended solids as coffee grounds and waste stream C with lower levels of suspended solids as coffee grounds, with the coffee grounds removed by settling.

2. The waste is discharged at greater than 55°C, so thermophilic operation would be possible without energy to heat the waste.

3. The waste is acidic (lower than pH 5) and has no bicarbonate alkalinity. Monitoring and addition of alkalinity will be required.

4. The waste has significant levels of lipid so problems may be experienced with handling and toxicity to the anaerobic process.

5. The waste seems to have low levels of nitrogen which may lead to problems with nutrient deficiency and low generation of alkalinity.

6. STUDIES ON WASTE CONTAINING COFFEE GROUNDS

6.1. Batch Biodegradability Studies

The mesophilic and thermophilic batch studies were monitored for gas production. The cumulative gas production of the mesophilic studies is shown in Figure. 6.1. The mesophilic experiment consisted of one batch digester acting as a control with inoculum and nutrients but no coffee waste and two batch digesters (Digester A and Digester B) with inoculum, sodium bicarbonate, nutrients and coffee waste. In the thermophilic study the control batch digester contained inoculum, sodium bicarbonate and nutrients with 7.3 gl^{-1} cellulose (Avicel) and 5.4 gl^{-1} D-glucose to determine if an active thermophilic culture was achieved. These values of cellulose and glucose were chosen to provide suspended solids and soluble COD similar to the coffee waste. The other two thermophilic batch digesters contained inoculum, sodium bicarbonate, nutrients and coffee waste.

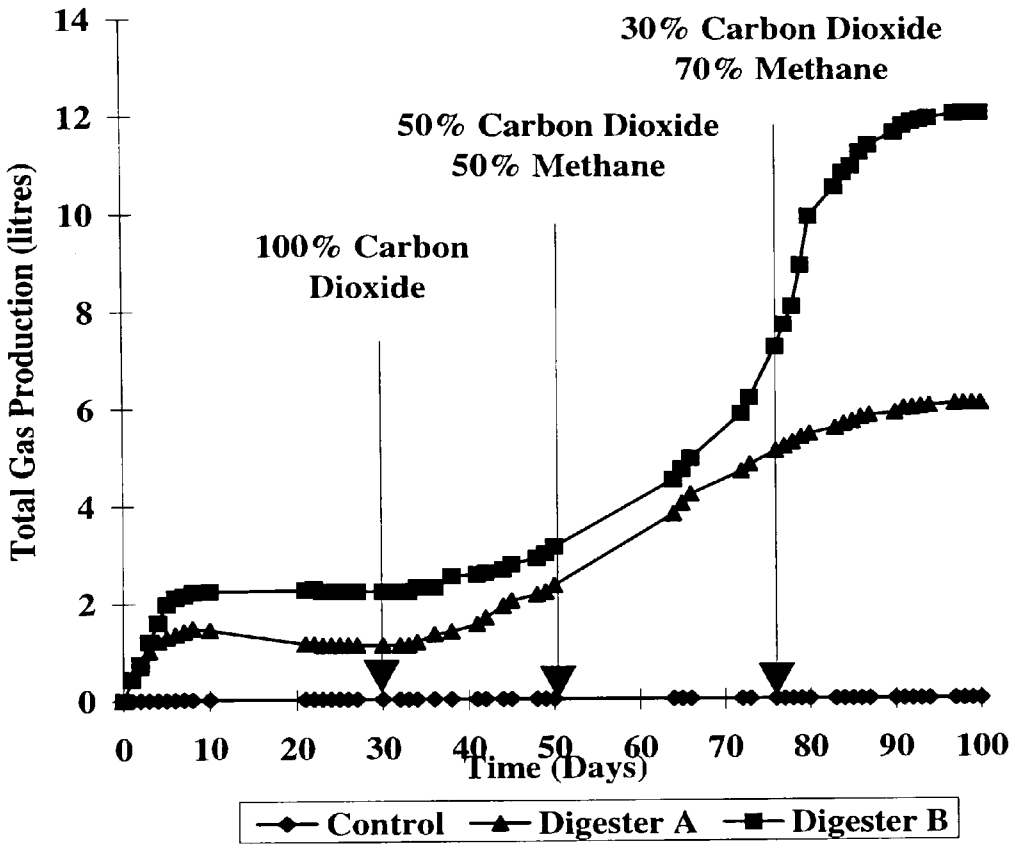

Figure 6.1. Cumulative Gas Production and Biogas Composition of Mesophilic Batch Studies

The electronic data collection system suffered from a variety of problems in collecting data which led to 90% of the data for gas production not being collected for the thermophilic system. In the mesophilic studies the biogas production started immediately and was found to be 100% CO_2 in composition at day 30. After 5 days biogas production halted but had started again by day 66 and then contained up to 50% methane. Biogas production was minimal from day 90 until day 115. Volatile fatty acid levels of mesophilic batch digester B are shown in Figure 6.2.

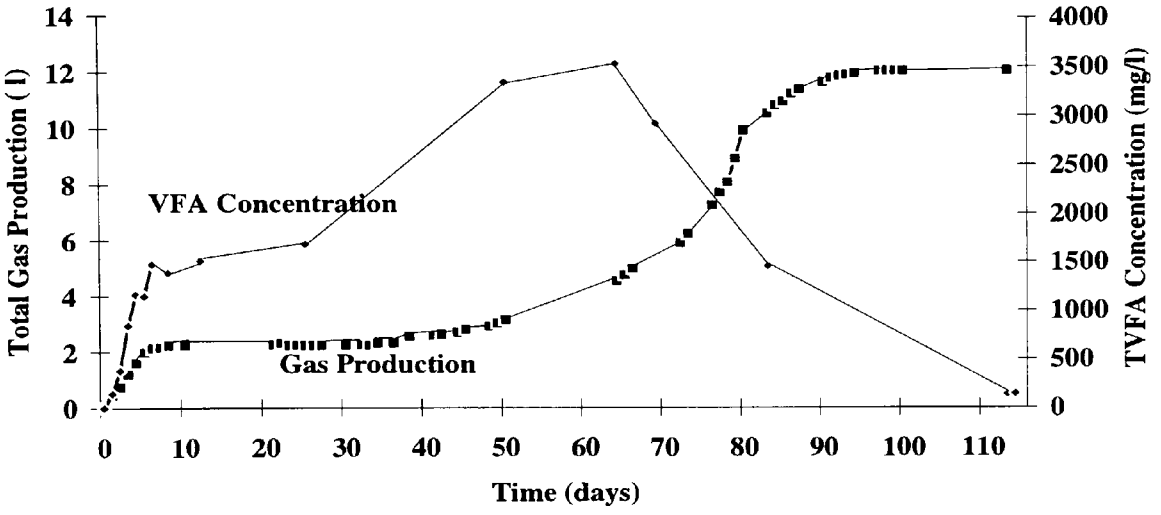

Figure 6.2. TVFA Concentration and Cumulative Gas Production of Batch Digester B

The total volatile fatty acid (TVFA) levels rose to rapidly to 1500 mgl^{-1} by day 10. A maximum level of 3500 mgl^{-1} was reached by day 70. The TVFA levels then declined to 150 mgl^{-1} by the end of the digestion. The pH did not fall below 6.9 throughout the digestion. In the duplicate of this reactor the TVFA rose to a maximum of 3200 mgl^{-1} on day 70 and fell to 260 mgl^{-1} at the end of the experiment. Levels of TVFA in the thermophilic digesters declined to 220 mgl^{-1} and 270 mgl^{-1} from levels of 2750 mgl^{-1} and 2610 mgl^{-1} respectively.

When the gas production was not detectable and TVFA levels had fallen to a low level it was decided that all the biodegradable substrate had been used. The batch studies were left to digest for 115 days and 85 days for the mesophilic studies and the thermophilic studies respectively.

The presence of methane and the increase and then reduction in TVFA levels indicated that a full anaerobic digestion process had been completed. The

thermophilic control reactor with glucose and cellulose gave a 95% VS destruction, showing a thermophilic culture had developed.

Raetz (1990) could not obtain significant methanogenesis due to pH problems. The problem Raetz experienced of low pH was also encountered in early studies. This was overcome by the addition of $9gl^{-1}$ sodium bicarbonate to provide sufficient buffering capacity.

At the end of the digestion period the degree of VS destruction and compositional changes in the contents of each reactor were determined. A 58% reduction in volatile solids was achieved in both the mesophilic and thermophilic batch studies. As the batch studies were left until no significant gas production was seen, this 58% reduction in volatile solids is an indication of the maximum volatile solids destruction. The initial coffee waste water had a lignin content of 9-11%, other plant materials with a similar lignin content such as wheat straw have a similar degree of degradation, (Jerger and Tsao, 1987). The amount of lignin remained virtually unchanged in both mesophilic and thermophilic batch digestions.

Raetz (1990), achieved a 60% reduction, however he did not achieve successful methanogenesis and calculated the reduction from the breakdown products, whereas in this study up to 70% methane gas concentration was achieved. In continuous studies Lane (1983) achieved a 86% VS destruction and Kostenberg (1993b) between 48-72% destruction.

The compositional results are shown in Figure 6.3.

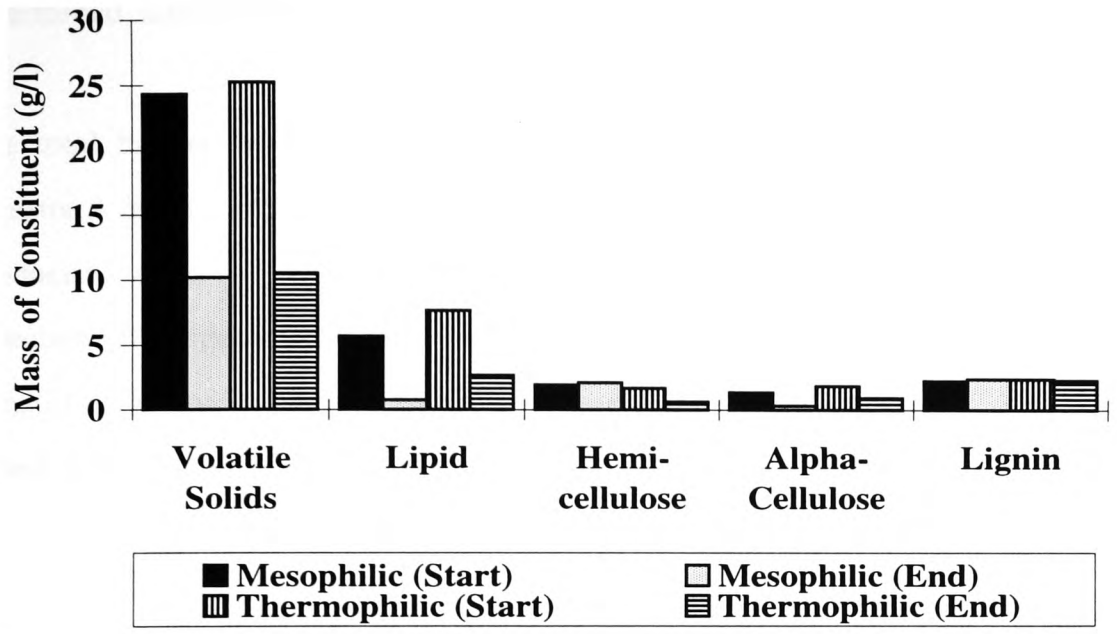

Figure 6.3. Composition Of Coffee Waste Before And After Thermophilic And Mesophilic Batch Digestion.

The largest identified component, comprising 26-33% of the volatile solids, was lipid. High levels of lipid (18% to 31% of total solids) were also reported by Raetz (1990). The degradation of the lipid varied from 87% in the mesophilic study to 65% in the thermophilic study. The proximate analysis divides the cellulosic fraction (holocellulose) into two components, α-cellulose and hemicellulose. The reduction in hemicellulose was 75% in the mesophilic batch studies and 64% in the thermophilic batch studies. There was a 51% reduction of α-cellulose in the mesophilic and thermophilic batch studies.

There remains a large (54%) unidentified component of volatile solids in the coffee waste. The high temperature processing of carbohydrate material results in the production of Maillard products. These are insoluble and soluble lignin-like compounds (Theander, 1980). A tannin and lignin (Folin-Ciocalteau) test showed a filtered sample of the coffee waste consisted of 80% tannin and lignin by weight. The

nature of the proximate analyses used means that no soluble components are determined, although they are included in TS and VS determinations.

The total biogas production in the mesophilic batch studies was 6.130 litres for digester A and 12.115 litres for digester B. The measurement of low quantities of gas production over long periods is difficult and prone to errors. A biogas yield was calculated for digester B of 0.25 m^3 kg^{-1} VS added and 0.500 m^3 kg^{-1} VS destroyed. Lane (1983) achieved a 86% VS destruction and a biogas yield of 0.25 m^3 kg^{-1} TS added and 0.704 m^3 kg^{-1} TS destroyed.

6.2. Continuous Digester Studies I

Figure 6.4. shows the gas production and TVFA levels of one of the mesophilic digesters. The mesophilic digesters were fed every day until day 35. Biogas production was 0.20 l $l^{-1}$$day^{-1}$ consisting of 65% CH_4 and 35% CO_2. The level of TVFA was 500mgl^{-1}.

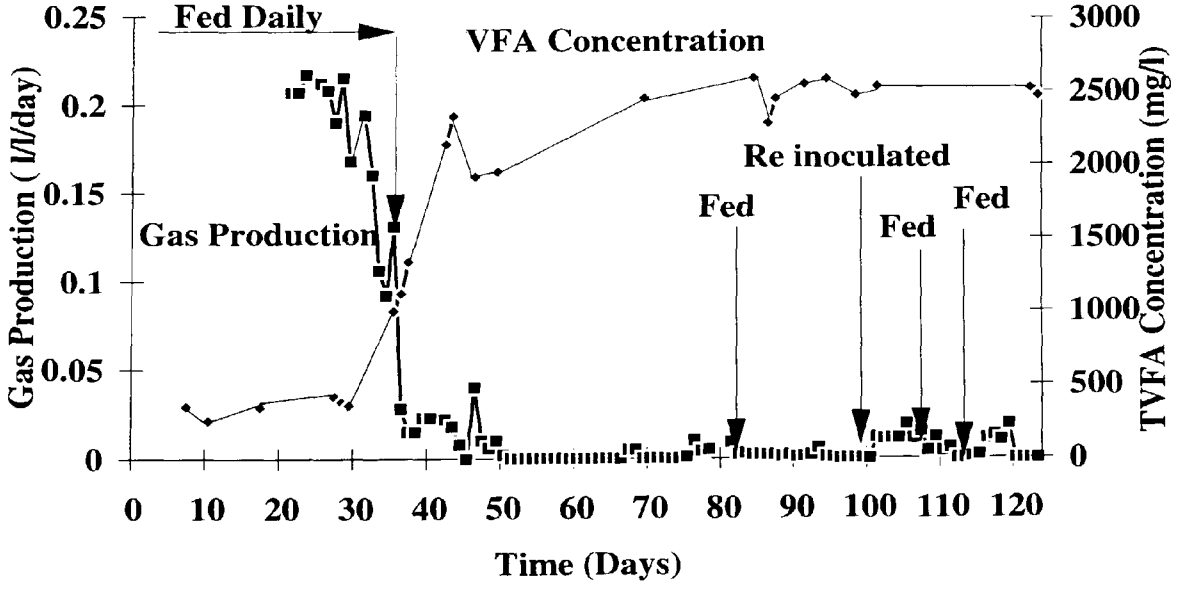

Figure 6.4. Mesophilic Continuous Digester Gas Production and TVFA Levels

At day 35 it was noticed gas production had fallen off in both digesters. A rise in TVFA occurred in the following days, from 500mgl^{-1} (day 30) to 1500mgl^{-1} on day 38 and then at least 2000 mgl^{-1} on day 45. The pH did no fall below 6.8 in this period, which is in the optimum range for methanogens and VFA levels were sufficiently high for gas production to occur. No further additions were made in the hope that gas production would recover. However gas production did not recover and TVFA levels remained high.

At day 100 an additional amount of the homogenised UASB granules was added. Sufficient inoculum was added to give an extra 5gl^{-1} VS of biomass in the digester. No gas production or reduction in TVFA levels was seen. Subsequent feeding at day 81, 105, 115 also saw no significant gas production or reduction in TVFA levels. Similar findings were reported by Lane (1983) who experienced a decline in gas production towards the end of a 80 day digestion. A longer digestion period was achieved probably due to solids recycling used by Lane (1983). This was not used in this study. Digester failure could be due to nutrient deficiency or build up of toxic compounds from the digestion of coffee grounds. The initial analysis of the waste shows low levels of nitrogen. Nutrient addition was used by Lane (1983) but without effect and Kostenberg and Marchaim (1993) saw no advantage in adding nitrogen during their studies.

A similar pattern in the thermophilic digestions can be seen. Figure 6.5 shows the gas production and TVFA levels of thermophilic digester 1 which suffered a 10°C fall in temperature at day 47. Gas production was maintained at 0.23 l l^{-1} day^{-1} with a composition of 64%CH_4 and 36%CO_2. TVFA levels fell to a level of 1000mgl^{-1}.

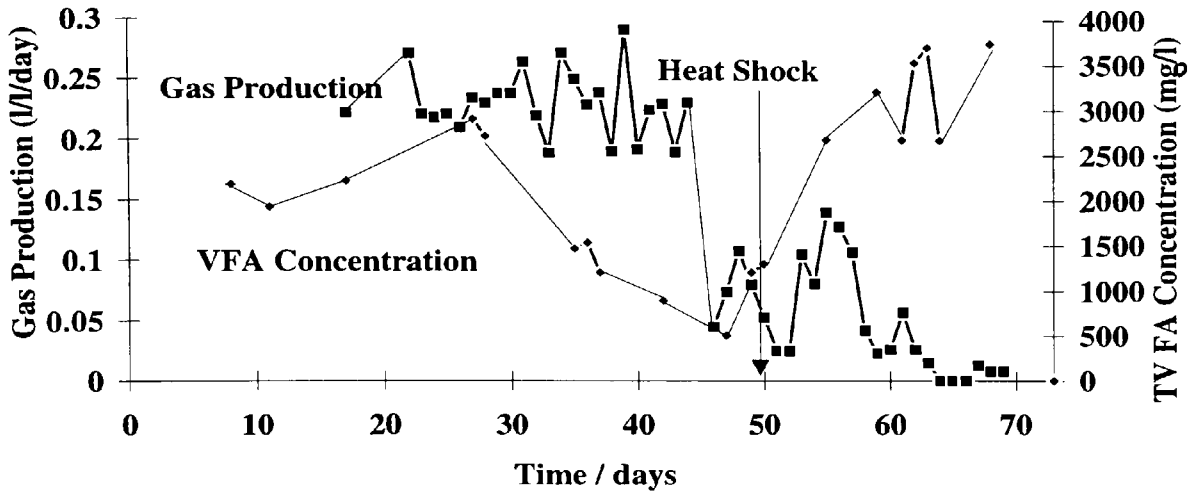

Figure 6.5. Daily Gas Production and Total Volatile Fatty Acid Levels of Thermophilic Digester 1

At day 47 both digesters suffered a heater malfunction, thermophilic digester 1 suffered a 10°C fall in temperature for 24 hours and digester 2 suffered an increase in temeperature to 90 °C for 24 hours. Thermophilic digester 1 and 2 did not recover. Thermophilic digester 1 gas production fell and did not recover to previous levels despite being fed on day 53-57. Total VFA levels by this time had risen to 2500-3600 mgl^{-1}. Reinoculation with sufficient inoculum to give 5 gl^{-1} as VS in the reactor did not see a recovery in gas production or fall in TVFA levels. After day 120 the study was terminated. The effect of the drop in temperature of 10°C to 45 °C for 24 hours was to result in ceasation of methanogenic activity.

Thermophilic digestion of coffee grounds was maintained for 60 days by Kostenberg and Marchaim (1993) although gas production may have been falling and TVFA levels rising towards the end of the digestion. Nitrogen addition was found not to be necessary in this study. The only significant difference between the study by Kostenberg and Marchaim (1993) and the study presented here was the use of $Ca(OH)_2$ to neutralise the feed stock. The addition of Ca^{2+} ions has been thought to aid stability of anaerobic digesters treating difficult wastes with high levels of lipid

(Angelidaki *et al.* 1990) by precipitating the LCFA for example. As the EPSRC pilot plant was also operating at the factory, bacteria from these reactors may have become acclimatised to the coffee waste. Use of these bacteria from the pilot-plant as inoculum may aid the start-up of the laboratory reactors.

6.3. Continuous Digester Studies II

To investigate the importance of nutrient and Ca^{2+} addition, a second study was initiated using the same equipment. The differences between the initial study and the second study were that the feed stock was neutralised with $Ca(OH)_2$ not sodium bicarbonate, was nutrient supplemented, and granules from the EPSRC pilot plant treating settled coffee waste were used as inoculum. The pilot plant reactor operated with TVFA levels of 700-1500 mgl^{-1}. This level of TVFA is higher than the usual levels of 500 mgl^{-1} TVFA found in anaerobic reactors (Sahm, 1984) indicating that the reactors are operating sub-optimally. However it was judged that any previous exposure to coffee waste would be advantageous in the start up of the laboratory reactors.

In the second continuous study, one mesophilic digester ran for 99 days and the other for 80 days, one digester operating for almost 4 HRTs and the other for more than 3 HRTs. They were shut down only because of a public holiday.

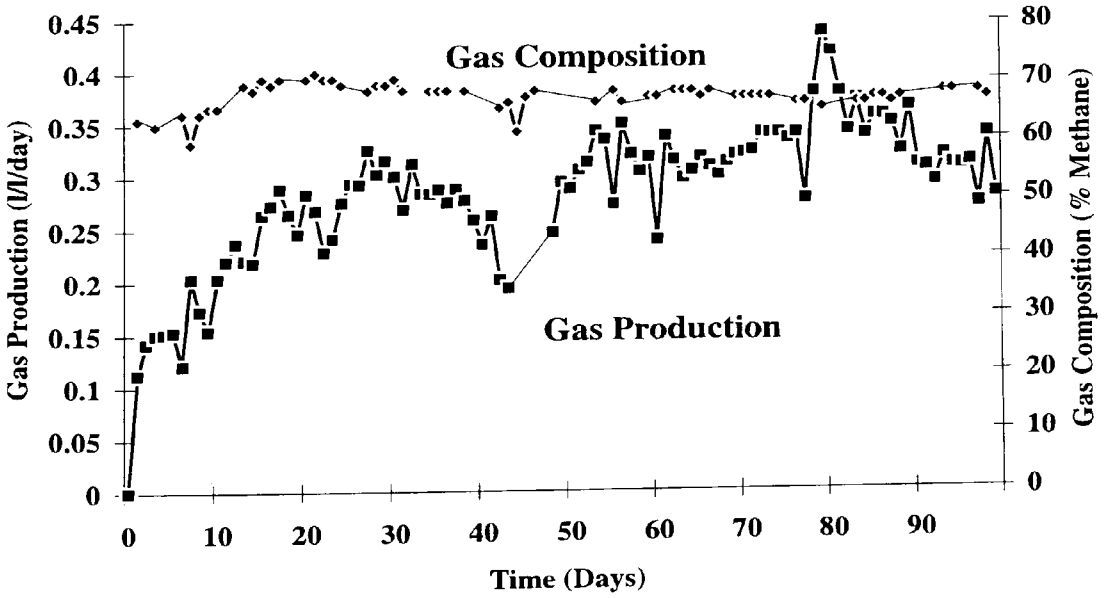

Figure 6.6. Gas Production and Gas Composition of the Mesophilic CSTR operated for 99 days.

In Figure 6.6 the daily gas production and gas composition (percentage methane) are shown for the mesophilic digester operated continuously for 99 days. Gas production rose to 0.30 l l^{-1} day^{-1} by day 20 and over the steady state period (day 75 to day 99) averaged 0.34 l l^{-1} day^{-1}. Gas composition varied from 65%-70% methane.

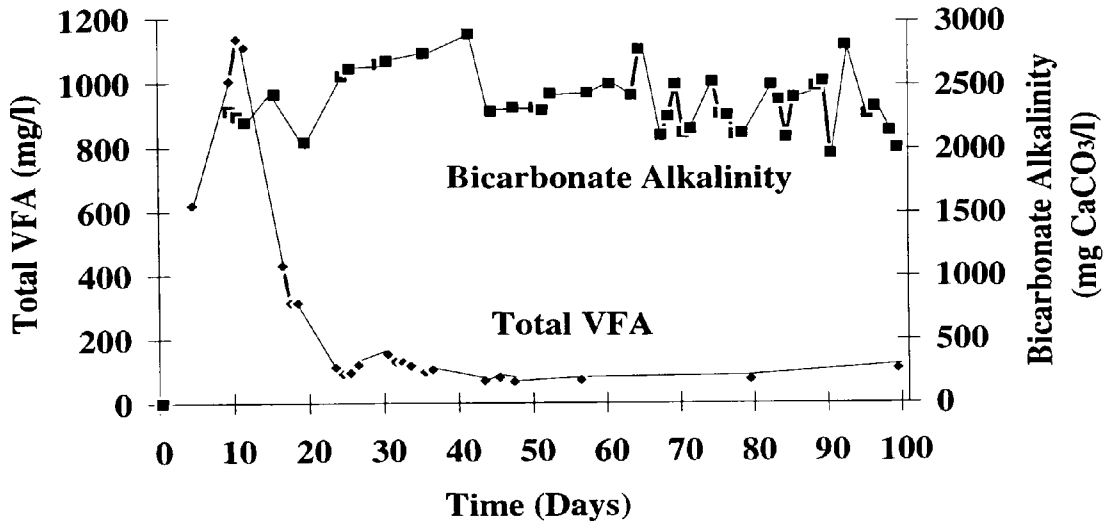

Figure 6.7. Bicarbonate Alkalinity and TVFA in Mesophilic CSTR operated for 99 days.

The TVFA and bicarbonate alkalinity of the 99 day mesophilic study are shown in Figure 6.7. The TVFA initially rose to 1140 mgl^{-1} by day 9, then fell to 110 mgl^{-1} on day 22. TVFA from day 22 to day 99 averaged 100 mgl^{-1} (18 samples SD = 24). The bicarbonate alkalinity was maintained at 2200-2400 $mgCaCO_3l^{-1}$. The low TVFA and high alkalinity level indicate that the various bacterial groups are in balance.

The TVFA levels are lower in this study than in the initial experiment, 100 mgl^{-1} compared to 500 mgl^{-1}. These results indicate that after almost 4 retention times this digester was working well, achieving good gas production and high methane content and a significant degree of COD and volatile solids removal.

Its duplicate ran for 80 days which is more than 3 HRT and was achieving similar COD and VS reductions, gas composition and bicarbonate alkalinity. This length of digestion (> than 3 HRT), could only be achieved if biomass growth was matching biomass removal. Therefore it can be concluded that stable long term mesophilic digestion was achieved. The mean VS destruction for the mesophilic digesters was 60% (SD = 2.5) and 62% (SD = 2.5) respectively. This compares with 70% reduction achieved by Lane (1983) and 47-72% reduction achieved by Kostenberg and Marchaim (1993, 1994). The 99 day digestion achieved a 63% COD reduction (SD = 4.3) and the 80 day digestion a 59% reduction (SD = 2.8).

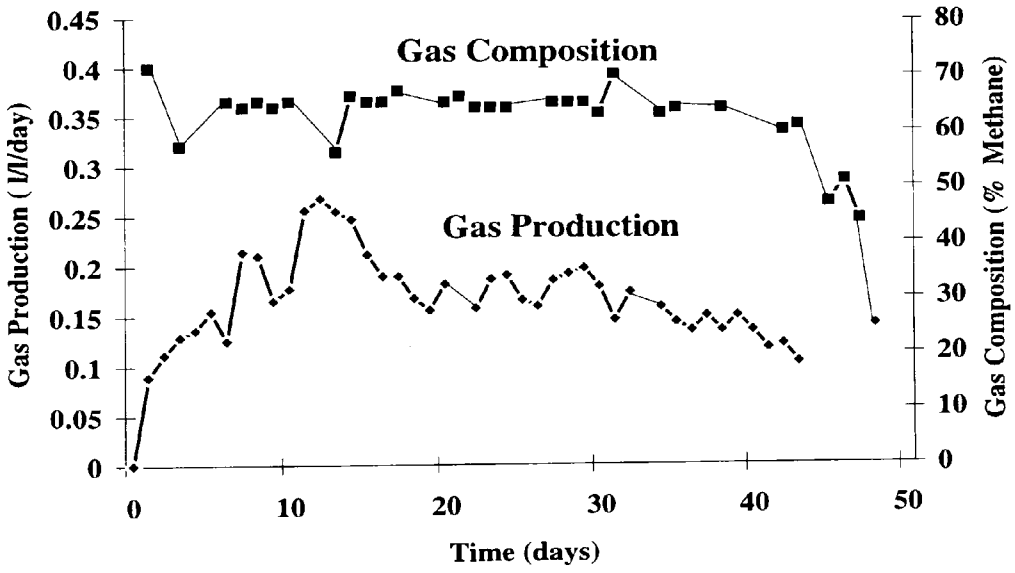

Figure 6.8. Gas Production And Biogas Composition For The Thermophilic Reactor.

The daily gas production and gas composition for one of the thermophilic digesters are shown in Figure 6.8. Gas production rose to 0.25 l $l^{-1}day^{-1}$ with a composition 60%-65% methane and 30%-35% carbon dioxide. TVFA and alkalinity for this digester are shown in Figure 6.9.

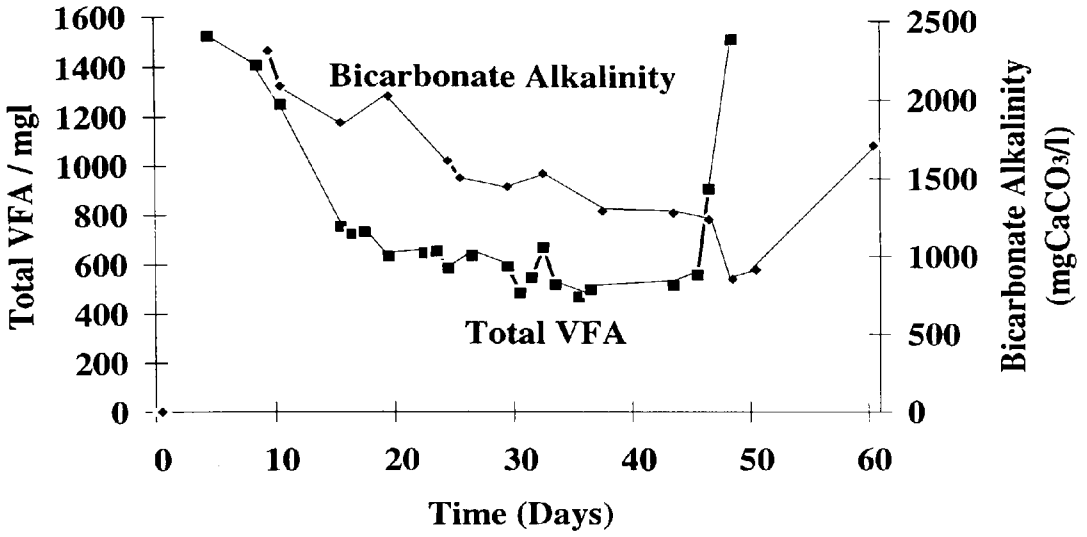

Figure 6.9. Total VFA and Bicarbonate Alkalinity In The Thermophilic Reactor.

The TVFA initially rose to 1500 mgl^{-1}, fell to 600 mgl^{-1} then rose again suddenly on day 48 to 1500 mgl^{-1}. The bicarbonate alkalinity gradually fell from a value of 2400 $mgCaCO_3l^{-1}$ to 1230 $mgCaCO_3l^{-1}$ on day 46. A sudden drop in alkalinity accompanied the rise in TVFA on day 48, and a drop in pH from 6.9 to 6.7 was also seen, indicating that the VFA producers were not in balance with the methanogenic bacteria. It was also noticed that large fatty deposits were building up on the surface of the digester. This would indicate that the lipid component was not being broken down in the digester. The increase in TVFA levels and bicarbonate alkalinity shows that this digester was not stable and on the verge of failure, therefore at day 48 feeding was halted. On day 50 0.36 gl^{-1} of $Ca(OH)_2$ was added to bring the pH to 7.2 and gas production, pH, and VFA levels were monitored until day 98. Gas production was negligible and the TVFA had fallen to 500 mgl^{-1} by day 98. The previous thermophilic study also failed at around 50 days (day 47) although this also coincided with a temperature shock. The use of nutrient addition in the second study would suggest that nutrient deficiency was not a cause of digester failure in the thermophilic study. A scum layer of fatty material was seen on the surface of the thermophilic digesters in both studies. A sample from the thermophilic digester was

taken on day 54 and extracted into ether as in the VFA analysis and analysed by GC mass spectroscopy. The mass spectra indicated large amounts of hexadecanoic acid (C_{16}, a long chain fatty acid), a greater concentration than the VFAs, such as acetic.

High lipid levels cause problems for mesophilic and thermophilic digestion (Angelidaki *et al.* 1990, Ahring, *et al.* 1991 and Hanaki, *et al.* 1981). The lipid is hydrolysed to long chain fatty acids (LCFA) which if allowed to accumulate become toxic to methanogenesis. Angelidaki, *et al.* (1990) had problems in digesting lipid in thermophilic digesters, however the digesters did acclimatise and Ca^{2+} ions aided digestion. However the lipid levels were lower than the levels found in coffee waste. In other studies (Ahring, *et al.*, 1991) neutral lipid levels of 2.5 gl^{-1} or free LCFA levels of 0.1-0.5 gl^{-1} were found to be inhibitory to methanogenic bacteria. Coffee waste has a high level of lipid (5-7 gl^{-1}) which may suggest this is the cause of the problem in the thermophilic anaerobic digestion of coffee waste.

Lipid toxicity can be alleviated in several ways. The first way would be to avoid shock loads to the system, (Hanaki, *et al.* 1981). Another way would be to reduce the toxicity of the LCFA until they are utilised in the anaerobic system. Angelidaki *et al.* (1990) reduced toxicity by the addition of bentonite which is thought to adsorb the LCFA. The addition of calcium ions has also been shown to reduce toxicity (Angelidaki *et al.* 1990,, Ahring, *et al.* 1991 and Hanaki, *et al.* 1981) by precipitating the LCFA.

Successful thermophilic anaerobic digestion of coffee grounds has been achieved by the addition of 0.3g CaO per gram VS added, (Kostenberg and Marchaim, 1993, 1994). The level used in this study was 0.05 $Ca(OH)_2$ per g VS added. Kostenberg and Marchaim, (1994) also tried sodium carbonate and ammonium bicarbonate at 0.3g buffering agent per gram VS added but with limited success. The pH levels remained at pH 7-7.5 but TVFA levels had risen to 2550 mgl^{-1} from 750 mgl^{-1}

(Kostenberg and Marchaim, 1994). This could also suggest that the ion type is responsible and not the buffering capacity as the pH remained at a suitable level for methanogenesis.

In the present study at mesophilic temperatures, the addition of 1 gl^{-1} $Ca(OH)_2$ to the feed was sufficient to maintain reactor stability at an OLR of 1.3 kgCOD $m^{-3}day^{-1}$ (25 day HRT). However at thermophilic temperatures at an OLR of 1.6 kgCOD $m^{-3}day^{-1}$ (20 day HRT) this level of calcium was insufficient to maintain reactor stability.

6.4. Conclusions From The Work On Coffee Waste Containing Grounds

1. Proximate compositional analysis showed that the coffee waste had a high lipid component (26 - 33%), hemicellulose (3.5 - 5.3%), α-cellulose (11.1 - 13.8%), lignin (9 - 11%), protein (1.7 - 1.1%) and ash (1.4 - 3.3%).

2. A 58% reduction in VS was seen in both mesophilic and thermophilic batch biodegradability tests. Greater lipid and hemicellulose degradation was observed in the mesophilic study, while α-cellulose was degraded equally at both operating temperatures. The lignin component was not reduced in either study.

3. Mesophilic continuous digestion was achieved for 99 days with the addition of $Ca(OH)_2$, nitrogen phosphorus, trace metals and using inoculum from a UASB fed on coffee waste. Addition of solely sodium bicarbonate was not sufficient for long term anaerobic digestion. A COD and VS removal of 60% was achieved.

4. Thermophilic digestion could be established at 1.6 kgCOD $m^{-3}day^{-1}$ (20 day HRT) with the addition of sodium bicarbonate, or $Ca(OH)_2$, nitrogen, phosphorus and trace metals. However long term digestion could not be established beyond 50 days without an increase in TVFA occurring.

