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A STUDY OF PRE-ACIDIFICATION REACTOR DESIGN FOR ANAEROBIC TREATMENT OF HIGH STRENGTH INDUSTRIAL WASTEWATERS

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ABSTRACT

Acidogenic activities, as part of anaerobic digestion, have been discovered since the beginning of the century. Still it was mid '60's when it was initially stated in the literature that engineered phase separation would increase stability in anaerobic reactors and possibly increase substrate digestion rates. Pioneering research in the early '70's, with the first report on two-phase digestion of sludges, came as practical proof of those past assumptions. Today phase separation is a proposed option to single-stage digestion, due to the many advantages over conventional operation. Such an application utilises the different steady-state kinetic rates in the two main bacterial groups in anaerobic digestion. Furthermore, the process benefits through differences of these two groups, in relation to changing conditions. The overall result of two-phase applications is lower operational costs, with higher treatment efficiency and energy recovery.

In recent decades much research work has created a positive image for two-phase applications, compared to single-stage digestion. Still until today, many consultants in the field of anaerobic processes, are not provided with sufficient knowledge to utilise fully the potential of the two-phase process. It seems often the case that leading companies in the design and construction of anaerobic plants, will design pre-acidification tanks without understanding the uncontrolled acidogenic activities taking place in them. Therefore, design is based on an empirical approach or lack of knowledge of the effects of reactor design parameters on acidogenesis.

Although data on acidification of industrial wastewaters is in high demand, few studies have been carried out previously to assess the effects of the whole range of engineered reactor design parameters on acidification of industrial wastewater. Out of these few studies none has examined the whole range of design parameters on freshly collected agro-industrial wastewater. Apparently, most studies have been made on synthetic versions of wastewaters or simple compounds. Additionally since the '80's anaerobic processes have been extensively applied for the treatment of agro-industrial wastewaters. Obviously the extent of information provided from this study, was particularly required to clarify many issues related to the role of acidification in the pre-treatment of agro-industrial wastewaters.

The research project presented in this thesis is based on a 3-year laboratory study. Some early conclusions of this study have been presented previously in a number of papers on pre-acidification discussing design guidelines, advantages of two-phase applications and methods to assess acidogenesis. This thesis is focused on the complete range of findings related to the effects of various reactor design parameters, namely: temperature (from ambient to thermophilic); pH (from 4.5 to 7.0); HRT (from 6 to 12 hrs, with and without variations in the organic loading rate); addition of commercial micro-nutrients; and mixing the reactor contents.

The two wastewaters studied are slaughterhouse, collected fresh each week; and synthetic instant coffee production. They are both considered as high strength wastewaters. Slaughterhouse wastewaters are found everywhere, as they are connected with daily human activities, while they are easily biodegradable wastewaters for high-rate digestion. On the other hand instant coffee production wastewaters, although not a common global industrial activity, involves more

complexity for high-rate digestion, due to various recalcitrant and inhibitory compounds present in the composition of coffee.

Results are based on analyses for: VFA concentration and composition (Acetic to Caproic acid). Tot. and Filt.COD, Tot.BOD, TS, VS, SS, VSS, TKN, NH_3-N, PO_4-P, gas composition and for slaughterhouse wastewaters protein concentrations. In particular, results on VFA are presented as concentration, COD of the acids, composition and in relation to the influent and effluent COD. Assessment of the effects of design parameters on the performance of acidogenic biomass are based on: VFA production and composition; acidified COD; and overall effluent quality in relation to methanogenic treatment requirements.

This study provides information on all design requirements needed to use acidogenic phenomena to convert organic matter into simple carbon source (i.e. VFA). Such a conversion appears to benefit biological wastewater treatment when used as pre-treatment for anaerobic digestion, but also for its potential in aerobic processes and nutrient removal processes. The process proves to have great low-cost pre-treatment potential, but can also be used for advanced wastewater treatment.

Finally, the extensive data collected is used to present various guidelines for process engineers, which should be considered in order to design anaerobic plants. Also, they should be even further used for the overall assessment of the treatment or pre-treatment potential of pre-acidification for agro-industrial wastewaters.

List of Abbreviations

ADP	Adenosine Diphosphate
AF	Anaerobic Filter
ATP	Adenosine Triphosphate
BOD_{5ATU}	Biological Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
COD	Chemical Oxygen Demand
CV	Coefficient of Variation
EVOP	Evolutionary Operation
Filt.COD	Filtered Chemical Oxygen Demand
HRT	Hydraulic Retention Time
MSW	Municipal Solid Waste
OLR	Organic Loading Rate
RT	Retention Time
SS	Suspended Solids
SRT	Solids Retention Time
TFM	Total Fatty Matter
TKN	Total Kjeldahl Nitrogen
TS	Total Solids
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
VTS	Volatile Total Solids
WTP	Wastewater Treatment Plant

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Chapter 1

INTRODUCTION

Humans, their daily domestic and industrial activities and their domesticated animals, have become a vast element in the pollution of Earth. Also, natural organic and inorganic resources are becoming increasingly important for the well-being of the whole planet. While reaching the end of this century, it is obvious that waste and wastewater management must set a major priority to recover all valuable elements and resources, present in our daily residues.

Waste and wastewater management involves various physical, chemical and biological processes for the removal and safe disposal of pollutants. In their effort to remove organic matter, engineers regarded microbes in biological methods as cheap catalyst-particles degrading biopolymers. However, biotechnological developments initiated cooperation of engineers, microbiologists and biochemists, in the effort to understand and optimise enzymic and microbial processes, used daily for biological treatment.

Biological wastewater treatment aims at the complete mineralisation of organic matter. It involves various microbial populations depending on the conditions applied, particularly with regard to available oxygen. Generally, biological methods utilise the metabolism of aerobic and anaerobic bacteria, while facultative bacteria are found in both types of processes and also in anoxic treatment methods.

Various technological applications can convert waste materials to produce usable products, but the process that is deemed practical in the short term to accomplish simultaneously energy recovery and waste treatment, is anaerobic digestion. Anaerobic digestion has been practised for many decades for the treatment of organic waste streams, especially for sewage sludges in the wastewater industry.

After the first half of this century, industrial pollution has been considered as the primary source of the most hazardous and unrecoverable form of pollution. Nowadays, industrial wastewater treatment with a high degree of operational reliability is in demand worldwide. Meanwhile, limitations like: sludge yield from biological treatment methods; energy consumption and space requirements for a treatment plant, are becoming more important design conditions. Also, energy reclamation is increasingly in demand. It is therefore no wonder that many organisations,

national authorities and research institutes are looking for a rapid development and optimisation of anaerobic digestion processes for waste and wastewater treatment.

Microbiological research on anaerobic digestion has come a long way since 1868, when the French chemist Bechamp recognised the microbial nature of the process (Schoberth, 1980). Anaerobic digestion, as known by the sanitary engineers, is a unique and robust process. It has proved to be a powerful tool in the stabilisation of strong organic wastes and wastewaters. Practically any type of organic matter can be decomposed to CH_4 and CO_2, as the principal end products, with very little in the way of toxic by-products being produced. Methane fermentation can be carried out on a mixed or enriched culture. So it is possible to maintain the process on any scale and continuously for apparently indefinite periods (Speece & McCarty, 1964).

Popel (1964) described how organic matter of fresh sludge is broken down into simple and stable end products in two-phases by two different types of bacteria. The first group of organisms,which he called acid producing bacteria, degrade organic matter into compounds like fatty acids, aldehydes and alcohols; whereas the second group (methane bacteria) convert the intermediate products into CH_4 and CO_2. In this degradation process the colloids and macromolecules are first rendered soluble by extracellular enzymes. After this process of liquefaction, the intermediate products pass the bacterial cell wall and are broken down by intracellular enzymes into gaseous end products (gasification). In the first phase facultative bacteria are active in a slightly acid environment; while in the second phase obligate anaerobic methane bacteria react in a slightly alkaline environment.

From this and other similar reports in the early '60's, some researchers started to consider utilising the two individual phases of anaerobic digestion, as two sequential processes occurring in different reactors. The main concern at that period was to optimise conventional anaerobic reactors treating sludges, while increasing loading rates for economical reasons, without facing process "failure" or in other words acid production. Until in the early '70's one of the first pioneering research projects on this application for the stabilisation of sludges, started a new era in anaerobic applications, namely the "two-phase concept" (Pohland & Ghosh, 1971).

Two-phase processes have not been applied to the expected extent commercially, although sufficient research proves high potential. The obvious and many advantages seem to be of limited benefit to process engineers, due to various misunderstandings surrounding their view of the process and lack of information for viable process design, especially for agro-industrial wastewaters. In particular parameters are missing related to the acidogenic reactor, both for process operation and performance. This lack of information is presented in the following literature review. Special sections were prepared regarding existing engineering aspects for acidogenic reactors, and the commercial approach in the design of the few full-scale applications of two-phase processes found around the world.

This study investigated a wide range of design parameters (i.e. temperature, pH, hydraulic retention time, etc.), in an attempt to provide information and optimise the effects of these parameters, on the acidification of two types of high strength agro-industrial wastewaters. Coffee

processing wastewater, has been selected for its recalcitrant and inhibitory nature. Although, it is generated only in certain parts of the world, where coffee is mainly produced, it carries its complex polluting characteristics round most of the world, where it is further processed as one of the most widely consumed hot drinks. Furthermore, slaughterhouse wastewaters are high strength pollutants, produced wherever there are human activities. As they are easily biodegradable, often disposed directly to the local sewer, acidification could be considered as low-cost high-rate pre-treatment option; providing at least colour removal, while converting biopolymers into simple acids.

Chapter 2

ANAEROBIC DIGESTION

2.1 General

Earth was originally anaerobic and methanogenesis has been assumed to be a very primitive metabolic activity (Hungate, 1987). Anaerobic digestion is metabolism in which bacterial groups co-operate to convert organic matter into biogas. It occurs naturally in river sediments, marshes and the rumen of herbivorous animals (e.g. cattle and sheep). Man-made habitats for anaerobic bacteria include sludge digestion tanks at sewage treatment plants and the anaerobic interiors of landfill sites. Because of increasing interest in anaerobic processes, this fermentation has been subjected to extensive scientific study. As an R&D result the process has been engineered into anaerobic reactors, capable of treating a wide range of wastes and wastewaters (Dunn et al., 1994).

Anaerobic digestion plays an important role in nature and it will keep on attracting an ever increasing number of scientists for several reasons (Schoberth, 1980):

- its global impact on stabilisation of organic matter, recycling carbon and minerals;
- its technical potential in waste/wastewater treatment and biogas production;
- intriguing inter-specific relationships between micro-organisms, involved in the process; and
- unique features of biochemistry and molecular biology in anaerobic bacteria.

There are various advantages of the process for the waste/wastewater industry. For example, the net sludge production of the anaerobic process in tandem with aerobic polishing was only 20-30% of that produced by aerobic treatment alone. Also, biomass solids production would be below 10% of the mass of removed organic matter. The conversion of organic matter to biogas yields little energy; hence growth rate is slow and the yield of organisms by synthesis is low. Therefore, anaerobic digestion results in much smaller sludge volumes than aerobic processes. Since there is less cell synthesis, nutrient requirements are less than in aerobic systems. Furthermore, sludge

produced in this process would be relatively more stable, consisting mainly of inert material and dead cells. Finally, the very strong feature of anaerobic digestion is that it stabilises most organic matter, by conversion to CH_4 fuel gas. The quantity of organic matter converted to gas will vary from 80 to 90%. This source of recycled energy will provide significant benefits in the economy of the overall treatment (Eckenfelder, 1989; Owen, 1982).

2.2 Microbiology

Anaerobic bacteria are placed into the most ancient line of descent, the archaebacteria, only distantly related to other living species, including most bacterial species (Schoberth, 1980). In 1868 Bechamp, a student of Pasteur, discovered that an "organism" was responsible for CH_4 production from ethanol (as cited in Zehnder et al., 1981). At the beginning of the century, research studies on CH_4 production assumed that biogas from fermentation of cellulose, was the activity of only one species of microorganism (*Bacillus omelianskii*). Microbiological research since that time, has shown that the production of CH_4 is the result of several microbial groups, occurring in several phases. Interrelationships between the bacteria of each phase can be defined as symbiotic, metabiotic or even antagonistic; depending on environmental conditions and substrate composition and concentration (Hausler, 1969).

Nowadays, digestion of organic matter is known to follow the simplified pathway presented in Figure 2.1. Organic compounds are utilised by the microbial population both as source of carbon, from which new cells can be synthesised and as source of energy (Hawkes, 1980).

In anaerobic biomass, some bacteria associated with hydrolysis are capable both of aerobic and anaerobic degradation, commonly referred to as facultative anaerobes. Other bacteria associated with the following phases, are active only under a strict anaerobic environment. These bacteria are known as obligate anaerobes. Obligate anaerobes are the ones most responsible for hydrolysis and acidification, while facultative bacteria have less input. Although some species are capable of both hydrolysis and acidification, some are only acid producers. All bacteria involved in methanogenesis are strict obligate anaerobes. At all stages of anaerobic digestion there are increases in cellular biomass (suspended solids) by biosynthesis (Anon., 1979). The COD of the anaerobic bacterial cells is approximately 1.21 kg/kg VSS (Eckenfelder, 1989).

Anaerobic digestion is brought about by a consortium of bacteria. It starts with hydrolysis of complex organics by common food-spoilage bacteria and ends with the evolution of CH_4-rich biogas, by specialist methanogens. In the process, large quantities of acidic intermediates are formed by one group of bacteria and then subsequently decomposed by another. Process management is often considering anaerobic digestion as a two-phase fermentation, comprising acid-formation (putrefaction) and acid-removal (CH_4 production), occurring simultaneously in one vessel. In practice, the process is more complex because methanogens have a very limited metabolic repertoire. Also, methanogens require the assistance of syntrophic bacteria to convert the complex mixtures of VFA into Acetic acid, CO_2 and H_2, which are their substrates.

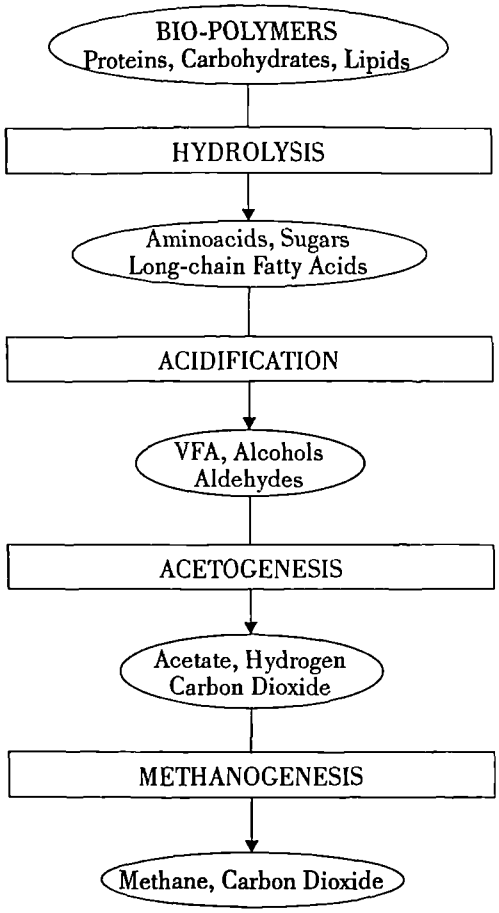


Figure 2.1: Simplified diagram of anaerobic digestion

More subtle interactions occur as active CH_4 production, allows preliminary metabolisms to be completed without product inhibition or production of alternative volatile and malodorous end products (Dunn et al., 1994).

It appears that degradation of biopolymers is a complex symbiotic interaction of various anaerobic and facultative bacteria. For simplicity decomposition of complex organic matter can be characterised as a sequential multi-phase process comprised mainly of hydrolysis, acidogenesis, acetogenesis and methanogenesis. When the process is stable these separate steps occur simultaneously at approximately equivalent degradation rates. There are numerous types of bacteria involved in anaerobic digestion, each characterised by its ability to use a relatively limited number of carbon compounds. Also, microbial growth rates and relative response to environmental conditions, vary among these bacterial groups. In particular methanogens have the slowest growth rate and are the ones most sensitive to environmental changes (Owen, 1982).

Biogas is the ultimate end product of digestion. It results from a series of very complex biochemical reactions in which a mixed population of bacteria form a food chain. Within this chain, the fermentation end products of one group are the starting growth medium for the next. This complex interdependency is simplified when considering the digester to contain four basic groups of micro-organisms, that sequentially degrade organic matter. Extensive reviews of this final approach for the microbiology of anaerobic digestion, have been recently reported by Hobson and Wheatley (1993) and Eckenfelder et al. (1992). For the contents of the following paragraphs these references were used, as well as the reviews provided by Hawkes (1980), Schoberth (1980) and Eckenfelder (1989).

The first group are the hydrolytic and acidogenic bacteria. For about 2.5 decades microbiologists have realized that the acid formation phase is carried out by two completely different groups of bacteria. Fermentative bacteria and acidogens hydrolyse polymers like polysaccharides, lipids, proteins and nucleic acids. Their fermentation results in oligo- and monomeric sugars, glycerol, amino acids and other simple nitrogenous compounds. In acidification it proceeds to a mixture of organic acids, alcohols, other simple solvents, H_2 and CO_2. Some of the acidogenic bacteria are capable of hydrolysing biopolymers, such as polysaccharides and proteins to monomers. But all of these bacteria are able to utilise monosaccharides or amino acids. This reduction results in no COD reduction. A few acidogens carry out homoacidic fermentations, e.g. the homolactic bacteria. The preferred substrates of homoacetic acid-producing bacteria are carbohydrates. Some of the last species may also grow on such simple compounds, such as methanol, formic or lactic acids. There are other species producing H_2 under certain conditions. Also some will obtain energy growing lithotrophically, reducing CO_2 and H_2 to acetic acid; or otherwise competing with methanogens for H_2. The ultimate end products of these various fermentations are VFA, H_2 and CO_2. The principal acids are Acetic, Propionic and Butyric with small quantities of Valeric acid. NH_3 and H_2S, both essential nutrients for methanogens, originate from amino acids and other simple nitrogenous compounds. In acidification there is minimal reduction of COD. When large amounts of H_2 and CO_2 occur, some COD reduction will also occur, but the reduction seldom exceeds 10%.

From the end products of acidification, acids higher than acetic can be utilised as an energy source by acetogenic bacteria. Only in the last decade it has become obvious that there is a fourth phase in the metabolic pathway of anaerobic digestion, called acetogenesis. The term acetogens, also known as obligate syntrophic acetogenic bacteria, may be often confused with obligate syntrophic acetogens. These homoacetic acid-producers should be better referred to as homoacetogens. Acetogenesis occurs only if H_2 concentration in the digester is very low. As a result, for thermodynamic reasons the obligate syntrophic acetogens can only grow in co-culture with H_2-consuming bacteria (interspecies hydrogen transfer), therefore distinguishing them from homoacetogens. From this reason acetogens are also called obligate proton reducers or H_2-producing acetogenic bacteria. Acetogens mainly degrade higher VFA than acetate and simple solvents. Generally, they convert compounds which can not be attacked by other groups in the anaerobic process. This group includes bacteria which metabolise long-chain fatty acids and probably those degrading aromatic compounds, since these two fermentative steps to acetate also depend on low H_2 concentration. The net result of the metabolism of propionate, butyrate, long-chain fatty acids and aromatic compounds by acetogens, is the production of acetate, CO_2 and H_2. In acetogenesis COD reduction does occur with formation of H_2.

Another population of bacteria in digesters are those responsible for conversion of formate or H_2 and CO_2 into acetate. These are the H_2-consuming acetogenic bacteria. They also have the capacity of fermenting monosaccharides to acetate without generating H_2 or CO_2 and are otherwise called homoacetogenic bacteria. A brief but very detailed description of the activities of homoacetogens and acetogens is presented by Li et al. (1994).

All biodegradable compounds in a substrate are ultimately converted to acetate, H_2 and CO_2. The end products of acetogens will be used as energy source by methanogens, which react to keep the H_2 concentration low so that the acetogens can continue to function. Methanogens, also known as methanogenic or methane bacteria, should not be confused with CH_4 oxidising bacteria. Formation of trace amounts of CH_4 may be found as side reactions in the activities of *Clostridia*, *Pseudomonas*, *Desulfovibrio* and *Desulfotomaculum*; but also under certain conditions in mammalian tissues. However, the vast amounts of biogenic CH_4 leaving anoxic environments are excreted by methanogens. Generally, methanogens are physiologically most active in pH range from 6.7 to 8.0. There are exceptions though. For example optimum pH for methanogenesis of a methanolic substrate lies between 5.5 and 6.0; while activity still occurs at pH values as low as 3.5. Some methanogens seem to be able to survive exposure to oxygen, being protected by natural micro-environments. However pure cultures of methanogens have the most stringent anaerobic requirements compared with other anaerobes. Oxygen has to be rigorously excluded and growth occurs only at redox potential below -330 mV. Methanogenesis occurs in nature at 0°C but some methanogens are also active near water boiling point (i.e. thermal springs). Most pure strains though, have their growth optimum around 35-40°C (mesophiles) or around 65-70°C (thermophiles). As a group methanogenic bacteria have a very limited choice of substrates to supply energy for metabolism. Only acetic acid, H_2 and C_1-compounds (e.g. CO_2), can be utilised by methanogens. In aqueous sediments and digesting sludges, about 70% of CH_4 originates from the methyl group of acetic acid; while the remainder is almost exclusively from

CO_2 and H_2. Although many methanogenic strains are stimulated by small amounts of acetic acid, yeast extract or other simple growth substrates, NH_3, H_2S or cysteine are the principle N and S sources for biosynthesis. Therefore, ancestral methanogens were very well endowed to grow in the primordial, O_2-free atmosphere of this planet; which contained only CO_2, H_2, NH_3, H_2S and minerals, thousands of years ago.

Although not dominating in environments low in sulphate, sulphate reducing bacteria may influence methanogenic systems in various ways. They are able to oxidise some organic acids and alcohols to acetic acid, with concomitant reduction of sulphate to H_2S. Since H_2S is an essential nutrient for methanogens, sulphate reduction is essential when no other sources of H_2S (e.g. amino acids) are available. However if the sulphate concentration is too high, the resulting H_2S levels may be toxic for methanogens and sulphate reducers may also compete successfully with methanogens for H_2. This may be a problem in a digester, but is useful in isolating syntrophic acetogens in co-culture with sulphate reducing bacteria. Two species in this group can degrade acetic acid completely to CO_2. In the absence of sulphate, sulphate reducing bacteria may act as syntrophic acetogens on compounds like lactic acid or ethanol, switching from sulphate reduction to H_2-formation, by proton reduction.

2.3 Biochemistry

Anaerobic treatment processes decompose organics in a controlled oxygen-free environment (Owen, 1982). Anaerobic digestion differs from simple putrefaction because of large populations of methanogens. It also differs due to the evolution of large quantities of CH_4, which provides a terminal electron acceptor for the overall fermentation, allowing the process to complete (Dunn et al., 1994). The rate limiting step in CH_4 conversion of solids, containing mainly carbohydrates and some proteins and lipids, is solubilisation of particulate matter (Eastman & Ferguson, 1981).

In order to degrade organic matter completely, the organic molecules must enter the bacterial cell. Macromolecules present in wastes or wastewaters, are too large to pass through the cell membranes. In order to achieve that, insoluble complex organics are initially hydrolysed by extracellular enzymes to smaller molecules, that are accessible to bacteria in the next stage. Those simpler compounds, occurring in hydrolysis, are converted in acidification. In this phase the absorbed molecules are further degraded, by metabolic activities within the bacterial cell. The smaller-molecule organic compounds are subsequently decomposed by acidogens mainly to simple acids, such as acetic, propionic and butyric acids. Short-chain fatty acids were first detected and determined by steam distillation from fermenting liquors. They became known collectively as volatile fatty acids (VFA). Other products of acidification, depending on the substrate, include gases like CO_2, H_2 and NH_3; and also small quantities of alcohols, aldehydes and ketones. VFA and other end products of acidification are converted by acetogens and methanogens, first to acetic and finally to biogas (Anon., 1979; Owen, 1982; Dunn et al., 1994).

The major common constituents in the composition of organic substrates stabilised in anaerobic reactors, are polysaccharides, lignin, proteins, other N-containing compounds and lipids. These are all biopolymers generally representing three major groups: carbohydrates, proteins and lipids (Hawkes, 1980). In terms of intermediates, carbohydrates (polysaccharides) degrade via saccharides and simple sugars, to produce VFA, alcohols and other acids like lactic and succinic. Fats (lipids) degrade to glycerol, glycerin and long-chain fatty acids and further to VFA and alcohols. While proteins degrade via peptides to amino-acids and VFA afterwards. Proteins contain N, S and P, all essential nutrients for methanogens (Popel, 1964; Dunn et al., 1994).

Extensive reviews regarding the biochemistry of anaerobic digestion have been recently reported by Hobson and Wheatley (1993) and Eckenfelder et al. (1992). For the contents of the following paragraphs these references were used, as well as reviews provided by Hawkes (1980), Schoberth (1980), Beccari et al. (1992), Dunn et al. (1994) and Li et al. (1994).

Glucose rings are responsible for the structure of cellulose and other polysaccharides. The enzyme or enzyme complex capable of hydrolysing glucose rings and producing short-chain, soluble oligo-saccharides (i.e. glucose), is cellulase. Cellulases from different sources have various abilities to degrade native cellulose, such as that found in plant cell walls. Some cellulases can only hydrolyse purified cellulose (e.g. filter paper or carboxymethyl cellulose). End products of cellulolysis may then be assimilated by bacteria which are not themselves cellulolytic, so that cellulolytic organisms have to compete for their own end products. Generally, hydrolysis of carbohydrates by extracellular enzymes is brought about by a small number of acidogens, which share hydrolysis end-products with other less capable acidogens. Cellulases also appear to hydrolyse xylose rings, found in hemicellulose. With some cellulosic substrates, hydrolysis is the rate limiting step of anaerobic digestion. Hydrolytic enzymes have difficulty penetrating cellulose and hemicellulose particles. This is due to various reasons such as, the crystallinity and poor surface extension of cellulose or the presence of a lignocellulosic shield around fibrous cellulose. The whole polysaccharide-content in wastes may be regarded as digestible. Hydrolysis of polysaccharides to their constituent monosaccharides, is a necessary prelude to the use of these sugars as a cellular energy source. The overall equation for hydrolysis and complete fermentation of a glucose based polymer to gaseous end products in anaerobic digestion, is:

$$(C_6H_{10}O_5)_n + (n-1)(H_2O) \rightarrow 3nCH_4 + 3nCO_2$$

Although the gas produced has 50% CH_4, factors affecting the solubility of CO_2 must be taken into account for the composition in digester biogas. From the above equation it can be calculated that the gas yield would be 0.75 m^3/kg VS destroyed, for a carbohydrate of general formula $C_nH_{2n}O_n$.

Lignin is a highly complex, branched, cross-linked polymer of derivatives of phenyl propane. The latter is an aromatic alcohol with a three-carbon side chain. Lignin is probably not degradable under anaerobic conditions, or only at very slow rates. This may be due to its random structure, which is different from other "regular" polymers, like polysaccharides and proteins. The inability

to digest lignin may cause problems in treating lignocellulosic substrates. Lignin sometimes shows some weight loss in digestion tests, which is mainly attributed to loosely linked aromatic acids removed from lignin. It is not the aromatic nature of lignin which stands in the way of digestion, since aromatic compounds expected to result from chemical delignification can be degraded anaerobically by enrichment cultures. It is possible that, like other species, the aromatic degraders may be inhibited by their own end products, particularly H_2.