7. COMPARISION OF SINGLE STAGE MESOPHILIC AND THERMOPHILIC UASB REACTOR OPERATION.

In this chapter the anaerobic digestion of a relatively coffee-ground-free waste was studied in laboratory-scale UASB reactors. The coffee waste was discharged at temperatures which would allow thermophilic operation without the extra cost of heating the waste to thermophilic temperatures. The conversion of mesophilic UASB granules to thermophilic operation and comparison of performance of the mesophilic and thermophilic UASBs on coffee waste was studied.

7.1. Feed Characteristics

The feed used in this chapter and Chapter 8 was prepared by settling for one hour at room temperature Waste Stream C. This procedure produced a feed relatively low in coffee grounds. Buffering capacity, as sodium bicarbonate and nutrient supplementation of nitrogen, phosphorus and trace metals were added in the amounts described in Chapter 4. The characteristics of the raw coffee waste produced after one hour`s settling, to remove most of the grounds, are presented in Table 7.1.

The waste from the Nestlé factory used in this study (described in Table 7.1) had a COD of between 7,400 and 18,000 mgO_2l^{-1}. This was up to 4 times stronger than the coffee waste waters used by other workers such as Hajipkkos (1992) and Lanting *et al.* (1989). The coffee waste used by Hajipkkos (1992) and Lanting *et al.* (1989) had a COD strength of 4,000 mgO_2l^{-1}

Table 7.1. Characteristics of Raw Settled Coffee Waste

Analysis	Range of Values
Total Solids gl^{-1}	8-11(3)
Non-Volatile Solids gl^{-1}	0.2(2)
Suspended Solids gl^{-1}	0.6-1.0 (2)
Total COD mgO_2l^{-1}	7,400-18,000 (15)
Total Lipid gl^{-1}	1.5 (1)
Calcium mgl^{-1}	70-90 (2)
Potassium mgl^{-1}	90-110 (2)
pH	4.1-4.6 (5)
Bicarbonate Alkalinity mg $CaCO_3l^{-1}$	0 (5)

() =Number of samples

These waste characteristics could lead to two main problems. This large variation in COD could adversely affect the digester performance by creating a fluctuating organic load; this in full scale could hopefully be overcome by the use of a balancing tank to reduce the variation in COD levels (Lettinga and Hushoff Pol, 1991). In the laboratory-scale work the raw coffee waste was diluted and blended to give a consistent feed strength from day 30 of the single stage experiment onwards.

The greater COD strength could lead to higher levels of inhibitory compounds being present in this particular waste water. Several workers have noted the possibility of toxic or inhibitory compounds being present in coffee waste (Lane, 1983, Fernandez and Forster 1993a and b and Shi and Forster, 1993). In particular the presence of potassium in the synthetic coffee waste was found to affect the performance of mesophilic and thermophilic filters (Fernandez and Forster 1993a and b). The effect of potassium on thermophilic operation was more pronounced leading to cessation of gas production. An adverse effect was also seen on the COD removal in thermophilic UASBs, also thought to be due to potassium in the feed (Shi and Forster, 1993). Analysis of the feed used in this study showed it had a relatively low level of potassium (90-110 mgl^{-1}) compared to the up to 1200 mgl^{-1} found in the synthetic wastes (Fernandez and Forster, 1993a). Levels of 30 mgl^{-1} potassium were found in real coffee waste by Kostenberg (1993a), although potassium is found in significant quantities in the coffee bean and in synthetic waste produced from infusions of coffee beans. In real wastes the potassium levels are lower, probably because in the instant coffee production process the potassium is leached out into the instant coffee.

Also present in the waste used in this study were 70-90 mgl^{-1} levels of calcium. In synthetic feed studies this level of calcium was found to counteract the effect of potassium inhibition (Shi and Forster, 1993).

The settling process removed the majority of the coffee grounds although the waste still had levels of 0.6-1.0 gl^{-1} suspended solids. However this level of suspended solids is within the maximum limit issued as a guide for treatment in retained biomass reactors (10% suspended solid content of the feed) (Mergaert *et al.*, 1992).

The pH of the feed was acidic (pH 4.1-4.6) and therefore sodium bicarbonate was added to the feed to aid stable reactor operation. With the addition of sodium bicarbonate and the nutrients the feed was found to have an average pH of 6.8 (pH6.7

and pH 7.0) and an average bicarbonate alkalinity of 530 mg $CaCO_3l^{-1}$(430 and 630 mg $CaCO_3l^{-1}$).

7.2. Reactor Start-up

From the literature survey, significant levels of VFA were expected at reactor start up, during conversion to thermophilic operation and possibly from the presence of inhibitory compounds in the waste. In this study the VFA levels were used as the principal means of determining the health status of the reactor. During the majority of the study the principal acids were acetic and propionic acid. The levels of acetic and propionic acids and total volatile fatty acids (TVFA) for the mesophilic UASB and thermophilic UASB used over the 109 days of single stage operation are shown in Figure 7.1. and Figure 7.2 respectively. The duplicate reactors were sampled at a lower frequency than the reactor results presented here in graphical form.

Each of the pair of mesophilic and thermophilic reactors behaved similarly over the 28 day start-up period with initially high levels of TVFA which became lower over time. After day 30 one thermophilic and one mesophilic reactor were maintained at a 2 day HRT to provide a back-up in case of failure of the experimental reactor.

High TVFA levels were experienced in both the mesophilic and thermophilic reactors in start up (see Fig. 7.1 and 7.2). In Figure 7.1 and 7.2 the numerals indicate the OLR at which the reactors were operating in that particular time band (indicated by the vertical bars). Where there is no numeral on the graph the reactor operation is described in the text.

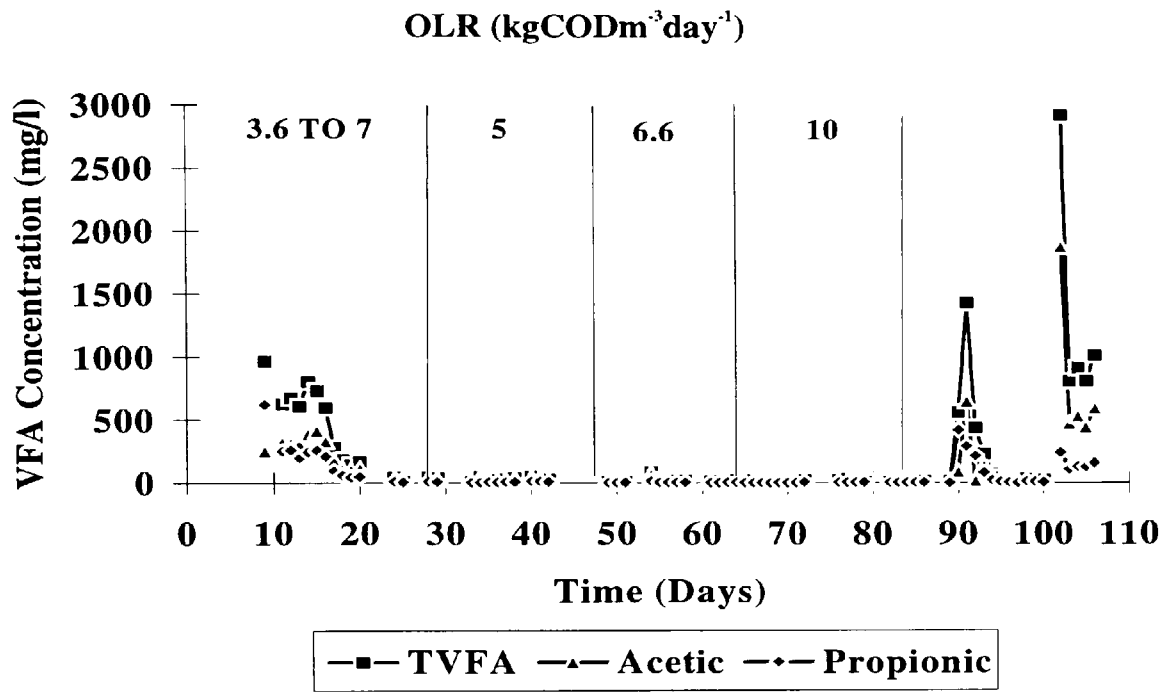

Figure 7.1. Levels Of The Principal VFA In The Mesophilic UASB.

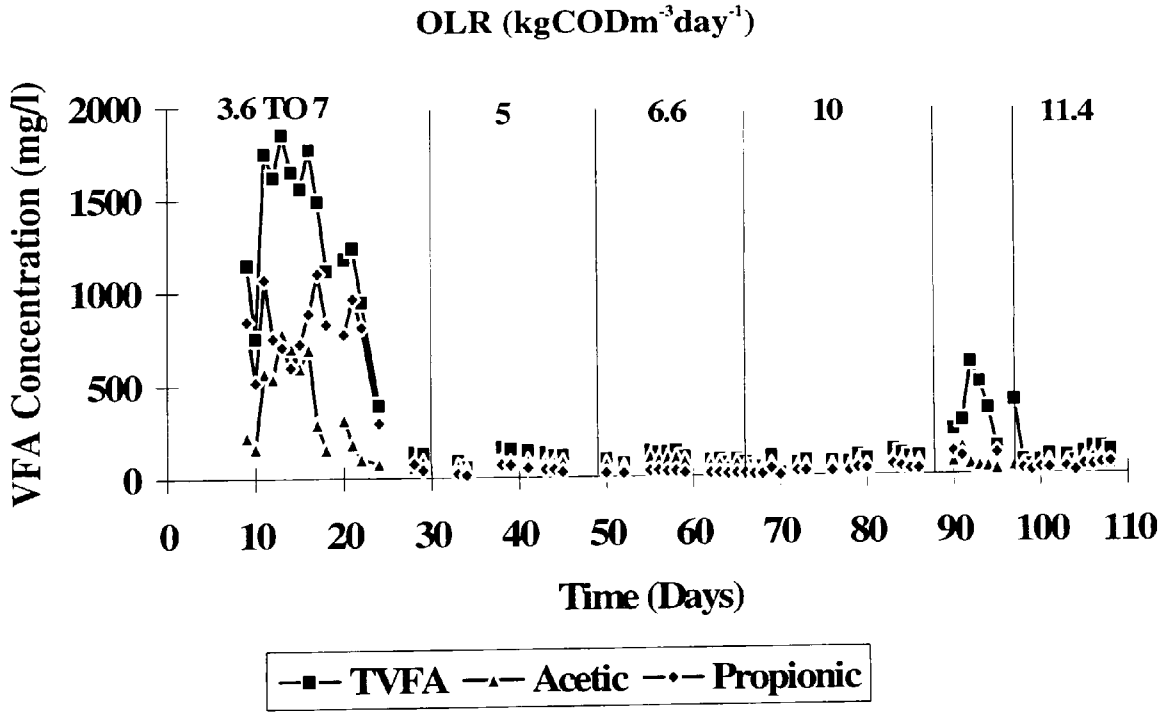

Figure 7.2. Levels Of The Principal VFA In The Thermophilic UASB

During the first 25 days of operation, levels of up to 970 mgl^{-1} TVFA occurred in the mesophilic reactor (Figure 7.1) and 1850 mgl^{-1} TVFA in the thermophilic reactor

(Figure 7.2). Despite the high TVFA levels, the mesophilic UASB maintained bicarbonate alkalinity at between 1590-2980 $mgCaCO_3l^{-1}$ and pH at 7.0-7.4 over days 0-25 (See Figure 7.3).

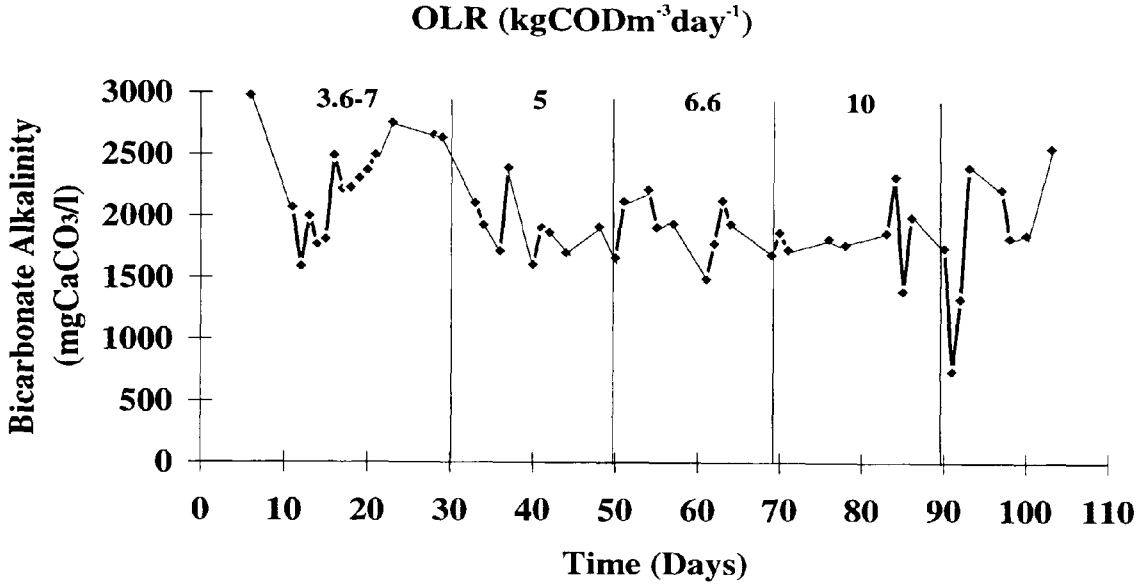

Figure 7.3. Levels Of Bicarbonate Alkalinity In The Mesophilic UASB

Bicarbonate alkalinity remained at 1030-1940 $mgCaCO_3l^{-1}$ and pH 6.75 - 7.45 in the thermophilic UASB except on day 15 where bicarbonate alkalinity dropped to 710 $mgCaCO_3l^{-1}$ and pH dropped to pH 6.70, requiring the addition of extra sodium bicarbonate (at 2.85 gl^{-1}) to maintain the bicarbonate alkalinity at 1000 $mgCaCO_3l^{-1}$.

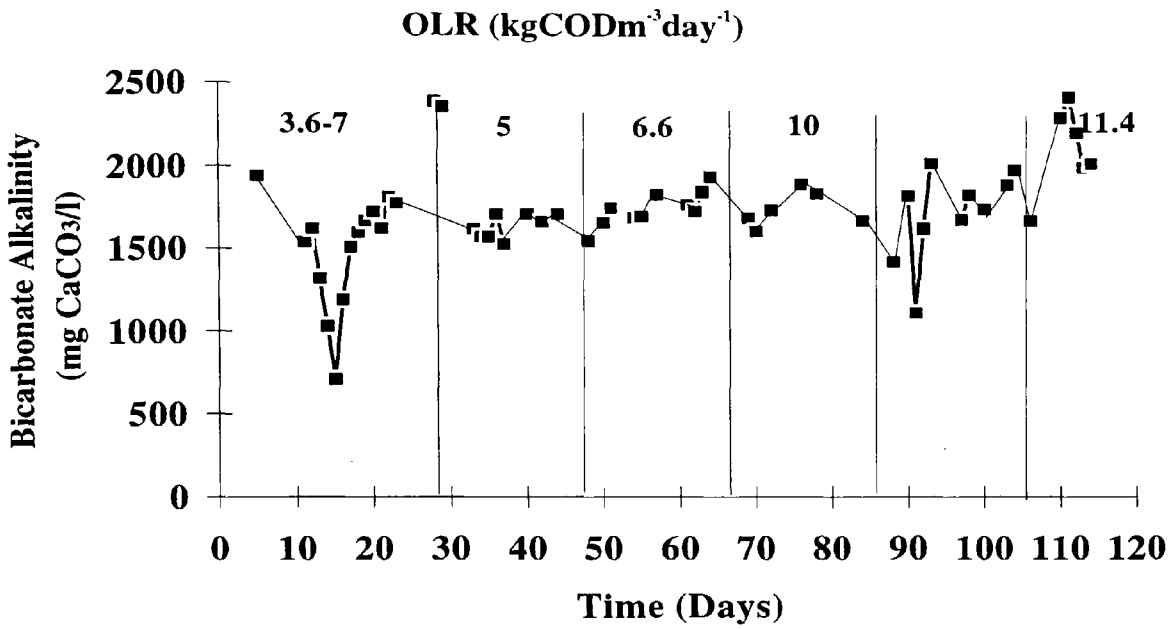

Figure 7.4. Levels Of Bicarbonate Alkalinity In The Thermophilic UASB

The strategy of monitoring bicarbonate alkalinity prevented pH shocks occurring while keeping sodium ion levels to a minimum, despite high TVFA levels related to the high organic strength of the wastewater (see Figure.7.4, 7.5, 7.6)

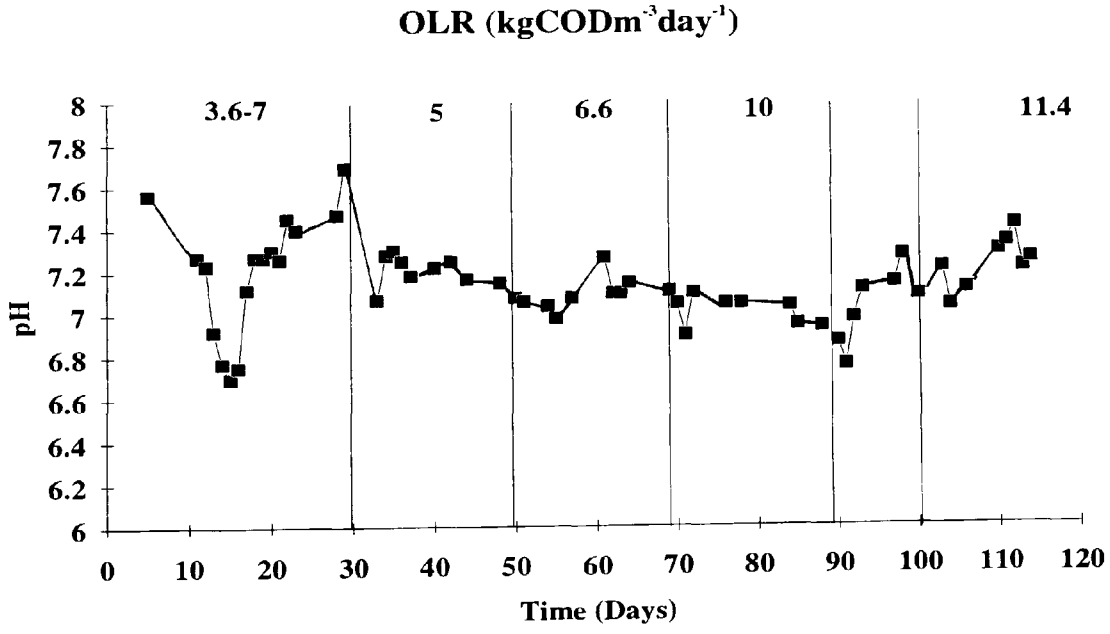

Figure 7.5. Levels Of pH In The Thermophilic UASB

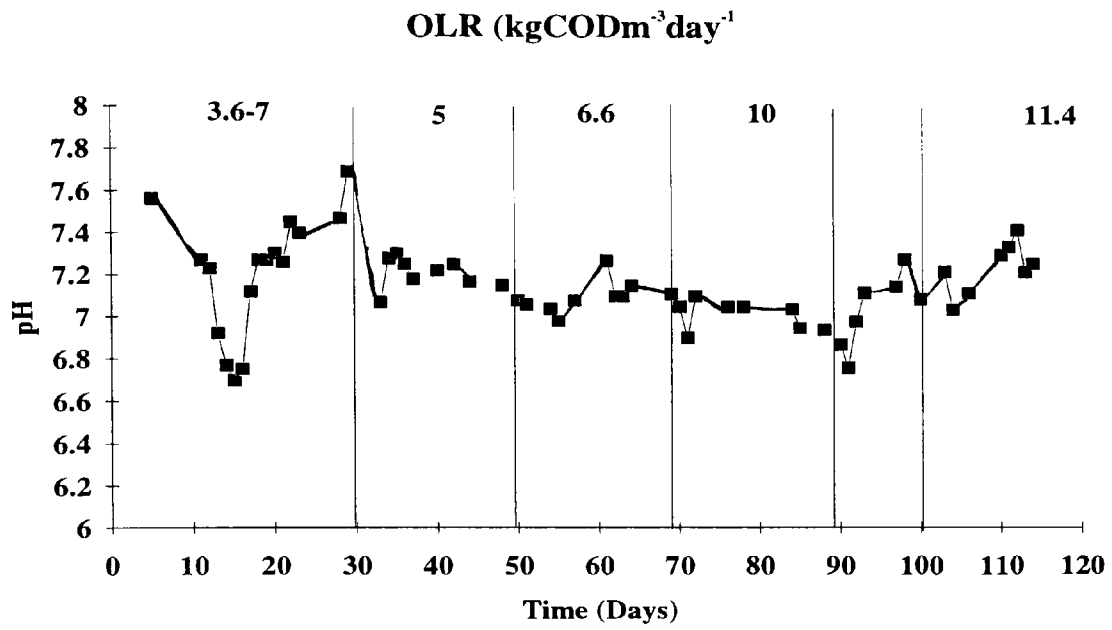

Figure 7.6. Levels Of pH In The Mesophilic UASB

The percentage of COD removal for the mesophilic UASB is shown in Figure 7.7 and in Figure 7.8 the level of COD removal in the thermophilic UASB.

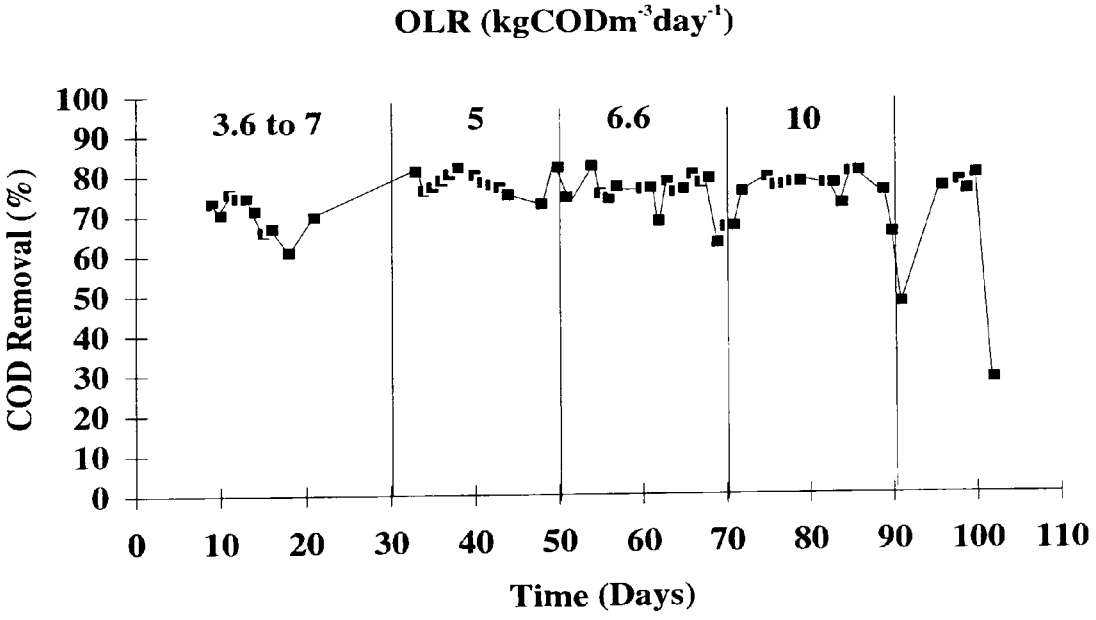

Figure 7.7. The % COD Removal In The Mesophilic UASB

By day 28 the TVFA levels had fallen to 130mgl^{-1} in the thermophilic reactor and by day 24 to 40 mgl^{-1} in the mesophilic reactor, with COD removal reaching 78% in the mesophilic digester and 70 % in the thermophilic digester. Subsequent levels of

TVFA and COD removal (see Figs. 7.1, 7.2, 7.7 and 7.8 and Table 7.2) showed no significant improvement over the rest of the experiment. This would indicate that after 28 days for the thermophilic UASB and 24 days for the mesophilic UASB acclimatisation was complete and steady state had been reached.

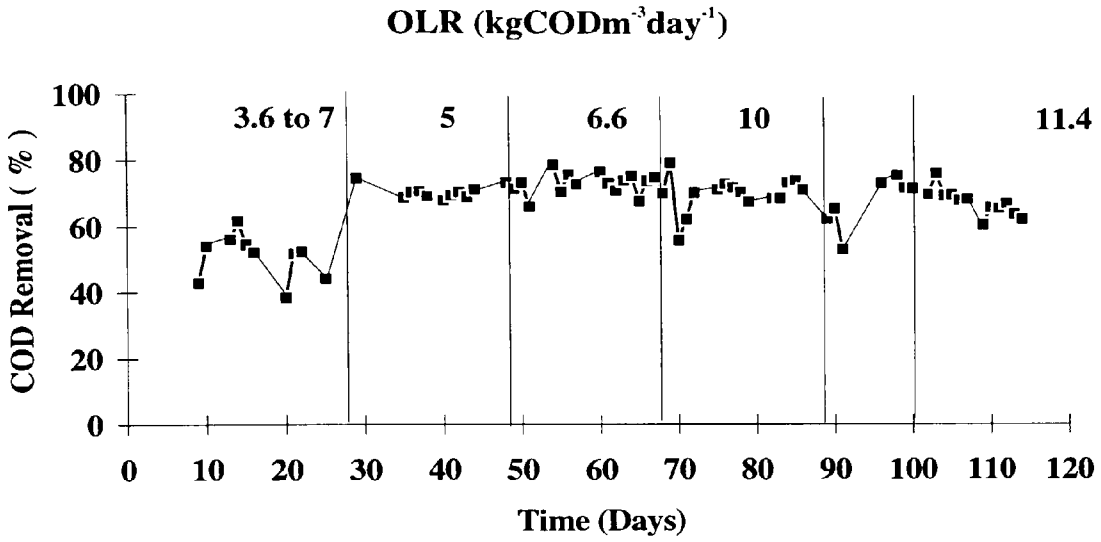

Figure 7.8. The % COD Removal In The Thermophilic UASB

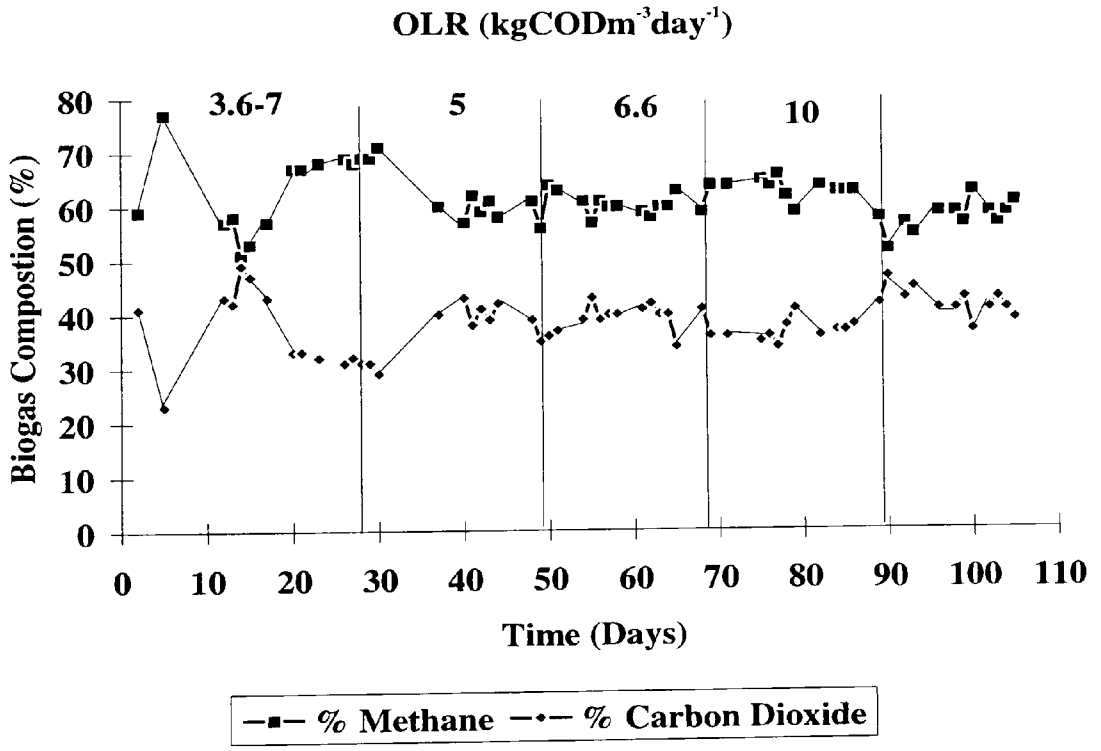

Figure 7.9. The Biogas Composition Of The Mesophilic UASB

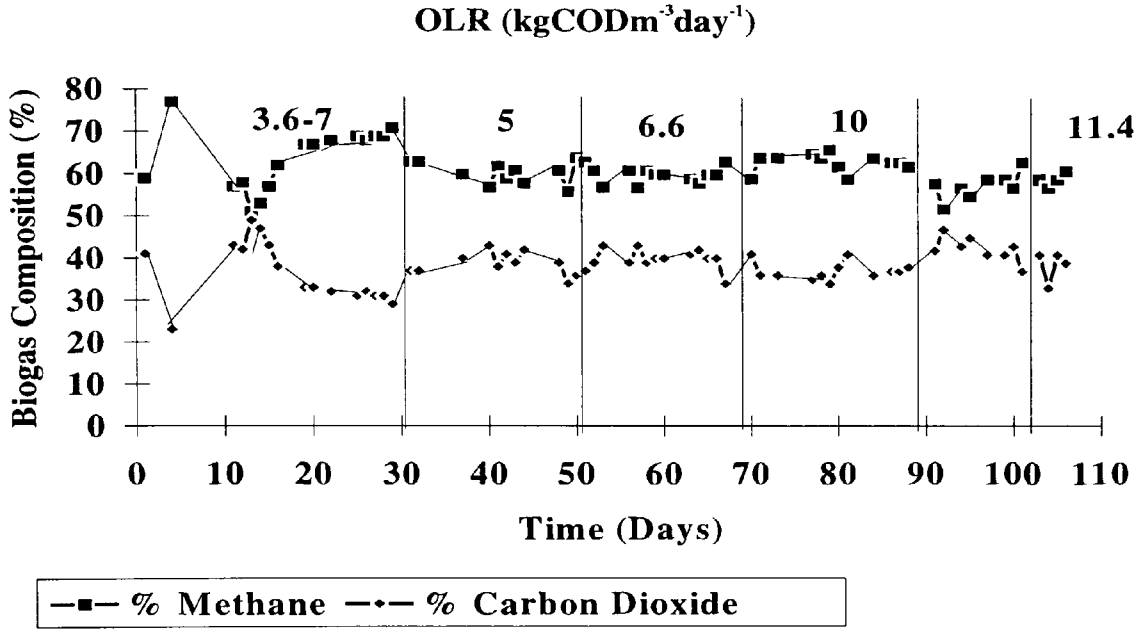

Figure 7.10. The Biogas Composition Of The Thermophilic UASB

Both reactors contained mesophilic granules from the EPSRC pilot plant adapted to treating instant coffee waste water at the Nestlé factory, and were subject to the same OLR regime at start-up. The higher levels of TVFA, particularly propionic acid, seen in the thermophilic reactor (Figure 7.2) were probably due to the conversion process from mesophilic to thermophilic operation.

In other studies on coffee waste, adaptation of mesophilic to thermophilic sludge was effected by small increases in temperature over a longer period, e.g. Lanting *et al.*, (1989) where the temperature was increased over a two month period. Alternatively granules were acclimatised to thermophilic temperatures in the presence of yeast extract and then acclimatised to coffee waste (Shi and Forster, 1993).

With other waste types conversion of mesophilic granules to thermophilic granules has taken between 25 and 100 days. Lepisto *et al.*, (1997) achieved up to 75% COD removal after 48 days after conversion to thermophilic operation on vegetable canning waste but this increased to 90% COD removal over the 70 day experiment.

Using sucrose and milk powder stable COD removals were achieved after 45 days of operation after conversion by Fang and Lau, (1996), while van Lier *et al.*, (1992) achieved stable operation after 2-3 weeks when operating on VFA.

In the present study a rapid rise to the thermophilic operating temperature led to satisfactory performance within 28 days. The granules still retained their activity and structural integrity after over 100 days of operation in this study and then over another 100 days after conversion to a two stage system.

7.3. Effect Of Retention Time And Loading Rate

The performance of the mesophilic and thermophilic UASBs was compared at HRTs of 48, 36, 24, 21 and 18 hours. The loading rates studied were 5, 6.6, 10, 11.4 and 13.3 $kgCODm^{-3}d^{-1}$ respectively. The reactors were operated for at least 3 HRT at each OLR. Where stable operation was achieved, the data are presented in Table 7.2. It was considered that stable operation was achieved once TVFA levels and % COD removals were obtained which were similar to those obtained at the lowest OLR of 5 $kgCODm^{-3}d^{-1}$.

Good COD removals of 70-78% were obtained under each of the steady-state operating conditions shown in Table 7.7 and in Figure 7.8. These COD removals of 70-78% compare favourably with other studies using real coffee waste, e.g. Hajipakkos (1992) achieved a 55% removal at mesophilic temperatures, compared with 78% in the present study, and Lanting *et al.*, (1989) achieved 60-70% at thermophilic temperatures. Low levels of suspended solids (measured on days 58 and 64) were found in the digester effluent. The mesophilic reactor had levels of 0.17 mgl^{-1} and 0.25mgl^{-1} and the thermophilic reactor 0.25 mgl^{-1} and 0.2 mgl^{-1} respectively.

After day 60, solids were removed periodically from the underside of the gas separator where a thick grey/brown sludge accumulated. Although there was no change in the volume occupied by the granular bed throughout the 100 day experimental period, a blanket of flocculating material built up gradually on its surface.

Table 7.2 Summary of Performance of Single Stage Mesophilic and Thermophilic UASBs

Parameter	HRT/hrs (OLR /kg COD $m^{-3}d^{-1}$)							
	48 (5)		36 (6.6)		24 (10)		18 (11.3)	
	MESO	THERM	MESO	THERM	MESO	THERM	MESO	THERM
No. of days operation	18	18	17	17	21	21	NA	13
No. of HRT	9	9	11	11	21	21		15
COD Removal /%	78	70	78	73	77	70		68
	(10)	(11)	(13)	(13)	(13)	(12)	NA	(12)
	SD=2	SD=2	SD=3	SD=4	SD=3	SD=6		SD=4
TVFA	35	122	26	99	14	80		78
mgl^{1}	(9)	(11)	(14)	(12)	(16)	(14)	NA	(10)
	SD=16	SD=32	SD=4	SD=27	SD=7	SD=33		SD=35
% CH_4	64	63	60	60	63	63		59
	(12)	(12)	(13)	(14)	(12)	(13)	NA	(7)
	SD=5	SD=5	SD=2	SD=2	SD=2	SD=2		SD=2
Bicarbonate	1900	1600	1900	1800	1800	1700		2100
Alkalinity	(8)	(9)	(10)	(9)	(9)	(7)	NA	(8)
$mgCaCO_3l^{-1}$	SD=240	SD=100	SD=230	SD=100	SD=250	SD=100		SD=250
Gas Production	1700	1480	2170	2120	3300	2920		3120
$l\,l^{-1}d^{-1}$	(15)	(15)	(16)	(16)	(19)	(19)	NA	(13)
	SD=150	SD=150	SD=200	SD=180	SD=330	SD=270		SD=300

() =No. of Samples SD = Standard Deviation NA = Not Available

In the mesophilic UASB the biogas contained 60-64% methane and 59-63 % methane in the thermophilic reactor (see Figure 7.9 and 7.10). The biogas was also analysed for hydrogen sulphide content on days 44, 60, 69 and 77. For the thermophilic UASB H_2S levels of 200ppm, 200ppm, 100ppm and 100ppm and for the mesophilic UASB H_2S levels of 500ppm, 100ppm, 100ppm and 40ppm were found. This gives an average H_2S content of 185ppm and 163ppm for the mesophilic and thermophilic UASBs respectively. There have been a number of reports referring to high levels of H_2S in the biogas of reactors treating actual coffee waste (Hajipakkos, 1992 and Gornall, 1991). Gornall and Rippey (1996) had levels of H_2S of up to 3000 ppm which required a modified engine to utilise the biogas.