Proteins are polymers of around 20 naturally occurring amino acids, linked by peptide bonds. Proteolytic enzymes produced by certain species of bacteria, hydrolyse these bonds to liberate free amino acids, which may be fermented to yield energy. Prior to fermentation, N is removed from the amino acids generating ammonia, while the main fermentation end products are acetate, propionate and butyrate. The microbial population in digesters is therefore able to convert proteins to gaseous end products plus ammonia. Fermentative and acidogenic bacteria can metabolise both protein and non-protein N sources, while methanogenic bacteria utilise only ammonia, as N source. The overall equation for protein digestion, is:

$$2C_5H_7NO_2 + 6H_2O \rightarrow 5CH_4 + 5CO_2 + 2NH_3$$

Variations of this formula occur as different proteins may be the starting point, e.g. $C_6H_{12}N_2O_3$; with a different combination of amino acids. An average value of 1 g of N from 6.5 g of protein can be calculated by the Kjeldahl method. From the above equation gas yield of 0.99 m^3/kg VS destroyed can be calculated. Also, the increase in NH_4^+ concentration in the digester contents as proteins ferment, will increase the solubility of CO_2, so that the amount of CH_4 in biogas will be greater than 50%.

With respect to other N-containing compounds the major mammalian nitrogenous end product is urea, i.e. $CO(NH_2)_2$. In a mixed anaerobic population urea is converted to ammonia plus CO_2, according to the equation:

$$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$$

Urea is soluble in water to such an extent that N may be lost from wastes if the liquid fraction is not retained for digestion. Such is the case for domestic sewage and manure. In birds the main nitrogenous end product is uric acid. Deamination of uric acid to liberate free ammonia may occur through bacterial activities during storage of litter or manure; which reduces the N-content, since ammonia is volatile.

Lipids are a heterogeneous group of organic compounds defined as insoluble in water but soluble in organic solvents, such as ether, chloroform or methanol. Some lipids are not degradable in the rumen and animal intestines and probably not even in anaerobic reactors. These are the waxes, esters of long-chain fatty acids with long-chain primary alcohols. Triglycerides and membrane lipids, such as phosphoglycerides, are acted on by lipases to liberate free fatty acids which may be further digested. Fatty acids are thought to be degraded by the classical pathway

for fatty acid oxidation, β-oxidation. In this pathway, acetyl (two-carbon) groups are removed sequentially from the -COOH end of the acid, so that acetic acid and H_2 are the end products. Although saturated and unsaturated fatty acids of chain length C_{14} to C_{18}, could be degraded to biogas with acetate as an intermediate, long-chain fatty acid degradation to acetate may be a rate-limiting step in complete digestion. As an example the complete digestion of stearic acid to biogas, is:

$$CH_3(CH_2)_{16}COOH + 8H_2O \rightarrow 13CH_4 + 5CO_2$$

which indicates gas composition with 72% CH_4 and gas yield of 1.42 m^3/kg VS destroyed.

Energy for all bacterial processes is supplied by hydrolysis of ATP to ADP and phosphate. ATP is the universal energy currency of living cells. The synthesis of ATP from ADP and phosphate is the objective of energy yielding catabolism. Biochemists distinguish two mechanisms by which cells may synthesise ATP. The first one, named substrate level phosphorylation, involves synthesis of ATP while organic compounds are being metabolised in accordance to a metabolic pathway. This is the mechanism by which ATP is synthesised in fermentation reactions and is likely to be the chief source of ATP in digesters. The second, named electron transport phosphorylation, involves the coupling of ATP synthesis to the transfer of electrons (or hydrogens) from a reducing electron donor to an oxidising electron acceptor, through a chain of redox compounds sited in a membrane. This latter mechanism is likely to occur in the methanogens.

Overall in the biochemical pathways of anaerobic digestion, acidogens provide important substrates for methanogens (i.e. acetic and formic acids, H_2 and CO_2). Methanogens in turn act symbiotically removing H_2, therefore diverting acidogenic energy metabolism towards the production of more H_2 and acetic acid, which is more ATP. For example, biochemical pathways show that if the digester H_2 concentration is low, a bacterium responsible for the metabolism of glucose to acetate, CO_2 and H_2, will maintain this pathway to obtain ATP as energy. If the digester H_2 concentration rises, in the case of decreased methanogenic activities, then this bacterium will cease to produce acetate and shift to the production of butyrate, as a preferred end product. From this example it is obvious that efficient utilisation of H_2 by methanogens, will bias the flow of carbon towards acetate formation. Since acetogenic bacteria degrade propionate, butyrate, long-chain fatty acids and probably aromatic compounds, all these will accumulate if the H_2 concentration rises. Therefore the net effect of lowering methanogenic activity will be to raise the concentration of formate, acetate, propionate and butyrate in the digester. It would be most useful to monitor changes in H_2 levels in the digester. Unfortunately, the normal concentration is below 10^{-4} atm, making the possibility to trace unstable operation by H_2 levels, extremely difficult.

At high growth rates (short SRT), 30-40% of the carbohydrate COD can be converted to biological solids, which reduces CH_4 yields considerably. When the partial pressure of H_2 is around 10^{-5} bar (concentration about 0.2×10^{-6}l H_2/l), the reaction responsible for the conversion of butyrate to acetate is reasonably exergonic. For example, when a methanogenic food chain has

been established in a digester on cellulose (hydrolysis eventually being the rate limiting step) and sudden loads of easily degradable acidogenic monomers (i.e. glucose) are applied, acidogens carry out very fast mixed acid fermentations. Although the ATP yield per mole substrate converted, is at that point less (compared to complete oxidation to acetic acid), ATP yield per unit time is higher. Acidogens gain more energy and outgrow the subsequent slower, with less energy, groups of acetogens and methanogens. This leads conventional digesters to severe souring. Therefore, great care has to be taken when changing loads to a digester. It is advisable to do OLR changes slowly, giving the food chain a chance to adjust relative activities and bacterial numbers, according to the new conditions. Meanwhile calculations show that even at reduced H_2 concentrations, the free energy of acetogenic processes is not exergonic enough to yield one mole ATP per mole substrate to be converted. In other words, more than one mole substrate has to be converted to yield one mole ATP.

Various metabolic pathways have been identified and are still undergoing research, for the fate of different VFA, during acidification and acetogenesis. Generally, acetic, propionic and butyric acids are produced by the fermentation of sugars and other monomers. Acidogens can produce high concentrations of propionic and acetic acids at pH as low as 4.1, utilising sugars. Approximately 2 moles of propionic acid and 1 mole of acetic acid are produced per 1.5 mole of glucose or galactose (common compounds in whey) (Tyagi et al., 1991).

Furthermore, Samain et al.(1987) reported synthesis of butyrate and acetate during batch degradation of propionate, either using anaerobic sludge or a highly purified culture. Especially, with regard to their findings with pure cultures, they observed propionate fermentation to CO_2 and CH_4 through a significant accumulation of acetate and butyrate. The latter VFA salt was only degraded when complete consumption of propionate and acetate was achieved, which may be explained by the synthesis of butyrate during propionate degradation. They concluded that although indications are that bacterial species or consortia are able to synthesise butyrate from propionate, still the metabolic pathways remain unclear.

n-Butyrate is another intermediate of carbohydrate, protein or lipid degradation; while iso-butyrate is mainly produced from fermentation of the branched amino-acid known as valine (Allison, 1978). Angelidaki and Ahring (1985) studied the isomerization of n- and iso-butyric acids. They reported that degradation rates of n-butyric were generally higher than those for iso-butyric acid.

Also, Lin and Hu (1992) reported that isomerization between iso- and n-butyric occurs during the degradation of butyric acid. The formation of iso-butyric from a system degrading n-butyric was proved pH independent (pH ranging from 4.5 to 8.1). On the contrary, the formation of n-butyric from iso-butyric degradation has been pH dependent, with pH 5.7 having the maximum production (up to 11 times more from pH above 7.2). Both iso- and n-butyric degradation produced minimal amounts of propionic acid, but only n-butyric acid produced n-caproic acid. The production of n-caproic from n-butyric was also pH dependent (maximum production at pH 7.2), with higher or similar concentrations to iso-butyric production. Additional results were presented for the degradation of butyric acid in relation to high levels of acetic acid, in order

to examine potential inhibition. They showed that accumulation of acetic acid (up to 3 g/l), would shift acidogenic activities to the formation of higher carbon acids. Furthermore, their data showed that degradation of n-butyric is less sensitive to high acetic concentration, than degradation of iso-butyric. In fact at an acetic acid concentration of 3 g/l the rate of iso-butyric converting to n-butyric increases by almost 4 times, compared with that at 1 g/l. Overall during their studies, relative formation levels for iso- and n-butyric, based on added concentration of butyric acid, were in the range of 6.0% and 2.3% respectively.

Hausler (1969) reported that VFA degradation in relation to methanogens proceeds according to the following pattern:

- Acetic acid is readily decomposed by methanogens;
- Butyric acid is decomposed by β-oxidation to acetic acid and further decomposed;
- Propionic acid is the last one to be decomposed, out of the three major VFA, to acetic acid by the activity of slow-growing bacteria; and finally
- Valeric acid is first oxidised to propionic acid and then further decomposed.

Additionally Buswell stated, in his discussion of the paper presented by Speece and McCarty (1964), that propionic acid has been extensively reported to be the acid that digests much more slowly than acetic and butyric. He went on to state that research findings, as well as full-scale experiences with "sour" digesters, indicated that anaerobic organisms were more tolerant to acetic than propionic acid. Furthermore Andrews and Pearson (1965) reported that the rate of metabolism of individual acids by anaerobic bacteria, varied in the order: Acetic $\simeq$ Butyric > Valeric > Propionic.

Biogas from complete anaerobic digestion consists of a mixture of gases, mainly CH_4 and CO_2. Small fractions (<1%) of other gases, like NH_3, H_2, H_2S, H_2O and N_2, are also found in biogas (Kostenberg & Marchaim, 1992). The content of hydrogen sulfide (H_2S) in biogas is a function of wastewater composition. Any nitrates (NO_3) or sulfates (SO_4) in the wastewater, will be converted to gaseous products (N_2 and H_2S, respectively). Therefore the ratio of sulfate-sulfur to biodegradable organic matter, will determine the H_2S content in biogas. Higher content of sulfates in wastewaters results in higher content of sulfide in biogas (Owen, 1982). In sludge digestion, trace amounts of NH_3 and H_2 are produced. In hydrolysis and acidification the main gases produced from carbohydrates and fats are CO_2 and H_2. While for proteins apart from these two gases, NH_3 and H_2S are also produced (Popel, 1964).

CO_2 is far more soluble in water than CH_4. For example at 35°C and partial pressure of 760 mm Hg; 1.17 g of CO_2 and 18 mg of CH_4 are soluble in 1 l of water (CO_2 65 times more soluble than CH_4). Also CO_2 can be converted to CH_4, provided that sufficient amount of H_2 is available (Popel, 1964). So due to solubility of CO_2, biogas may contain more CH_4 under working conditions than the theoretical composition value (Schoberth, 1980). CO_2 solubility is further explained by Sawyer and McCarty (1978).

2.4 Treatment processes

2.4.1 General

In order to obtain better performance and control of anaerobic bacteria, a number of process designs have been designed and installed. Anaerobic digestion is the oldest biological process for the stabilisation of sewage sludges. Nowadays, the process is successfully applied in the treatment of municipal solid waste (MSW) and various types of industrial wastewaters.

The first digester designs used for sludge treatment, are now mostly referred to as conventional digesters. These early digester designs are of the continuous stirred tank type and treatment is achieved in lengthy retention times. Most continuously stirred tanks are operated as chemostats. Such operation means that substrate concentration is controlling microbial growth rate, which ensures a dilution rate that precludes biomass washout. In the last 3 decades, various reactor designs and modifications have been developed; all are based on the concept of high-rate digestion. The main aspect of this concept is to achieve separation between SRT and HRT (Iza et al., 1991; Dunn et al., 1994).

2.4.2 Wastewaters

The main concept of high-rate reactors in wastewater treatment is based on three fundamental aspects. The most important is biomass accumulation within the reactor, by means of natural settling, attachment to media and/or recirculation. The second is improved contact between substrate and biomass. Finally, enhanced activity of the biomass is achieved by acclimatisation, growth and well designed process control (Iza et al., 1991).

There is a great variety of reactor designs used as high-rate systems with great success for wastewaters. These designs have been reported to treat successfully many types of industrial wastewaters, with more than 500 full-scale reactors constructed worldwide (Anderson & Donelly, 1977; Iza et al., 1991; Ross & Strohwald, 1992). In the last decade, these reactors are also being applied in full-scale for the treatment of raw domestic wastewaters (Schellinkhout & Osorio, 1994; Draaijer et al., 1994).

The reactor designs mostly used in practice are:

- **CSTR.** This process, Continuous Stirred Tank Reactor, is also known as anaerobic activated sludge or simply the contact process. In this process the biomass is suspended in the reactor. In order to increase SRT, it is connected with a secondary settling tank or even a centrifugal separating device. A modification of this design is the ADUF (anaerobic digestion ultra filtration) process, where a membrane is used as a separating unit to recycle washed out biomass (Iza et al., 1991; Nahle, 1991; Ross & Strohwald, 1992).
- **Anaerobic Filter (AF).** In the anaerobic filter process, biomass is attached on inert support material in biofilm form. The material can be arranged in various configurations,

made out of different material and can be packed in two configurations (loose or modular). The reactors can be operated in upflow or downflow feed mode. These reactors are also referred to as fixed-bed or packed-bed reactors (Anderson & Donelly, 1977; Iza et al., 1991; Young, 1991; Kennedy & Droste, 1991).

- **UASB.** One of the most used processes in wastewater treatment is the Upflow Anaerobic Sludge Blanket. The process relies on the unique tendency of the anaerobic bacteria to form granules. The phenomenon of granulation has been extensively studied and reviewed (Hulshoff Pol, 1989; Noyola & Moreno, 1994). The reactor retains and aids the formation of granules by an efficient gas/solid/liquid separation device at the top of the reactor. Various applications and reviews prove the success of the UASB, while providing sufficient design configurations (Iza et al., 1991; Lettinga & Hulshoff Pol, 1991; Wentzel et al., 1994).

- **Fluidised Bed.** The fluidised bed reactor contains fine carrier particles for the bacteria. In this process biomass is retained in the reactor by the formation of biofilm around the carrier particles. This formation results from well designed upflow liquid velocities, which also enable the bed to expand. These velocities are a combination of flows from the influent substrate and the recycled effluent. The degree of bed expansion will determine whether the operation of the process is as a fluidised or expanded bed reactor (Iza et al., 1991; Iza, 1991; Ehlinger, 1994).

Various other configurations in the design of an anaerobic reactor, can be found mainly as combinations of these four types. Hybrid reactors are often considered to be the ones involving an Anaerobic Filter and a UASB. Also, the ABR (Anaerobic Baffled Reactor) is a combination of UASB in series (Iza et al., 1991).

2.4.3 Sludges

Sludge treatment with anaerobic processes has been practiced for more than 100 years. The process is well established for the treatment of various types of sludges, especially those from sewage treatment plants (Brade, 1995). Digestion of sewage sludges reduces the organic content by 30-50%, converting it into biogas. The process also removes grease and reduces the number of pathogens (Anon., 1979).

Aspects related to modern practices in sludge digestion, especially the high-rate conventional process, have been reviewed by Ghosh (1987), Pitt et al. (1992), Brade (1995) and Kidson (1996). Originally the process was applied in tanks without any process control, commonly designed for very long retention times (up to 60 days). After the '60's attempts to apply process control, by heating and mixing the reactor, allowed the application of the conventional high-rate process. The CSTR process design was used which reduced retention times to 15-30 days.

In order to use more concentrated feed sludges, subsequently reducing RT, reactor volume and sludge heating expenses, the use of two-phase sludge digestion is necessary. Otherwise, an increase in the loading of single-stage mixed reactors, results to an imbalance between the

phases of anaerobic digestion and a shift to acidogenic fermentation. Apparently, single-stage conventional digesters are even nowadays most often commercially proposed for sludge treatment. A process configuration utilising upflow acid- and methane-phase digesters was demonstrated to be considerably superior to conventional single-stage digestion, in terms of CH_4 yield and production rate, net energy production and overall treatment efficiency (Ghosh, 1987).

2.4.4 Solid Waste

Since the beginning of the '80's, anaerobic digestion for municipal solid waste (MSW) has been extensively studied, due to the growing "Energy from Waste" recycle concept. Three basic approaches have been explored for the digestion of MSW, some of which have also been commercially applied. They are listed below with examples of pilot or full-scale applications representing each approach (Dunn et al., 1994):

- Conventional "low solids" slurry digestion, for example the RefCom and Cal Recovery processes in USA, WMC process in UK and WBG process in Sweden.
- Dry digestion or "high solids", for example VALORGA process in France, DRANCO process in Belgium and BioCel process in the Netherlands.
- Two-phase digestion, for example Hitachi process in Japan, IBVL process in the Netherlands, BTA process in Germany and Leach-bed two-phase process in USA.

Extensive literature reviews for MSW digestion have been reported by Clausen and Gaddy (1987), Ghosh (1987), Dunn et al. (1994) and Zatari (1996). An interesting point to derive from these reviews is that the main rate limiting step in the digestion of MSW is hydrolysis and solubilisation of organic solids. This problem becomes apparent in considering the financial viability of the application. This is the reason why two-phase processes have received greater interest for the treatment of MSW.

For example a down-flow leach-bed and an acid-phase fermenter was utilised to liquefy dry MSW, generating a high VFA content bioleachate product. The latter was separately gasified, as acidified leachate, in an upflow methane digester. The process operated under ambient temperatures for the leach-bed and acid-phase fermenter. Also, it recycled indigenous nutrients in MSW and did not require addition of external water for moisture. It achieved CH_4 yield up to 80% of the theoretical value of the MSW treated, in a mesophilic AF. Various advantages have been proposed for this process compared to slurry-MSW digestion or dry digestion processes. The process proves very beneficial, especially for in-situ acceleration in biodegradation of landfill sites with control and utilisation of biogas production (Ghosh, 1987).

In a similar mode, Anderson and Saw (1992) reported results from a lab-scale leach-bed process in tandem with a UASB, treating the putrescible fraction of MSW. The process was reported to treat MSW completely and succesfully within 100 days. Hydrolysis and acidification in the

leach-bed achieved high acid production (concentrations up to 26 g/l). When the UASB reactor had significantly reduced VFA in the overall system, methanogenesis could proceed rapidly in the leach-bed. Furthermore in Munich, the BTA mesophilic two-phase system was developed in an R&D plant for commercial purposes, during the second part of the '80's. This application has been operated full-scale since 1991, by the Danish Energy Agency in the Centralised Biogas Plant in Helsingor, Denmark (Anon., 1992).

2.5 Process Control/Operation

2.5.1 General

Process design is directed to maintain a large and stable population of methanogens. The environmental and operational factors known to influence digestion performance are presented below (Owen, 1982):

- Environmental factors: these are pH, alkalinity, temperature, nutrient availability and concentration of potential toxic compounds; and
- Operational factors: these are Solids Retention Time (SRT), Hydraulic Retention Time (HRT), Organic Loading Rate (OLR) and substrate characteristics (composition, biodegradability and concentration).

The main concern of process management is ensuring steady flow of intermediate products from acidogens to methanogens. This flow has to be controlled without overloading the natural pH buffer with VFA, which would cause the digester to fail (Dunn et al., 1994). Generally, the specific activity of typical anaerobic processes treating soluble industrial wastewaters is approximately 1 kg COD/kg biomass·d.

2.5.2 Temperature

The degree but also the rate of digestion of organic matter will be influenced by increasing temperatures. Anaerobic processes are typically operated in mesophilic ranges, although higher temperatures in the thermophilic range are often used to achieve higher degradation rates. Lower ambient temperatures are used when longer RT are not considered a drawback. Generally within the mesophilic range higher temperatures result in more rapid decomposition of biopolymers, decreasing the digester volume to achieve a required degree of efficiency. The high-rate process requires elevated temperatures and the use of heated reactors. CH_4 gas produced can be used to provide this heat. Conventional process design and wastewaters of low COD concentration will not provide sufficient biogas for heating and a supplementary source may be necessary (Popel, 1964; Owen, 1982; Eckenfelder, 1989).

Steiner et al. (1985) reported the effects of mesophilic (35°C) to thermophilic (55°C) temperatures, from studies on slaughterhouse wastes with CSTR. They observed no apparent differences neither with VS or COD reductions, nor with biogas production or CH_4 yield. But they reported better decomposition for proteins at 35°C, while for fats at 55°C. They concluded that thermophilic treatment was far better for removal of pathogenic organisms (tests for *E.coli* and *Salmonella*). Furthermore, Ahring (1994) reviewed the status of thermophilic high-rate digestion, concluding that thermophilic reactors are as stable as mesophilic ones. She stated that the main cause of instability, often reported in literature regarding thermophilic digestion, is improper start-up design. Optimal temperatures from full-scale experience in Denmark were between 52-56°C.

System performance can be affected significantly by relatively small changes in temperature. For example in conventional digesters, stabilisation rates are altered by temperature fluctuations in the order of 2-3°C (Owen, 1982). Biothane reported, out of long-term operational experience with full-scale UASB, that temperature variations and shocks are not as harmful as expected from literature studies. They point out that bacterial activities can adapt from mesophilic to thermophilic temperatures, as long as temperature changes are gradual and do not happen rapidly (Anon., 1988).

2.5.3 pH and Alkalinity

An anaerobic process should provide the preferred pH range for methanogenic activities. This is 6.8 to 7.5, although systems operate effectively in a broader range from 6.0 to 8.0. Hydrogen ions, recorded as pH, are closely related to alkalinity in reactors. Alkalinity drops as bicarbonate reacts to buffer increased VFA production. pH may drop if the alkalinity is not sufficient to buffer excess VFA. To establish stability between high-rate VFA production and maintain neutral pH, bicarbonate alkalinity should be kept in a range from 2.5 to 5.0 g/l. As pH drops slowly, it is considered a poor parameter to assess digester overload and forthcoming failure. On the other hand, the VFA to alkalinity ratio is of greater importance to detect instability (Owen, 1982; Anon., 1988).

Additionally, Spicka (1969) reported in the discussion of Hausler's presentation (1969), that the most suitable indication of the need for remedial measures in the operation of a digester, was the ratio VFA to total alkalinity. From full-scale experiments (all presented in Czechoslovakian literature) artificially producing "acid digestion" with digester overloading, he concluded that the ratio should not exceed 0.2 to 0.3 mg VFA as Acetic per mg $CaCO_3$. Furthermore, when the ratio VFA to alkalinity is higher than 0.8, the concentration of VFA is too high to be equalised by existing alkalinity. The latter results in unbalanced conditions and phase separation develops in the digester. In cases where the ratio is below 0.4 with the addition of alkali, it is indicated that there is excess use of lime (Vlissidis & Zouboulis, 1993).

Alkali addition for pH control and buffer potential is important for substrates with alkalinity below 1 gr $CaCO_3$/l. In proper system design provision must be made to supplement alkalinity

whenever the substrate composition indicates that alkalinity might be less than 3.0 g/l in the digestion process. If sufficient alkalinity is not present in the substrate, then alkalinity can be controlled by reducing OLR or supplementing alkalinity as an operational activity. Lime is commonly used to maintain neutral pH in digesters. Caution must be used though, since excess lime addition results in precipitation of calcium bicarbonate. Sodium bicarbonate can be used as an alternative for pH adjustment (Owen, 1982; Eckenfelder, 1989; Vlissidis & Zouboulis, 1993). Finally, Biothane suggests that monitoring digester performance should include, on a daily basis, the volumetric load of NaOH used. Reporting alkali requirements, should be also in terms of volume added per substrate COD (Anon., 1988). A similar suggestion is made by ETC Ltd, in UK (Anon., 1993).

Furthermore, in order to avoid pH decrease, sufficient alkalinity must be present to compensate for high CO_2 content. For example at 30% CO_2 content in biogas 1.5 g/l of alkalinity is necessary (Eckenfelder, 1989). Also, pH is buffered by the production of ammonium bicarbonate, with ammonium ions formed from the deamination of proteins and bicarbonate produced from the solubility of CO_2 (Dunn et al., 1994). CO_2 is used to establish alkalinity as an important stability indicator. The role of CO_2 solubility is closely related to reactor alkalinity (Sawyer & McCarty, 1978).

Steiner et al. (1985), studying digestion of slaughterhouse wastes, reported that pH remained at 7.7 even at OLR 8.75 g VS/l·d due to the buffering effect of NH_4^+. At a higher OLR of 10.5 g VS/l·d the system failed due to phase separation, which allowed an increase of VFA exceeding the concentration of ammonia. In addition, Speece and McCarty (1964), reported that buffer addition was unnecessary in the digestion of proteinaceous matter, as sufficient buffer was produced by the ammonium bicarbonate end product. In their case it was necessary to neutralise with HCl the excess ammonium bicarbonate alkalinity created by the digestion of proteins operating at longer SRT. Otherwise the resulting increase in pH led to ammonium toxicity. Furthermore, Bloodgood stated in his discussion of the paper presented by Speece and McCarty (1964), that ammonium bicarbonate in the digester is necessary to react with VFA produced in the first phase, so that the environment is never unfavourable to methanogens. Also, he stated that in two full-scale applications, "sour" digesters have been restarted adding anhydrous ammonia, in a quantity that was slightly in excess of the amount needed to react with the VFA present. He concluded that the concentration of the natural buffer in a digester is a function of the amount of protein in the substrate, the rate of break-down to ammonia and the HRT.

2.5.4 SRT, HRT and OLR

Solids retention time (SRT), hydraulic retention time (HRT) and organic loading rate (OLR) are all operational factors that are closely related to the substrate composition and concentration. Higher solid content or concentration strength in the substrate requires higher RT for digestion. In a similar way, more complex substrate characteristics increase RT. Furthermore high-rate

designs are based on the ability to retain higher concentrations of biomass in order to increase SRT and reduce HRT. Loading rates are mostly defined by biodegradation and growth rates of anaerobic bacteria. Therefore such factors imply the required RT and digester volume for the expected degree of treatment (Hobson & Wheatley, 1993).

In conventional single-phase digesters SRT is equivalent to HRT. Therefore the degree of stabilisation is a function of SRT (or HRT) and not the concentration of influent organic matter. Recognition of this fact changed the philosophy of digester design as practised prior to 1950, which was based on OLR. This concept achieves more efficient utilisation of reactor volume, simply by concentrating the substrate. The main rate-limiting step in conventional digestion of most substrates, is the conversion of long- and short-chain fatty acids to CH_4. This is observed by the build-up of lipids and VFA under unstable conditions, causing foaming problems in the digester. Accordingly, the main objective of conventional anaerobic process design is to provide conditions which are conducive to decomposition of VFA by methanogens. Apparently, odours are often observed due to incomplete digestion of sludges, when operating simple conventional digesters at RT below 20 days (Owen, 1982).

Recycling of bacteria in a continuous process is the most common method used to retain cells and thereby increase biomass concentration in the reactor. Also, recycling reduces substrate concentration in the digester influent. In accordance with the smaller reactor volume, heating requirements would be also reduced due to a reduction of heat losses to surroundings (Owen, 1982; Tyagi et al., 1991). Meanwhile all high-rate designs for wastewater treatment utilise natural and/or immobilisation methods (e.g. granulation, biofilm attachment) to achieve an increase in SRT (Iza et al., 1991).

At mesophilic temperatures Biothane suggests that treatment of industrial wastewaters should be designed at HRT and OLR, operating the digester at average sludge loading (F/M ratio) of 0.5 kg COD/kg VSS·d (Anon., 1988). UASB has been successful with OLR up to 96 kg COD/m^3·d in certain wastewaters. Generally in pilot-scale studies OLR of 15-40 kg COD/m^3·d and HRT of 3 to 8 h can successfully treat high-strength wastewaters in a UASB (Eckenfelder, 1989). General information about OLR and HRT applied on various high-rate designs for the treatment of various types of wastewaters can be found in the literature (Hickey et al., 1991; Hobson & Wheatley, 1993).