The methane yield per kg COD removed, measured at ambient temperature and pressure, was 0.29 m^3kgCOD^{-1} removed for the mesophilic reactor and 0.27 m^3kgCOD^{-1} removed for the thermophilic reactor. Both of these are below the theoretical value of 0.35 m^3kgCOD^{-1} removed at STP. The need to remove sludge regularly from the gas separator would suggest that the COD removal had been exaggerated either due to a component in the coffee waste or biomass sticking to the gas separator.

Studies using synthetic wastes achieved up to 70 % COD removal in mesophilic and thermophilic operation. However problems were experienced in achieving stable thermophilic digestion. Fernandez and Forster, (1993 a and b) experienced reactor failure and McDougall *et al.* (1993) after 41 days of operation had TVFA levels of 1000 mgl^{-1}. Synthetic feeds were found to have levels of K^+ between 125 mgl^{-1} and 1200 mgl^{-1} for instant coffee and coffee bean extract respectively (Fernandez and Forster, 1993a and b, Shi and Forster, 1993); these levels were found to reduce the efficiency of mesophilic and thermophilic operation. The above workers found that calcium addition alleviated the effects of K^+ toxicity. In the present study, analysis by

flame photometry showed an average of 100 mgl^{-1} of potassium and 80 mgl^{-1} of calcium respectively in the actual factory effluent (Table 7.1). Thus the levels of Ca^{2+} needed to alleviate any effects of K^{+} were already present. Kostenberg and Marchaim (1993) reported low potassium levels of 30 mgl^{-1} in instant coffee processing wastewater. Local conditions and factory operation may influence these ion levels and should be determined for each factory.

Figures 7.1, 7.2, 7.7 and 7.8 show data for TVFA levels and % COD removal for both mesophilic and thermophilic reactors. Steady state was achieved for both reactors at HRTs of 48, 36 and 24 hours (OLRs of 5, 6.6 and 10 $kgCODm^{-3}d^{-1}$). However, when the HRT was decreased to 18 hours (OLR of 13.3 $kgCODm^{-3}d^{-1}$) over days 89 and 90, a rise in TVFA in both the mesophilic and thermophilic UASBs followed. The rise in TVFA in the mesophilic UASB was more pronounced, increasing from 16 $mgl^{-1}$to 1430 mgl^{-1} (see Figure 7.1). This was associated with a reduction in bicarbonate alkalinity and pH to 750 $mgCaCO_3l^{-1}$ and pH 6.4 respectively. Feeding was therefore stopped at day 91 and 5gl^{-1} sodium bicarbonate added to restore the pH and bicarbonate alkalinity to previous levels. A reduction in COD removal from 77% to 48% also occurred (Figure 7.9).

Once the OLR was increased to 13.3 $kgCODm^{-3}d^{-1}$ over days 89 and 90, the thermophilic reactor saw a rise in TVFA from previous levels of 80 mgl^{-1} to 600 mgl^{-1} (Figure 7.2). The level of COD removal also fell from 70% to 53% by day 91 (Figure 7.8). The pH and alkalinity decreased from pH 6.9 to pH 6.8 and from 1830 $mgCaCO_3l^{-1}$ to 1120 $mgCaCO_3l^{-1}$ respectively. On decreasing the OLR to 10 $kgCODm^{-3}d^{-1}$ on day 93 previous performance levels were regained. The mesophilic reactor also saw a return to previous levels of performance when feeding was restarted at an OLR of 10 $kgCODm^{-3}d^{-1}$ on day 95.

The last HRT tried was 21 hours (OLR 11.4 $kgCODm^{-3}d^{-1}$) at days 100 and 101. The mesophilic UASB had a more severe reaction to this change (see Figure 7.2) than the previous increase in OLR. The TVFA levels rose from 27 mgl^{-1} to 2930 mgl^{-1} with a sharp rise in acetic acid content. The pH fell to 4.3 and bicarbonate alkalinity was reduced to zero.

These results would therefore indicate that using this feed stock a mesophilic UASB could achieve a OLR of up to 10 $kgCODm^{-3}d^{-1}$. However if the loading rate was increased to 11.4 $kgCODm^{-3}d^{-1}$ then the mesophilic UASB suffered failure. This OLR value is significantly higher than the maximum OLR achieved in studies using synthetic coffee effluent, which achieved OLR of between 3-4 $kgCODm^{-3}d^{-1}$, although these systems were not tested to failure (McDougall *et al.* 1993, Fernandez and Forster, 1993a and b and Shi and Forster, 1993).

Lanting *et al.* (1989) in two pilot-scale studies found that mesophilic UASBs failed at around 7 weeks (50 days) at loading rates of up to 12-13 $kgCODm^{-3}d^{-1}$ in the first study and in the second study as the OLR was increased up to 10-11 $kgCODm^{-3}d^{-1}$. Although Lanting *et al.* (1989) did not use nutrient addition, it perhaps suggests that loading rates above 10 $kgCODm^{-3}d^{-1}$ are not achievable in mesophilic UASBs with this waste. Lanting *et al.* 1989) found that longer term operation ie. longer than 50 days would be better achieved in thermophilic digestion. In the work reported here, mesophilic operation was maintained for up to 100 days, as long as the OLR did not exceed 10 $kgCODm^{-3}d^{-1}$, which is significantly longer than the 50 days achieved by Lanting *et al.* (1989). The existence of up to 3 full-scale mesophilic UASBs treating instant coffee waste waters up to OLRs of 6 $kgCODm^{-3}d^{-1}$ supports the proposition that long term digestion is feasible in mesophilic UASBs (Lettinga and Hulshoff Pol, (1991), Hajipakkos, (1992).

This study found that the thermophilic UASB could treat coffee waste at higher OLR than the equivalent mesophilic system, at an OLR of 11.4 $kgCODm^{-3}d^{-1}$ compared to 10 $kgCODm^{-3}d^{-1}$. The thermophilic system was not tested to failure. An increase in TVFA from 80 mgl^{-1} to 600 mgl^{-1} as the OLR was increased to 13.3 $kgCODm^{-3}d^{-1}$, (on days 89-90) would suggest that the limit was being approached between 11.4 and 13.3 $kgCODm^{-3}d^{-1}$. Lanting *et al.* (1989) operated a thermophilic UASB at a OLR of 10 $kgCODm^{-3}d^{-1}$ but did not exceed this OLR

Although the thermophilic UASB could treat a somewhat higher OLR than the mesophilic UASB (11.3 compared to 10 $kgCODm^{-3}d^{-1}$), the COD removal was less (68% compared to 77% respectively) and the levels of TVFA higher (see Table 7.2.). However the other performance parameters were similar for both UASBs. An initial review of the literature suggested that thermophilic UASBs offered the prospect of significantly higher OLRs than typical mesophilic systems (Weigant and Lettinga 1985). These high OLRs have been matched by UASBs operating in the mesophilic range (Fang and Chui, 1993). The work presented here would suggest that thermophilic systems can cope with higher OLRs but the advantage is not that great.

As seen in Table 7.2, at each organic loading rate the thermophilic UASB operated at a higher TVFA level than the mesophilic reactor. This effect has been noted previously (van Lier *et al.* 1993). In all cases the absolute TVFA levels were low (15-122 mgl^{-1}). Thermophilic systems tend to accumulate high levels of propionic acid (Wiegant *et al.* 1986). In this study this occurred during conversion from mesophilic operation but once steady state operation had been achieved the percentage of TVFA which was propionate was similar in both mesophilic (24%) and thermophilic (32%) reactors.

The coffee waste has a high level of lipid, 1.5 gl^{-1} (12% of VS). Lipid material has been shown to cause inhibition problems due to the long chain fatty acids produced

in the hydrolysis process (Rinzema, *et al.* 1994). Thermophilic organisms are more sensitive to certain inhibitory compounds than mesophilic (Fernandez and Forster (a and b) 1993, Shi and Forster, 1993) and this could explain the higher TVFA levels and lower COD removal in the thermophilic system. However thermophilic systems tend to operate at higher VFA levels no matter what the feedstock is (van Lier *et al.* 1993, Wiegant and Lettinga, 1985, Wiegant *et al.* 1986, Souza *et al.* 1992). The maximum OLRs reached in this study are lower than OLRs achieved with other wastes in UASBs, for example of 104 $kgCODm^{-3}d^{-1}$ with sugars (Weigant and Lettinga 1985). In a recent review of full scale UASBs the UASBs treating coffee waste were found to be operating at the lowest OLRs (Lettinga and Hulshoff Pol, 1991). The levels of lipid or other inhibitory compounds found in coffee waste could be responsible for this.

In Chapter 6 it was shown that mesophilic anaerobic digestion in a CSTR of the instant coffee factory effluent without prior settling to remove solids could give up to a 60% reduction in total COD at an OLR of 1.3kg total $CODm^{-3}d^{-1}$. With further solids separation, giving solids which may be used to raise steam in the factory, a waste was produced which was treated in either mesophilic or thermophilic UASBs at 300% greater loading rates and significantly shorter HRT, with a significantly better effluent quality.

7.4. Conclusions From The Single Stage UASB operation

1. Mesophilic granules from a pilot-plant treating instant coffee waste were successfully converted to thermophilic operation by raising the temperature in one step. The high TVFA levels experienced could be managed by monitoring bicarbonate alkalinity and adding sodium bicarbonate to maintain the bicarbonate alkalinity.

2. Both mesophilic and thermophilic UASBs could be operated for at least 100 days with low TVFA and good COD removal. The slow decline in performance reported in the literature was not seen.

3. The mesophilic reactor achieved a marginally better effluent quality, with average COD removal of 78% and TVFA of 25 mgl^{-1} compared to a 70% COD removal and 100 mgl^{-1} TVFA level in the thermophilic UASB under the same conditions.

4. The maximum OLR at which the thermophilic UASB could maintain steady-state operation was marginally higher than the mesophilic (11.4 $kgCODm^{-3}d^{-1}$ compared to 10 $kgCODm^{-3}d^{-1}$). Higher OLRs for either reactor led to digester failure with increasing TVFA and decreasing bicarbonate alkalinity.

5. High levels of lipid (up to 12% of VS) were found in the waste and may account for the relatively low OLR achieved. Unlike some previous studies with synthetic instant coffee wastes, potassium was not present at levels to cause inhibition problems.

8. THE OPERATION OF MESOPHILIC AND THERMOPHILIC UASBS WITH THERMOPHILIC PRE-ACIDIFICATION

8.1. Thermophilic Pre-acidification

The thermophilic pre-acidification reactors, acidification reactor 1 (AC 1) and acidification reactor 2 (AC 2) were operated for a period of 126 days as described in Table. 8.1.

Table. 8.1. Operating Conditions of the Thermophilic Pre-acidification Stage

HRT	AC1	AC2
24 hour HRT with pH control	day 1 to day 67	day 1 to day 58
24 hour HRT without pH control	day 67 to day 100	day 58 to day 100
18 hour HRT without pH control	day 100 to day 112	day 100 to day 112
15 hour HRT without pH control	day 112 to day 122	/
12 hour HRT without pH control	day 122 to day 126	day 112 to day 126

The level and distribution of volatile fatty acids in the acidification reactors is shown in Table 8.2. The level of volatile fatty acids found in the feed before feeding to the

acidification reactor and in the feed fed to the single stage UASBs in Chapter. 7.0 are shown in the first column of Table 8.2.

The levels of VFA are usually quoted as mgl^{-1}, however this makes comparison with other acidification studies difficult. The level of TVFA present will be influenced by the initial concentration of the feedstock and the VFA distribution as well as the effectiveness of acidification influenced by the operating conditions and the biodegradability of the waste. For example the degree of acidification in a waste of 5000 $mgCODl^{-1}$ with 500 mgl^{-1} acetic acid after acidification, is the same as a 10000 $mgCODl^{-1}$ waste with a 500 mgl^{-1} valeric acid after acidification. This is because although the 10000 $mgCODl^{-1}$ waste is twice the strength of the 5000 $mgCODl^{-1}$ waste valeric acid has twice the COD per mg VFA than acetate. As a percentage of COD, both wastes have undergone the same degree of acidification (5%). By quoting values as % COD of feedstock as VFA, a more valid determination of the level of effectiveness of acidification can be reached (Alexiou and Anderson, 1994, Eastman and Ferguson, 1981). Factors to convert mgl^{-1} VFA to mgl^{-1} COD were as used by these authors (shown in Table 8.3). The degree of acidification is shown in Figure 8.1.

Table 8.2. Summary of The Performance of Thermophilic Pre-Acidification Reactors

Parameter	HRT /HRS				
	24	24	18	15	12
OLR / kgCOD m^{3} d^{-1}	10	10	13.3	16	16
No. of days operation					
AC1	69	19	12	12	5
AC2	58	42	12	0	5
No. of HRT	127	61	32	19	20
pH	6.0 (58) SD=0.2	5.2 (34) SD=0.2	5.0 (21) SD=0.2	5.1 (8) SD=0.1	5.5 (8) SD=0.2
TVFA mgl^{-1}	2298 SD=322	2600 SD=265	2373 SD=341	1947 SD=302	1452 SD=35
Acetic mgl^{-1}	1234 SD=243	1150 SD=140	1256 SD=175	994 SD=140	831 SD=280
Propionic mgl^{-1}	220 SD=104	208 SD=67	194 SD=41	125 SD=25	218 SD=51
i-Butyric mgl^{-1}	12 SD=3	7 SD=5	7 SD=3	8 SD=38	7 SD=3
n-Butyric mgl^{-1}	797 SD=355	1208 SD=158	881 SD=191	800 SD=220	457 SD=155
i-Valeric mgl^{-1}	28 SD=19	27 SD=6	19 SD=7	12 SD=6	10 SD=3
n-Valeric mgl^{-1}	10 SD=7	13 SD=7	17 SD=6	7 SD=3	14 SD=5
No.of VFA samples	58	34	11	8	6

() =No. of Samples SD = Standard Deviation NA = Not Available

Table 8.3 Conversion Factors For VFA to COD

VFA	COD Equivalent ($gCODgVFA^{-1}$)
Acetic	1.066
Propionic	1.512
i-Butyric	1.816
n-Butyric	1.816
i-valeric	2.036
n-valeric	2.036

Values from Alexiou and Anderson, (1994), Eastman and Ferguson, (1981).

These values are calculated from the g of O_2 required to oxidise 1 g of VFA.

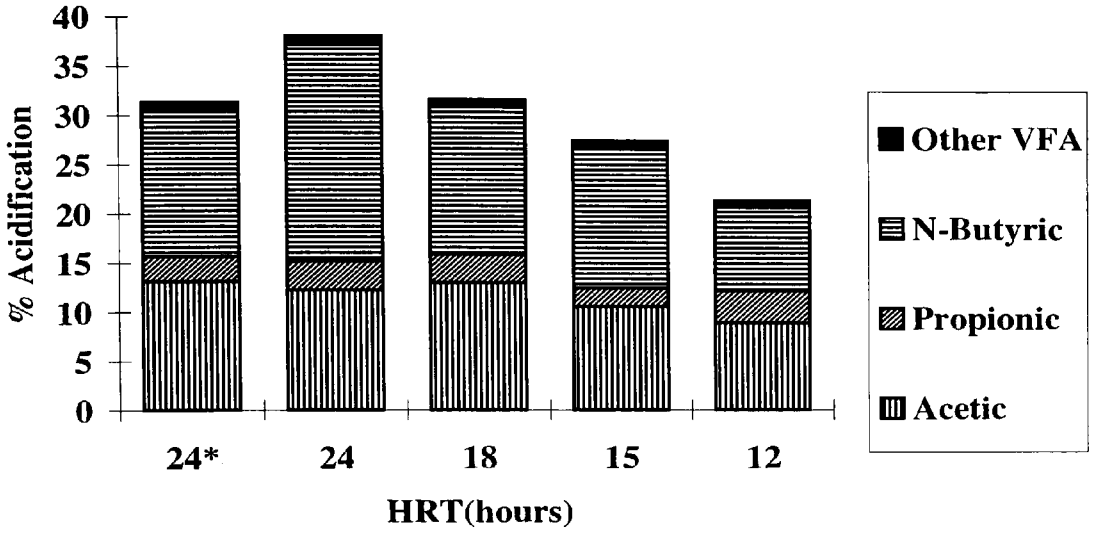

Figure 8.1. The Percentage Acidification In The Pre-Acidification Reactors.

Over a 67 day period in (AC 1) and 56 days in (AC 2) with pH control to pH 6.0 (OLR of 10 $kgCODm^{-3}d^{-1}$) an average of 2298 mgl^{-1} TVFA was found with values ranging from 1700 to 3000 mgl^{-1} TVFA (SD=322). Levels of up to 3000 mgl^{-1} TVFA were reported for the acidification at 37°C, pH 6.0, HRT 24 hours and OLR 10 $kgCODm^{-3}d^{-1}$ of a synthetic wastewater (instant coffee), whilst at 55°C using the same conditions 40% less VFA were found (McDougall *et al.* 1993).

In the feed fed to the acidification reactors the VFA levels corresponded to 4% of the COD of the feed. The VFA values as % COD of the feedstock for AC1 and AC2 are presented in Figure 8.1. For AC1 and AC2 at 24 hour HRT at pH 6.0 this gave 32% of total influent COD converted to TVFA. This level of acidification is within the range recommended by Lettinga and Hulsoff-Pol (1991), between 20 and 40% of total COD to be acidified.

At all operating conditions all C_2-C_5 VFAs were detected; the predominant VFAs were *n*-butyric, acetic and propionic (Table 8.2). As percentage of initial COD, at a 24 hour HRT and pH6.0, the acids were *n*-butyric (15%), acetic (13%), propionic (3%) with i-butyric, *i*-valeric and *n*-valeric at less than 1% (Figure 8.1). These

correspond to absolute values of 797 mgl^{-1} of *n*-butyric, 1234 mgl^{-1} acetic, 220 mgl^{-1} propionic and less than 28 mgl^{-1} for *i*-butyric, *i*-valeric and *n*-valeric acids respectively. McDougall *et al.* (1993) for the mesophilic pre-acidification of a synthetic instant coffee waste at pH 6.0 (24 hr HRT) found that acetic (1,500 mgl^{-1}), propionic (800 mgl^{-1}) and then *n*-butyric (400 mgl^{-1}) were the predominant VFAs. Zoetemeyer *et al.* (1982a) using glucose as substrate at 30^{0}C (pH 5.8) found that butyrate>acetate>ethanol (as % mg carbon l^{-1}) were the most common liquid phase products while at 55^{0}C (pH 5.8) ethanol>acetate>propionate were the most common.

Mosey (1983) predicted that in acidification most products would be acetic and propionic acids, however in a more recent paper (McCarty and Mosey, 1991), this model was modified, so that butyrate can replace propionic at lower pHs (pH 6 and below).

A review of pre-acidification papers would suggest that as well as loading rate (Zoetemeyer *et al.*,1982) and feed composition (Elefinositis and Oldham, 1995) that temperature and pH, influence product distribution and degree of acidification. Zoetemeyer *et al.* (1982a) found equally good acidification at mesophilic and thermophilic temperatures while McDougall *et al.* (1993) did not. McDougall *et al.* (1993) and Bull *et al.* (1984) showed the same upper and lower pH limit (between pH 5.0 and 6.0) for optimum acidification although absolute values vary somewhat.

From the literature it would seem there is some debate over what conditions produced the optimum conditions for acidogenesis, since good levels of acidification have been achieved under a wide range of conditions. It has also been reported that the advantages of pre-acidification can be achieved at relatively low levels of acidification, 3-10% (Cohen *et al.*, 1985). This would indicate that a lower level of management of the acidification reactors is required compared to the methanogenic stage.

The pH controllers were removed from AC1 and from the pH adjustment chamber feeding the mesophilic UASB on day 69 and from AC2 and the pH adjustment chamber feeding the thermophilic UASB on day 58. The only pH controlling agent was 1.5 gl^{-1} sodium bicarbonate added to the feedstock. The removal of the pH controllers while continuing to operate the reactors at a 24 hour HRT saw the pH drop in the pre-acidification reactors from pH 6.0 to pH 5.2. The degree of acidification as % of total COD increased from 32% to 38% (see Figure 8.1). The TVFA rose to 2600 mgl^{-1} (Table 8.1). The principal VFAs remained unchanged but with *n*-butyric increasing to 22%, acetic at 12% and propionic remaining at 3% of total COD.

At an HRT of 24 hours, allowing the pH to float at around pH 5.2 instead of being controlled at pH 6.0 increased the TVFA and the degree of pre-acidification. McDougall *et al.* (1993) found for mesophilic operation that control at pH 6.0 was the optimum for TVFA production. Zoetemeyer *et al.* (1982b) using mesophilic conditions found that pH 6.0 was optimal for the growth rate ($\mu_{max.}$) although product formation and distribution were similar between pH 5.0 and 6.0. At values below and above this range significant changes in product distribution were seen. Indeed Bull *et al.* (1983) at mesophilic temperatures achieved stable pre-acidification at between pH 3 and 5 (typical value of pH 3.5) and at HRTs of 10 to 1.66 hrs.

A reduction in HRT in AC1 and AC2 to 18, 15 and then 12 hours gave an operating pH of 5.0, 5.1 and 5.5 respectively (Table 8.1). If the pH had been maintained at pH 6.0, all this alkalinity would have to be added from external sources at extra cost as the coffee waste is acidic in nature (pH 4.3-4.6) and low in sources of potential alkalinity such as ammonia. Zoetmeyer *et al.* (1982b) found that 44% more NaOH was required to control the pH at pH 6.0 than at pH 5.0 for a synthetic glucose feed. The present study has shown that at thermophilic temperature and 24 hour HRT, maintenance of pH at pH 5.2 was superior to control at pH 6.

A reduction in HRT in AC1 and AC2 from 24 hrs to 18, 15 and then 12 hrs saw a steady reduction in the degree of pre-acidification with 32%, 28% and 21% being achieved respectively. At 12 hrs HRT the level of pre-acidification had dropped towards the lower level of optimum pre-acidification suggested by Lettinga and Hulsoff-Pol (1991), although there could be other products of acidification present such as ethanol, formate and lactate which were not measured in this study (Zoetemyer *et al.*, 1982a and b). Stable acidification at shorter retention times could be possible as the critical dilution rate thermophilic pre-acidification is around $0.71h^{-1}$ (an HRT of 1.41hours) (Zoetemyer *et al.*,1982a). Bull *et al.* (1983) achieved stable acidification down to 1.66 hr HRT in a mesophilic system and still found the system operational. Other factors apart from the degree of acidification may influence the choice of HRT for the acidification stage. One of the hoped for benefits of thermophilic pre-acidification was greater resistance to inhibitory compounds such as lipid in the coffee waste. Komatsu *et al.*, (1991) found that a minimum 8 hr HRT was required to overcome the inhibitory effect of lipids in the methanogenic stage.

At 18 and 15 hours HRT in the acidogenic stage, the predominant VFAs as % Total COD were still *n*-butyric>acetic>propionic (Figure 8.1). However at the 12 hour HRT the *n*-butyric and acetic acids as % Total COD had fallen to 8% and 9% respectively.

Only CO_2 was detected in the headspace of AC2 from day 1 to day 126. Some methane was detected in AC1 between day 26 and 35, the residual gas being CO_2.

8.2. Effect of Pre-acidification on the Methanogenic Reactors

The level of VFA (TVFA, acetic and propionic acids) from day 0 to 126 in the mesophilic and thermophilic UASBs are shown in Figure 8.2 and 8.3 respectively. For the start up phase, in the mesophilic UASB the TVFA increased to 164 mgl^{-1} on day 2 but subsequently decreased to 25 mgl^{-1} on day 3. From day 5 to day 64 the average level of TVFA was 17 mgl^{-1} (SD 10, number of samples 27). This would indicate that despite not being fed for 10 days and started up at an OLR of 10kgCODm^{-3} d^{-1} the mesophilic granules adapted quickly to the acidified feed.

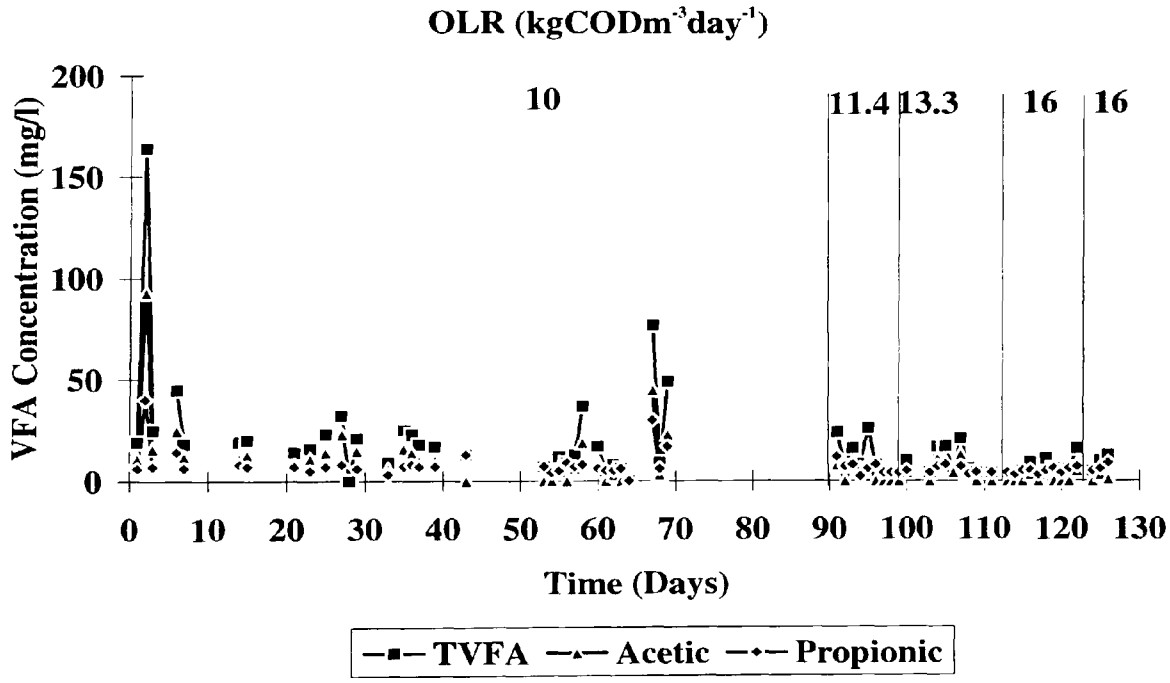

Figure 8.2. Levels of VFA in Mesophilic UASB Reactor (pH controller removed on day 67)

In contrast the thermophilic reactor reached TVFA levels of 1022 mgl^{-1} on day 5, levels dropping to 156 mgl^{-1} on day 8, but subsequently rising to 840 mgl^{-1} by day 58. On day 59 the pH controller was removed from the acidification reactor and the pH adjustment chamber.

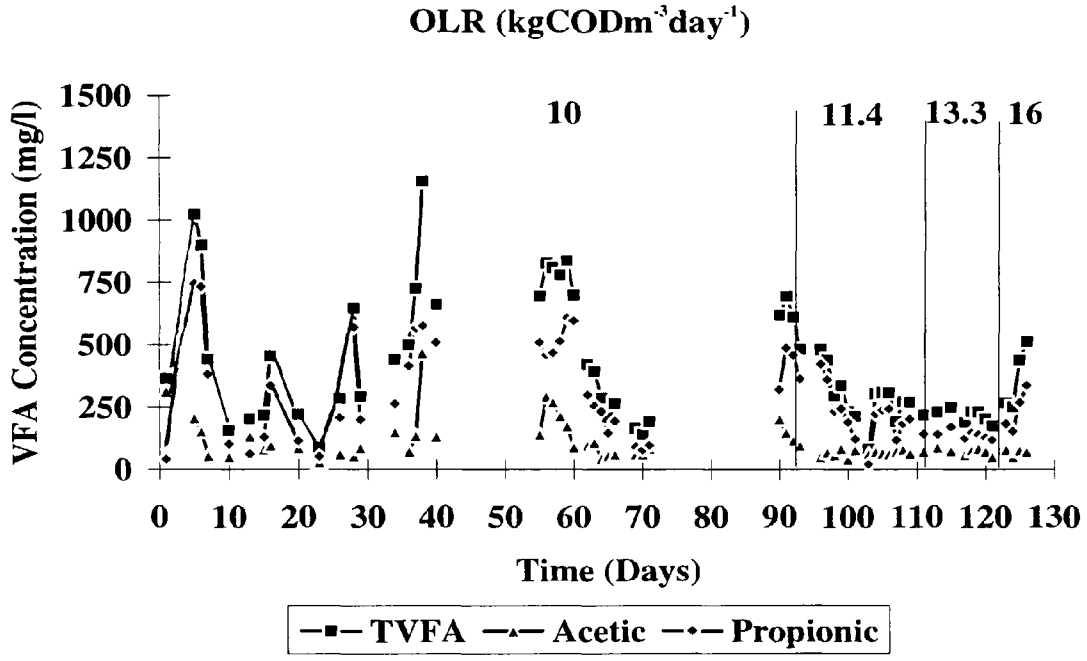

Figure 8.3. Levels of VFA in Thermophilic UASB Reactor (pH controller removed on day 59)

On day 62 the TVFA had fallen to 423 mgl^{-1} and from day 64 to day 71 the average TVFA was 210mgl^{-1}. For technical reasons (factory maintenance interrupted effluent supply) the reactors were without feed from day 70 to day 90. An increase in VFA levels seen in the thermophilic UASB on days 91-98 was assumed to indicate metabolic stress as feeding resumed. The increasing levels of TVFA in the thermophilic UASB when the pH controllers were in use, and the subsequent reduction in TVFA levels when the pH controller was removed, would suggest that the use of the pH controllers was adversley influencing the operation of the thermophilic UASB, perhaps through the addition of NaOH. However this effect was not seen in the mesophilic UASB. Greater sensitivity of thermophilic anaerobic reactors to ions such as potassium has been reported by Fernandez and Forster, (1993a and b).

Studies on thermophilic sodium toxicity suggest that total inhibition of activity can occur at 1150 $mgl^{-1}$of Na^{+} (Ahring *et al.* 1991). However small amounts of Mg^{2+}

($1.2 mgl^{-1}$) can alleviate this toxicity, although no reason was given (Ahring *et al.*1991) so that 8 gl^{-1} of sodium was needed to give total inhibition. No Mg^{2+} was added in this study and the amount present in the coffee feedstock was not determined. The level of Na^+ determined in the feed (10,000 mgO_2l^{-1} COD) with no additions was 100 mgl^{-1}. From this value and the additions of Na^+ added in the form of either sodium bicarbonate or sodium hydroxide, the level of Na^+ in the feedstock to the UASB was calculated. When the pH controllers were in use (adding NaOH) the level of Na^+ in the feedstock was calculated to be 1270 mgl^{-1}. When sodium bicarbonate was added instead, the level of 460 mgl^{-1} Na^+ would be expected. It has been shown that Na^+ levels in mesophilic studies on granular sludge do not exhibit inhibition below 5000 mgl^{-1} (Rinzema *et al.*, 1988). Hence the maximum levels used here are well under the inhibitory values for mesophilic reactors , but close to those which may give maximum inhibition for the thermophilic reactors with Mg^{2+} levels below 1.2 mgl^{-1}. Different batches of waste were used through the study but the waste was fed simultaneously to the mesophilic and thermophilic systems. So any change in feed composition would affect both thermophilic and mesophilic reactors. Thermophilic reactors have been reported to be more sensitive to temperature changes than mesophilic systems. Offline determination of reactor temperature did not vary more than $55^{\circ}C \pm 1^{\circ}C$.

Over the duration of the experiment at OLRs of 10 $kgCODm^{-3}d^{-1}$ (24 hr HRT) increasing to 16 $kgCODm^{-3}d^{-1}$ (12 hr HRT) low levels of TVFA were seen in the mesophilic UASB (Figure 8.2) with the highest value being 45 mgl^{-1}. For example, from day 3 to day 126 the average TVFA value was 13 mgl^{-1}(SD=9.2, number of samples 58). Higher than average values were seen on days 67 and 69, coinciding with removal of the pH controller. The otherwise low and consistent levels of TVFA would suggest that stable operation of the mesophilic UASB was obtained over a range of loading rates and HRTs.

In an equivalent single stage system it was found that above OLRs of 10kgCODm^{-3}d^{-1} (24 hr HRT) stable mesophilic digestion was not possible. When the OLR was increased to 11.4 kgCODm^{-3}d^{-1}(21 hr HRT) TVFA levels rose to 2930 mgl^{-1} in the single stage studies (chapter 7). This would indicate that thermophilic pre-acidification allowed stable operation of mesophilic UASBs at significantly higher OLRs (60% higher) and shorter (100% shorter) than the equivalent single stage system.

Table 8.4 Summary of Performance of Mesophilic and Thermophilic UASBs Operating With Thermophilic Preacidification

Parameter	Mesophilic	Thermo.	Mesophilic	Thermo.	Mesophilic	Thermo.	Mesophilic	Thermo.	Mesophilic	Thermo.
OLR kgCOD $m^{-3}d^{-1}$	10	10	11.4	11.4	13.3	13.3	16	16	16	16
HRT (hours)	24	24	21	21	18	18	15	15	12	12
No. of days operation	71	8	10	14	11	10	10	OLR Not attempted	5	Steady state not achieved
No. of HRTs	71	8	11	15	15	13	16	NA	10	NA
%COD Removal	75 (11) SD=9	63 (7) SD=4	75 (7) SD=5	62 (5) SD=5	69 (7) SD=10	68 (7) SD=10	77 (4) SD=4	NA	77 (2) SD=5	NA
TVFA (mgl^{-1})	20 (29) SD=16	210 (6) SD=59	10 (7) SD=8	241 (10) SD=7	10 (8) SD=7	217 (7) SD=28	7 (10) SD=4	NA	9 (3) SD=4	NA
% CH_4	79 (37) SD=2	74 (4) SD=2	71 (10) SD=2	73 (2) SD=13	71 (10) SD=2	75 (5) SD=4	73 (2) SD=5	NA	74 (4) SD=3	NA
Gas Production $l\,l^{-1}d^{-1}$	3.6 (4) SD=0.210	3.3 (3) SD=0.230	3.6 (4) SD=0.170	3.3 (2) SD=0.190	4.2 (3) SD=0.200	3.9 (3) SD=0.220	5.5 (3) SD=0.34	NA	5.0 (1)	NA

() =No. of Samples SD = Standard Deviation NA = Not Available

In the two stage system studied here, TVFA levels of the thermophilic UASB were much higher (see Figure 8.3) than the mesophilic UASB (see Figure 8.2) at equivalent loading rates even when the pH controller was removed, with levels of 220 mgl^{-1} compared to 17 mgl^{-1}(SD=10, 27 samples) in the mesophilic UASB. These levels were also higher than the equivalent single stage system of 15-35 mgl^{-1} TVFA for the mesophilic single stage reactor and 80-122 mgl^{-1} seen in the thermophilic single stage reactor (Chapter 7). The main component of the TVFA in the thermophilic UASB was propionic acid which formed over 50% of the TVFA by weight, an absolute value of 177 mgl^{-1} (SD=104, 24 samples). Thermophilic UASBs have a tendency to operate at higher levels of propionic acid even in apparently stable operation (Weigant *et al.* 1986). The latter authors found that a system consisting of two methanogenic stages was required to improve the anaerobic digestion of feedstock containing propionic acid. It appears that the obligate hydrogen-producing acetogens present under thermophilic conditions have a lower ability to metabolise propionic acid (Schimdt and Ahring, 1993). In the single stage UASBs studied previously, in Chapter 7, it was found that a thermophilic UASB operating on instant coffee waste waters had only slightly higher proportion of propionic acid, 32% of TVFA, (an absolute value of 36 mgl^{-1}) than the equivalent mesophilic systems 24% of TVFA (an absolute value of 6 mgl^{-1}).

The performance of the methanogenic reactors at steady state is summarised in Table. 8.4. Steady state was defined as where the TVFA levels remained at low levels. The removal of COD was found to be entirely due to the action of the methanogenic phase: no COD removal was seen in the acidification stage. The mesophilic UASB achieved good %TCOD removals ranging between 68% and 77% at all OLRs and HRT (See Table 8.4.). The COD removal was still high (77%) at the highest OLR (16 $kgCODm^{-3}d^{-1}$). The level of COD removal was comparable to the COD removal of 77% achieved in a mesophilic single stage system used in Chapter 7

but the two stage system could operate at a significantly higher OLR, 16 $kgCODm^{-3}d^{-1}$ compared to 10 $kgCODm^{-3}d^{-1}$. The COD removals of the two stage thermophilic UASB ranged between 63% and 68%. This level was slightly lower than the 68 to 77% seen in the mesophilic two-stage UASB and the 68 to 70% seen in the single stage thermophilic system described in Chapter 7. The slightly poorer COD removal in the thermophilic two stage system was reflected in the higher TVFA (210-241 mgl^{-1}) in the thermophilic single-stage (80-122mgl^{-1} TVFA). Both values of TVFA are above those found for mesophilic reactors, for the mesophilic single stage system (15-35 mgl^{-1}) or the mesophilic two stage system (7-20 mgl^{-1}). An increase in COD removal of 13% was reported by McDougall *et al.* (1993) by utilising a mesophilic two stage system instead of a single stage system. In this study similar %COD removals were seen in both the mesophilic two stage and single stage system.

High % methane and good gas production were seen in both the thermophilic and mesophilic two-stage system. The % methane in the mesophilic system ranged from 71 to 79%, and from 73-75% in the thermophilic system. These levels were greater than the 59-64% seen in the single-stage mesophilic and thermophilic system.

Most full-scale plants use a buffering tank to equalise effluent flows and strengths. Pre-acidification in this buffering tank, could be encouraged, although a potential disadvantage of using a buffering tank for pre-acidification is that high levels of VFA have a strong and offensive odour (Thacker and Evans, 1986). The present studies suggest that using pre-acidification a substantial increase in OLR and in biogas methane content can be achieved when compared with operation on unacidified feed, and that smaller methanogenic reactors could result.

8.3. Conclusions From Two Stage UASB operation

1. Thermophilic pre-acidification was maintained for more than 100 days with up to 37% pre-acidification being achieved.

2. Control of acidogenic reactors to pH 6.0 did not give better acidification than allowing the pH to float at pH 5 approximately.

3. Removal of pH control in the system saw an improvement in the operation of the thermophilic UASB. Levels of TVFA which had reached 800 mgl^{-1} fell to 200 mgl^{-1} 6 days after the pH controller was removed. Thermophilic methanogenic reactors may be more susceptible than mesophilic reactors to Na^{+} inhibition.

4. The mesophilic UASB achieved stable anaerobic digestion at OLR up to 16 $kgCODm^{-3}d^{-1}$ at an HRT of 12 hours with COD removals of up to 77% with TVFA levels of 7-20 mgl^{-1}. This was a significantly higher OLRs (60% higher) and shorter HRTs (100% shorter) than the equivalent single stage system.

5. The thermophilic UASB exhibited lower COD removals of and higher TVFA levels than either the two-stage or single-stage mesophilic UASB.

9. CONCLUSIONS.

The coffee factory's physical waste treatment systems and their resulting waste streams were identified. Samples of the wastewaters were analysed to determine the physical and chemical characteristics relevant to anaerobic digestion. The wastewater discharged from the factory was found to have high levels of coffee grounds (up to 27 gl^{-1}) as suspended solids), high lipid content (up to 33% of total solids) and a low pH (4.3-4.5). The wastewaters are discharged at high temperatures (68°C) so thermophilic digestion could be achieved without further heating being required .

Two wastewater types were chosen for treatment by anaerobic digestion, a wastewater stream with a high level of suspended solids and a wastewater with the majority of the suspended solids removed by one hour`s settling at room temperature.

The anaerobic digestion of waste with a high level of coffee grounds was studied in mesophilic and thermophilic batch studies and mesophilic and thermophilic CSTR reactors fed daily. Proximate compositional analysis showed that the coffee waste had a high lipid component (26 - 33%) with lower levels of hemicellulose (3.5 - 5.3%), α-cellulose (11.1 - 13.8%), lignin (9 - 11%), protein (1.7 - 1.1%) and ash (1.4 - 3.3%).

A 58% reduction in VS was seen in both mesophilic and thermophilic batch biodegradability tests. Greater lipid and hemicellulose degradation was observed in the mesophilic study, while α-cellulose was degraded equally at both operating temperatures. The lignin component was not reduced in either study.

Thermophilic digestion could be established at an organic loading rate (OLR) 1.6 $kgCODm^{-3}d^{-1}$ (20 day hydraulic retention times (HRT)) with the addition of sodium bicarbonate, or $Ca(OH)_2$, nitrogen, phosphorus and trace metals. However long term digestion could not be established beyond 50 days without an increase in total volatile fatty acid (TVFA) occurring. Mesophilic continuous digestion was achieved for 99 days with the addition of $Ca(OH)_2$, nitrogen phosphorus and trace metals at an OLR of 1.3 $kgCODm^{-3}d^{-1}$ (25 HRT). Addition of sodium bicarbonate alone was not sufficient for long term anaerobic digestion. A chemical oxygen demand (COD) and volatile solids (VS) removal of 60% was achieved.

The coffee waste low in suspended solids was treated in single stage mesophilic and thermophilic UASB reactors. Using the same UASBs the effect of thermophilic pre-acidification was also studied.

Stable anaerobic digestion of settled instant coffee wastewater was achieved for over 100 days in mesophilic (35°C) and thermophilic (55°C) UASB reactors. Thermophilic UASBs were seeded with mesophilic granules and converted to thermophilic operation by raising the temperature to 55°C in one step. Successful thermophilic operation was achieved within 28 days. Both mesophilic and thermophilic UASBs achieved stable digestion at OLRs of 5, 6.6 and 10 $kgCODm^{-3}d^{-1}$, corresponding to a HRT of 48, 36, 24 hours respectively. Higher OLRs to the mesophilic UASB of 11.4 and 13.3 $kgCODm^{-3}d^{-1}$ (21 and 18 hours HRT) resulted in reactor failure due to increasing TVFA levels. The thermophilic UASB achieved stable operation at a OLR of 11.4 $kgCODm^{-3}d^{-1}$ (21 hours HRT) but a OLR of 13.3 $kgCODm^{-3}d^{-1}$ (18 hours HRT) saw a rise in TVFA from $80mgl^{-1}$ to $600mgl^{-1}$. COD reduction in the thermophilic UASB was slightly lower at all OLRs, achieving a 70% COD reduction compared to a 78% COD reduction in the mesophilic UASB. The TVFA levels were low, but somewhat higher in the thermophilic UASB than the mesophilic UASB ($80mgl^{-1}$ compared to $25mgl^{-1}$). It is concluded that either mesophilic or thermophilic

digestion could be used successfully to treat settled instant coffee production wastewater in UASB reactors. Maximum OLRs in the system used here were 10kgCODm^{-3}d^{-1} for mesophilic operation and 11.4 kgCODm^{-3}d^{-1} for thermophilic operation.

The thermophilic and mesophilic UASB reactors used in single-stage operation were operated with thermophilic pre-acidification and studied over a period of more than 120 days. The thermophilic pre-acidification stage was operated with pH control or with 1.5gl^{-1} $NaHCO_3$ added to the feed, at retention times of 24, 18, 15 and 12 hours. Up to 38% of the total influent COD was converted to TVFA at a 24 hour HRT, dropping to 21% at a 12 hour HRT. The principal VFAs were *n*-butyric, acetic and propionic acid respectively. It was found that control with NaOH to pH 6.0 at an HRT of 24 hours was not required for efficient acidogenesis. The effluent from the acidogenic stage at pH 5.2 did not require prior neutralisation with NaOH before feeding to the methanogenic stage. The absence of neutralisation improved the performance of the thermophilic UASB reactor. Thermophilic digestion may be more sensitive to Na^+ toxicity than mesophilic digestion. The thermophilic/mesophilic two stage system gave a consistent improvement in performance over the thermophilic/thermophilic two stage system especially at higher organic loading rates. The thermophilic/mesophilic achieved greater COD removal (75%) and lower TVFA (11mgl^{-1}) than the 64% COD removal and 223 mgl^{-1} TVFA achieved by the thermophilic/thermophilic two stage system. Thermophilic pre-acidification gave an increase of 60% in the loading rate achievable to the mesophilic methanogenic stage and a 100% reduction in HRT compared with the single stage system.

Successful long term anaerobic digestion of instant coffee wastewater with and without coffee grounds was achieved with the addition of alkalinity and nutrients. Removing the grounds by settling resulted in a waste which could be treated at higher OLRs. The use of thermophilic pre-acidification further increased the OLR.

10. FUTURE WORK

10.1. Waste Containing Coffee Grounds

Successful anaerobic digestion of waste containing coffee grounds was achieved in a CSTR reactor at 1.3 $kgCODm^{-3}d^{-1}$ (25 day HRT) by using an acclimatised inoculum, nutrients and $CaOH_2$. The changes in composition and degree of VS destruction in the waste could be studied at shorter HRTs and higher OLRs. By performing these experiments a model of the anaerobic degradation of the coffee waste could be developed (Romero *et al.* 1988). This could allow sizing information for a full-scale plant to be derived i.e. the minimum HRT at which the coffee waste can be treated and how well the coffee waste can be digested at these HRTs. Although 58% of the VS in the coffee waste was converted to methane gas this still leaves large amount of waste to be disposed of. One option could be to sell the treated grounds as a plant compost. Kostenberg *et al.* (1994) have found that thermophilically digested coffee grounds when used as a compost, aid in plant growth and disease resistance. Investigation of the compost properties of mesophilically digested coffee grounds, if found to have similar properties as the thermophilically digested coffee grounds would be a positive selling point for a product which at the moment has a negative value.

The thermophilic digestion of waste containing coffee ground at a OLR 1.6 $kgCODm^{-3}d^{-1}$ (20 day HRT) was found not to be sustainable. Other techniques to develop a thermophilic culture could be attempted. It should be noted that Kostenberg and Marchaim (1993a, 1993b and 1994) who successfully digested coffee grounds thermophilically utilised a inoculum which was already able to operate thermophilically on cow manure. Using thermophilic inoculum could be a successful way of seeding a digester to treat the coffee waste. Problems were experienced with the lipid content of the waste, two-stage digestion is one way of

overcoming problems of inhibition with lipid containing wastes. Two-stage digestion could not only be used to establish thermophilic digestion but to improve the stability of mesophilic digestion.

10.2. Waste Containing Low Levels Of Coffee Grounds

Both single-stage mesophilic and thermophilic UASBs achieved stable digestion at OLR of 10 $kgCODm^{-3}d^{-1}$, corresponding to HRT of 24 hours. The thermophilic UASB achieved stable operation at an OLR of 11.4 $kgCODm^{-3}d^{-1}$(21 hours HRT). The mesophilic UASB achieved stable operation at an OLR of 16 $kgCODm^{-3}d^{-1}$(12 hours HRT) by utilising thermophilic pre-acidification. The limit of UASB or acidification reactor operation regarding the minimum level of HRT or maximum OLR was not studied. This work could be continued to determine at which level of OLR and HRT the reactors began to fail. This information could then possibly be used to size a full scale plant.

The effluent after anaerobic treatment still has a strong brown colour. Other treatment options and combination of treatment options such as aerobic polishing or advanced oxidation systems could be utilised to reduce the colour and the residual COD.

The pre-acidification system could be examined further. For example other products of the acidogenic stage such as gaseous hydrogen, biomass, organic acids and alcohols could be measured. Changes to specific components of the coffee waste such as lipids to LCFA need to be examined since LCFA is more toxic but more biodegradable in the methanogenic stage.

Other types of acidogenic reactors such as UASBs could be compared with the CSTR type. The UASB acidogenic reactor may have advantages in biomass retention or reduction in biomass production compared to the CSTR (Zoetemeyer *et al.*, 1980).

APPENDIX

1. Total Solids

Total solids (TS) were determined by drying to constant weight a known volume of sample at 105±2^{0}C (APHA, 1989). The residual mass is defined as the total solids of the sample. A 100 ml Pyrex beaker was placed in a "Eurotherm" electric furnace (Carbolite Ltd., Sheffield) for at least 30 minutes at 500±50^{0}C. The beaker was then cooled in a desiccator and the beaker mass determined with a 4 decimal point balance. A 20 ml volume of well mixed sample was placed in this pre-weighed beaker and then dried to constant weight in a Hot Box convection oven (Grant Instruments Ltd., Cambridge). For some total solids determinations the samples were dried in a Hitachi microwave set at low power. When 10 samples were compared using this method and the standard method the Students T-Test at 5% confidence limits showed no significant difference in the two methods. The total solids were calculated as in equation 9.

$$\text{Total solids } (gl^{-1}) = (A - B) * \frac{1000}{\text{sample volume}} \quad (9)$$

where : A = mass of beaker and total solids

B = mass of beaker

In 5 replicates of a typical sample an average of 10.47 gl^{-1} TS (Standard deviation =0.16) was achieved.

2. Volatile Solids

The level of volatile solids was determined by incinerating at 500±50^{0}C to constant weight a sample which had undergone total solids analysis as described in **4.1.2.** (APHA, 1989). After the total solids determination the samples were placed in a "Eurotherm" electric furnace (Carbolite Ltd., Sheffield) for at least 30 minutes at 500±50^{0}C. The beakers were then cooled in desiccator and weighed using a four decimal place balance to determine the residual sample mass (non-volatile solids or fixed solids). The solids "lost" were designated "volatile solids" although some structural water is probably lost at 500^{0}C. The volatile solids was calculated by taking away the mass of the fixed solids from the total solids value. The fixed solids were calculated as in equation 10.

$$\text{Fixed solids } (gl^{-1}) = (A - B) \quad * \quad \frac{1000}{\text{sample volume}} \qquad (10)$$

where : A = mass of beaker and fixed solids

B = mass of beaker

In 5 replicates of a typical sample an average of 10.22 gl^{-1} VS (Standard deviation =0.14) was achieved.

3. Suspended Solids

Suspended solids were determined as described in Standard Methods (APHA, 1989). Suspended solids is the sample component which cannot pass through a Whatman G/FC filter paper. A 70 mm Whatman G/FC filter paper was placed in a 100ml Pyrex

beaker and then placed in a "Eurotherm" electric furnace (Carbolite Ltd., Sheffield) for at least 30 minutes at 500±50^{0}C. The filter and watch glass was then cooled in a desiccator and weighed with a 4 decimal point balance. A known volume of sample was then pipetted on to the filter paper held in Hoffman filter unit. A positive pressure was maintained with the use of a Buchner flask and a water vacuum pump. The filter paper was then washed with 3 deionised water aliquots of 3 times the initial volume of sample. The filter paper was then placed in the 100 ml beaker and dried to constant weight in a Hot Box convection oven at 105±2^{0}C (Grant Instruments Ltd., Cambridge) or in a Hitachi microwave set at low power.

$$\text{Suspended solids } (gl^{-1}) = (A - B)^* \frac{1000}{\text{sample volume}} \qquad (11)$$

where : A = mass of beaker, suspended solids and filter

B = mass of beaker and filter

In 5 replicates of a typical sample an average of 3.68 gl^{-1} VS (standard deviation =0.20) was achieved.

4. Volatile Suspended Solids

A sample which had undergone suspended solids analysis as described in **3** was incinerated at 500±50^{0}C to constant weight (APHA, 1989). The residual mass on the filter paper is the fixed suspended solids or the non volatile suspended solids. The volatile suspended solids was calculated by subtracting the fixed suspended solids from the suspended solids value. The fixed suspended solids were determined as equation 12.

$$\text{Fixed Suspended solids } (gl^{-1}) = (A - B) * \frac{1000}{\text{sample volume}} \quad (12)$$

where : A = mass of beaker, fixed suspended solids and filter.

B = mass of beaker and filter.

5. Settleable and Non-settleable Solids

Settleable and non-settleable solids were determined according to Standard Methods, APHA, (1989). A sample was placed in a 1 litre beaker and allowed to stand at room temperature for 1 hour. A 100 ml sample was siphoned from the 500ml beaker graduation point. Aliquots of this sample was then analysed to determine the suspended solids level as in Section **4.1.4.** This value was defined as the non-settleable solids i.e. the sample solids which do not settle after 1 hours quiescence. Subtracting this value from the suspended solids level of the well mixed sample gives the settleable solids value.

6. Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) was determined using the sealed tube method and mercury free reagents as described in Standard Methods (HMSO, 1986). The COD is a measure of the oxygen demand of a sample when oxidised by a strong chemical oxidant. In standard methods for determining COD, the oxidant is a standard sulphuric acid, potassium dichromate and silver sulphate mix (HMSO, 1986, APHA, 1989).

A 5 ml aliquot of proprietary COD reagent (Ficodox plus, Fisons Ltd., Loughborough) was placed in a PTFE lined screw top Pyrex incubation tube (Fisons Ltd., Loughborough). This proprietary COD mixture, as well as the oxidising agents contains chromium (III) potassium sulphate instead of mercuric sulphate to suppress possible chloride interference. The chromium (III) potassium sulphate has disposal and safety benefits over mercuric sulphate (HMSO, 1986). Then 2.5 ml of sample was added to the COD reagent, the incubation tube sealed and inverted to mix the sample and COD reagent. As well as sample incubation tubes, a set of tubes which contain 2.5 ml of deionised water to act as a "blank" for the COD test were also prepared. The tubes were then heated at 150±5°C for 2 hours in a DriBlock heating block (Techne Ltd., Cambridge, UK) placed in a fume cupboard. The tubes were then cooled and the residual dichromate determined by titration.

The contents of a tube were emptied into a conical flask containing three drops of 1,10-phenanthroline-ferrous sulphate (ferroin). The tube was then rinsed 3 times by filling to the top with deionised water and then emptying the rinse water into the conical flask. This solution was then titrated with 0.0122 M ferrous ammonium sulphate (FAS) until the orange endpoint was reached. The titration volume was then used to calculate the COD value from equation 13.

$$\text{COD } mgO_2l^{-1} = \frac{(A - B) \times M \times 8000}{\text{sample size}} \times \text{dilution factor} \qquad (13)$$

where :A = ml FAS used for blank

B = ml FAS used for sample

M = molarity of FAS

The COD determination is most accurate when approximately 50% of the dichromate is reduced. This occurs when the wastewater sample contains 200 mg COD l^{-1}, if the wastewater sample is of greater COD strength then it must be diluted accordingly to approach the ideal COD strength.

A number of different COD parameters were determined depending on the sample pre-treatment.

Total COD = analysed from well mixed sample.

Settled COD = analysed from sample left to stand in a 250 ml beaker at room temperature for one hour. Sample taken from 100 ml mark on beaker.

Filtered COD = analysed from filtrate after filtering with GF/C filter.

The FAS was made up in 5 litre batches by weighing out 24.5 g of Analar ferrous ammonium sulphate (Fisons Ltd., Loughborough). This was then dissolved in 1 litre of deionised water, 50 mls of concentrated H_2SO_4 added and then made up to 5 litres with deionised water. A standard 0.0417 M $K_2Cr_2O_7$ solution was made up by dissolving 12.259 g of Analar $K_2Cr_2O_7$ and then making up to 1 litre with deionised water. The FAS was then standardised by titrating against a standard $K_2Cr_2O_7$solution to the orange end point. This was prepared by adding 2.5ml 0.0417 M $K_2Cr_2O_7$ to 25

ml deionised water, 7 ml conc. H_2SO_4 and 3 drops of "ferroin" in a conical flask. The FAS molarity was calculated from Equation 14.

$$\text{Molarity of FAS} = \frac{\text{volume of 0.0417 M } K_2Cr_2O_7 \text{ (ml)}}{\text{volume of FAS used in titration (ml)}} \times 0.25 \qquad (14)$$

A sample of standard potassium phthalate solution (theoretical COD of 2000mgCODl^{-1}) analysed in a number of duplicate analyses gave a mean COD of 1920 mg COD l^{-1} (SD=40). The test value of 1920 mg COD l^{-1} was 96 % of the expected value.

7. pH

The pH (hydrogen ion activity) was determined by electrometeric measurement Standard Methods (APHA, 1989). A Kent instruments EIL1940 pH meter utilising temperature compensation with glass combination electrode was used (ABB Kent-Taylor Ltd, Stonehouse, Gloucestershire). The pH meter was calibrated daily before use with standard buffer solutions of pH 4.0 and pH 9.2 (Fisons Ltd., Loughborough). A 25 ml sample was agitated by the use of a magnetic stirrer to establish equilibrium between sample and electrodes. The analysis was performed within one minute so that minimum interference occurred from the loss of carbon dioxide.

In a triplicate analysis of reactor effluent a mean pH of 7.71 (SD= 0.08) was found.

8. Bicarbonate Alkalinity

Bicarbonate alkalinity was determined by titration as in Jenkins *et al.* (1983). This method differs from the Standard Methods (APHA, 1989) determination of bicarbonate alkalinity as a titration endpoint of pH 5.75 is used instead of a endpoint of pH 4.3. A compensation factor is required to compensate for the higher pH endpoint. However the higher pH endpoint ensures there is no or little interference from the buffering capacity of volatile fatty acids (Jenkins *et al.*, 1983).

A 25 ml sample was placed in a 100 ml beaker containing a PTFE coated stirring bar. The beaker was then placed on a magnetic stirrer to ensure good mixing. The pH of the sample was continuously monitored using a Kent Instruments EIL1940 pH meter utilising temperature compensation with glass combination electrode. The pH meter had been calibrated as in section **4.1.8**. The sample was then titrated with 0.05 M HCl from a self filling burette. The sample was titrated until the endpoint of pH 5.75 had been overshot by 0.02 to 0.05 pH units. The "true bicarbonate alkalinity" (TBA) was then calculated from the following equation :

$$TBA_{5.75}\ mgCaCO_3l^{-1} = 1.25 \times ALK_{5.75} \qquad (15)$$

where $ALK_{5.75} = \frac{\text{ml standard HCl titrated} * \text{M HCl} * 50\,000}{\text{ml sample}}$

In a triplicate set of coffee digester effluent bicarbonate analyses a mean of 2040 $mgCaCO_3l^{-1}$ (SD =50) was found.

9. Gas Composition

The methane and carbon dioxide content of the biogas was determined by GC (gas chromatography) as described in Peck *et al.* (1986). Either a Varian 6000 or a Varian 3400 GC (Varian Ltd, Walton-upon-Thames, UK) fitted with a TCD (thermal conductivity detector) and a 2 feet stainless steel column packed with Porapak Q 80-100 (Supelco Ltd, Poole, Dorset, UK) was used. The injector temperature was set at 70°C, the column was maintained at 60°C and the detector at 200°C. Chromatography grade helium (MG gas Products, Cardiff) at a flow rate of 30 ml min^{-1} was used as the carrier gas. A sample of gas was taken by drawing slowly, approximately 15 ml^3 of gas into a 25 ml gas tight syringe fitted with a 3 way valve. To ensure a representative sample the first sample drawn was returned to the reactor and the second sample drawn was analysed. The sample was then injected into the GC sample loop and then the loop contents was injected onto the column by a manually operated valve. The carbon and methane percentages were then calculated by either a Varian 401 Datastation or Viglen PC running Varianstar software from the integration of the peak area. Using a 60:40 % mix of a methane and carbon dioxide (BOC Gases, Guildford) a single point calibration of the GC was performed daily prior to use.

In 5 samples of the standard gas mixture a mean of 60% methane (SD=1) was found.

10. Volatile Fatty Acid Analysis

Individual volatile fatty acid levels (C_2-C_5) were determined by GC fitted with FID after extracting the VFA with diethyl ether from an acidified sample as described by

Peck *et al.* (1986). The advantage of the diethyl ether extract method is that column fouling from components in the waste is reduced.

The VFAs were extracted by placing a 5ml aliquot of sample in a 12.5 mm screw capped incubation tube (Fisons Ltd., Loughborough). The sample was then acidified to below pH 2 by the addition of 0.75 mls of 50% Analar phosphoric acid. Then 5mls of Chromatography Grade diethyl ether containing 0.1 ml l^{-1} *i*-caproic acid was added to the tube contents. The *i*-caproic acid acted as a internal standard for the GC analysis. The tube was then sealed and inverted 10 times to extract the VFA in to the ether. Pressure from the tube was then released and resealed The tube was inverted a further 10 times and left to stand for 3-5 minutes. In most aqueous samples this results in a distinct ether layer appearing. However in some coffee samples, centrifugation for a few seconds at 3000 rpm was required. A portion of the diethyl ether layer (upper layer) was pipetted into a 2.5 ml Varian autosampler vial and then sealed with a PTFE covered septum and lid.

The VFA concentrations were determined using a Varian 6000 or a Varian 3400 GC (Varian Ltd, Walton-upon-Thames, UK) fitted with a FID (flame ionisation detector). A 1.5 µl sample was injected onto a 6ft (4mm ID) glass column packed with 15% SP1220/1%H_3PO_4 on 100/120 Chromosorb W/AW (Supelco, Poole, Dorset). The injector was held at 135 C and the detector at 155 C. The column was initially held at 115 C for 5 minutes, a temperature ramp was then initiated to increase the column temperature to 130 C at a rate of 10 C min^{-1} the column was maintained at 130 C until the end of the run at 16 minutes. The nitrogen carrier gas was maintained at a

flow rate of 30 ml min^{-1}, the air and hydrogen gases at a rate of 300 ml^{3} min^{-1} and 25 ml min^{-1} respectively to the FID.

The autoinjector was controlled, the VFA peak areas integrated and VFA concentration calculated by the Varian 401 datastation or Viglen PC running Varianstar software. The Varian 401 and Viglen PC was calibrated with a standard VFA mix prior to analysing each batch of samples.

The Varian 401 was calibrated with a single point calibration of the VFA mix. This mix contained 100 mg l^{-1} acetic, 40 mg l^{-1} propionic, 20 mg l^{-1} of *i* and *n*- butryic and 10 mg l^{-1} of *i*-valeric and *n*-valeric. The Viglen PC running Varianstar software was calibrated with a 4 point calibration using a 1000 mg l^{-1} acetic, 400 mg l^{-1} propionic, 200 mg l^{-1} of *i* and *n*- butyric and 100 mg l^{-1} of *i*-valeric and *n*-valeric. This mixture was diluted 75%, 50% and 10% with deionised water to provide the calibration standards. The initial calibration standard was made up from Analar VFA standards in 5 litre flask with deionised water.

A Varian 8000 autosampler was used to inject the sample on the column, as part of the autosampler program a 2 µl aliquot of 10% formic acid/90% diethyl ether was injected on to the column between each sample to prevent "ghosting".

11. Total Lipid Determination

Total lipid was determined gravimetrically after chloroform/methanol extraction (Bligh and Dyer, 1959). To prevent oxidation of the extract an antioxidant, BHQ (Butyrated Hydroxy Quinoline) was used as well as evaporation of the chloroform with N_2 was employed. The BHQ was added to the chloroform used in the assay to produce a concentration of 5 mg l^{-1} BHQ. All apparatus used in the assay was washed with chloroform to remove any lipid before coming into contact with the extract.

The extraction procedure was as follows, a 4 ml aliquot of sample was placed in a boiling tube with 10 ml of Analar grade methanol and 5 ml of chloroform. The tube contents were then homogenise for 30 seconds with a Whirli mixer. A further 5 ml of chloroform and then 5 ml of deionised water was added. The sample was homogenised for 30 seconds after each addition using the Whirlimixer.

The sample was then filtered using a Buchner funnel fitted with a Whatman G/FC filter to remove the particulate material. The liquid was then transferred to a 250 ml separating funnel. The filter paper under went the extraction procedure as above and the liquid added to the liquid already in the separating funnel. The lower chloroform layer was then placed in to a preweighed beaker. The majority of the chloroform was evaporated by bubbling GC grade N_2 through the chloroform layer. Any residual chloroform was allowed to a evaporate in a fume cupboard over night. The beaker was then dried for 15 minutes in a microwave oven set at defrost setting. The mass of lipid was calculated as in equation 16.

$$\text{Total Lipid } (gl^{-1}) = (A - B) * \frac{1000}{\text{sample volume}} \quad (16)$$

where : A = mass of beaker and lipid solids

B = mass of beaker

A set of four analyses showed an average Total Lipid of 7.08 gl^{-1} (SD=0.16).

12. Holocellulose (Hemicellulose and α-Cellulose) Determination

The amount of hemicellulose and α-cellulose was determined as in a method described by Allen (1974). The first step in the analysis was the delignification stage. A 30 ml sample containing no more than 0.5 gl^{-1} TS was placed in a boiling tube. To the tube was added 0.3 g of GPR grade sodium chlorite and 1 ml of 10 % v/v analar acetic acid. The tube contents were then mixed with a Whirli mixer for 30 seconds and tube was then placed in a water bath maintained at 75 ±1°C for four hours. At 1, 2 and 3 hour intervals further aliquots of sodium chlorite and acetic acid were added. Any water loss due to evaporation was made up with deionised water. After 4 hours the tube and contents were cooled in iced water. After cooling the tube was placed in the centrifuge at 4000 rpm for 15 minutes. The supernatant was then poured off leaving the pellet at the bottom of the boiling tube. The pellet was then washed with deionised water by resuspending the pellet with a Whirlimixer and 30 ml of deionised water and centrifuging at 4000 rpm for 15 minutes. This washing procedure was then repeated with deionised water (once), Analar grade acetone (twice) and analar diethyl ether (once). The total solids and volatile solids content of the pellet were then determined as in Section **4.1.2.** and **4.1.3.**. Prior to total solids

determination, care was taken to ensure that all the diethyl ether had evaporated from the holocellulose pellet. The volatile solids of the holocellulose pellet was deemed the holocellulose component of the coffee waste ie α-cellulose + other polysaccharides (hemicellulose), (Allen, 1974).

To determine the level of α-cellulose in the holocellulose, a sample which had undergone the analysis for holocellulose was treated with potassium hydroxide to break down hemicellulose leaving the α-cellulose (Allen, 1974). The holocelluolose pellet was incubated in a water bath at 20±1°C with 20 ml of 24 %w/v analar potassium hydroxide in a boiling tube for two hours. After 2 hours the breakdown products were removed by centrifuging at 4000rpm and pouring off the supernatant. This pellet was the washed by resuspension and centrifuging at 4000 rpm, first with deionised water (twice), acetone (twice) and diethyl ether (once). After allowing the ether to evaporate the total solids and volatile solids of the α-cellulose pellet was determined as in section **4.1.2.** and **4.1.3.**. The hemicellulose was derived by subtracting the α-cellulose value from the holocellulose value.

13. Lignin Determination

The lignin content of a sample was determined gravimetrically after removing all other organic compounds (Allen, 1974).

To a pre-weighed bag made from 15 µm Nytal fabric (Sericol Industrial Fabrics Ltd., Broadstairs, Kent) approximately 1 g of sample was added and then weighed. This was then placed in a extraction thimble and a Soxhlet extraction performed for 6

hours using petroleum ether as the solvent. This extraction removed the lipid and other solvent soluble components. After extraction the sample was left overnight in a fume cupboard while the ether evaporated. The bag contents were then placed in a tall 1 litre Pyrex beaker with 400 mls of deionised water and boiled gently to remove the soluble carbohydrates. Further aliquots of deionised were added to replace any water lost by evaporation. After 3 hours of gentle boiling, 22 ml of 10% H_2SO_4 was added and the sample boiled for a further hour to remove the protein component. After settling the supernatant was removed with a No. 3 sintered filter stick (Fisons Ltd., Loughborough). The settled solids were then transferred with the sintered filter to a 250 ml beaker. Deionised water was used to rinse residual solids into the 250 ml beaker. The solids and the sinterstick were dried overnight. The holocellulose component was then hydrolysed by the addition of 72% and 3% acid. To this residue in the 250 ml beaker 15 ml of 72 % H_2SO_4 was added. This was incubated at 18±1°C for two hours with occasional stirring with the sintered filter stick. After two hours the 250 ml beaker contents were washed in to a tall 1 litre Pyrex beaker with 560ml of deionised water. The addition of the water effectively reduced the acid strength to 3%. The sintered filter stick was then washed by using the stick to stir the 1 litre beaker contents, the filter stick was then discarded. The beaker was then boiled gently for 4 hours with additions of deionised water to maintain the liquid volume. The contents were then allowed to cool for 15 minutes. The beaker contents were filtered through a preweighed No.3 sintered glass crucible. Hot water used to rinse out any remaining beaker contents was utilised to wash through the sintered crucible to remove any residual acid. The sintered crucible was then dried at 105±2°C to determine the dry weight of the extract and subsequently at 500±50°C to determine

the ash content of the extract. The volatile solids fraction of the extract was deemed to be the lignin component of the sample.

14. Soluble Tannin and Lignin Determination

The level of soluble tannin and lignin was analysed according to the method described by the APHA, (1989). This is a spectrophotomic method based on the Folin-Ciocalteau reaction and registers all hydroxylated aromatic compounds including tannin, lignin, phenol and cresol. The reagents used were those supplied in the Hach Tannin and Lignin Test kit (Camlab Ltd., Cambridge).

A sample was filtered with a GFC/C filter paper to remove any particulate matter. The filtrate was then diluted with deionised water such that the anlaysis method did not overshoot. To 25 ml of sample in a Hach Spectrophotometer cell was added 0.5 ml of Hach TanniVer3 Tannin and lignin reagent (Camlab Ltd., Cambridge) and 5 ml of sodium carbonate solution (Camlab Ltd., Cambridge). The cell was then swirled to mix the reagents and allowed to stand for 25 minutes for the reaction to take place. The level of tannin and lignin in the sample was then determined by using the Hach DR/2000 (Camlab, Cambridge) spectrophotometer set at method 720 and the light wavelength at 720 nm. The level of tannin and lignin (mgl^{-1}) was the adjusted for the dilution factor.

15. Ammonia Nitrogen Determination

Ammonia nitrogen was determined by distillation and titration as in Standard Methods (APHA, 1989). A sample of waste was placed in a 1 litre Pyrex Quick fit round bottom flask with 400 ml deionised water. This solution was then made alkali

(>pH 9) by the addition of 10 ml of concentrated Analar NaOH solution (240 gl^{-1} NaOH). The round bottom flask was connected to a distillation apparatus consisting of a condenser, splash head and delivery adapter. The delivery adapter was placed in a 250 ml conical flask containing 50 ml of indicating boric acid solution. The indicating boric acid solution was made by dissolving 20 g Analar H_3BO_3 in deionised water, adding 10 ml of mixed indicator solution and making up to 1 litre with deionised water. The indicator solution was made by combining the following: 200 mg of methyl red indicator dissolved in 100 ml of 95% ethyl alcohol and 100 mg of methylene blue dissolved in 50 ml 95% ethyl alcohol.

The delivery adapter was positioned such that the boric acid solution covered the bottom of delivery adapter. The round bottom flask was heated until 200 ml of its contents had been distilled over into the conical flask. The presence of ammonia caused a colour change in the indicating boric acid solution which turned the from blue to green. To determine the amount of ammonia this solution was titrated with 0.01 M H_2SO_4 until the colour changed from green to blue. The amount of ammonia was calculated as in equation 17.

$$\text{Ammonia (mg N } l^{-1}) = \frac{(A - B) * 0.28 * 1000}{\text{sample volume (ml)}} \qquad (17)$$

where : A = sample titre ml H_2SO_4

B = blank titre of deionised water ml H_2SO_4

0.28 mg N equivalent to 1 ml 0.01M H_2SO_4

16. Total Kjeldahl Nitrogen Determination

Kjeldahl nitrogen was determined using a mercury free method with a copper catalyst, (HMSO, 1986), the subsequent ammonia produced was determined by distillation and titration, (APHA, 1989). Organic nitrogen in the tri-negative state is broken down in to ammonia by digesting the sample with acid and a catalyst at elevated temperatures. A copper based catalyst was used consisting of 600 g anhydrous sodium sulphate and 18 g copper sulphate pentahydrate.

A sample of waste was placed in a digestion tube with 6.5 ml of 90% ethanol, 0.5 g of catalyst and 6 ml of concentrated sulphuric acid. The digestion tube contents were then mixed and gently heated to around 200°C for 15 minutes to drive off the water. The temperature was then increased to around 370°C so that the sulphuric acid refluxed in the digestion tube. The refluxing was continued for 45 minutes. After the digest had cooled, the contents were rinsed into a 1litre round bottom flask with 400 ml deionised water. The pH of the flask contents were then adjusted to greater than pH 9 by adding concentrated NaOH (240 gl^{-1} NaOH). The ammonia determination was then completed as in Section **4.1.18**.

11. REFERENCES

Ahring B.K., Alatriste-Mondragon F., Westermann P. and Mah R.A. (1991) Effect of cations on *Methanosrcina thermophila* TM-1 growing on moderate concentrations: production of single cells. *Appl. Microbiol Biotechnol.* 35:686-689.

Alexiou, I.E. and Anderson, G.K. (1994). Pre-acidification: Concepts, guidelines and parameters for application. Proc. 7th Int. Symp. on Anaerobic Digestion, Cape Town, South Africa. p668-677.