An interesting study on the relationship between OLR increase due to an increase in HRT and an OLR increase due to changing wastewater concentration, was presented by Steiner et al. (1985). They evaluated the effects of OLR on slaughterhouse wastes, maintaining stable HRT and diluting to the desired substrate concentration. Also, they tried changes in HRT to obtain different OLR values. Their study on OLR was detailed, proving that no significant changes were observed from 2.9 to 8.1 g VS/l·d, in the performance of a CSTR. But at 10.5 g VS/l·d the system failed due to overloading and phase separation. Their study on HRT did not give major conclusions because only 3 different HRT were examined. These results were derived from experiments on different waste characterisation (as COD and VS) as two sets of combinations. The first combination was a reduction in HRT from 10 to 7 days, without any

effect on digester efficiency and CH_4 yield or content, but a 40% increase in gas production. The second combination was done between 12 and 10 days, but the digester failed due to overloading and operated on critical stability with 12 days. An interesting point in this study is a combination of two experiments with similar OLR (8.6 and 8.75 g VS/l·d) but different HRT (7 and 12 days); due to different waste characterisation (77% and 75% higher as COD and VS respectively). These two experiments gave significant differences in the performance with a 11% reduction in BOD removal; 24% reduction in biogas production; 25% reduction in CH_4 yield; but no difference in CH_4 content.

With the results of that study and considering that the performance at 12 days was maintained at critical stability, it could be assumed that HRT changes affect OLR in relation to performance and overloading of a specific design. On the contrary, waste concentration changes, with subsequent OLR changes, do not represent HRT changes towards overloading. This interesting example proves the difference of the two parameters, namely OLR and HRT, especially when assessing overloading. Therefore, both waste strength and HRT can be responsible for overloading. Meanwhile different patterns are observed in loading from HRT (mainly responsible for bacterial wash-out and conversion rates), than waste strength (representing only substrate conversion and accumulation). Finally, it seems necessary when assessing OLR effects in the performance of a digester to consider changing both waste strength and HRT to obtain more conclusive and realistic results.

2.5.5 Nutrients and Toxicity

Anaerobic processes are important for many treatment applications, which often involve nutrient deficient substrates. Anaerobic processes require lower amounts of N and P than aerobic processes. Due to lower biomass yields, nutrient addition can be reduced up to 5 times, compared to aerobic treatment (Owen, 1982). Generally, industrial wastewaters are less nutritionally balanced for digestion than sewage sludge. An analysis assessing the treatment benefits from the addition of nutrients in the reactor, in relation to subsequent operational costs of the anaerobic process; will determine the use of nutrients for anaerobic treatment. The engineering decision is to determine whether to accept the increased capital cost of a digester with nutrient limitations and decreased utilisation rates and stability or proceed with added operational cost for nutrient supplies. Determination of nutrient requirements should be part of process design. In some cases substrates may satisfy these requirements, but in many cases certain nutrients may have to be specifically supplemented, to insure adequacy for N, P, S, and trace metals (Speece & Parkin, 1987).

N and P are major elements which most often limit digestion efficiency in nutrient deficient substrates. Nitrogen requirements can be determined by cell yields and the fraction in the cell. Based on a typical elemental analysis for anaerobic bacterial cells, i.e. $C_5H_9O_3N$, nitrogen requirement appears to be approximately 11% of the cell volatile suspended solids. N:P ratio is approximately 5:1-7:1, or 2% of the cell VSS weight. For every 1,000 kg BOD digested,

macro-nutrient requirements are approximately 6 to 10 kg of N and 1 to 2 kg of P (depending on waste characteristics). The minimum requirement for N and P is about 2.5% and 0.5% of the dry organic matter respectively. Sulfur requirements are partly a complex case. They can be found presented in a range of sulphide concentrations, as S source, from 25 to 280 mg/l; with concentrations depending on COD converted to CH_4 ranging from 1 to 50 g/l respectively (Speece & McCarty, 1964; Anon., 1979; Owen, 1982; Speece & Parkin, 1987; Eckenfelder, 1989).

Speece and McCarty (1964) reported with studies on nutrient requirements on digestion of simple carbohydrates, a marked decrease in N requirements as SRT increased. This was due to conservation of nutrients as a result of endogenous respiration. According to Buswell, as cited by Popel (1964), organic matter can be digested only if at least 6 mg of N are present for every 1 g of organic matter. This leads to a C:N ratio of less than 65 to allow for anaerobic decomposition of a substrate. Popel (1964) went on to present results which concluded that maximum gas production could be produced by sludge only if C:N ratio was 13-14. Digester failure in the treatment of MSW was observed when the C/N ratio was 52:1 (Diaz et al., 1987).

The overall nitrogen balance is an important consideration in anaerobic digestion. For all practical purposes N is conserved in most anaerobic applications, although any nitrates (NO_3) present will be reduced to nitrogen gas and exit as biogas. Since biomass yields are very low, only a small fraction of the biodegradable nitrogen compounds will be converted to biomass. Most of the biodegradable nitrogen (i.e. proteins and other organic nitrogen compounds) is converted to ammonia in aqueous solution. Accordingly ammonia concentration in the effluent of anaerobic reactors is generally higher than the influent concentration (Owen, 1982).

Microbial generation time is a function of nutrients present in the substrate. It is difficult to identify that one nutrient is more important than another, because all of the required nutrients are essential and should be supplied. Effects of nutrient limitations will range from either prolonging microbial growth and conversion rates to, complete cessation of bacterial activities. Also, toxicity response is compounded by nutrient limitations. Furthermore, methanogenic bacterial changes can occur due to nutritional changes. The increase observed in utilisation rates was attributed to changes in bacterial population, mainly groups with rates 4 to 8 times greater (from a *Methanobacterium soehngenii* culture to a predominant *Methanosarcina mazei* or *M. barkeri* culture) (Speece & Parkin, 1987).

Speece and McCarty (1964) concluded from studies on nutrient requirements for digestion that high rates of acetate digestion could be achieved by additions of combinations of Fe, Co, thiamine and components of vitamin B_{12}. Generally trace nutrients like Fe, Co, Ni, Mo, Se, Ca, Mg and microgram-levels of vitamin B_{12}, are necessary for high-rate digestion. Most often alkalinity, N and P are the only supplemented chemicals in anaerobic processes. Heavy metals are relatively inexpensive to supplement and methanogens have the following concentration requirements: iron 10 mg/l; cobalt 5 mg/l; nickel, molybdenum and selenium 0.1 mg/l (Speece & Parkin, 1987; Eckenfelder, 1989).

On a commercial level, Biothane suggests that N and P (referred to as macro-nutrients) should be

present in wastewaters, at COD:N:P ratio of 500:5:1 for highly acidified wastewaters and 350:5:1 for low VFA-content wastewaters. In relation to micro-nutrients the following concentrations are proposed, for wastewaters with COD of 1-10g/l: N:K in the range 14:1; Fe and Mg 5 mg/l; Ca 1 mg/l; Zn 0.1 mg/l; Cu, Mn, Ni and Al 0.05 mg/l; Co 0.01 mg/l and Mo 0.001 mg/l. In order to assess excess addition or further need of macro-nutrients, the effluent of the reactor should have N and P values of 15-20 and 10-15 mg/l respectively (Anon., 1988). Accordingly a mixture of trace elements involving Fe, Ni, Se, Cr, Mo, Cu and Co are dosed in Biothane-UASB reactors treating soft-drinks wastewaters at "Coca Cola & Schweppes Beverages Ltd" in Wakefield, UK (Anon., 1993).

Generally recalcitrant, inhibitory and toxic compounds and concentrations are those with difficulty in biodegradation. It is often the conversion of these complex compounds to VFA that appears to be the rate limiting step in the overall process (Speece & Parkin, 1987). In addition, the most sensitive trophic group in digestion are the methanogens. The next most sensitive group are the acetogens, often found in syntrophic associations with the methanogens. There are a number of compounds reported as toxic or inhibitory, listed in six main groups as follows: a) disinfectants; b) heavy metals; c) ammonia and other cations; d) pesticides; e) chlorinated hydrocarbons and f) hydrocarbons and other complex organic compounds. Hydraulic, organic or toxic compound overloading are the causes for digester instability. However it is important not to generalise, as the effects of substrate inhibition depend greatly on the characteristics and concentrations of the wastewaters and the conditions in the reactor (Dunn et al., 1994).

The term toxic is relative, as this is always the case for all conditions and mostly compounds characterised as toxic or even inhibitory for anaerobic digestion. In fact at very low concentrations, many so-called toxic compounds are considered necessary as micro-nutrients to increase or achieve certain treatment efficiencies. Toxic compounds alter microbial metabolism and tend to increase replication time, which reflects in decrease of overall removal efficiency. However, proper attention to SRT can offset these adverse effects and it is common that acclimatization of the biomass and adaptation of new operational conditions will resolve toxicity problems. In fact, an underlying principle of wastewater treatment is that maintenance of longer SRT will compensate for less ideal environmental conditions in temperature, pH and also toxic substrates. In addition proper acclimatisation periods can significantly increase a reported threshold of a toxic concentration at which inhibition occurred. Efficient stabilisation has been achieved in the presence of 20 to 50 times higher concentrations of toxic substances, that have exhibited up to 50% inhibition of methanogenic activities for less acclimatised bacteria (Owen, 1982).

Owen (1982) prepared an extensive list for various compounds and selected organics, presenting concentrations and their relative toxicity. In addition, various aromatic compounds, pesticides and even higher molecular weight hydrocarbons that make up oil, are decomposable by anaerobic bacteria. Generally, significant inhibition of methanogenesis was observed with additions of cations in the following concentrations: sodium and calcium 8 g/l; potassium 12 g/l; magnesium and ammonium 3 g/l. It appears that cation toxicity is increased with increasing atomic weight and valency. The presence of antagonistic ions may sharply reduce the inhibitory effect of specific

cations (Eckenfelder, 1989; Dunn et al., 1994).

At a biochemical level ammonia can cause potassium depletion in some species. The toxicity of dissolved ammonia and ammonium ions are different, as the toxicity of ammoniacal nitrogen is pH dependent increasing at increased pH. Toxicity occurs at concentrations of ammonium ions in excess of 3 g/l and inhibition above 1.5 g/l. Meanwhile, dissolved ammonia concentrations are inhibitory in excess of a range between 0.15-3 g/l. These concentrations are dependent on digestion conditions and substrates. Some degree of self regulation can be expected in single-phase reactors, as inhibition results in increased VFA. The latter in return depresses the pH and converts dissolved ammonia into ammonium ions, alleviating the inhibition (Dunn et al., 1994).

Finally, it has been reported by van den Heuvel (1985) and in literature studies by Dunn et al. (1994), that VFA have been shown to inhibit methanogenesis at concentrations above 10 g/l and 6 g/l for acetate and propionate respectively.

2.5.6 Mixing

Mixing is essential for good conversion of solid substrates in a reasonable and cost-effective RT (Clausen & Gaddy, 1987). Efficient mixing of the contents of digesters is essential to maximise loading, while producing a stable product. Especially, efficient mixing of sludges seems to be of critical importance, as digester mixing significantly influences treatment efficiency. Meanwhile, inadequate mixing with poor volume utilisation jeopardises the quality of effluent sludge. Therefore in the design of digesters it is specified that contents are completely mixed (Hobson & Wheatley, 1993).

Supplemental mixing, in addition to that caused by biogas evolution, is necessary to increase the "effective" digester volume and permit high-rate digestion. Mixing digester contents increases the rate of stabilisation substantially. Digester mixing is accomplished by "pumping" or recirculating the reactor contents. This is generally done by gas recirculation, liquid recirculation using external pumps or internal, impeller mixers. Generally, digester mixing is not well understood. There is confusion and great disparity in mixing design and in perceived effect. In most full-scale instalations mixing is relatively ineffective, with less than 50% of the total volume effectively utilised. Mixing though is necessary to provide homogeneity and prevent stratification of contents (Owen, 1982).

The performance relationships found with completely mixed anaerobic reactors operating under ideal laboratory conditions can not be practically achieved in full-scale plants. Particularly mixing is substantially less in full-scale reactors than the rates used in laboratory to identify digestion performance. Better mixing can be achieved by more and better consideration for the design of the mixing system (i.e. using mixing models, pilot or tracer studies, etc.); ensuring in the meantime that overdesign of the system is avoided. A vortex or baffles may be used to increase mixing. Regular cleaning of the digester proves useful and operation at a higher flowrate (i.e. increase in OLR) ensures better volume utilisation of the digester (Owen, 1982; Hobson &

Wheatley, 1993).

Various figures have been reported for the mixing of CSTR at laboratory scale. For example Steiner et al. (1985) reported using mechanical mixing with propellers at 75 rpm in lab-scale studies treating slaughterhouse slurries. Kostenberg and Marchaim (1992) reported mixing reactors at 150 rpm with a timer, for 2 min every 20 min; in lab-scale treatment of coffee slurries. Also, Hulshoff Pol (1989) applied mixing at 140 rpm for 15 seconds every 10 minutes, in lab-scale batch-fed reactors used for specific methanogenic activity tests. Additionally he applied 30 rpm for 5 seconds every 30 minutes during the initial start-up of UASB reactors. After start-up the UASB appeared to produce sufficient amounts of biogas to effectively mix the system naturally without the need of mechanical support. Furthermore, D'Addario et al. (1992) reported mixing batch and CSTR systems with 0, 50 and 100 rpm for the acidification of MSW. They also produced comparative results between 0 and 100 rpm. Their batch system operated at pH 5.5, HRT 12 days, 35°C and 15% TS in the feed. Without mixing the system decreased VFA yield only 6%, compared to mixing with 100 rpm, while the marginal CH_4 yield decreased 67%.

On the contrary Ghosh (1987), describing an upflow two-phase system, stated that the two digesters were neither mixed mechanically nor by compressed biogas, for the treatment of high solids-content sludges. He suggested adequate mixing achieved by the following process, on a lab-scale application: "The liquid fraction of sludge travelled upwards, towards an overflow port on the surface. Meanwhile, incoming solids were deflected downwards to increase SRT, affecting the fermentation of retained solids. The overflowing supernatant, with concentrated VFA-content from the acidogenic reactor, enters the bottom of the methanogenic reactor. The latter has similar mixing operation with the first-phase reactor."