Allen, S.G. (1974) *Chemical Analysis of Ecological Materials.* Blackwell, Oxford, UK.

APHA. (1989) *Standard Methods for the Examination of Waste and Wastewater.* (17th Ed.). Washington, USA.

Angelidaki, I., Petersen, S.P., and Ahring, B.K., (1990) Effect of lipids on thermophilic digestion and reduction of lipid inhibition upon the addition of bentonite. *Appl. Microbiol. Biotechnol.* ***33,*** 469-472.

Beaubien, A., Jolicoeur, C., and Alary, J.F. (1988) Automated high sensitivity gas metering for biological processes. *Biotechnol. and Bioeng.****32***, 105-109.

Bligh, E. G. and Dyer, N. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911-917.

Box, J.D. (1983). Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Wat. Res.* ***17*** (5), 511-525.

Bull, M. A, Sterritt, R. M. and Lester, J. N. (1984). An evaluation of single and separated-phase anaerobic industrial wastewater treatment in fluidized bed reactors. *Biotech. Bioeng*. **26,** 1054-1065.

Buhr, H.O and Andrews, J.F. (1977). The thermophilic anaerobic digestion process. *Wat. Res.* **11**,129-143.

Caine, M., Anderson, G.K., Donnelly, T. (1991). A study into the effect of a series of shocks on a pilot-scale anaerobic filter.*45th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers Inc., Michigan, pp. 451-463.

Calzada J.F., de Leon, O.R., de Arriola M.C. ,Rolz, C., de Leon, R., and Menchu, J.F. (1981). Biogas production from coffee pulp juice. *Biotechnology Letters* **3**, (12) 713-716.

Calzada, J.F., de Porres E., Yurrita, A., de Arriola, M.C. de Micheo, F.,Rolz, C., Menchu, J.F.and Cabello, A. (1984) Biogas production from coffee pulp juice: One and Two-Phase Systems. *Agricultural Wastes* **9**, 217-230.

Calzada, J.F., R.A. Garcia, A., Porres, C.A., Rolz, C.E. (1989). Integrated utilization of coffee processing by-products and wastes. In: International Biosystems Wise, D.L (Ed).Vol. 2 pp. 41-51.

Clark, R.J., and Macrae, R. (1985) Coffee Volume 1. Chemistry. Elsevier Publishers, London and New York.

Cohen, A., Breure, A.M., van Handel, J.G., van Deursen, A. (1980). Influence of phase separation on the anaerobic digestion of glucose-I. Maximum COD turnover rate during continuous operation. *Wat. Res.* **14** (9), 1439-1448.

Cohen, A., Breure, A.M., van Handel, J.G., van Deursen, A. (1982) Influence of phase separation on the anaerobic digestion of glucose-II. Stability, and kinetic responses to shock loadings. *Wat. Res.* **16** (4), 449-455.

Cohen, A., Breure, A.M., Schmeddling, D.J.M., Zoetemeyer, R.J. and van Handel, J.G. (1985). Significance of partial pre-acidification of glucose for methanogenesis in an anaerobic digestion process. *Appl. Microbiol. Biotechnol.* **21**, 404-408.

Conrad, R., Phelps, T.J. and Zeikus, J.G. (1985). Gas metabolism evidence in support of the juxtaposition of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments. *Appl. and Environ. Microbiol.* **50,** 595-601.

Council of European Communities (1991). Directive concerning urban waste water treatment (91/271/EEC). Official journal L135/40, 30 May 1991.

Danish Energy Agency (1992). Update on Centralized Biogas Plants. Biomass section of Danish Energy Agency, Copenhagen.

Dinopoulou, G. and Lester, J.N. (1989). Optimization of a two-phase anaerobic system treating a complex wastewater. *Environmental Technology Letters.* **10**, 799-814.

Disley, R.S., Walmsley, M.J. and Forster, C.F. (1992). Inhibition of gas production by thermophilic anaerobic sludges:The effect of organic compounds. *Environmental Technology,* **13**, 1153-1159.

Eastman, J.A. and Ferguson, J.F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. Wat. Pollut. Contr. Fed.* **53**, 352-366.

Elefsiniotis, P. and Oldham, W.K. (1994). Influence of pH on the acid-phase anaerobic digestion of primary sludge. *J. Chem. Tech. Biotechnol.* **60**, 89-96.

Fang, H. H. P. and Chui, H. K. (1993). Maximum COD loading capacity in UASB reactors at 37°C . *Envir. Engrg.,* ASCE, **119** (1) 103-119.

Fang, H.H.P. and Lau, I.W.C. (1996). Startup of thermophilic (50°C) UASB reactors using different mesophilic seed sludges. *Wat. Sci. Tech.* **34**, (5-6) 445-452.

Fernandez, N. and Forster, C.F. (1993a). A study of the operation of mesophilic and thermophilic anaerobic filters treating a synthetic coffee waste. *Bioresource Technology* **45,** 223-227.

Fernandez, N. and Forster C.F. (1993b). The anaerobic digestion of a simulated coffee waste using thermophilic and mesophilic upflow filters. *Trans IChemE* **72** Part B, 15-20.

Fernandez, N. and Forster C.F. (1994). A comparative examination of the start-up of a mesophilic and thermophilic anaerobic filter treating a synthetic coffee. waste. *Rev. Tec. Ing. Univ. Zulia* **17** (2),59-66.

Ghosh, S., Ombreght, J.P. and Pipyn, P. (1984). Methane production from industrial wastes by two-phase anaerobic digestion. *Wat. Res.* **19** (9), 1083-1088.

Ghosh, S. (1991). Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge. *Wat. Sci. Tech.* **23** ,1179-1188.

Goodwin, J. A. S., Wase, D. A. J. and Forster C. F. (1992). Pre-granulated seeds for UASB reactors: How necessary are they ? *Bioresource Technology* **41,** 71-79.

Gornall, L.K., (1991). Making money out of muck. In *ETSU Anaerobic Digestion Seminar,* (Edited by Richards, G.E., Mistry, P.), 91-113, Harwell Laboratories, Harwell, UK.

Gornall, L.K. and Rippey, B. (1996). Corrosion resistant engine for odour control with electricity generation. *Wat. Res.* **30** (2) 351-356.

Gujer, W. and Zehnder, A.J.B. (1983). Conversion processes in anaerobic digestion. *Wat. Sci. Tech.* ***15***, 127-167.

Guwy, A.J. (1995). Monitoring the stability of anaerobic digestion using a novel on-line bicarbonate alkalinity monitor. PhD Thesis, University of Glamorgan.

Guwy, A. J., Hawkes, D. L. and Hawkes, F. R. (1995). On-line low flow high-precision gas metering systems. *Wat. Res.* **29** (3),977-979.

Graef, S.P. and Andrews, J.P. (1974). Stability and control of anaerobic digestion. *J. Wat. Pollut. Control Fed.* ***46*** (4), 666-683.

Hajipakkos, C. (1992). The application of a full-scale UASB plant for the treatment of coffee waste. *Wat. Sci. Tech.* ***25*** (1), 17-22.

Hanaki, K., Matsuo T. and Nagase, M. (1981). Mechanism of inhibition caused by long chain fatty acids in anaerobic process. *Biotech. Bioeng.* **23,** 1591-1610.

Hanaki, K., Matsuo, T., Nagase, M. and Tabata, Y. (1987). Evaluation of effectiveness of two-phase anaerobic digestion process degrading complex substrate. *Wat. Sci. Tech.* **19**, 311-322.

Harper, S.R. and Pohland, F.G. (1986). Recent developments in hydrogen management during anaerobic biological wastewater treatment. *Biotech. Bioeng.* **28,** 585-602.

Harris, W.L. and Dague, R.R.(1993). Comparative performance of anaerobic filters at mesophilic and thermophilic temperatures. *Water Environment Research* **55** (5) 764-771.

Hawkes, F.R and Hawkes, D.L. (1994). Priorities in the anaerobic digestion of food industry wastewaters. In Biogas Technolgy as an Environmental Solution to Pollution. Ed. Marchaim, U., and Ney, G. FAO, Rome.

Hawkes, F.R., Donnelly, T. and Anderson, G.K. (1995). Comparative performance of anaerobic digesters operating on ice cream waste waters. *Wat. Res.* **29** (2),525-534.

Hickey, R.F., Wu, W.M., Veiga, M.C. and Jones, R. (1991). Start-up, operation, monitoring and control of high-rate anaerobic treatment systems. *Wat. Sci. Tech.* **8**, 207-255.

HMSO, (1985). Total Nitrogen and Total Phosphorous in Sewage sludge. HMSO, London, UK.

HMSO, (1986). Chemical Oxygen Demand (Dichromate Value) Of Polluted And Wastewaters, London, UK.

HMSO, (1988). The Assessment of Biodegradabilty in Anaerobic Digesting Sludge, HMSO, London.

Jenkins, S.R., Morgan, J.M. and Sawyer, C.L. (1983). Measuring anaerobic sludge digestion and growth by a simple alklimetric titration. *J. Wat. Pollut. Control Fed.* ***55,*** 448-453.

Jerger, D. E. and Tsao, G. T., (1987) Feed Composition. In *Anaerobic Digestion of Biomass* Ed Chynoweth, D. P. and Isaacson, R., Chap 5, Elsevier Applied Science Publishers, Barking, UK.

Kaiser, S.K. and Dague, R.R. (1994). The temperature-phased anaerobic biofilter process. *Wat. Sci. Tech.* **29** (9) 213-223.

Kida, K., Ikbal and Sonada, Y. (1992). Treatment of coffee waste water by slurry state anaerobic digestion. *Journal of Fermentation and Bioengineering.* ***73*** (5), 390-395.

Kinnerlsey, D. (1994) Coming Clean: The politics of water and the environment. Penguin Books, London.

Komatsu, T., Hanaki, K., and Matsuo, T. (1991) Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Wat. Sci. Tech.* ***23***, 1189-1200.

Kostenberg, D. and Marchaim, U. (1993a) Solid waste from the instant coffee industry as a substrate for anaerobic thermophilic digestion. *Wat. Sci. Tech.* ***27***, 97-107.

Kostenberg, D. and Marchaim, U. (1993b) Anaerobic digestion and horticultural value of solid waste from manufacture of instant coffee. *Environ. Techn.* **14**, 973-980.

Kostenberg, D., Marchaim, U., and Epstein, E. (1994). Anaerobic digestion of instant coffee waste as an essential process for production of plant growth medium. In Biogas Technolgy as an Environmental Solution to Pollution. Ed. Marchaim, U., and Ney, G. FAO, Rome.

Koster, I.W. and Cramer, A. (1987). Inhibition and methanogenesis from acetate in granular sludge by long-chain fatty acids. *Appl. and Environ. Microbiol.* **53,** 403-409.

Lane, A.G., (1983). Anaerobic digestion of spent coffee grounds. *Biomass 3*, 247-268.

Lanting, J., Jordan, J.A., Schone, M.T., Kull, A., Carey, W.W. and Kitney , B.L., (1989). Thermophilic anaerobic digestion of coffee waste water. *43rd Purdue Industrial Waste Conference Proceedings*, Lewis Publishers Inc., Michigan, pp.513-527.

Lepisto, S.S. and Rintala, J.A. (1997) Start-up and operation of laboratory scale thermophilic upflow anaerobic sludge blanket reactors treating vegetable processing wastewaters. *J.Chem.Tech. Biotechnol.* **68**, 331-339.

Lettinga, G. and Hulshoff Pol, L.W. (1991) UASB process design for various waste waters. *Wat. Sci. Tech.* ***24*** (8), 87-107.

van Lier, J.B. (1996) Limitations of the thermophilic anaerobic wastewater treatment and the consequences for process design. *Antonie van Leeuwenhoek* **69**, 1-14.

van Lier, J.B., Groenveld, N., and Lettinga, G. (1996) Development of thermophilic sludge in compartmentalized upflow reactors. *Biotech. and Bioeng.* **50** 115-124.

van Lier, J.B., Grolle, K. C. F., Frijters C. T. M. J., Stams A. J. M. and Lettinga G. (1993) Effect of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludges and defined cultures. *Appl. and Environ. Microbiol.* **59** 1003-1011.

van Lier, J.B., Sanz Martin, J.L. and Lettinga, G. (1996) Effect of temperature on the anaerobic thermophilic conversion of of volatile fatty acids in dispersed and granular sludge. *Wat. Res.* **30,** 199-207.

van Lier, J.B., Grolle, K. C. F., Stams, A.J.M., de Macario, E.C., and Lettinga, G. (1992) Start-up of a thermophilic upflow anaerobic sludge bed (UASB) reactor with mesophilic granular sludge. *Appl. Microbiol. Biotechnol.* **37,** 130-135.

Macleod, C. and Forster, C. (1988) Heavy metal inhibition of gas production by thermophilic anaerobic sludges. *Microbios,* **54**, 31-40.

McCarty, P.L. and Mosey, F.E. (1991) Modelling of anaerobic digestion processes (discussion of concepts). *Wat. Sci. Tech.* **24**, 17-33.

McDougall, F.R, Anderson, G.K., Evison, L.M. (1993). Two-phase anaerobic digestion of coffee wastewater.*48th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers Inc., Michigan, pp. 677-683.

Mergaert, K., Vanderhaegen, B. and Verstraete, W. (1992) Applicability and trends of anaerobic pre-treatment of municipal wastewater. *Wat. Res.* ***26*** (8), 1025-1033.

Mathrani, I.M. Johansen, K. and Ahring, B.K. (1994) Experiences with thermophilic anaerobic digestion of manure, organic industrial and household waste at the large scale biogas plant at Vegger, Denmark.. Proc. 7th Int. Symp. on Anaerobic Digestion, Cape Town, South Africa. P365-375.

Molletta, R. and Albagnac, G. (1982) A gas meter for low rates of gas flow: Applications to methane fermentations. *Biotechnol. Lett.* ***4*** (5), 319-322.

Mosey, F.E. (1983) Mathematical modelling of the anaerobic digestion process :regulatory mechanisms for the formation of short chain volatile acids from glucose. *Wat. Sci. Tech.* **15**, 209-232.

Nanninga, H.J. and Gottschal, J.C. (1986) Anaerobic purification of waste water from a potato-starch producing factory. *Wat. Res.* **20** (1), 97-103.

Ohtsuki, T., Watanabe, M. and Miyaji, Y.(1992) Start-up of thermophilic UASB (upflow anaerobic sludge blanket) using micro-carrier and mesophilic granular sludge. *Wat. Sci. Tech.* ***26*** (3-4), 877-886.

Oi, S., Tanaka, T. and Yamamoto, T. (1981) Methane fermentation of coffee grounds and some factors to improve the fermentation. Agric.Biol. Chem. **45** (4) 871-878.

Pavolostathis, S.G. and Giraldo-Gomez, E. (1991) Kinetics of anaerobic treatment: A critical review. *Critical Reviews in Environmental Control.* **21** (5,6), 411-490.

Peck, M.W., Skilton, J.M., Hawkes, F.R. and Hawkes, D.L., (1986) Effects of temperature shock treatments on the stability of anaerobic digesters operated on separated cattle slurry. *Wat. Res.*. ***20*** (4), 453-462.

Quarmby, J. and Forster, C. F. (1995). An examination of the structure of UASB granules. *Wat. Res.* **29**, (11), 2449-2454.

Raetz, E. (1990) Anaerobic Digestion of Wastes; Spent Coffee Grounds. *Nestlés Research Centre.* No. 492.

Rinzema, A., Alphenaar, A., and Lettinga, G. (1989). The effect of lauric acid shock loads on the biological and physical performance of granular sludge in UASB reactors digesting actate. *J. Chem. Tech. Biotechnol.* **46** 257-266.

Rinzema, A., Boone, M., Kniippenberg, K., and Lettinga, G. (1994). Bactericidal effect of long chain fatty acids in anaerobic digestion. *Water Environ. Res.* **66** 40-49.

Rinzema, A., van Lier, J., and Lettinga, G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme Microbiol. Tech.* **10**, 24-32.

Romero, L.I, Sales, D., Cantero, D., and Galan, M.A.(1988) Thermophilic anaerobic digestion of winery waste (vinasses): kinetics and process optimisation. *Process Biochemistry* **8**, 119-125.

Ross, J. (1994) Personal Communication, Binnie Consultants.

Rozzi, A. (1983). Physico-chemical equilibria in anaerobic digesters. In Biomethane, production and uses. Turret-Wheatland Ltd., Rickmansworth.

Sahm, H. (1984). Anaerobic wastewater treatment. *Adv. Biochem. Eng.* **29**, 83-115.

Schmidt, J. E. and Ahring B. K. (1994). Granules from thermophilic upflow anaerobic sludge blanket (UASB) reactors. Poster Paper 7th Int. Symp. on Anaerobic Digestion, Cape Town, SA. pp.35-40

Shi, D. and Forster, C.F. (1993). An examination of the start-up of a thermophilic upflow anaerobic sludge blanket reactor treating a synthetic coffee waste. *Environ. Techn.* **14**, 965-971.

Shi, D. and Forster, C.F. (1994). Inhibitory effects in the thermophilic anaerobic treatment of a simulated coffee waste. *Environ. Techn.* **15**, 287-292.

van Soest, P.J. and Mason, V.C. (1991) The influence of the Maillard reaction upon the nutritive value of fibrous foods. *Animal Feed Science and Technology* **32** 45-53.

Souza, M.E., Fuzaro, G., Polegato, A.R. (1992). Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. *Wat. Sci. Tech.* ***25*** (7), 213-222.

Speece, R.E. (1983) Anaerobic biotechnology for industrial wastewater treatment. *Environmental Science and Technology.* **17,** 416-427.

Speece, R.E. (1996) Anaerobic biotechnology for industrial wastewater treatment, Archae Press, Nashville, Tennessee.

Stronach, S.M., Rudd T and Lester J.N. (1986) Anaerobic Digestion Processes in Industrial Wastewater Treatment. Springer-Verlag, Berlin.

Switzenbaum, M.S. (1994) Obstacles to the implementation of anaerobic treatment technology for industrial wastewater treatment. In Biogas Technolgy as an Environmental Solution to Pollution. Ed. Marchaim, U., and Ney, G. FAO, Rome

Switzenbaum, M.S., Giraldo-Gomez, E. and Hickey, R.F. (1990). Monitoring of anaerobic methane fermentation process. *Enz. Microb. Technol. 12,* 722-730.

Thacker F E and Evans M R. (1986) BOD_5, TOA and odour offensiveness. In: Odour prevention and control of organic sludge and livestock farming. Nielsen,V.C., Voorburg, J.H. and L'Hermite, P.L. (eds). Elsevier Applied Science Publishers, London. 307-320.

Thauer, R.K., Jungermann, K. and Decker, K. (1977) Energy conversion in Chemotrophic anaerobic bacteria. Bacteriol. Rev. 41, 100-180.

Theander, O. (1980) Sugars in Thermal Processes. *In Carbohydrate Sweetners in Foods and Sweetners.* Ed. Koivistoinen, P. and Hyvönen, L., 185-199, Academic Press, London, UK.

Varel, V.H., Hasihmoto, A.G. and Chen, Y.R. (1980) Effect of temperature and retention time on methane production from beef cattle waste. *Appl. and Environ. Microbiol.* **40**, (2), 217-222.

van Vooren, L., Willems, P., Ottoy, J.P., Vansteenkiste, G.C. and Verstraete, W. (1996) Automatic buffer capacity based sensor for effluent quality monitoring. Wat. Sci. Tech. **33** (1) 81-87.

Water Facts `95.(1995) Water Services Association Publications, Sheffield, UK.

Wheatley, A.D., Fisher, M.B. and Grobicki, A.M.W. (1997) Applications of anaerobic digestion for the treatment of industrial wastewaters in Europe. *J.CIWEM.* 11, 39-46.

Wiegant, W.M. and Lettinga, G. (1985). Thermophilic anaerobic digestion of sugars in upflow anaerobic sludge blanket reactors. *Biotech. and Bioeng.* **27,** 1603-1607.

Wiegant, W.M. and De Man, A.W.A. (1986) Granulation of biomass in anaerobic sludge blanket reactors treating acidified wastewaters. *Biotech. and Bioeng.* **28,** 718-727.

Wiegant, W.M., Hennick, M., and Lettinga, G. (1986) Separation of propionate degradation to improve the efficiency of anaerobic treatment of acidified wastewaters. *Wat. Res.* **20**,517-524.

Young, J.C. (1991). Factors effecting the design and performance of upflow anaerobic filters. *Wat. Sci. Tech.* ***24***, (8)133-155.

Zinder, S.H. (1986). Thermophilic waste treatment systems. In Brock, T.D . Thermophilies: general, molecular and applied biology. p 257-277. Wiley, New York.

Zoetemeyer, R.J., Arnoldy, P., Cohen, A. and Boelhouwer, C. (1982a) Influence of temperature on the anaerobic acidification of glucose in a mixed culture forming part of a two stage digestion process. *Wat. Res.* **16**, 313-321.

Zoetemeyer, R.J., van den Heuvel, J.C.A. and Cohen, A. (1982b) pH influence on acidogenic dissimilation of glucose in an anaerobic digester. *Wat. Res.* **16**, 303-311.

Zoetemeyer, R.J., Matthijsen, A.J.C.M., van den Heuvel, J.C.A., Cohen, A. and Boelhouwer, C. (1980) Anaerobic acidification of glucose in an upflow reactor. *Trib. Cebedeau*. 455,34, 443-450.

Pergamon

0043-1354(95)00157-3

Wat. Res. Vol. 30, No. 2, pp. 371–377, 1996

0043-1354/96 $15.00 + 0.00

THE MESOPHILIC AND THERMOPHILIC ANAEROBIC DIGESTION OF COFFEE WASTE CONTAINING COFFEE GROUNDS

RICHARD M. DINSDALE[1]Ⓜ, FREDA R. HAWKES[2]Ⓜ* and DENNIS L. HAWKES[1]Ⓜ

[1]Department of Mechanical and Manufacturing Engineering, and [2]School of Applied Sciences, University of Glamorgan, Pontypridd, Mid Glamorgan CF37 1DL, Wales

(First received November 1994; accepted in revised form May 1995)

Abstract—The anaerobic digestion of waste water containing significant levels of coffee grounds was assessed in mesophilic and thermophilic batch studies and CSTRs fed daily. A 58% reduction in VS was seen in both batch studies. Proximate compositional analysis showed that the waste had a high lipid component (26–33%). Levels of lipid, hemicellulose, α-cellulose and lignin were determined before and after digestion. These components were reduced as follows: lipid by 87% in the mesophilic study and 65% in the thermophilic study, α-cellulose by 51% in both mesophilic and thermophilic batch studies, hemicellulose by 22% in the mesophilic studies and 64% in the thermophilic studies. The lignin component was not reduced in either study. Mesophilic continuous digestion was achieved at a loading rate of 1.3 kg COD m^{-3} day^{-1} (25 day HRT) for 99 days. Addition of sodium bicarbonate alone was not sufficient for long term anaerobic digestion. Addition of $Ca(OH)_2$, nitrogen, phosphorus and trace elements, however, gave successful digestion with COD and VS removal of 60% and a gas production rate of 0.34 l l^{-1} day^{-1} (65–70% methane). Low levels of TVFA and high levels of bicarbonate alkalinity were present. Thermophilic digestion could be established at 1.6 kg COD m^{-3} day^{-1} (20 day HRT) with the addition of sodium bicarbonate alone, or $Ca(OH)_2$ with nitrogen, phosphorus and trace elements. However long term digestion could not be established beyond 50 days without a increase in TVFA occurring.

Key words—anaerobic digestion, instant coffee waste water, thermophilic, mesophilic, lipids

INTRODUCTION

Instant coffee production results in large amounts of waste water possessing significant polluting power, varying from 4 to 60 g l^{-1} COD. The waste water varies in strength and composition due to the number and effectiveness of the processes employed to remove the spent coffee grounds before discharge. Although plant materials have been widely treated by anaerobic digestion, coffee grounds have been roasted in air reaching temperatures of up to 540°C (Clark and Macrae, 1985). Heating has been found to have significant effects on the composition and digestibility of animal feedstuffs (van Soest and Mason, 1991) and the anaerobic digestion of heat roasted plant material has not been studied extensively.

The waste can be discharged at high temperatures (~70°C) so either mesophilic or thermophilic anaerobic digestion could be used. Laboratory scale anaerobic digestion of coffee grounds has been attempted at mesophilic temperatures by Lane (1983) and Raetz (1990) and at thermophilic temperatures by Kida *et al.* (1992), Raetz (1990) and Kostenberg and Marchaim (1993). Biodegradation varied from 20 to 87% reduction in volatile solids. Raetz and Kida *et al.*, however found problems in achieving stable gas production either due to pH problems or inhibition in their batch studies. Lane in a mesophilic continuous study found a decline in gas production after 80 days due to some inhibitory compound in the coffee waste. Kostenberg and Marchaim (1993) operated thermophilic continuous studies for long periods, achieving stable digestion at a variety of loading rates. However they found that some studies began to have increasing levels of volatile fatty acids (VFA) after a certain time of operation.

The review of the literature suggests that the anaerobic digestion of coffee grounds is possible but long term stability is a problem. The aim of this work was to assess the anaerobic digestion of coffee waste containing coffee grounds using mesophilic and thermophilic reactors in batch and continuous digestion studies. The continuous studies were used to assess the long term stability of coffee ground digestion. Proximate analysis was performed to determine the principal components in the coffee waste before and after digestion.

*Author to whom all correspondence should be addressed.

MATERIALS AND METHODS

Feed source

The coffee waste was obtained from one of the waste streams at Nestlé, Hayes, Middlesex, U.K. The waste was frozen until required.

Analyses

Total solids were determined by using a microwave set at low power. When compared using Student's *t-test* at 5% probability level with total solids determined according to *Standard Methods* (APHA, 1989) no significant difference was seen. Volatile solids, suspended solids, settleable solids, colour, pH, tannin and lignin determination and temperature were performed as described in *Standard Methods* (APHA, 1989). Bicarbonate alkalinity (partial alkalinity) was determined by titration to pH 5.75 (Jenkins *et al.*, 1983). Total chemical oxygen demand (COD), soluble COD and settled COD were determined using the sealed tube method with the mercury free reagents as described in *Standard Methods* (HMSO, 1986).

Ammonia nitrogen was determined by distillation and titration as in *Standard Methods* (APHA, 1989). Kjeldahl nitrogen was determined using a mercury free method with a copper catalyst (HMSO, 1985), the subsequent ammonia was determined by distillation and titration, (APHA, 1989). Protein was determined by multiplying the Kjeldahl nitrogen value by 6.25 (Allen, 1974). Holocellulose, hemicellulose, α-cellulose, lignin and lipid were determined as in Allen (1974). Biogas composition and volatile fatty acid levels were determined by gas chromatography as described by Peck *et al.* (1986).

Analysis of waste stream

Samples from the factory were taken on two different occasions, 2 months apart. The data is presented in Table 1. For each sample each test was performed in at least triplicate. The standard deviation for each sample is shown in parentheses after the mean value.

Batch study experiments

Six 5 litre vessels were used. The digesters were incubated at mesophilic (35°C) or thermophilic (55°C) temperatures. Four vessels were filled with 2 litres of waste, 3 litres of deionized water and sufficient homogenized UASB granules (from a full scale reactor treating paper mill effluent) to provide inoculum of 3 g l^{-1} as volatile solids. For the mesophilic study a control digester was set up containing 2 litres of deionized water instead of coffee waste but with all other additions. In the thermophilic study the control digester was supplemented with 7.3 g l^{-1} cellulose (Avicel) and 5.4 g l^{-1} D-glucose to determine if an active thermophilic culture was achieved. These values of cellulose and glucose were chosen to provide suspended solids and soluble COD similar to the coffee waste. All reactors had nutrient supplementation. Nitrogen and phosphorus were added to give a COD:N:P ratio of 400:7:1. Trace elements and metals were supplied in the amounts as described in *Standard Methods* (HMSO, 1988). Sodium bicarbonate was added at concentration of 9 g l^{-1} to maintain the digestion at the optimum pH for anaerobic digestion.

Table 1. Analysis of coffee waste stream

Analysis	Sample date 12.12.91	31.1.92
Total solids (g l^{-1})	23.9 (0.6)	27.5 (0.8)
Volatile solids (g l^{-1})	23.1 (0.6)	27.1 (0.8)
Suspended solids (g l^{-1})	14.1 (0.5)	18.3 (0.7)
Non-settled suspended solids (g l^{-1})	1.6 (0.04)	1.7 (0.12)
Total COD (mg O_2 l^{-1})	35,900 (1400)	40,000 (1400)
Soluble COD (mg O_2 l^{-1})	9600 (400)	6400 (200)
Nonsettleable COD (mg O_2 l^{-1})	13,000 (900)	11,500 (300)
Holocellulose (g l^{-1})	3.5 (0.2)	5.3 (0.4)
α-cellulose (g l^{-1})	5.8 (0.2)	5.8 (0.3)
Total lipid (g l^{-1})	7.1 (1.2)	7.3 (1.1)
Total Kjeldahl nitrogen (g l^{-1})	0.12 (0.01)	0.08 (0.01)
Protein (g l^{-1})	0.4 (0.01)	0.3 (0.01)
pH	4.6	4.3
Temperature (°C)	68	67
Bicarbonate alkalinity (mg $CaCO_3$ l^{-1})	0	0

The progress of the digestion was determined by monitoring gas production by wet type gas meter and gas composition, pH and VFA levels. When gas production had ceased to be measurable and VFA levels had fallen to <300 mg l^{-1}, the digestion was left for a further 2 weeks and then deemed to have been completed. One litre of well mixed digester contents was then removed for analysis along with a sample taken prior to digestion to determine the compositional changes after digestion. The analyses performed were for volatile solids, lipid, α-cellulose, hemicellulose and lignin. The volatile solids reduction calculation was adjusted for the initial inoculum which was assumed to be not degraded over the experiment. The lipid values were adjusted for lipid found in the inoculum which was assumed to remain constant over the duration of the experiment. Lignin, α-cellulose and hemicellulose were assumed not to be present in the inoculum.

Continuous study experiments

Four 5 litre stirred digesters were constructed. The main vessel was a modified 5l Quickfit vessel with a outflow port fitted by a glassblower. Two digesters were maintained at mesophilic temperature (35°C) and two at thermophilic temperature (55°C).The digesters were stirred at 300 rpm for 5 min every 6 h.

The vessels were seeded by adding to each digester sufficient homogenized UASB reactor granules to give a volatile solids level due to the inoculum of 5 g l^{-1} in the final volume. To each digester was added 1.3 litres of coffee waste, 9 g l^{-1} sodium bicarbonate and the volume was made up to 5 litres with deionized water. The digesters were left to acclimate for 20 days, after which feeding was commenced at day zero. To provide as stable conditions as possible and due to the particulate and oily nature of the waste each digester was fed daily by hand (7 days a week). Initial retention times were chosen which were typical for the start-up of thermophilic and mesophilic CSTRs. The mesophilic digesters were fed at a loading rate of 1.3 kg COD m^{-3} day^{-1} (25 day HRT) and the thermophilic digesters at a 1.6 kg COD m^{-3} day^{-1} (20 day HRT). Gas production was monitored as in Hawkes *et al.* (1994). Gas composition and bicarbonate alkalinity, pH and TVFA levels were monitored on a regular basis.

Two studies were performed. In the first continuous study inoculum was taken from a UASB reactor treating paper mill effluent and was from the same batch used to seed the EPSRC Anaerobic Facility pilot plant. The feed had 9 g l^{-1} sodium bicarbonate added and was not supplemented in any other way. To investigate the importance of nutrient and calcium addition, a second study was initiated using the same apparatus. In the second continuous study the feed was neutralized by the addition of 1 g l^{-1} $Ca(OH)_2$ and nutrient supplemented. Nitrogen and phosphorus were added to give a COD:N:P ratio of 400:7:1. Trace metals were supplied by the addition of 2 cm^3 per litre of trace metal solution as described in *Standard Methods* (HMSO, 1988). The inoculum was taken from a UASB reactor of the EPSRC Anaerobic Facility pilot plant, located at Nestlé, Hayes, Middlesex, U.K., which had been fed on settled coffee waste.

In the second study volatile solids destruction was determined for both mesophilic reactors. Samples were analysed in triplicate on six separate occasions. Samples were taken on days 50, 65, 71, 78, 85, 92 and 99 for the 99 day mesophilic digester and on days 30, 45, 51, 58, 65, 72 and 79

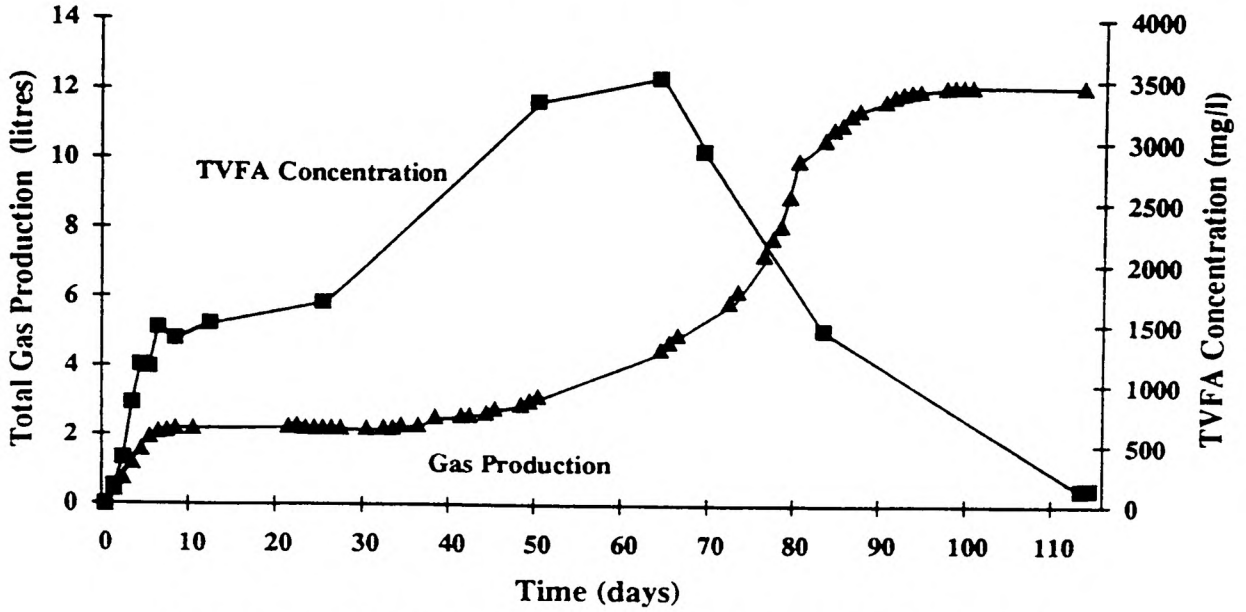

Fig. 1. Cumulative gas production and TVFA levels in a mesophilic batch study.

for the 80 day mesophilic digester. COD reduction was determined on samples taken on eight separate occasions which were analsed in triplicate. The COD samples were taken on days 45, 51, 73, 78, 79, 80, 84 and 92 for the 99 day digestion and on days 25, 31, 53, 58, 59, 60, 64 and 72 for the 80 day digestion.

RESULTS AND DISCUSSION

Batch studies

The mesophilic control reactor produced a negligible amount of gas, while the thermophilic control reactor with glucose and cellulose gave a 95% VS destruction, showing an effective thermophilic culture had developed.

The batch studies except the mesophilic control exhibited production of methane and an initial rise in TVFA which was followed by a fall in TVFA. The batch studies were left to digest for 115 and 85 days for the mesophilic and thermophilic studies respectively. The cumulative gas production and VFA levels for one of the mesophilic batch reactors is shown in Fig. 1. At the end of the digestion period the composition of the contents of each reactor was determined. The compositional results are shown in Fig. 2. A 58% reduction in volatile solids was achieved in the mesophilic and thermophilic batch studies. The coffee waste water had a lignin content of 9–11%, other plant materials with a similar lignin content such as wheat

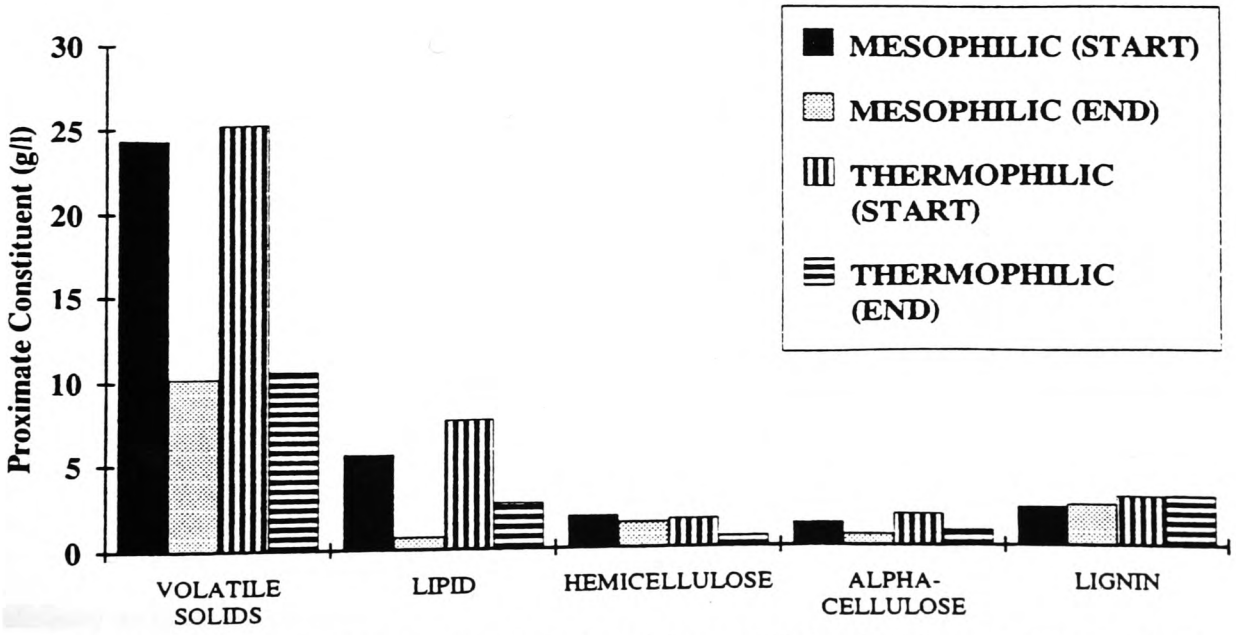

Fig. 2. Compositional changes in the mesophilic and thermophilic batch studies. Expressed as g/l of undiluted coffee waste.

straw have a similar degree of degradation (Jerger and Tsao, 1987). The amount of lignin remained virtually unchanged in both mesophilic and thermophilic batch digestions.

The largest identified component, comprising 26–33% of the volatile solids, was lipid. High levels of lipid (18–31% of total solids) were also reported by Raetz (1990). The degradation of the lipid varied from 87% in the mesophilic study to 65% in the thermophilic study. The reduction in hemicellulose was 22% in the mesophilic batch studies and 64% in the thermophilic batch studies. There was a 51% reduction of α-cellulose in the mesophilic and thermophilic batch studies.

There remains a large unidentified component in the coffee waste, 54% of the volatile solids remains unidentified. The high temperature processing of carbohydrate material results in the production of Maillard products. These are insoluble and soluble lignin like compounds (Theander, 1980). A tannin and lignin (Folin–Ciocalteau) test showed a filtered sample (sample date 12.12.91, see Table 1) consisted of 80% tannin and lignin by weight. The nature of the proximate analyses used means that no soluble components are determined, although they are included in TS and VS determinations.

First continuous study

In the first continuous study successful digestion was initially established in the mesophilic and thermophilic digesters. The mesophilic digesters were fed every day until day 35. Until day 35 biogas production was 0.20 l l^{-1} day^{-1} (10 analyses, SD = 0.019), consisting of 65% CH_4, the level of TVFA was 500 mg l^{-1}. At day 35 it was noticed that gas production had fallen off in both digesters. A rise in TVFA occurred in the following days rising from 500 to 1500 mg l^{-1} and then to at least 2000 mg l^{-1}. The pH did not fall below 6.8 in this period; as this pH level was in the optimum range for methanogens and VFA levels were sufficiently high for gas production to occur, no further additions were made in the hope that gas production would recover. However gas production did not recover and TVFA levels remained high. At day 100 an additional amount of the homogenized UASB granules was added to give an extra 5 g l^{-1} VS of biomass in the digester. No gas production or reduction in TVFA levels was seen. Subsequent feeding at day 81, 105, 115 also saw no significant gas production or reduction in TVFA levels. Similar findings were reported by Lane (1983) who experienced a decline in gas production towards the end of a 80 day digestion. Lane utilized solids recycling which could explain the longer period of operation achieved compared to the 35 days in this study. Digester failure could be due to nutrient deficiency or build up of toxic compounds from the digestion of coffee grounds. The initial analysis of the waste shows low levels of nitrogen. Nutrient addition was used by Lane (1983) but without effect and Kostenberg and Marchaim (1993) saw no advantage in adding nitrogen.

A similar pattern in the thermophilic digestions was seen. Gas production was maintained at 0.23 l l^{-1} day^{-1} with a composition of 64% CH_4 until day 45. TVFA levels fell to a level of 1000 mg l^{-1} over this period. At day 47 both digesters suffered a heater malfunction for 24 h, thermophilic digester 1 suffered a 10°C fall in temperature and digester 2 suffered a increase to 90°C. Gas production fell and did not recover to previous levels despite being fed on day 53–57. Total VFA levels had risen to 2500–3600 mg l^{-1}. Reinoculation at day 100 with sufficient inoculum to give 5 g l^{-1} as VS, did not give a recovery in gas production or fall in TVFA levels.

Second continuous study

In the second continuous study, one mesophilic digester was still running well at 99 days and the other at 80 days (almost 4 HRT and more than 3 HRT respectively).

In Fig. 3 the daily gas production and gas composition (percentage methane) are shown for the mesophilic digester operated continuously for 99 days. Gas production rose to 0.30 l l^{-1} day^{-1} by day 20 and over the steady state period (day 75–99) averaged 0.34 l l^{-1} day^{-1}. Gas composition varied from 65 to 70% methane.

The TVFA and bicarbonate alkalinity of the 99 day mesophilic study are shown in Fig. 4. The TVFA initially rose to 1140 mg l^{-1} by day 9, then fell to 110 mg l^{-1} on day 22. TVFA from day 22 to day 99 averaged 100 mg l^{-1} (18 samples SD = 24). The bicarbonate alkalinity was maintained at 2200–2400 mg l^{-1} $CaCO_3$. The low TVFA and high alkalinity level indicate that the various bacterial groups are in balance. The TVFA levels are lower in this study than in the initial experiment, 100 mg l^{-1} compared to 500 mg l^{-1}.

Both reactors operated at low TVFA, achieving good gas production, high methane composition, bicarbonate alkalinity and a significant degree of COD and volatile solids removal. The 99 day digestion achieved a 60% VS reduction (SD = 2.5), a 63% COD reduction (SD = 4.3) and the 80 day digestion a 62% VS reduction (SD = 2.5), a 59% COD reduction (SD = 2.8). This compares with 70% reduction achieved by Lane (1983) and 47–72% reduction achieved by Kostenberg and Marchaim (1993). This length of digestion (> 3 HRT), could only be achieved if biomass growth was matching biomass removal. Therefore it can be concluded that stable long term mesophilic digestion was achieved.

The daily gas production and gas composition for the thermophilic digester is shown in Fig. 5. Gas production rose to 0.25 l l^{-1} day^{-1} with a composition 60–65% methane and 30–35% carbon dioxide. TVFA and alkalinity for this digester are shown in Fig. 6. The

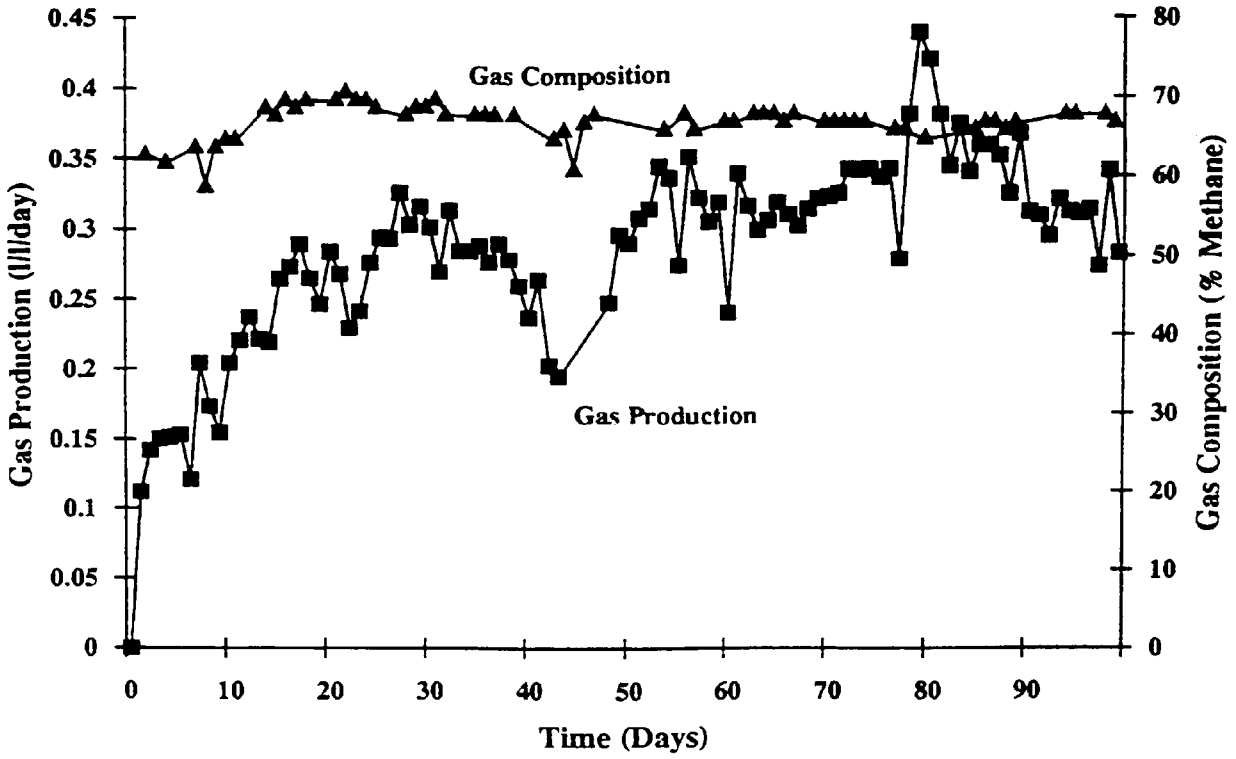

Fig. 3. Second mesophilic continuous study: gas production and % methane.

TVFA initially rose to 1500 mg l^{-1}, fell to 600 mg l^{-1} then rose again suddenly on day 48 to 1500 mg l^{-1}. The bicarbonate alkalinity gradually fell from 2400 mg l^{-1} $CaCO_3$ to 1230 mg $l^{-1}CaCO_3$ on day 46. A sudden drop in alkalinity accompanied the rise in TVFA on day 48, and a drop in pH from 6.9 to 6.7 was also seen, indicating that the VFA producers were not in balance with the methanogenic bacteria. It was also noticed that large fatty deposits were building up on the surface of the digester, similar to those in the previous study. This would indicate that the lipid component was not being broken down in the digester. The increase in TVFA levels and decrease in bicarbonate alkalinity shows that this digester was not stable and on the verge of failure, therefore at day 48 feeding was halted. On day 50 0.36 g l^{-1} of $Ca(OH)_2$ was added to bring the pH to 7.2 and gas production, pH, and VFA levels were monitored until day 98. Gas production was negligible and the TVFA had fallen to 500 mg l^{-1} by day 98. The previous thermophilic study also failed at around 50 days (day 47) although this also coincided with a temperature shock. The use of

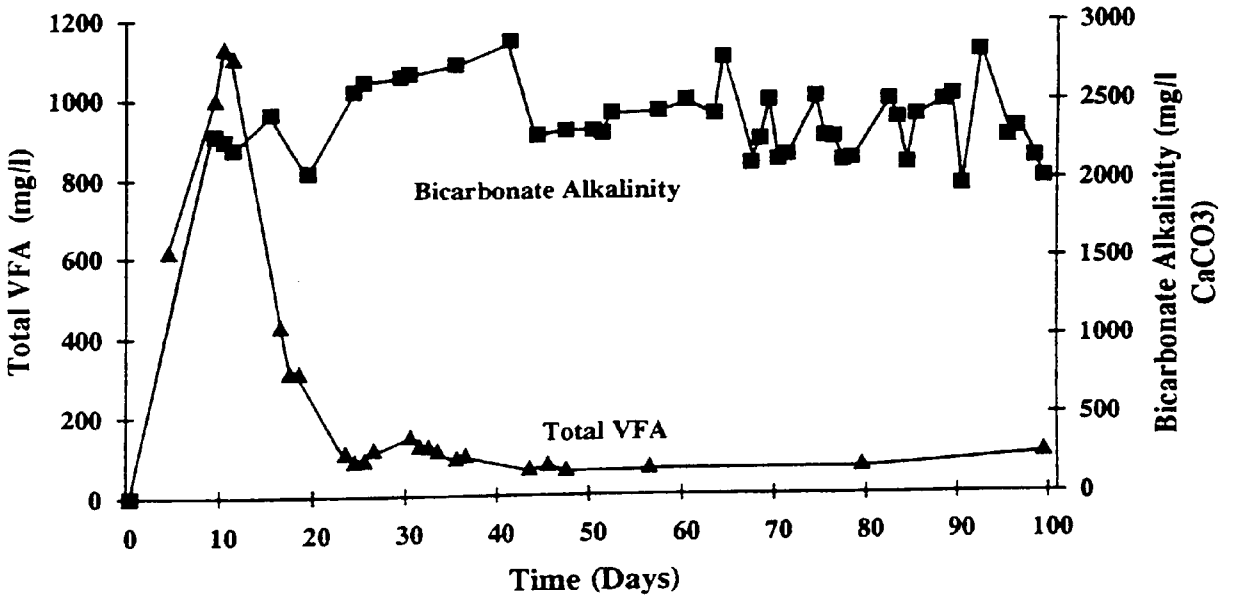

Fig. 4. Second mesophilic continuous study: TVFA levels and bicarbonate alkalinity.

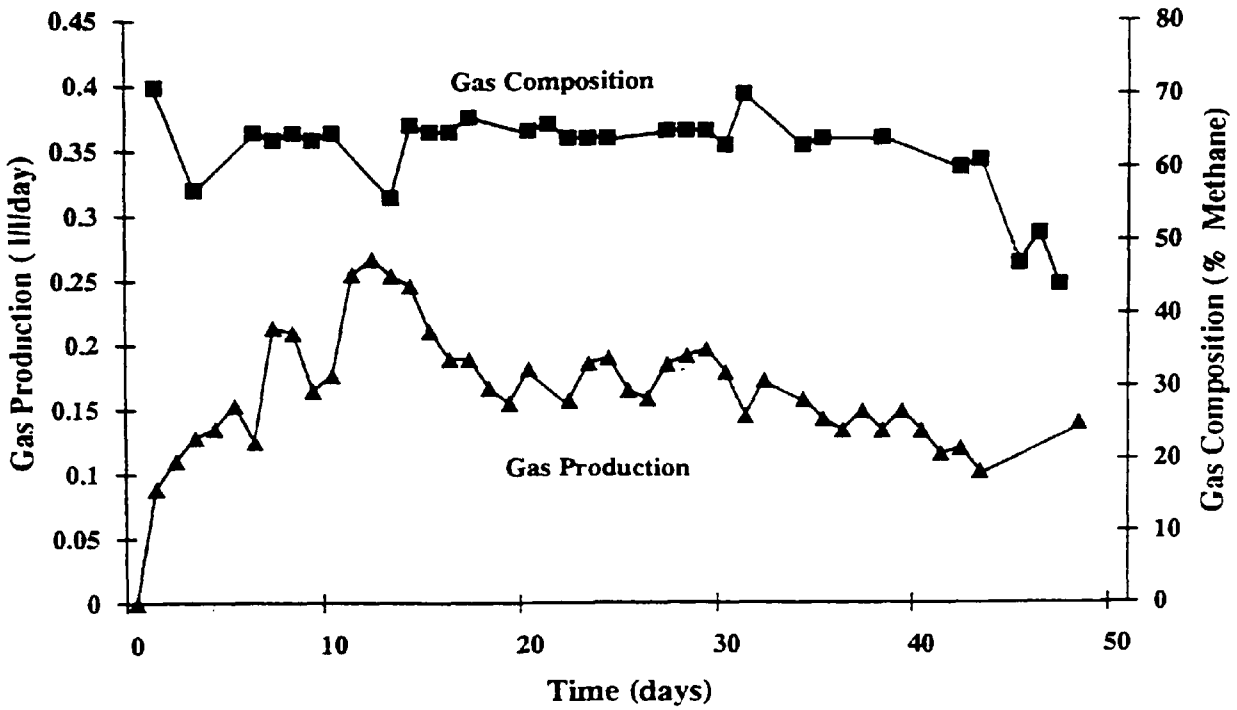

Fig. 5. Second thermophilic continuous study: gas production and gas composition during the period of daily feeding.

nutrient addition in the second study would suggest that nutrient deficiency was not a cause of digester failure in the thermophilic study.

High lipid levels cause problems for mesophilic and thermophilic digestion (Angelidaki *et al.*, 1990; Ahring *et al.*, 1991; Hanaki *et al.*, 1981). The lipid is hydrolysed to long chain fatty acids (LCFA) which if allowed to accumulate become toxic to methanogenesis. Angelidaki *et al.* (1990) had problems in digesting lipid in thermophilic digesters, however the digesters did acclimate and Ca^{+} ions aided digestion. However the lipid levels were lower than the levels found in coffee waste. In other studies (Ahring *et al.*, 1991) neutral lipid levels of 2.5 g l^{-1} or free LCFA levels of 0.1–0.5 g l^{-1} were found to be inhibitory to methanogenic bacteria. Coffee waste has a high level of lipid (5–7 g l^{-1}) which may suggest this is the cause of the problem in the thermophilic anaerobic digestion of coffee waste. This toxicity can be alleviated by avoiding shock loads to the system (Hanaki *et al.*, 1981), by the addition of bentonite (Angelidaki *et al.*, 1990) or the addition of calcium ions (Angelidaki *et al.*, 1990; Ahring *et al.*, 1991; Hanaki *et al.*, 1981).

Successful thermophilic anaerobic digestion of

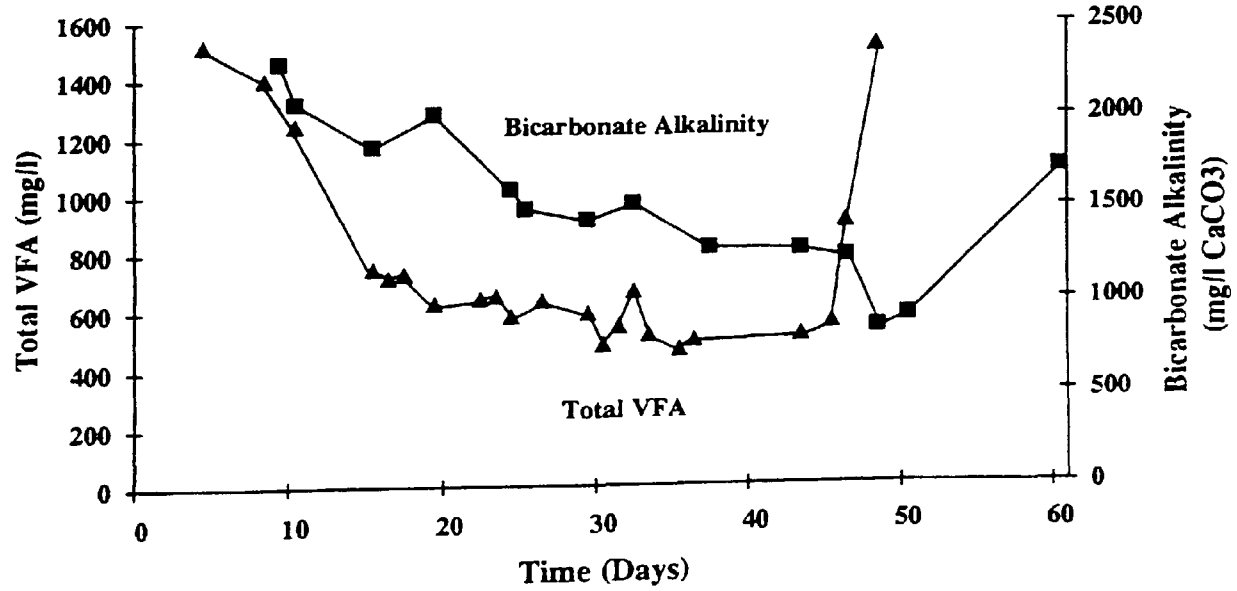

Fig. 6. Second thermophilic continuous study: TVFA and bicarbonate alkalinity (daily feeding up to day 48).

coffee grounds has been achieved by the addition of 0.3 g CaO/g VS added (Kostenberg and Marchaim, 1993), compared to 0.05 g $Ca(OH)_2$/g VS added in the present study. Kostenberg and Marchaim (1993) also tried sodium carbonate and ammonium bicarbonate at 0.3 g buffering agent/g VS added but with less success due to rising TVFA levels. This could suggest that the ion type is responsible and not the buffering capacity as the pH remained at a suitable level for methanogenesis.

Successful mesophilic digestion could have been due to the nutrient supplements or presence of Ca^{+} or use of acclimatized granules from the ESPRC pilot plant UASB fed on coffee ground-free waste. Although standard start up procedures were used, systems such as UASBs with inherently large SRTs, have superior acclimation ability compared to CSTRs (Lee Long-de Valliere *et al.*, 1989). However the suspended solids content of the waste necessitated the use of CSTRs for this study. Further work will be conducted using reactors with long SRT using coffee ground-free waste.

CONCLUSION

Proximate compositional analysis showed that the coffee waste had a high lipid component (26–33%) with lower levels of hemicellulose (3.5–5.3%), α-cellulose (11.1–13.8%), lignin (9–11%), protein (1.7–1.1%) and ash (1.4–3.3%).

A 58% reduction in VS was seen in both mesophilic and thermophilic batch tests. Greater lipid but lower hemicellulose degradation was observed in the mesophilic study, α-cellulose was degraded equally at both operating temperatures. The lignin component was not reduced in either study.

Mesophilic continuous digestion was achieved for 99 days using feed supplemented with $Ca(OH)_2$, nitrogen, phosphorus and trace metals and using inoculum from a pilot plant treating settled coffee waste. A COD and VS removal of 60% was achieved.

Thermophilic digestion could be established at 1.6 kg COD m^{-3} day^{-1} (20 day HRT) with the addition of sodium bicarbonate, or $Ca(OH)_2$, nitrogen, phosphorus and trace metals. However long term digestion could not be established beyond 50 days without a increase in TVFA occurring.

Acknowledgements—The work was funded by the U.K. Engineering and Physical Sciences Research Council EPSRC (formerly the Science and Engineering Research Council SERC) under grant No. GR/H18494. The authors would also like to thank Nestlé, Middlessex, U.K., for supplying the coffee waste.

REFERENCES

Ahring B. K., Angelidaki I. and Johansen K. (1991) Anaerobic digestion of manure together with industrial waste. *Proceedings of the Sixth International Symposium on Anaerobic Digestion*, Sao Paulo, Brazil, pp. 309–316.

Allen S. G. (1974) *Chemical Analysis of Ecological Materials*. Blackwell, Oxford.

APHA (1989) *Standard Methods for the Examination of Waste and Wastewater* (Edited by Clesceri L. S., Greenberg S. E. and Trussel R. R.), 17th edition. American Public Health Association, Washington, D.C.

Angelidaki I., Petersen S. P. and Ahring B. K. (1990) Effect of lipids on thermophilic digestion and reduction of lipid inhibition upon the addition of bentonite. *Appl. Microbiol. Biotechnol.* **33**, 469–472.

Clark R. J. and Macrae R. (1985) *Coffee, Volume 1 Chemistry*. Elsevier, London.

Hanaki K., Matasuo T. and Nagase M. (1981) Mechanism of inhibition caused by long chain fatty acids. In anaerobic digestion process. *Biotechnol. Bioengng* **23**, 1561–1610.

Hawkes F. R., Guwy A. J., Hawkes D. L. and Rozzi A. G. (1994) On-line monitoring of anaerobic digestion: application of a device for continuous measurement of bicarbonate alkalinity. *Wat. Sci. Technol.* **30**,(12), 1–10.

HMSO (1985) *Total Nitrogen and Total Phosphorus in Sewage sludge*. HMSO, London.

HMSO (1986) *Chemical Oxygen Demand (Dichromate Value) of Polluted And Wastewater*. HMSO, London.

HMSO (1988) *The Assessment of Biodegradabilty in Anaerobic Digesting Sludge*. HMSO, London.

Jenkins S. R., Morgan J. M. and Sawyer C. L. (1983) Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration. *J. Wat. Pollut. Control Fed.* **55**, 448–453.

Jerger D. E. and Tsao G. T. (1987) Feed Composition. In *Anaerobic Digestion of Biomass* (Edited by Chynoweth D. P. and Isaacson R.), Chap. 5. Elsevier Applied Science, Barking.

Kida K., Ikbal and Sonada Y. (1992) Treatment of coffee waste water by slurry state anaerobic digestion. *J. Ferment. Bioengng* **73**, 390–395.

Kostenberg D. and Marchaim U. (1993) Anaerobic digestion and horticultural value of solid waste from manufacture of instant coffee. *Envir. Technol.* **14**, 973–980.

Lane A. G. (1983) Anaerobic digestion of spent coffee grounds. *Biomass* **3**, 247–268.

Lee Long-de Valliere C., Petrozzi S., Zurrer D., Baier U. and Dunn I. J. (1989) Methods of anaerobic degradation of toxic compounds in chemical and industrial wastewaters. In *Biological Waste Treatment* (Edited by Mizrahi A.), pp. 35–72. Liss, New York.

Peck M. W., Skilton J. M., Hawkes F. R. and Hawkes D. L. (1986) Effects of temperature shock treatments on the stability of anaerobic digesters operated on separated cattle slurry. *Wat. Res.* **20**, 453–462.

Raetz E. (1990) Anaerobic digestion of wastes; spent coffee grounds. Nestlé Research Centre. No. 492.

van Soest P. J. and Mason V. C. (1991) The influence of the Maillard reaction upon the nutritive value of fibrous feeds. *Anim. Feed Sci. Technol.* **32**, 35–44.

Theander O. (1980) Sugars in thermal processes. In *Carbohydrate Sweeteners in Foods and Sweeteners* (Edited by Koivistoinen P. and Hyvönen L.), pp. 185–199. Academic Press, London.

Pergamon

PII: S0043-1354(96)00233-3

Wat. Res. Vol. 31, No. 1, pp. 163–169, 1997

0043-1354/97 $17.00 + 0.00

COMPARISON OF MESOPHILIC AND THERMOPHILIC UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS TREATING INSTANT COFFEE PRODUCTION WASTEWATER

RICHARD M. DINSDALE[1], FREDA R. HAWKES[2]* and DENNIS L. HAWKES[1]

[1]Department of Mechanical and Manufacturing Engineering and [2]School of Applied Sciences, University of Glamorgan, Pontypridd, Mid Glamorgan CF37 1DL, U.K.

(First received November 1995; accepted in revised form July 1996)

Abstract—Stable anaerobic digestion of settled instant coffee wastewater was achieved for over 100 days in mesophilic (35°C) and thermophilic (55°C) UASB reactors. Thermophilic upflow anaerobic sludge blankets (UASBs) were seeded with mesophilic granules and converted to thermophilic operation by raising the temperature to 55°C in one step. Successful thermophilic operation was achieved within 28 days. Both mesophilic and thermophilic UASBs achieved stable digestion at organic loading rates (OLR) of up to 10 kg COD $m^{-3} d^{-1}$ (hydraulic retention times (HRT) 24 h). Higher OLRs to the mesophilic resulted in reactor failure due to increasing total volatile fatty acid (TVFA) levels. The thermophilic UASB achieved stable operation at an OLR of 11.4 kg COD $m^{-3} d^{-1}$ (21 h HRT) but an OLR of 13.3 kg COD $m^{-3} d^{-1}$ (18 h HRT) saw a rise in TVFA from 80 to 600 mg l^{-1}. COD reduction in the thermophilic UASB was slightly lower at all OLRs, achieving a 70% COD reduction compared to a 78% COD reduction in the mesophilic UASB. The average TVFA levels were low, but somewhat higher in the thermophilic UASB than the mesophilic UASB (100 mg l^{-1} compared to 25 mg l^{-1}). It is concluded that either mesophilic or thermophilic digestion could be used successfully to treat settled instant coffee production wastewater in UASB reactors. Copyright © 1996 Elsevier Science Ltd

Key words—anaerobic digestion, instant coffee wastewater, thermophilic, mesophilic, UASB

INTRODUCTION

The production of instant coffee generates large volumes of high strength particle-bearing carbonaceous wastewater (Dinsdale *et al.*, 1996) which would seem, after reduction of suspended solids, to be ideally suited for treatment by anaerobic digestion. The waste is discharged at high temperature (~70°C), so the economic use of thermophilic anaerobic digestion would be possible. Thermophilic operation offers the potential advantage of higher loading rates and therefore smaller treatment plants than the equivalent mesophilic system (Wiegant and Lettinga, 1985; Wiegant *et al.*, 1986; Lanting *et al.*, 1989; Souza *et al.*, 1992).

The UASB process is widely used in wastewater treatment; however, reports of the treatment of instant coffee wastewater in USABs are conflicting. Successful full-scale mesophilic operation had been reported by Hajipakkos (1992) (using effluent from which particles has been removed by settling) and Lettinga and Hulshoff Pol (1991). In contrast Lanting *et al.* (1989), in a pilot plant study, concluded that mesophilic operation could not be sustained for more than 50 days without a rise in total volatile fatty acids (TVFA) leading to digester failure. Longer term stability (greater than 50 days) could only be achieved by conversion to thermophilic operation.

Studies on waste containing coffee grounds using CSTRs have also experienced problems in achieving long-term anaerobic digestion at mesophilic and thermophilic temperatures (Lane, 1981; Kostenberg and Marchaim, 1993; Hawkes *et al.*, 1994; Dinsdale *et al.*, 1996).

Laboratory scale experiments have been performed using various synthetic feed stocks to mimic instant coffee wastewaters. These include instant coffee itself (Fernandez and Forster, 1993a; McDougall *et al.*, 1993; Shi and Forster, 1993), and coffee bean extract (Fernandez and Forster, 1993b). McDougall *et al.* (1993) and Fernandez and Forster (1993a, b) used anaerobic filters while Shi and Forster (1993) operated a UASB. McDougall *et al.* (1993) also utilised a two phase system with an acidogenic CSTR.

McDougall *et al.* (1993) and Fernandez and Forster (1993a, b) could achieve stable mesophilic digestion at organic loading rates up to 4 kg COD $m^{-3} d^{-1}$. Problems were experienced in

*Author to whom correspondence should be addressed [Fax +44(0)1443 482285].

establishing stable thermophilic digestion; Fernandez and Forster (1993a, b) experienced reactor failure and McDougall *et al.* (1993), after 41 days of operation, had TVFA levels of 1000 mg l^{-1}. The general conclusion from the work using synthetic feeds was that mesophilic operation was superior to thermophilic operation. This is in contrast to Lanting *et al.* (1989) who found the reverse when using actual coffee waste. This work therefore used actual coffee waste to resolve this conflict. The aim of this study was to determine if long-term stable anaerobic digestion of high strength coffee wastewater could be achieved in UASB reactors. As the UASB process has become more common, faster start-up can be achieved by seeding from an established reactor rather than generating the granules from ungranulated inoculum (Goodwin *et al.*, 1992). Seed in this case was taken from a pilot plant UASB operating mesophilically on instant coffee wastewater (Quarmby and Forster, 1995). As thermophilic inoculum is rarely available, the work also provided information on the feasibility of converting mesophilic granules to thermophilic operation, the time scale over which this could be achieved and what precautions were required.

MATERIALS AND METHODS

Feed stock

Coffee waste containing coffee grounds was obtained from one of the waste streams at the Nestlé instant coffee factory, Hayes, London, UK. The waste was frozen until required, after which the coffee grounds were removed by settling at room temperature for 1 h and siphoning off the top layer. This procedure resulted in coffee waste free of coffee grounds with total COD ranging from 7400 to 14,000 mg O_2 l^{-1}. From day 30 of the experiment the coffee waste was blended or diluted with deionised water to produce a feed with COD of 10,000 mg $O_2 l^{-1}$. Nitrogen and phosphorus (as urea and diammonium hydrogen orthophosphate) were added from day 1 to give a COD:N:P ratio of 400:7:1. Trace elements were supplied by the addition of 2 cm^3 l^{-1} of trace element solution as described in Standard Methods (HMSO, 1988). Sodium bicarbonate was also added at 1.5–2.0 g l^{-1}.

Analyses

Total solids and suspended solids were determined in triplicate by a convection oven at 105°C (American Public Health Association, 1989) or a microwave set at low power. When both methods were compared using Student's *t*-test at 5% confidence limits no significant difference was seen. Volatile solids determination was performed in triplicate as described in Standard Methods (American Public Health Association, 1989). The pH was determined using a pH probe as Standard Methods (American Public Health Association, 1989). Bicarbonate alkalinity (partial alkalinity) was determined by titration to pH 5.75 (Jenkins *et al.*, 1983).

Total chemical oxygen demand (COD) was determined almost daily using the sealed tube method as Standard Methods (American Public Health Association, 1989) with the mercury-free reagents as described in Standard Methods (HMSO, 1986). Each COD measurement is the average of three analyses of the same sample. Calcium and potassium levels were determined in triplicate on filtered samples using a Corning 400 flame photometer. Lipid levels were determined in triplicate by a chloroform/methanol extraction (Bligh and Dyer, 1959). Samples were taken daily for analysis of VFA, and biogas composition and volatile fatty acid levels were determined by gas chromatography as described by Peck *et al.* (1986). Samples for pH and bicarbonate alkalinity were taken from the sample port of the UASB reactors. All other analyses were performed on effluent collected from the outflow port.

Reactor apparatus

Four 5 l perspex UASB reactors were constructed. The main body consisted of a 64 cm long perspex tube of 10 cm i.d., 11 cm o.d. Feed was pumped through a T piece situated at the bottom of the reactor. Effluent exited at the top of the reactor via a U-bend, which was arranged such that the reactor had 4.8 l liquid volume. The sample port position used for pH and bicarbonate alkalinity was 6 cm below the liquid level in the reactor. The gas separator consisted of a perspex baffle and a polypropylene funnel. Reactor temperature was maintained using a water jacket, two UASB reactors being maintained at mesophilic temperature 35°C, and the other two at thermophilic temperature 55°C. After start-up, one mesophilic and one thermophilic reactor were used to provide the experimental results below and the remaining two maintained on a 2 day HRT as backup reactors in case of failure.

Biogas volume was measured continuously by an electronic low flow gas meter and data logging system described by Guwy *et al.* (1995), which averaged counts over a 10 min period. The feed was supplied by peristalic pump from two continually mixed 20 l containers. After day 31 the feed was maintained at 5°C by placing the feed container in a domestic larder refrigerator. This reduced the amount of biomass growth in the peristaltic tubing and prevented any significant pre-acidification.

Reactor operation

All reactors were seeded with 1.6 l of mesophilic granules from the SERC pilot plant Anaerobic Facility operating at the Nestlé instant coffee factory, Hayes, London, UK, treating instant coffee wastewater (Quarmby and Forster, 1995). The granules had been washed and sieved to remove any non-granular material. The inoculum gave a volatile solids concentration of 14.5 g l^{-1} and a sludge bed height of 21 cm. The reactors were warmed up rapidly to the required temperature (35 or 55°C) and 4 days later feeding was started (day 0) at a 2 day hydraulic retention time (HRT) and an organic loading rate (OLR) of 3.6–7 kg COD m^{-3} d^{-1}. During start-up, to minimise the addition of sodium ions, levels of bicarbonate alkalinity were monitored and extra sodium bicarbonate was added if the bicarbonate alkalinity level dropped below 1000 mg $CaCO_3$ l^{-1}. After start-up, changes in OLR were affected by changes in feed flow rate (i.e. in HRT) introduced over a 2 day period.

Table 1. Characteristics of raw settled coffee waste

Analysis	Range of values
Total solids (g l^{-1})	10.4–13.2 (3)
Volatile solids (g l^{-1})	10.2–13.0 (3)
Suspended solids (g l^{-1})	0.6–1.0 (2)
Total COD (mg O_2 l^{-1})	7400–18,000 (15)
Total lipid (g l^{-1})	1.5 (1)
Calcium (mg l^{-1})	70–90 (2)
Potassium (mg l^{-1})	90–110 (2)
pH	4.1–4.6 (5)

( ) = Number of separate samples taken from the effluent stream for analysis.