2.5.7 Acclimatisation

In all experiments it is found that acclimatisation of the digester culture to the substrate treated and the operational conditions applied is a prerequisite to successful performance (Diaz et al., 1987). It is known that one has to allow sufficient time (sometime weeks) to acclimatise bacteria in digesting various compounds. If one is not patient enough, misleading results may be collected. The following critical stages in the process should be considered: hydrolysis of insoluble polymers (e.g. cellulose); interspecies hydrogen-transfer reactions; and methanogenesis (especially of acetic acid). Depending on the particular conditions, one or another of these stages will be rate limiting in anaerobic digestion (Schoberth, 1980).

For start-up of digestion, fresh sewage sludge should be seeded with an appropriate amount of seed sludge, which should be rich in solids, contain small amounts of organic acids and have an age of about 10-15 days of digestion (Popel, 1964). Commercially Biothane considers for UASB start-up that parameters used to evaluate the performance should be observed stable within a determined range for at least 3 days (HRT up to a day for their reactor designs) (Anon., 1988). Apparently most commercial and lab-scale applications as a "rule of thumb" utilise operation

of 3 RT before assuming acclimatisation. Extensive reviews on start-up of different high-rate systems for wastewater treatment have been reported by Hickey et al. (1991) and Weiland and Rozzi (1991)

Various researchers appeared to report different ways to assess acclimatisation. For example, Clausen and Gaddy (1987) reported steady-state data for kinetic studies on solid waste digestion after visually observing steady gas production for 2 to 3 retention times. Initially they would operate the digesters for 2.5 RT before sampling at steady-state. Additionally, Myburg and Britz (1993), mention that during their study on the effect of OLR on the digestion of leachates in a hybrid reactor, their data collection period would be at "stable state" conditions for 7 HRT. They assumed "steady state" to be achieved after 5 volume turnovers (i.e. HRT), when parameters would show a variation of less than 10%. Furtermore, Vlissidis and Zouboulis (1993) reporting the performance of a full-scale thermophilic anaerobic digestion plant, treating distillery slops, described acclimatisation occurring within two months from restarting operations (winery closed for two months). This suggests acclimatisation periods of 5.5 times and around 3 times of the presented HRT and SRT respectively.

The periods required for acclimatisation range even more widely when considering treatment of high solids-content. Kostenberg and Marchaim (1992) reported starting their studies on coffee slurries with HRT 40 days, after acclimatisation of 30 days. The same acclimatisation period was used for the second set of experiments, with HRT 20 days. Also D'Addario et al. (1992) reported that their data on acidogenesis of MSW for VFA recovery were obtained after approximately three RT (HRT 12 days for the CSTR used). While for a Multistage Counter-Flow Reactor (MCFR), they reported substantially stable steady conditions were reached after approximately 30 days (system operated with RT 12 and 21 days). Finally, Lin and Hu (1992) reported acclimatization of more than one year for their studies on butyric acid degradation. They operated fill-and-draw type digesters at HRT of 10 days on the seed sludge obtained from this acclimatisation.

Chapter 3

TWO-PHASE PROCESS

3.1 General

The concept to develop anaerobic digestion as a two-phase process, originated from the view that it is generally a process involving two different sets of activities. In the first phase hydrolysis and acidification takes place at rapid rates, so that under high-rate digestion the activities of methanogens are precluded for conventional processes. In the second part of the process the methanogens operate in parallel to acetogens. This second group of bacteria has slower growth and degradation rates, while it is less tolerant towards changing conditions (Verstraete et al., 1981). Such biological conditions and the need to apply high-rate digestion of sludges, led to the practice of two-phase process by the research of Pohland and Ghosh (1971), Fan et al. (1973) and Ghosh et al. (1975).

Overall, the two-phase process takes advantage of the phase separation phenomenon, occurring naturally from different kinetic rates, providing separate acidogenic and methanogenic reactors to increase the economy, treatment efficiency, energy production and process stability of anaerobic applications. Various operating modes and reactor designs can be envisaged within the broad framework of the two-phase digestion concept (Ghosh, 1987).

3.2 History & Development

Over the years the two-stage hypothesis for methane fermentation has been proposed many times. Maze stated in 1903 (broad translation from French document, cited by Hungate, 1987): "It is in old cultures that the *pseudosarcina* are best seen. The bacteria which accompany fermentation are there of course, but they ferment the initial substrate, producing as gas only carbonic acid and hydrogen, whereas in the cultures producing methane, hydrogen is always absent. Analysis of the H_2 cultures discloses acetate and butyrate." Furthermore, Sohngen, in his doctorate thesis in 1906, reviewed the literature taking into account the direction pursued by Maze. He obtained CH_4 from enrichments of fatty acids (with carbon atoms up to caproic acid), but also from sugar, starch, cellulose and pectin. The acids from these, he regarded as

substrate for the CH_4-producing bacteria, according to the two-phase concept (Hungate, 1987).

Speece and McCarty (1964) reported from their studies that the most significant observation was the discovery of an exponential high net synthesis and a corresponding reduced CH_4 production from carbohydrates. This observation occurred with the application of short SRT and merits caution from those responsible for process design of treatment facilities. They stated that anaerobic digestion is considered to take place in two stages. They concluded that: "Two stages exist in anaerobic digestion of complex substrates, one in which BOD remains constant and another in which BOD is reduced due to the production of CH_4". In addition Heukelekian, in his discussion on the paper of Speece and McCarty (1964), agreed with all comments made by the authors and tried to explain further their observations. Similar statements about the two phases of anaerobic digestion and the possible engineering potential from the phenomenon of separate phases, were made by Andrews and Pearson (1965).

A very interesting moment in the development of the engineering aspects of phase separation can be found in Hausler's presentation and group discussion, at the 4th International Conference on "Advances in Water Pollution Research" in Prague, Czechoslovakia, in 1969. In his paper on the succession of microbial processes in the anaerobic decomposition of organic compounds, he concluded with a new method to utilise the potential of phase separation. Following these conclusive remarks and due to the complexity of substrates from industrial wastewaters compared to sewage sludge digestion, he proposed a method for engineering anaerobic digestion consisting of "two or even more physiological stages which maintain optimum conditions for the bacteria of the individual phases, especially the methane bacteria. The principle of the new method is that the methane digestion takes place in two or more separate digesters, depending on the composition of the wastewater. In the first digester, hydrolysis of high molecular organic compounds and formation of volatile organic acids occurs. Since the process takes place at optimum conditions, the time of formation of volatile acids is considerably shortened. In the second digester, with a specific community of active forms of methane bacteria, wastewaters are treated which have already undergone the first stage (i.e. the formation of volatile acids). In the second stage the pH has been adjusted to 7.2, with lime addition. Thus the optimal conditions are maintained even for the second microbial community and the whole process is considerably accelerated."

In summary, he stated that: i) Based on results on the succession of microbial processes, a new method has been proposed for anaerobic treatment of industrial wastes with high carbohydrate content. ii) The method was experimentally tested in an anaerobic pilot plant, treating wastewaters from the production of citric acid. In the first stage, the concentration of VFA increased to 10-12 g/l. iii) A new treatment plant based on this method has been built (1969) for the "Chemical Works" (producing citric acid) at Kaznejov, Czechoslovakia. Unfortunately no further literature has been found relating either to the pilot or the full-scale applications mentioned in this paper, of this first operation of two-phase process for industrial wastewater treatment.

Apparently, McCarty in his discussion of Hausler's presentation (1969), argued against the proposed two-phase treatment method. He stated that "greater advantages of two-stage treatment,

than those indicated by Hausler, seem necessary if the proposed method of treatment is to be attractive."

Meanwhile Kollatsch reported, as cited in his discussion of Hausler's paper (1969), the development of a two-phase digestion in a single reactor operated as part of a two-stage (anaerobic and aerobic activated sludge) process, for the treatment of the wastewaters at the "Sehnde" sugar factory in Germany. The application based on "several years laboratory and large scale research", was backed by a number of hypotheses proving the benefits of acidogenic fermentation to provide simple acids for the treatment with activated sludge. The process was operating full-scale in 1968, after pilot trials in 1967. One of the advantages claimed for this two-stage process, was that filamentous growths were not observed after the application, although they were a major problem before. Also, the degree of treatment achieved the required standards. Although other literature presented by the same author at the same period is published in German, it is evident that this speaker reported the application of pre-acidification in an uncontrolled anaerobic environment, prior to aerobic treatment. It is, according to personal knowledge, the first description of its kind about studies on laboratory and full-scale plants, utilising some of the benefits of acidification in tandem with activated sludge treatment, which, even nowadays, are not fully exploited.

Finally, in the '70's the first acclaimed pioneering paper was published on the two-phase concept, presenting results proving the benefits of phase separation for the digestion of sludge (Pohland & Ghosh, 1971). In the '80's two-phase processes, physically separating hydrolysis and acidogenesis from acetogenesis and methanogenesis, gained increasing interest (Schoberth, 1980). In the '90's guidelines have been suggested for process engineers, to design acidogenic reactors for pre-treatment of high-strength industrial wastewaters (Alexiou & Anderson, 1994).

Overall, two-phase processes should not be mixed with two-stage ones, also found in the literature; which operate two different single-phase digesters in tandem e.g. a UASB and a batch digester-flocculator-precipitator (Vlissidis & Zouboulis, 1993). Generally, two-stage digestion is referred to as a combination of mesophilic and thermophilic digesters or anaerobic and aerobic processes. Also two-stage digestion is a combination of a primary digester operating as a mixed high-rate system and a second digester, which is not mixed. The purpose of the second digester is to store and concentrate the sludge prior to ultimate disposal (Owen, 1982). Furthermore Popel (1964) described the use of two- and three-stage digestion, as sequential operation of anaerobic reactors. The same approach was adopted by Kubler and Schertler (1994), who used three-phase digestion to treat organic industrial wastes.

3.3 Advantages/Disadvantages

One advantage of two-phase digestion is operation at shorter HRT and more concentrated feed. This results in significant reduction in plant capital cost and enhancement of net energy production, when applying the two-phase process on soft-drinks wastewaters (Ghosh & Henry, 1981).

Similar advantages were presented by Ghosh (1987) with his studies on sewage sludges. Using a hypothetical example to compare energy requirements and operation of a conventional sludge digester with a two-phase one, he proved the significant difference of the application of the two systems. With 68% higher solid-content sludge, 64% higher OLR and 39% reduction in total reactor volume, the two-phase process achieved 3 times better VS reduction. Regarding energy benefits, the two-phase produced 4 times more CH_4 than the conventional system, resulting in net energy production of 823 GJ/d for the two-phase plant, instead of 20 GJ/d net energy consumption with the conventional. An additional economical benefit could be the cost of digested sludge handling and disposal, which in the two-phase process is far less (3 times better treatment efficiency) than in the conventional process.

Also, improvement in gas quality is achieved with two-phase applications. This is mainly due to pH differences naturally occurring between the two phases. As a liquid stream passes through the acid phase, excess VFA decrease the pH to 5.0-6.5, so dissolved CO_2 shifts towards the formation of H_2CO_3 and CO_2 gas. When this stream passes through the methanogenic reactor, VFA convert to biogas causing an increase to pH from 7.0-8.0. This pH increase causes dissolved CO_2 to predominate in the HCO_3^- form and increase the absorption capacity of the liquid stream for the produced CO_2. These phenomena result in CH_4 enrichment in the produced biogas. With the two-phase process in the first reactor there is low solubility for CO_2 and all the CO_2 produced is released in the gas phase. While in the second reactor a lot of CO_2 has already been released and removed, therefore most of the CO_2 produced is absorbed in the liquid stream due to high CO_2 solubility in this stage (Hayes & Isaacson, 1987).

Acidified carbohydrate wastewaters have up to six times less macro-nutrient requirements than in the conventional process, for the provision of stable methanogenic activities (Speece & Parkin, 1987). Furthermore another advantage of two-phase leach-bed digestion of MSW or sludges is the leaching of heavy metals from the solid substrates, resulting in a digested residue which contains low levels of heavy metals (Dunn et al., 1994).

Finally, Kollatsch stated (as cited in his discussion of Hausler's paper, 1969), two major advantages with a full-scale application of an uncontrolled acidogenic reactor in tandem with an activated sludge process, at a sugar factory in Germany in 1968: i) The activated sludge process did not have any more bulking problems (filamentous growths were not observed), in comparison to the period when only activated sludge process was used. Also an improvement in settling qualities was observed. ii) The overall treatment significantly increased. (With activated sludge between 1960 and 1966, effluent quality was on average 400-500 mg BOD/l; after using pre-acidification the figure obtained was below 30 mg BOD/l).

Overall, some of the main advantages of the two-phase process over conventional digestion are listed below (Alexiou & Kamilaki, 1996):

- faster start-up of high-rate systems
- increased process stability

- enhanced process efficiency
- better conversion of solids
- better biogas quality
- better pathogen removal
- better colour removal
- reduction in the overall capital and operational costs

3.4 Balancing/Equalisation Tanks

The objective of balancing/equalisation is to minimise or control fluctuations in wastewater characteristics, in order to provide optimum conditions for subsequent treatment processes. The size and type of equalisation tanks provided vary with the quantity and variability of the wastewater stream. The tank should be of sufficient size to adequately absorb wastewater fluctuations, caused by variations in plant-production scheduling for industrial wastewaters, while dampening concentrated batches periodically dumped or spilled to the sewer (Eckenfelder, 1989).

The following advantages would be provided by the use of a balancing/equalisation tank in industrial wastewater treatment (Alexiou et al., 1993):

- equalisation of the flow;
- homogeneity of temperature, pH, nutrients and organic matter;
- dilution of high concentrations or potential inhibitory or toxic compounds; and
- a degree of pre-acidification would occur, unless aeration is used for mixing purposes.

Biothane always applies an equalisation/acidification tank prior to the UASB, with the primary purpose to condition the wastewaters before entering the digester. This tank is designed to balance and mix influent and recycled wastewaters, with chemicals added for nutrients and alkalinity (Anon., 1988). Although it is referred to as acidification tank, no microbial activities are promoted, allowing only uncontrolled acidogenesis to occur, with unpredicted results.

The use of a balancing tank for pre-acidification of soft-drinks wastewaters was reported by Kamilaki and Alexiou (1998). They reported that more than 40% Acidified COD could be the result of minimally controlled balancing tanks. The objective was to acidify these high strength wastewaters (COD above 20 g/l) for treatment in a thermophilic high-rate AF.

3.5 Process Control/Operation

3.5.1 General

Process control in two-phase anaerobic digestion is similar to single-phase digestion, with respect to the control required by the methanogenic reactor. Different requirements are thought necessary for the acidogenic reactor in a number of aspects referring to hydrolysis and acidification of organic compounds. These relate to the subsequent biochemical pathways that will in effect optimise the performance of this reactor, both in effluent products and economy in operation. The limited literature for acidogenic reactors treating industrial wastwaters refers mainly to basic aspects in the operation of an anaerobic reactor, i.e temperature, pH, HRT and minimal information on nutrients and toxicity.

Regarding the degree of acidification that should be achieved in the acidogenic reactor in order to benefit the methanogenic reactor, very little information can be found. Lettinga and Hulshoff (1991) reported that for UASB treatment of wastewaters 20-40% should not be exceeded, due to detrimental effects on granulation of biomass in the methanogenic UASB reactor. Later, Alexiou and Anderson (1994) reported that pre-acidification could be as high as 50% for high strength industrial wastewaters treated in AF. Their comments were based on experience on two-phase operation with AF as methanogenic reactors, in the laboratory of Environmental Engineering in Newcastle University. More recently, Zatari (1996) observed acidogenesis reaching up to 60%, in a novel single-phase reactor for high-rate dry solids digestion of MSW. His observations during the HRT of 24 days, for the first part of the HRT duration in the reactor, appeared without any unbalance in the performance or digester failure for the methanogenic activities.

3.5.2 Temperature

Zoetemeyer et al. (1982b) contacted the most detailed study on the effects of temperature on acidification of a synthetic substrate based on glucose. The temperature range was 20 to 60°C, HRT between 8 and 10 h and pH 5.8. They reported that the optimum temperature for acidogenesis of glucose was 37°C for mesophilic ranges and 52°C for thermophilic. Although they observed slightly higher degradation rates at thermophilic ranges, they suggested that mesophilic ranges should be applied due to the advantages of even greater stability. Acetic, Propionic and n- Butyric acids were the main detected VFA, with the content of n-Butyric being the highest in mesophilic ranges and decreasing rapidly at thermophilic ranges. Also they observed that CO_2 and H_2 were the main gases produced and no CH_4 was traced in their studies. Finally at high thermophilic temperatures (>55°C) they found high concentrations of Ethanol and Lactate which were proportionally higher than the different VFA produced.

Furthermore van Lier et al. (1990) studied short-term shock effects on mesophilic digester populations using synthetic substrates, with temperature changes from 37 to 60°C. They reported that temperature shocks did not seriously affect acidogenic bacteria.

McDougall (1996) reported a comparison between 37, 45 and 55°C for the acidifcation of synthetic coffee wastewaters at pH 6.0 and HRT 24 h. He found that 37°C was the optimum temperature for his application and that 45°C and 55°C were producing very erratic and unstable results even after steady-state was achieved. However Kozuchowska (1992) reported that 45°C was a better option than 37, 55 and 60°C on her lab-scale studies on synthetic coffee wastewater acidification, with no pH control (pH around 4.3) and short HRT (<12 h).

Aoki and Kawase (1991) reported that acidification of sludge (2% TS) without pH control, was reducing 58% of VSS at 70°C at RT 5 d but only 49% of VSS at 55°C and RT 6 d. Also they reported that under the applied conditions at 70°C no CH_4 was detected.

Shin et al. (1992) reported the effects of temperature on acidification of food wastes from restaurants. They operated laboratory scale reactors with 2.75 l volume, HRT 5.5 days, 4% TS in the substrate and a sequential batch mode to increase SRT. pH was uncontrolled, with starting values of 6.0 to 6.7 in the different studies, while values for the final pH ranged from 5.2 to 5.8. The greatest decreases in pH were observed for the mesophilic system. Their results for the mesophilic acidogenic reactor, indicated that about 50% of the soluble intermediate materials were acidified. They concluded that solubilisation efficiency in a thermophilic acidogenic reactor was about 57% higher than that of a mesophilic digester.

3.5.3 pH and Alkalinity

At a pH of 7.0 to 8.0, soluble CO_2 exists predominately in the bicarbonate ion form (HCO_3^-). As pressure increases more CO_2 is solubilised from the digester gas phase into the liquid phase causing a pH decrease. In two-phase digestion much of the alkali demand for pH control can be supplied through the biological conversion of VFA to biogas. Since acids of 1 to 4 carbon atoms exist predominately in the ionic form at pH above 5.5 and also as they need to be in the protonated form before they can be biologically metabolised to CH_4 and CO_2, one mole of hydroxide is released per mole of monoprotic acid converted to biogas (Hayes & Isaacson, 1987).

Zoetemeyer et al. (1982a) has also presented the most detailed study on the effects of pH on acidification of glucose. They studied a pH range from 4.5 to 7.9, at 30°C and HRT 3 to 9 h. They found that optimum pH for acidogenesis of glucose was around 5.7-6.0. In their studies n-Butyric acid, Acetic acid, Ethanol and Propionic acid were the main intermediates, with Lactic and Formic acids also present at smaller proportions. They also reported that the only gases detected were CO_2 and H_2, adding up to a 100% of the gas composition, with H_2 content increasing with the decrease in HRT values.

Genschow et al. (1996) reported data on sulfate removal from tannery wastewaters with two-phase anaerobic digestion. The main purpose of their long-term pilot studies was to optimise pH for acidification, desulfurisation and methanogenesis, as the wastewaters have pH 8-11. They studied pH values from 5 to 7, with temperature of 34°C and overall mean RT for the system of 3.5 days. From their results they concluded that an adjustment of the influent to pH 7, would

be the best to achieve the desired removal and reduce overall treatment costs.

D'Addario et al. (1992) reported that pH-control at 6.5 compared to an uncontrolled pH in the range of 5.5, resulted in 23% increase in VFA yield and reduced by almost 4 times the minimal CH_4 yield. The results were reported for a semi-fed CSTR treating MSW mixed with fresh water with 15% TS, operated at 35°C and HRT 12 days.

Negri et al. (1992) presenting a model for VFA production from fruit and vegetable solid wastes, concluded that pH is a critical parameter. It was found that the plug-flow reactor was very sensitive towards pH. With the use of the model, they found that variations in the order of 8% in the pH of the process gave variations of 50% in VFA production.

3.5.4 HRT and OLR

Ghosh and Henry (1981) reported comparative studies between single and two-phase digestion evaluating a range of different HRT in the treatment of soft-drinks wastewaters. Their aim was to assess the effects of OLR on the overall process. The conventional anaerobic reactor was a CSTR with 5 l working volume, while the two-phase system was a CSTR of 2.5 l working volume for acidogenesis and an upflow anaerobic filter of 5.5 l working volume for methanogenesis. All units were operated at 35°C. The wastewater used was dilutions of concentrated soft-drink wastewaters with the addition of essential nutrients, in which the wastewaters were deficient. The conventional system failed with a drop to pH 5.0 when it reached its highest OLR at 2.0 kg VS/m^3·d with HRT 10 days and influent COD 26 g/l. In comparison the two-phase system was successfully operated with OLR reaching up to 16.0 kg VS/m^3·d and influent COD 45 g/l in the acidogenic reactor, with HRT 7.4 days for the whole system. In the acidogenic reactor, under the highest OLR, pH was 4.7, HRT 2.2 days and Acidified COD was around 46% (VFA around 8 g/l as Acetic acid).

Dinopoulou and Lester (1989) reported studies on two-phase digestion of a synthetic wastewater based on meat extract, in which they evaluated the acidification phase on two OLR values (5.28 and 10.5 kg COD/m^3·d), two substrate COD concentrations (3.0 and 5.9 g/l) and HRT values ranging from 1.7 to 13.5 h. They operated CSTR units for acidificiation with no pH control (pH around 6.0) and 37°C. With OLR 5.28 kg COD/m^3·d and COD 5.9 g/l they observed an 18% increase in Acidified COD with the change of HRT from 3.4 to 6.8 h, and a minor increase with the change in HRT from 6.8 to 13.5 h. Similar but smaller increases in Acidified COD they observed for OLR 5.26 kg COD/m^3·d and COD 3.0 g/l with HRT increasing from 1.7 to 6.8 h, and for OLR 10.5 kg COD/m^3·d and COD 5.9 g/l. Small differences in Acidified COD also appear in the application of the two different OLR values and the two different COD strengths. Acidified COD values, calculated from their data and based on Filt.COD, were around 44 to 64% with most values around 52%.

D'Addario et al. (1992) used a multistage counter-flow reactor for the production and recovery of VFA from MSW. They presented a comparison between RT of 12 and 21 days. The system

was operated at 35°C and no pH-control (around 5.5), while fresh water was added to dilute the MSW to 20% TS. With 3 l H_2O added/kg TS to leach the reactor contents, they reported that increasing RT resulted in an increase of 21% and 5% for VFA concentration and yield respectively, but also an increase of 39% in the minimal CH_4 yield. Additionally with 6 l H_2O added/kg TS, VFA concentration and yield increased 34% and 38% respectively, with CH_4 yield increasing 25%.

Sanders et al. (1996) reported results on the treatment of waste activated sludge, in an anaerobic Hydrolysis Upflow Sludge Blanket (HUSB). They studied the effect of various SRT (from 1.4 to 10.6 days) and HRT (from 0.4 to 3.0 days) on acidification. They concluded that at SRT above 1.4 days (HRT of 0.4 days) no better performance can be obtained, apart from the improvement in sludge filterability. Their studies were carried out at 20 and 30°C.

Also Tseng (1992) found that short HRT or high OLR resulted naturally in phase separation, in the treatment of hog wastewaters with an improved plug-flow reactor design.

Negri et al. (1992) introducing a mathematical model for acidification of MSW, suggested that several factors appear to influence VFA production. Amongst them they selected SRT as an important one because of the direct effect on process economy. They also considered the recirculation ratio of liquids, to inoculate incoming solids and potentially increase SRT in the system. VFA production was found to be proportional to SRT at low SRT values, while at increased SRT, methanogenesis reduces VFA production. The effect of recycle was negligible for a plug-flow reactor under the operational conditions used to acidify fruit and vegetable wastes.

3.5.5 Nutrients and Toxicity

The nutrient requirements of methanogens and H_2-producing acetogens are not well documented, but even less is understood or defined about the hydrolytic/acidogenic group. In addition for carbohydrate wastewaters, N, P and S requirements may be as much as six times more than for fatty acid wastewaters, due to the increased synthesis of hydrolytic/acidogenic bacteria. This has significant impact, particularly on nitrogen deficient substrates (Speece & Parkin, 1987).

Long-term anaerobic digestion tests on leach-bed two-phase digestion of MSW, showed CH_4 yield of 0.26 m^3/kg VS added without external nutrient addition, but recycle of indigenous MSW nutrients. With added external nutrients it increased to 0.29 m^3/kg VS added, while MSW biodegradability became 64% from 56% without nutrient addition (Ghosh, 1987). Unfortunately no description of the nutrients was presented in the paper.

Substrate and product inhibition kinetics for acidification of glucose have been reported by van den Heuvel (1985). He found that free Butyric aid inhibited acidification while free Acetic acid did not, at pH 5.8-6.0 and 30°C. The lethal concentration of free Butyric acid was found to be above 4.0 g/l, while concentrations above 1.8 g/l were found inhibitory.

Furthermore Ghosh and Lall (1988) based on their studies on MSW (25% TS) digestion using a

two-phase process operation reported inhibition of hydrolysis and acidification at VFA concentration above 20 g/l, but their studies did not report if inhibition was related with the free form of any of the produced acids.

However D'Addario et al. (1992) reported that the high level of non-ionised VFA, generated in their studies on VFA recovery from MSW, contributed to the inhibition of acidogenesis in batch reactors. The maximum VFA concentrations presented were above 24 g/l. Inhibition was deduced with the visual aid of the VFA graphs plotted versus time, where the maximum VFA concentrations for higher amounts of solids (from 5%-20% TS) were reached at longer RT. No further information was provided as proof about the inhibition from high levels of non-ionised VFA presented in these studies. Meanwhile inhibition could be equally attributed to acclimatisation of the acidogenic bacteria to higher TS content. In addition they stated that acidogenesis was also retarded by other inhibitory or toxic compounds in the substrate (e.g. dissolved phenolic and humic acids related to lignin, heavy metals, etc.), or even metabolites of acidification (e.g. partially reduced organic molecules, ammonia, etc.).

3.6 General Laboratory and Pilot Scale Case Studies

ETC Ltd operated two two-phase systems in parallel to evaluate their performance in the treatment of real soft-drinks wastewaters from the "Coca Cola & Schweppes Beverages Ltd" plant in Wakefield (Anon., 1993). Both involved simplified acidification tanks, controlled under different modes, in tandem with UASB reactors operated at HRT 11 hrs, 33-35°C and pH 7.0. One acidification tank (referred to as AT1) was operated in 33°C, neutral pH and recycled effluent from the UASB; while the other (referred to as AT2) was run at 20°C, pH 6.0 and no recycle. Both tanks had HRT of 4 hrs, addition of macro-nutrients for COD:N:P ratio of 350:5:1 and 0.05 ml trace element "cocktail" per liter of wastewater. The trace element "cocktail" was the same as that used in the full-scale treatment plant for the UASB. AT1 had significantly lower COD and VFA than AT2, due to methanogenic bacteria entering with the recycled UASB effluent. The efficiency of pre-acidification in AT2 varies from 30-95%, with an average of 50%. Additionally AT1 was producing up to 17% of the total CH_4 produced by this system, while AT2 did not produce CH_4. The overall COD removal efficiencies and CH_4 yields of the two systems were quite similar and both achieve adequate treatment. Obviously the process with AT1 was a more expensive (pH and temperature control, effluent recycle) and less practical design (CH_4 produced in AT1 was uncontrolled and needed to be collected) than AT2 which utilised more simplified phase separation.