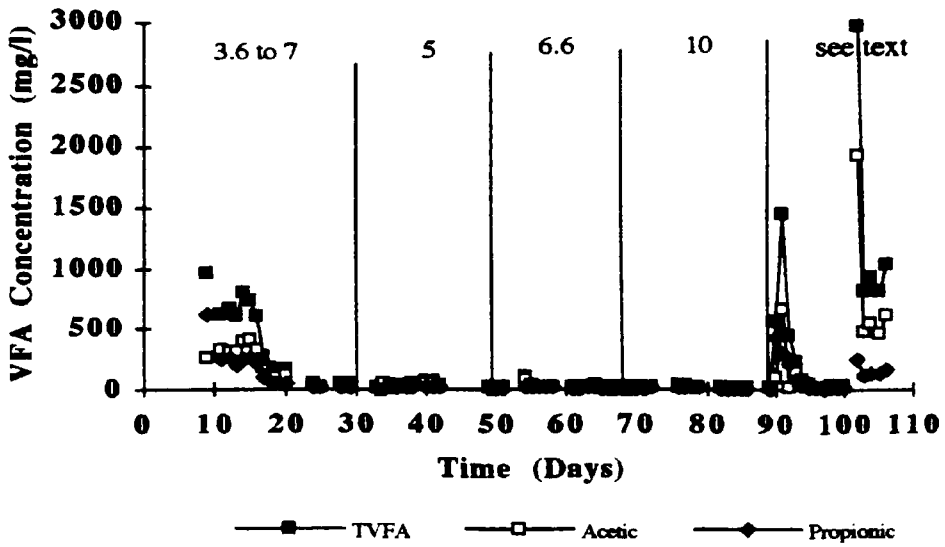

Fig. 1. Mesophilic UASB volatile fatty acid (VFA) levels. Organic loading rates of 3.6–7, 5, 6.6 and 10 kg COD $m^{-3} d^{-1}$ were used and after day 89, 13.3, 10 and 11.4 kg COD $m^{-3} d^{-1}$ (see text).

RESULTS AND DISCUSSION

Feed analysis

Table 1 shows representative data giving the general characteristics of the waste as sampled on five separate days over a period of 8 months. Each value given is the average of triplicate assays. The waste from the Nestlé factory was up to four times stronger than the coffee wastewaters used by Hajipakkos (1992), having a COD of 7400–18,000 mg $O_2 l^{-1}$ compared to 4000 mg $O_2 l^{-1}$. This could lead to much higher levels of inhibitory compounds being present in this particular wastewater.

Start-up

High TVFA levels were experienced in all reactors at start-up. The results for one mesophilic and one thermophilic reactor are given in Figs 1 and 2. During the first 25 days of operation levels of up to 970 mg l^{-1} TVFA occurred in the mesophilic reactor (Fig. 1) and 1850 mg l^{-1} TVFA in the thermophilic reactor (Fig. 2). Despite the high TVFA levels, the mesophilic UASBs maintained bicarbonate alkalinity at between 1590 and 2980 mg $CaCO_3 l^{-1}$ and pH at 7.0–7.4 over days 0–25. Bicarbonate alkalinity remained at 1030–1940 mg $CaCO_3 l^{-1}$ and pH 6.75–7.45 in the thermophilic UASBs except on day 15 where bicarbonate dropped to 710 mg $CaCO_3 l^{-1}$ and pH dropped to pH 6.70, requiring the addition of extra sodium bicarbonate to maintain the bicarbonate alkalinity at 1000 mg $CaCO_3 l^{-1}$. The strategy of monitoring bicarbonate alkalinity prevented pH shocks occurring while keeping sodium ion levels to a minimum, despite high TVFA levels related to the high organic strength of the wastewater.

All reactors contained mesophilic granules from the SERC pilot plant adapted to treating instant

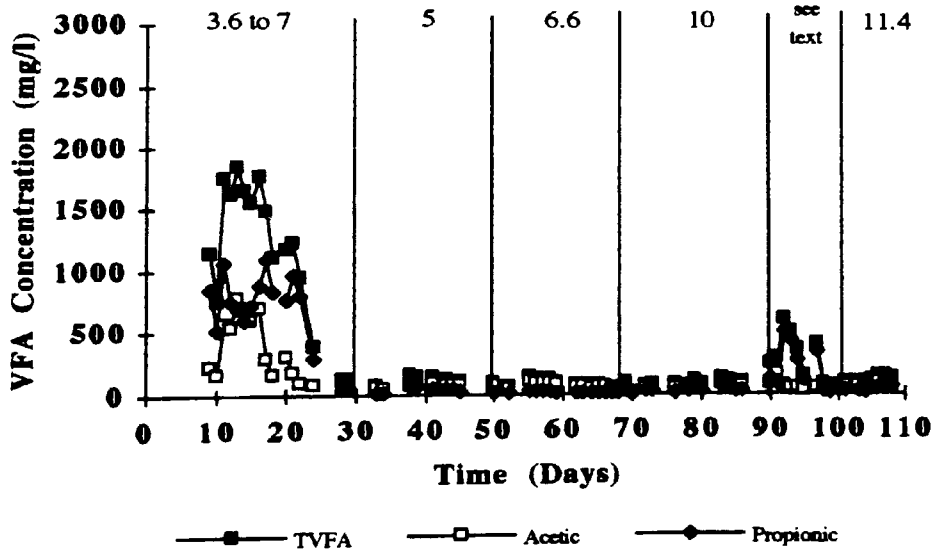

Fig. 2. Thermophilic UASB volatile fatty acid (VFA) levels. Organic loading rates of 3.6–7, 5, 6.6, 10 and 11.4 kg COD $m^{-3} d^{-1}$ were used and between days 89–100, 13.3 and 10 kg COD $m^{-3} d^{-1}$ (see text).

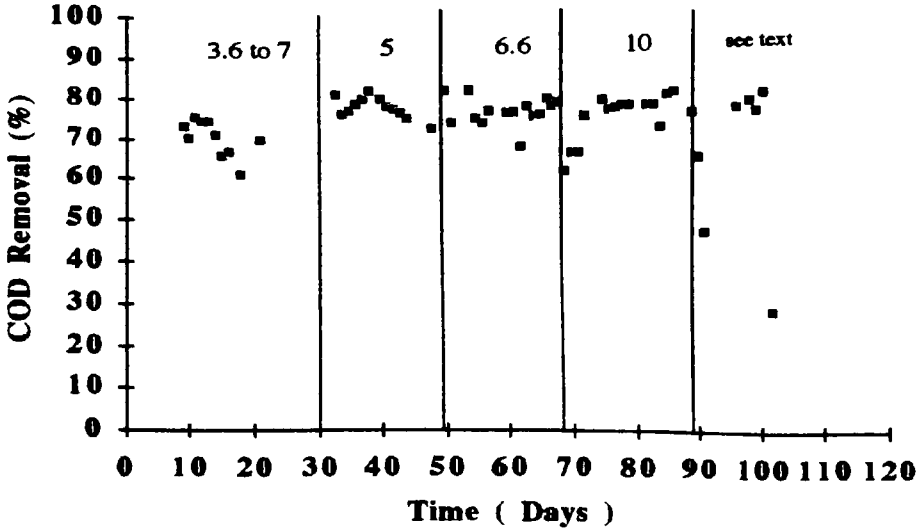

Fig. 3. Mesophilic COD removal. Organic loading rates of 3.6–7, 5, 6.6 and 10 kg COD $m^{-3} d^{-1}$ were used and after day 89, 13.3, 10 and 11.4 kg COD $m^{-3} d^{-1}$ (see text).

coffee wastewater at the Nestlé factory, and were subject to the same OLR regime at start-up. The higher levels of TVFA, particularly propionic acid, seen in the thermophilic reactors (Fig. 2) were probably due to the conversion process from mesophilic to thermophilic operation. In other studies on coffee waste, adaptation of mesophilic to thermophilic sludge was effected by small increases in temperature over a longer period, e.g. Lanting *et al.* (1989) where the temperature was increased over a 2 month period. Alternatively granules were acclimatised to thermophilic temperatures in the presence of yeast extract and then acclimatised to coffee waste (Shi and Forster, 1993). In the present study a rapid rise to the thermophilic operating temperature led to satisfactory performance within 28 days. The granules still retained their activity and structural integrity over more than 100 days of operation in this study.

By day 28 the TVFA levels had fallen to 130 mg l^{-1} in the thermophilic reactor and by day 24 to 40 mg l^{-1} in the mesophilic reactor, with COD removal reaching 78% in the mesophilic digester and 70% in the thermophilic digester. Subsequent levels of TVFA and COD removal (see Figs 1–4 and Table 2) showed no significant improvement over the rest of the experiment.

Effect of retention time and loading rate

The performance of the mesophilic and thermophilic UASBs was compared at HRTs of 48, 36, 24, 21 and 18 h. The loading rates studied were 5, 6.6, 10, 11.4 and 13.3 kg COD $m^{-3} d^{-1}$, respectively. The reactors were operated for at least three HRT at each OLR. Where steady state was achieved, the data is presented in Table 2. It was considered that steady state was achieved once three consecutive readings of TVFA levels and % COD removal were obtained

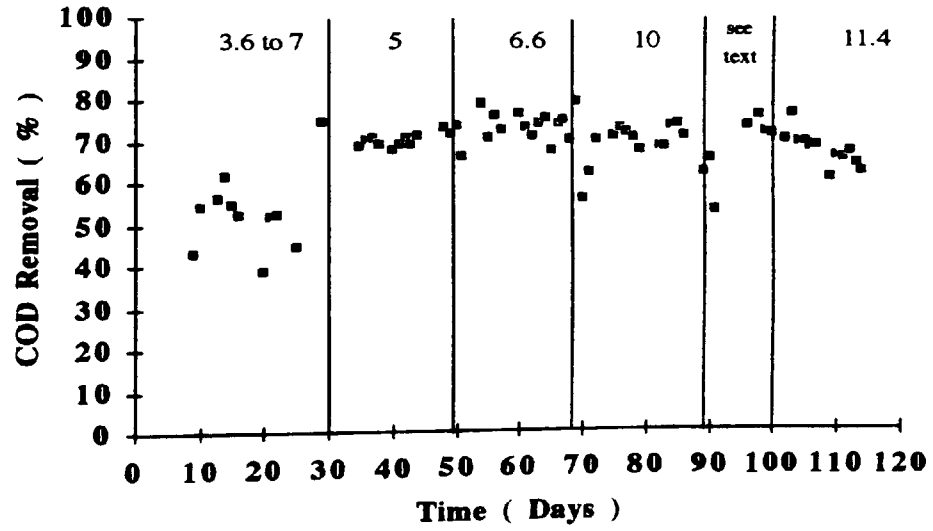

Fig. 4. Thermophilic COD removal. Organic loading rates of 3.6–7, 5, 6.6 and 10 kg COD $m^{-3} d^{-1}$ were used and between day 89–100, 13.3 and 10 kg COD $m^{-3} d^{-1}$ (see text).

Table 2. Summary of performance of single stage mesophilic and thermophilic UASBs

Parameter	Mesophilic	Thermophilic	Mesophilic	Thermophilic	Mesophilic	Thermophilic	Mesophilic	Thermophilic
Organic loading rate (kg COD $m^{-3} d^{-1}$)	5	5	6.6	6.6	10	10	11.4	11.4
Hydraulic retention time (h)	48	48	36	36	24	24	18	18
No. of days operation	18	18	17	17	21	21	Failed after 2 days	13
No. of HRTs	9	9	11	11	21	21	NA	15
%COD removal	78	70	78	73	77	70	NA	68
	(10)	(11)	(13)	(13)	(13)	(12)		(12)
	SD = 2	SD = 2	SD = 3	SD = 4	SD = 3	SD = 6		SD = 4
TVFA (mg l^{-1})	35	122	17	99	15	80		97
	(9)	(11)	(14)	(12)	(16)	(14)	NA	(10)
	SD = 16	SD = 32	SD = 4	SD = 27	SD = 7	SD = 33		SD = 35
$\%CH_4$	64	63	60	60	63	63		59
	(12)	(12)	(13)	(14)	(12)	(13)	NA	(7)
	SD = 5	SD = 5	SD = 2	SD = 2	SD = 2	SD = 2		SD = 2
Bicarbonate alkalinity	1900	1600	1900	1800	1800	1700		2100
(mg $CaCO_3$ l^{-1})	(8)	(9)	(10)	(9)	(9)	(7)	NA	(8)
	SD = 240	SD = 100	SD = 230	SD = 100	SD = 250	SD = 100		SD = 250
Gas production ($l^{-1} d^{-1}$)	1.8	1.6	2.5	2.3	3.5	3.0		3.2
	(15)	(15)	(16)	(16)	(19)	(19)	NA	(13)
	SD = 0.14	SD = 0.15	SD = 0.2	SD = 0.16	SD = 0.34	SD = 0.25		SD = 0.27

( ) = Number of samples; SD = standard deviation; NA = not available.

which were similar to those obtained at the lowest OLR of 5 kg COD $m^{-3} d^{-1}$.

Good COD removals of 70–78% were obtained under each of the steady-state operating conditions shown in Table 2. These COD removals compare favourably with other studies using real coffee waste; e.g. Hajipakkos (1992) achieved a 55% removal at mesophilic temperatures, compared with 78% in the present study, and Lanting *et al.* (1989) achieved 60–70% at thermophilic temperatures. Low levels of suspended solids (measured on days 58 and 64) were found in the digester effluent. The mesophilic reactor had levels of 0.17 mg l^{-1} and 0.25 mg l^{-1} and the thermophilic reactor 0.25 mg l^{-1} and 0.2 mg l^{-1}, respectively. When compared with the levels of suspended solids entering the reactor (400–800 mg l^{-1}) it appears that some solids were retained rather than digested. After day 60, solids were removed periodically from the underside of the gas separator where a thick grey/brown sludge accumulated. Although there was no change in the volume occupied by the granular bed throughout the 100 day experimental period, a blanket of flocculating material built up gradually on its surface.

The methane yield per kg COD removed, measured at ambient temperature and pressure, was 0.29 m^3 kg COD^{-1} for the mesophilic reactor and 0.27 m^3 kg COD^{-1} for the thermophilic reactor which was below the theoretical value of 0.35 m^3 kg COD^{-1} at STP. The need to remove this sludge regularly from the gas separator would suggest that the COD removal had been exaggerated either due to a component in the coffee waste or biomass sticking to the gas separator.

Studies using synthetic wastes achieved up to 70% COD removal (Fernandez and Forster, 1993a, b) in mesophilic and thermophilic operation. However, problems were experienced in achieving stable thermophilic digestion. Fernandez and Forster (1993a, b) experienced reactor failure and McDougall *et al.* (1993) after 41 days of operation had TVFA levels of 1000 mg l^{-1}. Synthetic feeds were found to have levels of potassium between 125 mg l^{-1} and 1200 mg l^{-1} for instant coffee and coffee bean extract respectively (Fernandez and Forster, 1993a, b; Shi and Forster, 1993); these levels were found to reduce the efficiency of mesophilic and thermophilic operation. The above workers found that calcium addition alleviated the effects of K^+ toxicity. In the present study, analysis by flame photometry showed an average of 100 mg l^{-1} of potassium and 80 mg l^{-1} of calcium respectively in the actual factor effluent (Table 1). Thus the levels of Ca^+ needed to alleviate any effects of K^+ were already present. Kostenberg and Marchaim (1993) reported low potassium levels of 30 mg l^{-1} in instant coffee processing wastewater. Local conditions and factory operations may influence these ion levels and should be determined for each factory.

Figures 1–4 show data for TVFA levels and %COD removal for both mesophilic and thermophilic reactors. Steady state was achieved for both reactors at OLRs of 5, 6.6 and 10 kg COD $m^{-3} d^{-1}$ (HRTs of 48, 36 and 24 h). However, with the OLR of 13.3 kg COD $m^{-3} d^{-1}$ (HRT was decreased to 18 h) over days 89 and 90, a rise in TVFA in both the mesophilic and thermophilic UASBs followed. The rise in TVFA in the mesophilic UASB was more pronounced, increasing from 16 mg l^{-1} to 1430 mg l^{-1} (see Fig. 1). This was associated with a reduction in bicarbonate alkalinity and pH to 750 mg $CaCO_3$ l^{-1} and pH 6.4, respectively. Feeding was therefore stopped at day 91 and 5 g l^{-1} sodium bicarbonate added to restore the pH and bicarbonate alkalinity to previous levels. A reduction in COD removal from 77 to 48% also occurred (Fig. 3).

Once the OLR was increased to 13.3 kg COD $m^{-3} d^{-1}$ over days 89 and 90, the thermophilic reactor saw a rise in TVFA from previous levels of 80 mg l^{-1} to 600 mg l^{-1} (Fig. 2). The level of COD removal also fell from 70 to 53% by day 91 (Fig. 4). The pH and alkalinity decreased from pH 6.9 to pH 6.8 and from 1830 mg $CaCO_3$ l^{-1} to 1120 mg $CaCO_3$ l^{-1}, respectively. On decreasing the OLR to 10 kg COD $m^{-3} d^{-1}$ on day 93 in the thermophilic reactor previous performance levels were regained (Figs 2 and 4). The mesophilic reactor also saw a return to previous levels of performance when feeding was restarted at an OLR of 10 kg COD $m^{-3} d^{-1}$ on day 95 (Figs 1 and 3).

The last OLR tried was 11.4 kg COD $m^{-3} d^{-1}$ (HRT 21 h) at days 100 and 101. The mesophilic UASB had a more severe reaction to this change (see Fig. 2) than the previous increase in OLR. The TVFA levels rose from 27 to 2930 mg l^{-1} with a sharp rise in acetic acid content. The pH fell to 4.3 and bicarbonate alkalinity was reduced to zero.

These results would therefore indicate that using this feed stock, a mesophilic UASB could achieve an OLR of up to 10 kg COD $m^{-3} d^{-1}$. However, if the loading rate was increased to 11.4 kg COD $m^{-3} d^{-1}$ then the mesophilic UASB suffered failure. This OLR value is significantly higher than the studies of synthetic coffee effluent which achieved OLR of between 3 and 4 kg COD $m^{-3} d^{-1}$, although these systems were not tested to failure (Fernandez and Forster, 1993a, b; McDougall *et al.*, 1993; Shi and Forster, 1993).

Lanting *et al.* (1989) in two pilot scale studies found that mesophilic UASBs failed at around 7 weeks (50 days) at loading rates of up to 12–13 kg COD $m^{-3} d^{-1}$ in the first study and in the second study as the OLR was increased up to 10–11 kg COD $m^{-3} d^{-1}$. Although Lanting *et al.* (1989) did not use nutrient addition, it perhaps suggests that loading rates above 10 kg COD $m^{-3} d^{-1}$ are not achievable in mesophilic UASBs with this waste. Lanting *et al.* (1989) found that longer-term digestion would be better achieved in thermophilic digestion. In our study, mesophilic operation was maintained for up to 100 days as long as the OLR did not exceed 10 kg COD $m^{-3} d^{-1}$, which is significantly longer than the 50 days achieved by Lanting *et al.* (1989). The existence of up to 3 full-scale mesophilic UASBs treating instant coffee wastewaters up to OLRs of 6 kg COD $m^{-3} d^{-1}$ supports the proposition that long-term digestion is feasible in mesophilic UASBs (Lettinga and Hulshoff Pol, 1991; Hajipakkos, 1992).

This study found that the thermophilic UASB could treat coffee waste at higher OLR than the equivalent mesophilic system, at an OLR of 11.4 kg COD $m^{-3} d^{-1}$ compared to 10 kg COD $m^{-3} d^{-1}$. The thermophilic system was not tested to failure. An increase in TVFA from 80 to 600 mg l^{-1} as the OLR was increased to 13.3 kg COD $m^{-3} d^{-1}$ (on days 89–90) would suggest that the limit was being approached between 11.4 and 13.3 kg COD $m^{-3} d^{-1}$. Lanting *et al.* (1989) operated a thermophilic UASB at an OLR of 10 kg COD $m^{-3} d^{-1}$ but did not exceed this OLR.

Although the thermophilic UASB could treat a somewhat higher OLR than the mesophilic UASB (11.3 compared to 10 kg COD $m^{-3} d^{-1}$), the COD removal was less (68% compared to 77%, respectively) and the levels of TVFA higher (see Table 2). However, the other performance parameters were similar for both UASBs. An initial review of the literature suggested that thermophilic UASBs offered the prospect of significantly higher OLRs than typical mesophilic systems (Wiegant and Lettinga, 1985), however, these have been matched by USABs operating in the mesophilic range (Fang and Chui, 1993). The work presented here would suggest that thermophilic systems can cope with higher OLRs but the advantage is not that great.

As seen in Table 2, at each organic loading rate the thermophilic UASB operated at a higher TVFA level than the mesophilic reactor. This effect has been noted previously (van Lier *et al.*, 1993). In all cases the absolute TVFA levels were low (15–122 mg l^{-1}). Thermophilic systems tend to accumulate high levels of propionic acid (Wiegant *et al.*, 1986). In this study this occurred during conversion from mesophilic operation but once steady-state operation had been achieved the percentage of TVFA which was propionate was similar in both mesophilic (24%) and thermophilic (32%) reactors.

The coffee waste has a high level of lipid, 1.5 g l^{-1} (12% of VS). Lipid material has been shown to cause inhibition problems due to the long-chain fatty acids produced in the hydrolysis process (Rinzema *et al.*, 1994). Thermophilic organisms are more sensitive to certain inhibitory compounds than mesophilic (Fernandez and Forster, 1993a, b; Shi and Forster, 1993) and this could explain the higher TVFA levels and lower COD removal in the thermophilic system. However, thermophilic systems tend to operate at higher VFA levels no matter what the feedstock is (Wiegant and Lettinga, 1985; Wiegant *et al.*, 1986; Souza *et al.*, 1991; van Lier *et al.*, 1993). The maximum OLRs reached in this study are lower than OLRs achieved with other wastes in UASBs, e.g. 104 kg COD $m^{-3} d^{-1}$ with sugars (Weigant and Lettinga, 1985). In a recent review of full-scale UASBs, the UASBs treating coffee waste were found to be operating at the lowest OLRs (Lettinga and Hulshoff Pol, 1991). The levels of lipid or other inhibitory compounds found in coffee waste could be responsible for this.

A previous study (Dinsdale *et al.*, 1996) showed that mesophilic anaerobic digestion in a CSTR of the instant coffee factory effluent without prior settling to remove solids could give up to a 60% reduction in total COD at an OLR of 1.3 kg total COD $m^{-3} d^{-1}$. With further solids separation, giving solids which

may be used to raise steam in the factory, a waste was produced which was treated in either mesophilic or thermophilic UASBs at 300% greater loading rates and significantly shorter HRT, with a significantly better effluent quality.

CONCLUSIONS

Mesophilic granules from a pilot plant treating instant coffee waste were successfully converted to thermophilic operation by raising the temperature as rapidly as possible in one step. The high TVFA levels experienced could be managed by monitoring bicarbonate alkalinity and adding sodium bicarbonate to maintain the bicarbonate alkalinity.

Both mesophilic and thermophilic UASBs could be operated for at least 100 days with low TVFA and good COD removal. The slow decline in performance reported in the literature was not seen.

The mesophilic reactor achieved a marginally better effluent quality, with average COD removal of 78% and TVFA of 25 mg l^{-1} compared to a 70% COD removal and 100 mg l^{-1} TVFA level in the thermophilic UASB under the same conditions.

The maximum OLR at which the thermophilic UASB could maintain steady-state operation was marginally higher than the mesophilic (11.4 kg COD m^{-3} d^{-1} compared to 10 kg COD m^{-3} d^{-1}). Higher OLRs for either reactor led to digester failure with increasing TVFA and decreasing bicarbonate alkalinity.

High levels of lipid (up to 12% of VS) were found in the waste and may account for the relatively low OLR achieved. Unlike some previous studies with synthetic instant coffee wastes, potassium was not present at levels to cause inhibition problems.

Acknowledgements—This work was funded under SERC grant No. GR/H18494. We are grateful to Nestlé UK for supplying the waste, and staff at the SERC pilot plant for provision of UASB granules.

REFERENCES

American Public Health Association (1989) *Standard Methods for the Examination of Waste and Wastewater*, 17th edn. Washington, USA.

Bligh E. G. and Dyer N. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917.

Dinsdale R. M., Hawkes F. R. and Hawkes D. L. (1996) The mesophilic and thermophilic anaerobic digestion of coffee waste containing coffee grounds. *Wat. Res.* **30**(2), 371–377.

Fang H. H. P. and Chui H. K. (1993) Maximum COD loading capacity in UASB reactors at 37°C. *Envir. Engng ASCE* **119**(1), 103–119.

Fernandez N. and Forster C. F. (1993a) The anaerobic digestion of a simulated coffee waste using thermophilic and mesophilic upflow filters. *Trans IChemE* **72**, Part B, 15–20.

Fernandez N. and Forster C. F. (1993b) A study of the operation of mesophilic and thermophilic anaerobic filters treating a synthetic coffee waste. *Bioresour. Technol.* **45**, 223–227.

Goodwin J. A. S., Wase D. A. J. and Forster C. F. (1992) Pre-granulated seeds for UASB reactors: How necessary are they? *Bioresour. Technol.* **41**, 71–79.

Guwy A. J., Hawkes D. L. and Hawkes F. R. (1995) On-line low flow high-precision gas metering systems. *Wat. Res.* **29**(3), 977–979.

Hajipakkos C. (1992) The application of a full-scale UASB plant for the treatment of coffee waste. *Wat. Sci. Technol.* **25**(1), 17–22.

Hawkes F. R., Hawkes D. L. and Dinsdale R. M. (1994) Mesophilic and thermophilic digestion of effluent from an instant coffee processing plant. *Proc. 7th Int. Symp. on Anaerobic Digestion, Cape Town, South Africa*. Poster papers, pp. 291–294 (Jan. 1994).

HMSO (1986) *Chemical Oxygen Demand (Dichromate Value) of Polluted and Wastewaters*. London, UK.

HMSO (1988) *The Assessment of Biodegradability in Anaerobic Digesting Sludge*. HMSO, London.

Jenkins S. R., Morgan J. M. and Sawyer C. L. (1983) Measuring anaerobic sludge digestion and growth by a simple alkalimetric titration. *J. Wat. Pollut. Control Fed.* **55**, 448–453.

Kostenberg D. and Marchaim U. (1993) Solid waste from the instant coffee industry as a substrate for anaerobic thermophilic digestion. *Wat. Sci. Technol.* **27**, 97–107.

Lane A. G. (1983) Anaerobic digestion of spent coffee grounds. *Biomass* **3**, 247–268.

Lanting J., Jordan J. A., Schone M. T., Kull A., Carey W. W. and Kitney B. L. (1989) Thermophilic anaerobic digestion of coffee waste water. *43rd Purdue Industrial Waste Conference Proceedings*. Lewis Publishers Inc., Michigan, pp. 513–527.

Lettinga G. and Hulshoff Pol L. W. (1991) UASB process design for various waste waters. *Wat. Sci. Technol.* **24**(8), 87–107.

McDougall F. R., Anderson G. K. and Evison L. M. (1993) Two-phase anaerobic digestion of coffee wastewater. *48th Purdue Industrial Waste Conference Proceedings*. Lewis Publishers Inc., Michigan, pp. 677–683.

Peck M. W., Skilton J. M., Hawkes F. R. and Hawkes D. L. (1986) Effects of temperature shock treatments on the stability of anaerobic digesters operated on separated cattle slurry. *Wat. Res.* **20**(4), 453–462.

Quarmby J. and Forster C. F. (1995) An examination of the structure of UASB granules. *Wat. Res.* **29**(11), 2449–2454.

Rinzema A., Boone M., Kniippenberg K. and Lettinga G. (1994) Bactericidal effect of long chain fatty acids in anaerobic digestion. *Wat. Environ. Res.* **66**, 40–49.

Shi D. and Forster C. F. (1993) An examination of the start-up of a thermophilic upflow anaerobic sludge blanket reactor treating a synthetic coffee waste. *Environ. Technol.* **14**, 965–971.

Souza M. E., Fuzaro G. and Polegato A. R. (1992) Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. *Wat. Sci. Technol.* **25**(7), 213–222.

van Lier J. B., Grolle K. C. F., Frijters C. T. M. J., Stams A. J. M. and Lettinga G. (1993) Effect of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludges and defined cultures. *Appl. Environ. Microbiol.* **59**, 1003–1011.

Wiegant W. M., Hennick M. and Lettinga G. (1986) Separation of propionate degradation to improve the efficiency of anaerobic treatment of acidified wastewaters. *Wat. Res.* **20**, 517–524.

Wiegant W. M. and Lettinga G. (1985) Thermophilic anaerobic digestion of sugars in an upflow anaerobic sludge blanket reactors. *Biotech. Bioeng.* **27**, 1603–1607.

Pergamon

PII: S0043-1354(97)00041-9

Wat. Res. Vol. 31, No. 8, pp. 1931–1938, 1997

Printed in Great Britain
0043-1354/97 $17.00 + 0.00

MESOPHILIC AND THERMOPHILIC ANAEROBIC DIGESTION WITH THERMOPHILIC PRE-ACIDIFICATION OF INSTANT-COFFEE-PRODUCTION WASTEWATER

RICHARD M. DINSDALE[1], FREDA R. HAWKES[2]* and DENNIS L. HAWKES[1]

[1]Department of Mechanical and Manufacturing Engineering and [2]School of Applied Sciences, University of Glamorgan, Pontypridd, Mid Glamorgan, CF37 1DL, U.K.

(Received February 1996; accepted in revised form February 1997)

Abstract—The thermophilic and mesophilic digestion of instant-coffee-production wastewater in upflow anaerobic sludge blanket (UASB) reactors with thermophilic pre-acidification was studied over a period of more than 120 days. The UASB reactors had been seeded with granules adapted to this wastewater, and they previously operated in single-stage mode mesophilically or thermophilically. The thermophilic pre-acidification stage was operated with pH control or with 1.5 g l^{-1} $NaHCO_3$ added to the feed, at retention times of 24, 18, 15 and 12 h. Up to 38% of the total influent chemical oxygen demand (COD) was converted to total volatile fatty acids at a 24-h hydraulic retention time (HRT), dropping to 21% at a 12-h HRT. It was found that control with NaOH to pH 6.0 at an HRT of 24 h was not required for efficient acidogenesis. The effluent from the acidogenic stage at pH 5.2 did not require prior neutralisation with NaOH before feeding to the methanogenic stage. The absence of neutralisation improved the performance of the thermophilic UASB reactor. Thermophilic digestion may be more sensitive to Na^+ toxicity than mesophilic digestion. The thermophilic/mesophilic two-stage system gave a consistent improvement in performance (measured, for example, as % COD reduction) over the thermophilic/thermophilic two-stage system, especially at higher organic loading rates. Thermophilic pre-acidification gave an increase of 60% in the loading rate achievable with the mesophilic methanogenic stage (a 100% reduction in HRT) compared with the single-stage system. © 1997 Elsevier Science Ltd

Key words—anaerobic digestion, instant-coffee wastewater, thermophilic, pre-acidification, UASB

INTRODUCTION

The upflow anaerobic sludge blanket (UASB) process has been widely used to treat industrial wastewaters, with over 200 installations world-wide (Lettinga and Hulshoff Pol, 1991). Successful treatment of settled instant-coffee wastewaters has been reported in laboratory-scale single-stage mesophilic and thermophilic UASB reactors by Dinsdale *et al.* (1997). Stable digestion was achieved at organic loading rates of 10 and 11.4 kg COD m^{-3} d^{-1} in mesophilic and thermophilic UASBs, respectively. Hajipakkos (1992), in a two-stage full-scale UASB operating on coffee wastewater, achieved loading rates of up to 6 kg COD m^{-3} d^{-1}, although no information on the acidification stage was presented. Lanting *et al.* (1989), in two pilot-scale studies with coffee wastewater, found that the mesophilic UASB reactor failed at around 7 weeks (50 days) at loading rates of up to 12–13 kg COD m^{-3} d^{-1} in the first study and as the organic loading rate (OLR) was increased up to 10–11 kg COD m^{-3} d^{-1} in the second study. However, longer-term digestion was achieved in thermophilic digestion at OLRs up to 10 kg COD m^{-3} d^{-1}. The general conclusion from these works is that settled instant-coffee wastewater is treatable in mesophilic or thermophilic UASBs but that the maximum loading rate is relatively modest. It is suggested that this is because of inhibitory compounds present in the waste. A process modification which could increase these loading rates would be of obvious commercial advantage.

One possible method to increase the process efficiency is to use a two-stage system, with a first reactor optimised for hydrolysis/acidification and a second for acetogenesis/methanogenesis (Ghosh *et al.*, 1985; Ghosh, 1991). The two-stage concept has been promoted as offering the following advantages over a single-stage system: increased chemical oxygen demand (COD) removal (McDougall *et al.*, 1993), increased stability to shock loads (Bull *et al.*, 1983) and greater resistance to inhibitory compounds, e.g. lipid (Komatsu *et al.*, 1991). Instant-coffee wastewaters have been found to contain up to 12% (w/w) of lipid (Dinsdale *et al.*, 1997), so the two-stage system could offer significant advantages in the treatment of instant-coffee wastewaters. McDougall *et al.* (1993), using a synthetic coffee waste in a mesophilic two-stage system with an anaerobic filter,

*Author to whom all correspondence should be addressed [Fax: +44 1443 482 285].

achieved a 13% greater COD removal than the equivalent single-stage system.

The wastewater from instant-coffee production is discharged at high temperatures (~ 70°C). Therefore, operation at thermophilic temperatures may be the most likely option. However, the majority of the work on two-stage reactors has been conducted at mesophilic temperatures, using synthetic wastes. In this study, settled instant-coffee-production wastewater was used and the pre-acidification stage was operated thermophilically. A mesophilic and a thermophilic methanogenic stage were employed, and the influence of pH control and changes in OLR and hydraulic retention time (HRT) on both stages was investigated.

MATERIALS AND METHODS

Feed stock

Coffee waste containing coffee grounds was obtained from one of the waste streams at the Nestlé instant-coffee factory, Hayes, London. The waste was collected on five occasions and first used from days 0, 22, 46, 84 and 108. The waste was frozen until required, after which the coffee grounds were removed by settling at room temperature for 1 h and siphoning off the top layer. This procedure resulted in coffee waste free of coffee grounds with a total COD ranging from 7400 to 18,000 mg O_2 l^{-1}. To provide a consistent feed throughout the experiment the coffee waste was blended or diluted with de-ionised water to produce a feed with a COD of 10,000 mg O_2 l^{-1}. However, from day 120, feed COD of 8000 mg O_2 l^{-1} was supplied due to technical difficulties. Nitrogen and phosphorus were added to give a COD:N:P ratio of 400:7:1. Trace elements were supplied by the addition of 2 cm^3 l^{-1} of a trace element solution, as described in Standard Methods (HMSO, 1988). Sodium bicarbonate was added at 1.5 g l^{-1} when the pH controllers were removed. The feed was maintained at 5°C by placing the two continually mixed 20-l feed containers in a domestic refrigerator. This reduced the amount of biomass growth in the peristaltic tubing and prevented any significant pre-acidification.

Analyses

Total solids and suspended solids were determined in triplicate in a convection oven at 105°C (Standard Methods, APHA, 1989) or a microwave set at low power. When both methods were compared using Students *t*-test at 5% confidence limits, no significant difference was seen. Volatile solids (VS) determination was performed in triplicate as described in Standard Methods (APHA, 1989). The pH was determined using a pH probe as in Standard Methods (APHA, 1989).

COD was determined by using the sealed tube method as in Standard Methods (APHA, 1989) with the mercury-free reagents as described in HMSO (1986). Each COD measurement is the average of three analyses of the same sample. Total COD was determined on a well-mixed sample. Samples were taken daily for analysis of volatile fatty acids (VFA), and biogas composition and VFA levels were determined by gas chromatography as described by Peck *et al.* (1986). Samples for pH were taken from the sample port of the UASB reactors. All other analyses were performed on effluent collected from the outflow port.

Reactor apparatus

The two-stage reactor apparatus consisted of a continuously stirred thermophilic pre-acidification reactor which then supplied acidified feed to a methanogenic UASB reactor. Pre-acidification reactors (AC1 and AC2) were connected to a mesophilic and a thermophilic methanogenic UASB reactor, respectively, and operated simultaneously on the same feed.

Pre-acidification reactors. The two pre-acidification reactors each consisted of a Quickfit vessel (total volume 6.2 l, liquid volume 5.6 l) with a side arm. The reactors were maintained at 55°C and were continuously stirred at 100 rpm. Both reactors were fitted with an Ingold Xerolyte gel-filled electrode, type HA405-DXK-S/120, (Mettler Toledo, Leicester, U.K.) and a Kent EIL9142 pH meter/controller (ABB Kent-Taylor Ltd., Stonehouse, Gloucestershire, U.K.). The acidified effluent exited to a 150-ml pH adjustment chamber, continually mixed with a magnetic stirrer and fitted with another pH controller unit, and was then supplied to the UASB reactors.

UASB reactors. Two 5-l Perspex UASB reactors as reported by Dinsdale *et al.* (1997) were used. The main body consisted of a 64-cm-long Perspex tube of 10 cm i.d., 11 cm o.d. Feed was pumped through a T-piece situated at the bottom of the reactor. Effluent exited at the top of the reactor via a U-bend, which was arranged such that the reactor had a 4.8-l liquid volume. The sample port position used for pH and bicarbonate alkalinity samples was 6 cm below the liquid level in the reactor. The gas separator consisted of a Perspex baffle and a polypropylene funnel. Reactor temperature was maintained using a water jacket, one UASB reactor being maintained at mesophilic temperature (35°C) and the other at thermophilic temperature (55°C). Biogas volume was measured continuously by an electronic low-flow gas meter and data logging system described by Guwy *et al.* (1995), with counts averaged over a 10-min period.

Reactor operation

The pre-acidification reactors were inoculated with 310 cm^3 of homogenised UASB granules from a mesophilic UASB digester treating paper-mill effluent. This gave an initial inoculum concentration of 2.7 g VS l^{-1}. Feeding was started (day 0) at a 24-h HRT to the acidogenic stage and changes in OLR were effected by changes in feed flow rate introduced over a 2-day period. In AC1 from day 0 to day 69 and in AC2 from day 0 to day 58, the pH controller activated a pump to add 50 g l^{-1} NaOH when the pH dropped below pH 6.0. For AC1 after day 69 and for AC2 from day 58, no pH adjustment was made, but instead the feed to the system contained 1.5 g l^{-1} sodium bicarbonate. Thermophilic pre-acidification was studied with pH control at pH 6.0 at a 24-h HRT and an OLR of 10 kg COD m^{-3} d^{-1} and then without pH control at a 24-h HRT (an OLR of 10 kg COD m^{-3} d^{-1}), 18-h HRT (an OLR of 13.3 kg COD m^{-3} d^{-1}), 15-h HRT (an OLR of 16 kg COD m^{-3} d^{-1}) and 12-h HRT (an OLR of 16 kg COD m^{-3} d^{-1}).

The UASB reactors had been seeded, as previously reported (Dinsdale *et al.*, 1997), with 1.6 l of mesophilic granules from the SERC pilot-plant Anaerobic Facility operating at the Nestlé instant-coffee factory, Hayes, London, treating instant-coffee wastewater (Quarmby and Forster, 1995). The granules had been washed and sieved to remove any non-granular material. The inoculum gave a VS concentration of 14.5 g l^{-1} and a sludge bed height of 21 cm. For thermophilic operation, the mesophilic inoculum was adapted as described by Dinsdale *et al.* (1997). The laboratory mesophilic and thermophilic UASB reactors were operated in single-stage configuration for over a 100 days on wastewater from the instant-coffee-production process, and the results are given in Dinsdale *et al.* (in press). The same reactors and reactor contents were used, with a 10-day rest period when the reactors were maintained at either 35 or 55°C but not fed before the start of the experimental work reported.

A portion of the effluent from the pre-acidification reactors AC1 and AC2 was passed to the UASB reactors,

such that the HRT of both the acidogenic and methanogenic stages was the same despite the differences in reactor volume. Feeding was started on day 0 at a 24-h HRT to the methanogenic stage, giving a 48-h HRT in the whole system. Since there was minimal COD removal in the acidogenic stage, the OLR to both stages at this HRT was 10 kg COD m^{-3} d^{-1}.

From day 0 to day 69 for the mesophilic UASB and from day 0 to day 58 for the thermophilic UASB, the pH of the acidified feed was adjusted to pH 6.7 in the intermediate pH adjustment chamber by the addition of 50 g l^{-1} NaOH. After these time periods, no pH adjustment was made.

The mesophilic UASB was operated at 24-h HRT (OLR 10 kg COD m^{-3} d^{-1}), 21-h HRT (OLR 11.4 kg COD m^{-3} d^{-1}), 18-h HRT (OLR 13.3 kg COD m^{-3} d^{-1}), 15-h HRT (OLR 16 kg COD m^{-3} d^{-1}) and 12-h HRT (OLR 16 kg COD m^{-3} d^{-1}). The thermophilic UASB was operated at 24-h HRT (OLR 10 kg COD m^{-3} d^{-1}), 21-h HRT (OLR 11.4 kg COD m^{-3} d^{-1}), 18-h HRT (OLR 13.3 kg COD m^{-3} d^{-1}) and 12-h HRT (OLR 16 kg COD m^{-3} d^{-1}).

RESULTS

Thermophilic pre-acidification

The feed fed to the acidification stage contained on average 329 mg l^{-1} total VFA (TVFA), which consisted of 301 mg l^{-1} acetic, 18 mg l^{-1} propionic, 1 mg l^{-1} of *i*-butyric, 2 mg l^{-1} *n*-butyric, 5 mg l^{-1} *i*-valeric and 3 mg l^{-1} *n*-valeric acids ($N = 8$). The level and distribution of VFAs in the thermophilic pre-acidification reactors with and without pH control at a 24-h HRT and without pH control at lower HRTs are shown in Table 1. It can be seen that without pH control the preacidification reactors operated at a pH between 5.0 and 5.5.

By quoting values as % COD of feedstock, a more valid determination of the level of effectiveness of acidification can be reached (Alexiou and Anderson, 1994). Factors to convert mg l^{-1} VFA to COD were as used by these authors. In the feed fed to the acidification reactors, the VFA levels corresponded to 4% of the COD of the feed. The degree of acidification occurring in the acidification reactors as % COD of the feed influent is presented in Fig. 1.

Only CO_2 was detected in the headspace of AC2 from day 1 to day 126. Some methane was occasionally detected in AC1 (45% CH_4 detected in the headspace at day 26 and 27–44% CH_4 between days 27 and 35), the residual gas being CO_2.

Effect of pre-acidification on the methanogenic reactors

The levels of VFA (TVFA, acetic and propionic acids) from day 0 to 126 in the mesophilic and thermophilic UASBs are shown in Figs 2 and 3, respectively. For the start-up phase, in the mesophilic UASB the TVFA increased to 164 mg l^{-1} on day 2 but subsequently decreased to 25 mg l^{-1} on day 3. From day 5 to day 64, the average level of TVFA was 17 mg l^{-1} (SD = 10, $N = 27$).

In contrast, the thermophilic reactor reached TVFA levels of 1022 mg l^{-1} on day 5, levels dropping to 156 mg l^{-1} on day 8 but subsequently rising to 840 mg l^{-1} by day 58. On day 59, the pH controller was removed from the acidification reactor and the pH adjustment chamber. On day 62, the TVFA had fallen to 423; from day 64 to day 71, the average TVFA was 210 mg l^{-1}. For technical reasons (factory maintenance interrupted effluent supply), the reactors were without feed from day 70 to day 90. An increase in VFA levels to 700 mg l^{-1} in the thermophilic UASB on days 90–97 was assumed to indicate metabolic stress as feeding resumed.

Over the duration of the experiment, at OLRs of 10 kg COD m^{-3} d^{-1} (24-h HRT) increasing to 16 kg COD m^{-3} d^{-1} (12-h HRT), low levels of TVFA were seen in the mesophilic UASB (Fig. 2), with the highest value being 45 mg l^{-1}. For example, from day

Table 1. Performance of thermophilic pre-acidification reactors

	(h)				
	24-h HRT[a]	24-h HRT	18-h HRT	15-h HRT	12-H HRT
OLR (kg COD m^{-3} d^{-1})	10	10	13.3	16	16
No. of days operation	128	43	30	12	10
No. of HRT	128	43	40	19	20
pH	6.0	5.2	5.0	5.1	5.5
	($N = 58$)	($N = 34$)	($N = 21$)	($N = 8$)	($N = 8$)
	SD = 0.2	SD = 0.2	SD = 0.2	SD = 0.1	SD = 0.2
TVFA (mg l^{-1})	2298	2600	2373	1947	1452
	SD = 322	SD = 265	SD = 341	SD = 302	SD = 35
Acetate (mg l^{-1})	1234	1150	1256	994	831
	SD = 243	SD = 140	SD = 175	SD = 140	SD = 280
Propionate (mg l^{-1})	220	208	194	125	218
	SD = 104	SD = 67	SD = 41	SD = 25	SD = 51
i-Butyrate (mg l^{-1})	12	7	7	8	7
	SD = 3	SD = 5	SD = 3	SD = 38	SD = 3
n-Butyrate (mg l^{-1})	797	1208	881	800	457
	SD = 355	SD = 158	SD = 191	SD = 220	SD = 155
i-Valerate (mg l^{-1})	28	27	19	12	10
	SD = 19	SD = 6	SD = 7	SD = 6	SD = 3
n-Valerate (mg l^{-1})	10	13	17	7	14
	SD = 7	SD = 7	SD = 6	SD = 3	SD = 5
No. of VFA samples	58	34	11	8	6

[a]pH controlled to pH 6.0.
SD, standard deviation.

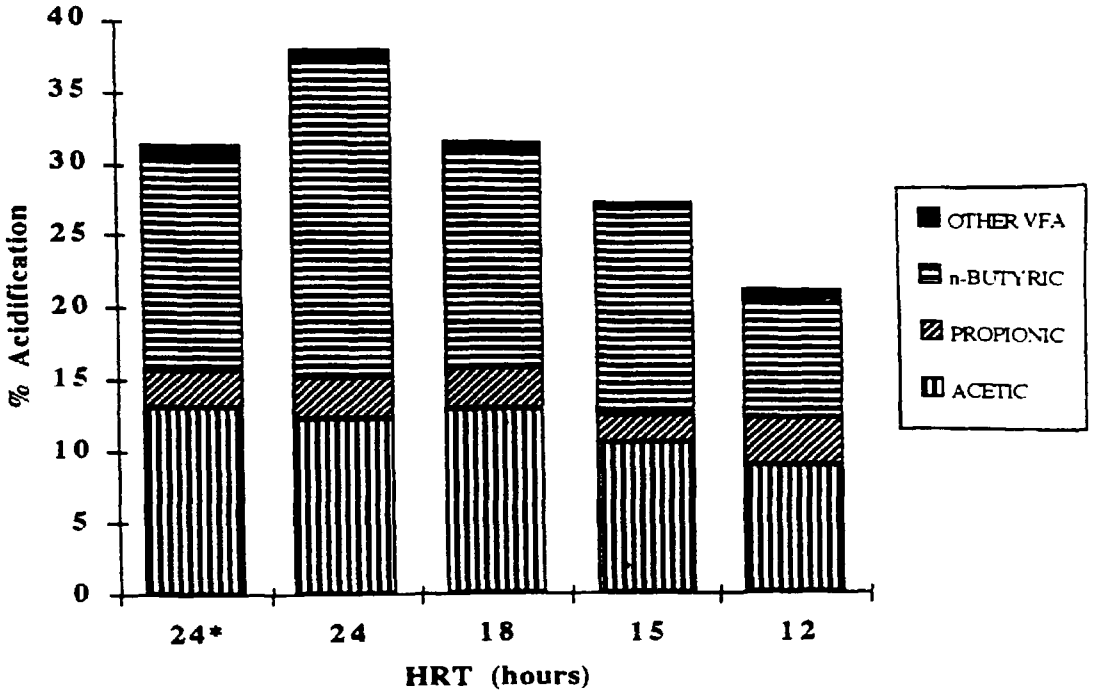

Fig. 1. Variation in percentage acidification of total COD at 55°C with HRT (*indicates pH control to pH 6).

3 to day 126 the average TVFA value was 13 mg l^{-1} (SD = 9.2, N = 58). Higher than average values were seen on days 67 and 69, coinciding with removal of the pH controller. The otherwise low and consistent levels of TVFA would suggest that stable operation of the mesophilic UASB was obtained over a range of loading rates and HRTs.

In the two-stage system studied here, TVFA levels of the thermophilic UASB were much higher (see Fig. 3) than the mesophilic UASB (see Fig. 2) at equivalent loading rates, even when the pH controller was removed, with levels of 210 mg l^{-1} (SD = 87, N = 6) compared to 17 mg l^{-1} (SD = 10, N = 27) in the mesophilic UASB. The main component of the TVFA in the thermophilic UASB was propionic acid, which formed over 50% of the TVFA by weight, with an absolute value of 177 mg l^{-1} (SD = 104, N = 24).

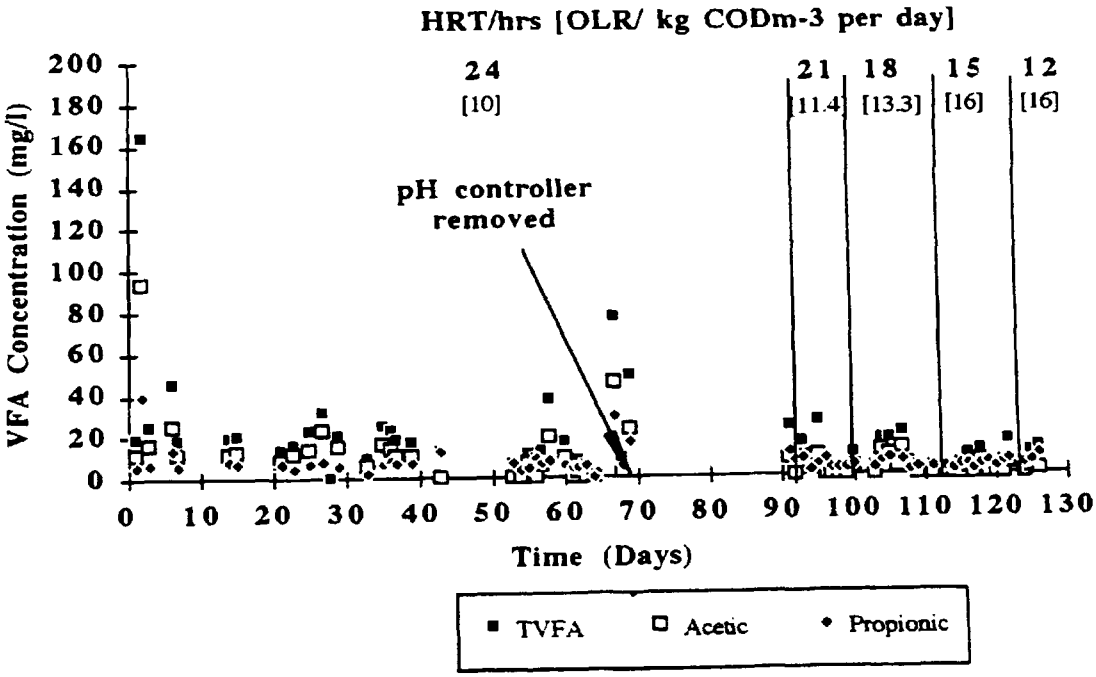

Fig. 2. Mesophilic UASB VFA concentration.

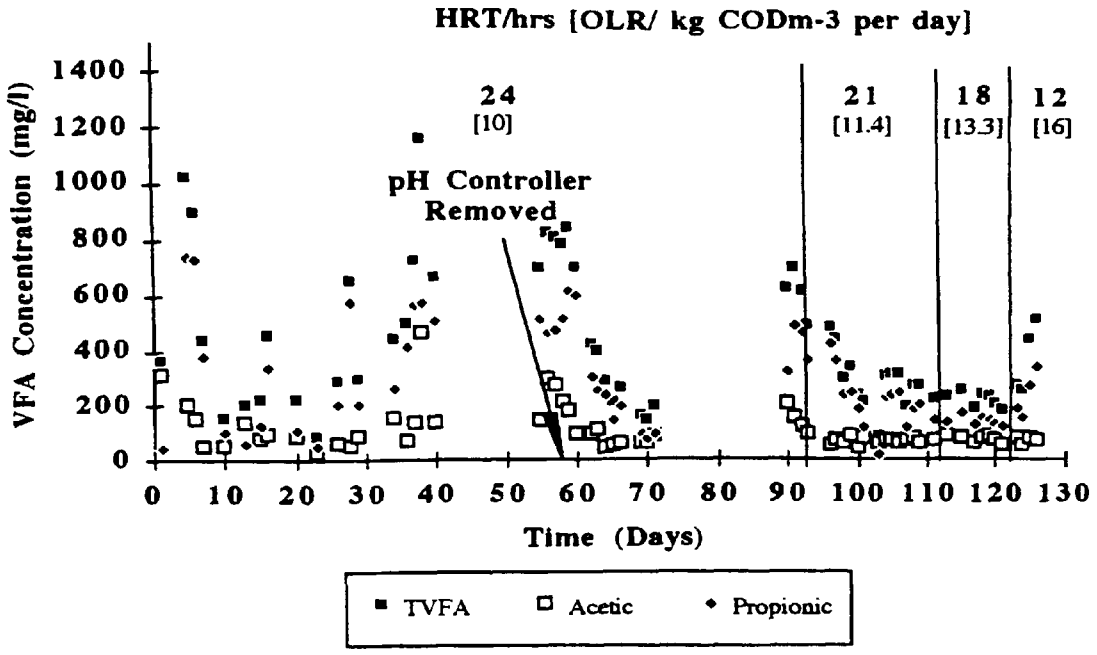

Fig. 3. Thermophilic UASB VFA concentration.

The performance of the methanogenic reactors at steady state is summarised in Table 2. Steady state was defined as at least three HRTs where the TVFA levels remained at constant low levels. Table 2 shows that COD removal was between 69 and 77% at OLRs up to 16 kg COD m^{-3} d^{-1} and did not appear to be falling with increased loading rate.

DISCUSSION

Thermophilic pre-acidification

A significant degree of acidification (22–38%) was seen at all the conditions studied. This level of acidification fell in the range of 20–40% of total COD acidification recommended by Lettinga and Hulshoff Pol (1991) for the operation of UASBs. At the shortest HRT (12 h) studied, the level of pre-acidification had dropped towards the lower level of this range.

McDougall *et al.* (1993) achieved 38% acidification at pH 6.0, with the reactor operating at 37°C at a HRT of 24 h and OLR of 10 kg COD m^{-3} d^{-1} using synthetic wastewater (instant coffee). When real-coffee waste was used, the level of acidification fell to 30% while operating under the same conditions.

However, whilst at 55°C at pH 6.0 and an HRT of 24 h and OLR of 10 kg COD m^{-3} d^{-1} using instant coffee, only 5% acidification was achieved. Zoetemeyer *et al.* (1982a) found equally good acidification at mesophilic and thermophilic temperatures.

At 12-h HRT, the level of pre-acidification had dropped towards the lower level of optimum pre-acidification suggested by Lettinga and Hulshoff Pol (1991), although there could be other products of acidification present such as ethanol, formate and lactate which were not measured in this study (Zoetemeyer *et al.*, 1982a, b). Stable acidification at shorter HRTs could be possible, as the critical dilution rate for thermophilic pre-acidification is around 0.71 h^{-1} (an HRT of 1.41 h) (Zoetemeyer *et al.*, 1982a). Bull *et al.* (1983) achieved stable acidification down to a 1.66-h HRT in a mesophilic system and still found the system operational. Other factors, apart from the degree of acidification, may influence the choice of HRT for the acidification stage. One of the hoped-for benefits of thermophilic pre-acidification was greater resistance to inhibitory compounds such as lipid in the coffee waste. Komatsu *et al.* (1991) found that a minimum 8-h HRT was required to overcome the inhibitory effect of lipids in the methanogenic stage.

A reduction in HRT in AC1 and AC2 to 18, 15 and then 12 h gave an operating pH of 5.0, 5.1 and 5.5, respectively (Table 1). If the pH had been maintained at pH 6.0, all this alkalinity would have to be added from external sources at extra cost, as the coffee waste is acidic in nature (pH 4.3–4.6) and low in sources of potential alkalinity such as ammonia (Dinsdale *et al.*, 1996). Zoetmeyer *et al.* (1982b) found that 44% more NaOH was required to control the pH at pH 6.0 than at pH 5.0 for a synthetic glucose feed. The present study has shown that, at thermophilic temperature and 24-h HRT, maintenance of pH at pH 5.2 was superior to control at pH 6. However, if the level of acidification was to be maintained above 20% at HRTs of less than 12 h, then pH control may have to be used.

At all operating conditions, all C_2–C_5 VFAs were detected: the predominant VFAs were *n*-butyric, acetic and propionic acids (Table 1). Zoetemeyer *et al.* (1982a), using glucose as substrate at 30°C (pH 5.8), found that butyrate then acetate followed

by ethanol were the most common liquid phase products, while at 55°C (pH 5.8) ethanol then acetate followed by propionate were the most common.

A review of the literature on pre-acidification would suggest that the following factors influence product distribution and degree of pre-acidification: temperature, Zoetemeyer *et al.* (1982a) finding equally good acidification at mesophilic and thermophilic temperatures, while McDougall *et al.* (1993) did not; loading rate (Zoetemeyer *et al.*, 1982); pH at mesophilic temperatures, McDougall *et al.* (1993), Bull *et al.* (1983) and Eastman and Ferguson (1981) all showing some upper and lower pH limit (e.g. pH 5.0–6.0) for maximum acidification, although the absolute values vary somewhat; and feedstock composition (Elefsiniotis and Oldham, 1994). Thus, it is recommended that the optimum conditions for acidification should be experimentally determined for each different type of feedstock.

Effect of pre-acidification on the methanogenic reactors

The initial low level of TVFA in the mesophilic UASB would indicate that, despite not being fed for 10 days and started up at an OLR of 10 kg COD m^{-3} d^{-1}, the mesophilic granules adapted quickly to the acidified feed. In contrast, the thermophilic UASB had higher levels of TVFA than the mesophilic UASB. Also, the TVFA levels in the thermophilic UASB in this study were also higher than when the same reactor operated thermophilically in single-stage mode at the same loading rate (Dinsdale *et al.*, 1997).

The increasing levels of TVFA in the thermophilic UASB when the pH controllers were in use, and the subsequent reduction in TVFA levels when the pH controllers were removed, would suggest that the use of the pH controllers was influencing the operation of the thermophilic UASB in some way. However, this effect was not seen in the mesophilic UASB.

Greater sensitivity of thermophilic anaerobic reactors to ions such as potassium has been reported by Fernandez and Forster (1993a, b). Studies on thermophilic sodium toxicity suggest that total inhibition of activity can occur at 1150 mg l^{-1} of Na^+ (Ahring *et al.*, 1991). However, small amounts of Mg^+ (1.2 mg l^{-1}) can alleviate this toxicity (Ahring *et al.*, 1991), so that 8 g l^{-1} of sodium was needed to give total inhibition. No Mg^+ was added in this study and the amount present in the coffee feedstock was not determined. The level of Na^+ determined in the feed (10,000 mg O_2 l^{-1} COD) with no additions was 100 mg l^{-1}. From this value and the additions of Na^+ added in the form of either sodium bicarbonate or sodium hydroxide, the level of Na^+ in the feedstock to the UASB was calculated. When the pH controllers were in use (adding NaOH), the level of Na^+ in the feedstock was calculated to be 1270 mg l^{-1}. When sodium bicarbonate was added instead, the level of 460 mg Na^+ l^{-1} would be expected. It has been shown that Na^+ levels in mesophilic studies on granular sludge do not exhibit inhibition below 5000 mg l^{-1} (Rinzema *et al.*, 1988). Hence, the maximum levels used here are well under the inhibitory values for mesophilic reactors but close to those which may give maximum inhibition for the thermophilic reactors with Mg^+ levels below 1.2 mg l^{-1}. Different batches of waste were used through the study but the waste was fed simultaneously to the mesophilic and thermophilic systems. So any change in feed composition would affect both thermophilic and mesophilic reactors. Thermophilic reactors have been reported to be more sensitive to temperature changes than mesophilic systems. Off-line determination of reactor temperature did not vary more than 55 ± 1°C.

Thermophilic UASBs have a tendency to accumulate propionic acid and other intermediates even in apparently stable operation (Souza *et al.*, 1992; Wiegant *et al.*, 1986; Lier *et al.*, 1993). Wiegant *et al.* (1986) found a system consisting of two methanogenic stages was required to improve the anaerobic digestion of feedstock containing propionic acid. It appears that the obligate hydrogen-producing acetogens present under thermophilic conditions have a lower ability to metabolise propionic acid (Schmidt and Ahring, 1993). In the single-stage UASBs studied previously, Dinsdale *et al.* (1997) found that a thermophilic UASB operating on instant-coffee wastewaters had only a slightly higher proportion of propionic acid (32% of TVFA, an absolute value of 36 mg l^{-1}) than the equivalent mesophilic systems (24% of TVFA, an absolute value of 6 mg l^{-1}). In the two-stage thermophilic UASB, propionic acid formed over 50% of the TVFA by weight, an absolute value of 177 mg l^{-1} (SD = 104, $N = 24$). In contrast, the two-stage mesophilic reactor had an absolute propionic level of 6 mg l^{-1} (SD = 2, $N = 53$), which formed 46% of the TVFA. This suggests that feed pre-acidification is not as suitable for thermophilic methanogenic reactors as it is for mesophilic.

The mesophilic UASB achieved good % TCOD removals, ranging between 68 and 77%, at all OLRs and HRTs (see Table 2). The COD removal was still high (77%) at the highest OLR (16 kg COD m^{-3} d^{-1}). The level of COD removal was comparable to the COD removal of 77% achieved in a mesophilic single-stage system (Dinsdale *et al.*, 1997). The removal of COD was found to be entirely due to the action of the methanogenic phase, no COD removal was seen in the acidification stage. An increase in COD removal of 13% was reported by McDougall *et al.* (1993) by utilising a mesophilic two-stage system instead of a single-stage system. In the results presented here, the two-stage mesophilic system could operate at a significantly higher OLR than the single stage, 16 kg COD m^{-3} d^{-1} compared to 10 kg COD m^{-3} d^{-1}. The COD removals of the two-stage thermophilic UASB ranged between 63 and 68%

removal. This level was slightly lower than the 68–77% seen in the mesophilic two-stage UASB and the 68–70% seen in the single-stage thermophilic system (Dinsdale *et al.*, 1997). The slightly poorer COD removal in the thermophilic two-stage system was reflected in the higher TVFA (210–241 mg l^{-1}) than in the thermophilic single-stage system (80–122 mg l^{-1} TVFA). Both values of TVFA are above those found for the mesophilic single-stage system (15–35 mg l^{-1}) or the mesophilic two-stage system (7–20 mg l^{-1}).

High % methane and good gas production were seen in both the thermophilic and mesophilic two-stage systems. The % methane in the mesophilic system ranged from 71 to 79%, and that in the thermophilic system from 73 to 75%. These levels were greater than the 59–64% seen in the single-stage mesophilic and thermophilic system. The methane yield per kg COD removal, measured at ambient temperature and pressure, was 0.30–0.32 m^3 kg^{-1} COD for the mesophilic reactor and 0.32 m^3 kg^{-1} COD for the thermophilic reactor, which was below the theoretical value of 0.35 m^3 kg^{-1} COD at STP.

In an equivalent single-stage system, it was found that, above OLRs of 10 kg COD m^{-3} d^{-1} (24-h HRT), stable mesophilic digestion was not possible. In the single-stage system, when the OLR was increased to 11.4 kg COD m^{-3} d^{-1} (21-h HRT), TVFA levels rose to 2930 mg l^{-1} (Dinsdale *et al.*, 1997). This would indicate that thermophilic pre-acidification allowed stable operation of mesophilic UASBs at significantly higher OLRs (60% higher) and shorter HRTs (100% shorter) than the equivalent single-stage system. A possible reason for this higher OLR is that two-stage systems can prevent inhibition by lipid in the waste (Komatsu *et al.*, 1991). The coffee waste in the present study contained 12% (w/w) (1.5 g l^{-1}), which can be sufficient for long-chain fatty acid (LCFA) toxicity to occur (Rinzema *et al.*, 1994). This greater resistance was thought to be due to the LCFA toxicity being reduced by the LCFA binding to the acidogenic biomass, not by breakdown in the acidogenic stage (Hanaki *et al.*, 1987).

As in the previous study (Dinsdale *et al.*, 1997), there was a need to remove sludge regularly from the gas separator, which would suggest that the COD removal had been exaggerated either due to a component in the coffee waste or biomass sticking to the gas separator.

Most full-scale plants use a buffering tank to equalise effluent flows and strengths. Pre-acidification in this buffering tank could be encouraged, although a potential disadvantage of using a buffering tank for pre-acidification is that high levels of VFA have a strong and offensive odour (Thacker and Evans, 1986). The present studies suggest that, by using pre-acidification, a substantial increase in OLR and in biogas methane content can be achieved over operation on un-acidified feed, and smaller methanogenic reactors could result.

CONCLUSIONS

1. Thermophilic pre-acidification was maintained for more than 100 days, with up to 37% pre-acidification being achieved.
2. Control of acidogenic reactors to pH 6.0 did not give better acidification than allowing the pH to float at approximately pH 5.
3. Removal of pH control in the system saw an improvement in the operation of the thermophilic UASB. Levels of TVFA which had reached 800 mg l^{-1} fell to 200 mg l^{-1} 6 days after the pH controller was removed.
4. The mesophilic UASB achieved stable anaerobic digestion at OLRs up to 16 kg COD m^{-3} d^{-1} at an HRT of 12 h, with COD removals of up to 77% with TVFA levels of 7–20 mg l^{-1}. These were significantly higher OLRs (60% higher) and shorter HRTs (100% shorter) than for the equivalent single-stage system.
5. The thermophilic UASB exhibited lower COD removals and higher TVFA levels than either the two-stage or single-stage UASB.

Acknowledgements—This work was funded under SERC grant No. GR/H18494. The authors are grateful to Nestlé UK for supplying the waste and to staff at the SERC pilot plant for the provision of UASB granules.

REFERENCES

Ahring B. K., Alatriste-Mondragon F., Westermann P. and Mah R. A. (1991) Effect of cations on *Methanosracina thermophila* TM-1 growing on moderate concentrations: production of single cells. *Appl. Microbiol Biotechnol.* **35**, 686–689.

Alexiou I. E. and Anderson G. K. (1994) Pre-acidification: concepts, guidelines and parameters for application. In *Proceedings of the 7th International Symposium on Anaerobic Digestion*, pp. 668–677.

APHA (1989) *Standard Methods for the Examination of Waste and Wastewater*, 17th edn. American Public Health Association, Washington, DC.

Bull M. A., Sterritt R. M. and Lester J. N. (1983) An evaluation of single and separated-phase anaerobic industrial wastewater treatment in fluidized bed reactors. *Biotech. Bioeng.* **26**, 1054–1065.

Dinsdale R. M., Hawkes F. R. and Hawkes D. L. (1996) The mesophilic and thermophilic anaerobic digestion of coffee waste containing coffee grounds. *Wat. Res.* **30**, 371–377.

Dinsdale R. M., Hawkes F. R. and Hawkes D. L. (1997) Comparison of mesophilic and thermophilic UASB reactors treating instant coffee production wastewater. *Wat. Res.* **31**, 163–169.

Eastman J. A. and Ferguson J. F. (1981) Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *J. Wat. Pollut. Contr. Fed.* **53**, 352–366.

Elefsiniotis P. and Oldham W. K. (1994) Influence of pH on the acid-phase anaerobic digestion of primary sludge. *J. Chem. Tech. Biotechnol.* **60**, 89–96.

Fernandez N. and Forster C. F. (1993a) The anaerobic

digestion of a simulated coffee waste using thermophilic and mesophilic upflow filters. *Trans. I. Chem. E.* **72**(Part B), 15–20.

Fernandez N. and Forster C. F. (1993b) A study of the operation of mesophilic and thermophilic anaerobic filters treating a synthetic coffee waste. *Bioresource Technol.* **45**, 223–227.

Ghosh S. (1991) Pilot-scale demonstration of two-phase anaerobic digestion of activated sludge. *Wat. Sci. Tech.* **23**, 1179–1188.

Ghosh S., Ombreght J. P. and Pipyn P. (1985) Methane production from industrial wastes by two-phase anaerobic digestion. *Wat. Res.* **19**, 1083–1088.

Guwy A. J., Hawkes D. L. and Hawkes F. R. (1995) On-line low flow high-precision gas metering systems. *Wat. Res.* **29**, 977–979.

Hajipakkos C. (1992) The application of a full-scale UASB plant for the treatment of coffee waste. *Wat. Sci. Tech.* **25**(1), 17–22.

HMSO (1986) *Chemical Oxygen Demand (Dichromate Value) of Polluted and Wastewaters*, HMSO, London.

HMSO (1988) *The Assessment of Biodegradability in Anaerobic Digesting Sludge*, HMSO, London.

Komatsu T., Hanaki K. and Matsuo T. (1991) Prevention of lipid inhibition in anaerobic processes by introducing a two-phase system. *Wat. Sci. Tech.* **23**, 1189–1200.

Lanting J., Jordan J. A., Schone M. T., Kull A., Carey W. W. and Kitney B. L. (1989) Thermophilic anaerobic digestion of coffee waste water. In *43rd Purdue Industrial Waste Conference Proceedings*, pp. 513–527, Lewis Publishers, Michigan.

Lettinga G. and Hulshoff Pol L. W. (1991) UASB process design for various waste waters. *Wat. Sci. Tech.* **24**(8), 87–107.

Lier J. B. van, Grolle K. C. F., Frijters C. T. M. J., Stams A. J. M. and Letting G. (1993) Effect of acetate, propionate, and butyrate on the thermophilic anaerobic degradation of propionate by methanogenic sludges and defined cultures. *Appl. Environ. Microbiol.* **59**, 1003–1011.

Hanaki K., Matsuo T., Nagase M. and Tabata Y. (1987) Evaluation of effectiveness of two-phase anaerobic digestion process degrading complex substrate. *Wat. Sci. Tech.* **19**, 311–322.

McDougall F. R., Anderson G. K. and Evison L. M. (1993) Two-phase anaerobic digestion of coffee wastewater. In *48th Purdue Industrial Waste Conference Proceedings*, pp. 677–683, Lewis Publishers, Michigan.

Peck M. W., Stilton J. M., Hawkes F. R. and Hawkes D. L. (1986) Effects of temperature shock treatments on the stability of anaerobic digesters operated on separated cattle slurry. *Wat. Res.* **20**, 453–462.

Quarmby J. and Forster C. F. (1995) An examination of the structure of UASB granules. *Wat. Res.* **29**, 2449–2454.

Rinzema A., van Lier J. and Lettinga G. (1988) Sodium inhibition of acetoclastic methanogens in granular sludge from a UASB reactor. *Enzyme Microbiol. Tech.* **10**, 24–32.

Rinzema A., Boone M., Kniippenberg K. van and Lettinga G. (1994) Bactericidal effect of long chain fatty acids in anaerobic digestion. *Wat. Environ. Res.* **66**, 40–49.

Schmidt J. E. and Ahring B. K. (1993) Effects of hydrogen and formate on the degradation of propionate and butyrate in thermophilic granules from an upflow anaerobic sludge blanket reactor. *Appl. Environ. Microbiol.* **59**, 2546–2551.

Souza M. E., Fuzaro G. and Polegato A. R. (1992) Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. *Wat. Sci. Tech.* **25**(7), 213–222.

Thacker F. E. and Evans M. R. (1986) BOD_5, TOA and odour offensiveness. In: *Odour Prevention and Control of Organic Sludge and Livestock Farming*, ed. V. C. Nielsen, J. H. Voorburg and P. L. L'Hermite, pp. 307–320, Elsevier Applied Science, London.

Wiegant W. M., Hennick M. and Lettinga G. (1986) Separation of propionate degradation to improve the efficiency of anaerobic treatment of acidified wastewaters. *Wat. Res.* **20**, 517–524.

Zoetemeyer R. J., Arnoldy P., Cohen A. and Boelhouwer C. (1982a) Influence of temperature on the anaerobic acidification of glucose in a mixed culture forming part of a two stage digestion process. *Wat. Res.* **16**, 313–321.

Zoetemeyer R. J., van den Hevvel J. C. A. and Cohen A. (1982b) pH influence on acidogenic dissimilation of glucose in an anaerobic digester. *Wat. Res.* **16**, 303–311.