Weiland (1992) reported a pilot-scale comparison of one- and two-phase digestion for the treatment of various agro-industrial wastes. The one-phase reactor was a completely filled mechanically mixed loop reactor with conical bottom and upper section, with 6.0 m^3 volume. The two-phase process used the same type of reactor for hydrolysis and acidification of the solids, with 2.5 m^3 volume and an anaerobic contact reactor for the methanogenic stage, with 1.0 m^3 volume. Both systems were operated at 35°C. The process was carried out in a semi-liquid

phase, where solids were mixed with recirculated process water or fresh water to achieve a solids content of 7-15%. Four types of wastes were treated during the two-year study, namely: sugar beet pulp, potato pulp, potato thick stillage and brewer's grains. The results for the sugar beet pulp showed that two-phase process increased by 8.5% and 9.5% the COD removal and CH_4 yield respectively. It should be pointed out however that these results were achieved at overall RT of 13 days for two-phase, compared to 10 days for one-phase. The effluent quality of the two processes differed considerably, as in one-phase it contained undigested SS, while in two-phase SS were adequately separated in the internal clarifier of the contact reactor. Therefore in one-phase Tot. COD was 10-50 g/l, compared to 1.0-1.4 g/l in two-phase. Protein rich wastes, like potato thick stillage and brewer's grains, with C:N ratio below 10, could not be treated at high OLR in one-phase. The process was unstable at COD loadings below 3 kg/m^3·d. At loadings above 5 kg/m^3·d complete process failure was observed with high VFA accumulation, which was caused by the toxic effect of accumulated ammonia. Ammonia concentrations at these loadings was above 5 g/l. At such concentration and a pH of 7.2-7.4 more than 50 mg/l of free ammonia were present, which were toxic for unadapted cultures. For these types of protein rich wastes the two-phase process could be operated with loadings above 10 kg/m^3·d, without any sign of process instability. Ammonia concentration under these conditions was still in the range of 5 g/l, but bacteria in the two-stage process proved more robust against toxicity or even inhibition. Ammonia accumulation in the acidogenic reactor promoted the formation of Propionic acid and inhibited acetogenesis. This resulted, in the studies on brewer's grains treatment, in VFA composition with higher Propionic (above 6 g/l) than Acetic acid (below 5 g/l). Nevertheless no problem was observed and the process reached good efficiency and high process performance. The author concluded that anaerobic processes could treat agro-industrial wastes with efficient solids stabilisation and energy recovery. However it was suggested that the applied process should be selected based on the waste C:N ratio, to avoid ammonia inhibition and digester failure.

D'Addario et al. (1992) used for VFA production from MSW, the following three types of reactors, listed below with the objectives for each type: i) Batch: 1) minimize addition of water to maximize VFA concentrations; 2) establish pH effects; and 3) reduce or avoid mixing. ii) semi-CSTR (fed once a day): 1) establish microbial concentrations effects; and 2) observe inhibition from accumulation of metabolites or intermediates of the process. iii) Multistage Counter-Flow Reactor (MCFR): 1) differentiate solid from liquid RT; 2) reduce effects from inhibitory compounds, maximising the kinetics of VFA production; 3) maintain dominant cultures of hydrolytic and acidogenic bacteria, preventing the proliferation of methanogens; 4) optimise solids-leachate separation; 5) maximise recovery of VFA; and 6) produce leachates that provide feasible recovery of VFA. MSW were mixed with fresh water from 5% to 20% of TS. All experiments were done at 35°C. They concluded that the MCFR system was most successful for the following reasons: i) production of leachates directly processable for acids recovery; ii) no consumption of electric power for mixing; iii) no need of biomass for inoculation; and iv) negligible methanogenic activities (1.8-2.5 l CH_4/kg VS).

Also Beccari et al. (1992) reported laboratory studies using chemical pre-treatment of MSW to

increase bioconversion to acids in acidogenic reactors, for VFA recovery. They studied the effects of various reagents for pre-treatment, namely NaOH, $Ca(OH)_2$, HCl, H_2SO_4 and H_2O. They achieved optimum yield at acidogenic reactors operated at 25°C and pH 6.0, after NaOH pre-treatment at room temperature. Moreover they observed that independent of the pre-treatment, Acetic and Butyric acids were mainly produced when the soluble fraction of the pre-treated MSW was the only substrate. In contrast, fermentation of substrate obtained by biohydrolysis of residual particulate fraction of pre-treated MSW, mainly produced Propionic acid.

Tseng (1992) reported the use of a plug-flow design to promote natural phase separation, for the treatment of wastewaters and sludges from hog farming. He used 3 different operating modes with two-phase plug-flow reactors. One was controlled at 35°C (filled with media) and two without temperature control (one with and one without media). The two-phase system was made out of a front tank (40 l, divided in 2 compartments) and a rear tank (20 l, divided in 4 compartments). The main function of the front tank was designed to be solid sedimentation, sludge digestion and acidogenesis of wastewater. The highly concentrated wastewaters were collected fresh and diluted to COD 10 g/l and BOD 3.6 g/l. HRT was controlled at 1, 3, 5 and 7 d to study changes in performance according to OLR. The wastewater entered the system in a semi-continuous mode. When OLR was between 1.6 and 10 kg COD/m^3·d, COD removal in the front tank with mesophilic operation was 62 to 80% (31 to 58% due to sedimentation); while the system filled with media and at ambient temperature, had COD removal 54 to 71% (45 to 52% by sedimentation). They reported that cross section observations in the digester on pH, soluble COD and VFA values, were proof of natural phase separation. At HRT above 5 d methanogens appeared in the front tank of the mesophilic system and absence of VFA accumulation represented poor phase separation. However at HRT below 3 d there was high content of VFA. Meanwhile the system filled with media and at ambient temperature could achieve phase separation even at HRT 5 d. The improved plug-flow designs (incorporating phase separation and filled with media), were concluded to achieve above 80% COD removal in OLR up to 10 kg COD/m^3·d treating hog wastewaters.

Orlandini and Furlan (1992) used the benefits of phase separation for the treatment of green macroalgae. These seaweeds were collected as eutrophication products from a natural lagoon and disposed as solid waste. Meanwhile their studies assessed the potential of energy recovery from these seaweeds with anaerobic digestion. They operated a series of lab-scale acidogenic reactors for hydrolysis of these complex solids in a brackish liquid medium (50% fresh and 50% sea water), with 5% TS at 35°C. Unfortunately their data only presented reduction in pH values as indication of the acidogenic activities and no further comments could be made. They concluded that this process increased hydrolysis rates and allowed a better process operation for biogas production, inhibition from accumulated VFA and high levels of H_2S and better pumping of substrates in the system. They also stated that if they operated a single-phase process, they would have faced difficulties from accumulated VFA and would need longer RT.

3.7 Modelling and Optimisation

Models could be further improved if there was better understanding of the kinetics of hydrolysis and how this affects reaction rates of other constituent processes. Three main factors must be considered when developing models describing hydrolysis: i) the nature of the enzyme, ii) the type of substrates and iii) the enzyme-substrate interactions. The understanding of the enzyme-substrate interaction is poorly developed and very little is known about its detailed mechanism (Dunn et al., 1994).

Modelling results of their studies on acidification of MSW was reported by Beccari et al. (1992). Their model showed that the higher the soluble fraction in the acidogenic reactor, the higher the VFA concentration. Also biomass recycle resulted in small differences in the acidogenic activities. which was also observed in their experiments. The small value obtained for the biohydrolysis rate constant showed that hydrolysis of particulate substrate controls the performance of the overall process.

Additionally, a mathematical model of a plug-flow reactor with liquid recycle, was elaborated by Negri et al. (1992) to simulate VFA production in the treatment of the organic fraction of MSW. An alternative hydrolytic reaction model (homogeneous-heterogeneous) was proposed. The effect of methanogenesis occurring simultaneously was also considered. Process parameters (SRT, pH and recycle ratio) affecting the performance were analysed, based on data from fruit and vegetable waste. The sensitivity of the model was evaluated towards changing fluidised and initial biomass concentrations, which were found to have negligible effects.

Dunn et al. (1994), reviewing literature of dynamic models that use the Monod expression for anaerobic digestion, stated that: "The hydrolysis step is either ignored or not considered in enough detail to enable the models to be used for prediction". They also mentioned that studies were undertaken to determine which factors had the most effect on hydrolysis rates, but conclusions were difficult to reach due to the interaction of factors involved.

The use of Evolutionary Operation (EVOP) for the assessment of the effects of multi- variable functions in the Chemical Industry, was introduced by Box and Draper (1969). The operation of the experimental work and the methodology of the statistical assessment required in order to derive the effects of various factorial designs have been described by Box and Draper (1969) and Box et al. (1978). The process has long been applied as a simplified tool for process engineers to operate and evaluate experiments in the Chemical Industry. Such experiments mostly have an objective to optimise various processes particularly in relation to various design parameters in chemical reactors, and the effects of these design parameters on various performance and quality criteria.

Although EVOP was designed as an experimental and statistical optimisation tool for the Chemical Industry, it has been used for a few years by the anaerobic digestion group in South Africa led by Prof. Britz (Britz, 1997). EVOP was recently introduced to anaerobic digestion researchers and professionals with a keynote presentation for the optimisation of anaerobic digestion phe-

nomena, at the last International Anaerobic Digestion Conference in Sendai, Japan (Britz et al., 1997).

EVOP has already been successfully used by various researchers associated with the South African group in the optimisation of the effects of design parameters on specific microbial species that acidified dairy and baker's yeast wastewaters (Britz et al., 1997, van der Merwe-Botha and Britz, 1997).

3.8 Treatment and Energy/Product Recovery

The only reliable and economic treatment process that can effectively stabilise organic matter, with simultaneous recovery of fuel gas or even chemicals, is anaerobic digestion (Ghosh, 1987). Furthermore, when considering various treatment processes for overall energy requirements it is common practice to exclude the potential for energy recovery from organic matter. Such a consideration can change substantially total specific energy costs. Generally the application of anaerobic digestion has energy recovery potential exceeding or at least equal to consumption demands. For example anaerobic digestion for sludge stabilisation requires only 65% of the total energy requirements for aerobic treatment, considering savings from biogas used for heating as the only energy recovery potential (Owen, 1982).

Additionally, viewing the end-products of anaerobic digestion, biogas is often considered most important. It can be burned, converted to methanol by chemical means, or temporarily stored. In addition when treating substrates, anaerobic digestion does not need intensive stirring and costly supply of O_2 and nutrients, as aerobic processes require. Such energy saving operational requirements in comparison to aerobic process, could be considered as a kind of "energy production". Furthermore the sludge residue may be used as an effective fertiliser and soil conditioner, since N, P and other bioelements present in the original digester substrate, are conserved during digestion to a high degree due to little cell formation by anaerobes. Process water may also be recycled back into the system, thereby recycling essential nutrients. Formation of organic acids instead of CH_4 is also possible, followed by photoelectrolysis to hydrocarbons directly in aqueous solution (Schoberth, 1980).

CO_2 removal, in order to achieve pipeline quality CH_4 gas (98% CH_4 in biogas volume), from digester biogas is a relatively expensive method. Most commonly it employs absorption techniques in an aqueous or chemical solution. Most processes result in concomitant H_2S removal (Owen, 1982). CH_4 enrichment in digester gas has not been of major concern, as anaerobic applications have emphasized treatment rather than energy production. A major economic consideration in the use of anaerobic digestion to convert wastes and wastewaters to pipeline quality CH_4 ($\geq$95%), is the cost of separating CO_2 from the produced biogas (Hayes & Isaacson, 1987).

As the solubility of CO_2 is up to 40 times greater than that of CH_4, it is possible to utilise reactor processes capable of achieving efficient separation of these two gases. Conventional digestion processes contain 55-65% CH_4, while two-phase ones appear to produce up to 70-80%

CH_4. Hayes and Isaacson (1987) utilising data from literature developed a model to predict CH_4 and CO_2 levels produced under digestion of organic sludges and wastes. Based on potential solubility levels of CO_2 and reactor operational conditions, they conceptualised a modification of two-phase process that has capacity to produce high quality biogas (CH_4 content of 90-95%). With their configuration of two-phase process design, a gas stream of more than 95% CO_2 can be generated from the acidogenic stage, raising the possibility of CO_2 recovery as a commercial by-product in addition to CH_4.

Ghosh and Henry (1981) proved that if the energy content of soft-drink wastewaters was recovered, it would displace all energy purchased by the U.S. soft-drink industry by 3.5 times. They also provided data to optimize anaerobic treatment with two-phase application. Comparing a hypothetical conventional CSTR with a two-phase system incorporating a CSTR and an upflow AF, they suggested that an increase of 3 times in the OLR (from 1.6 to 4.8 kg VS/m^3·d) with a subsequent halving of HRT (from 15 to 7.4 days), would allow for a 72% increase in the net energy production. Additional savings in capital costs were projected, estimating overall digester volume for the two-phase system only one-third of that needed for conventional high rate digestion. Also their comparison between the optimum examined OLR for the conventional system (0.6 kg VS/m^3·d) and the optimum overall OLR for the two-phase system (4.8 kg VS/m^3·d), presented marginally different values for CH_4 composition and yield but a 14% increase in COD removal (from 84% to 96%).

Ghosh (1987) proved that using a two-phase process would provide 83% net energy production from CH_4, while conventional sludge digestion would provide only 93% of the net energy requirements of the digestion facilities. Furthermore, he achieved high content of Acetate (3.3 g/l) and Propionate (8.2g/l) by liquefaction of MSW in a leach-bed. He pointed out that such peak concentrations, if maintained or even exceeded by avoiding gasification, would be worthwhile recovering as valuable chemicals for commercial purposes.

Additionally, shortage of fossil fuels has stimulated interest in the production of liquid fuels and valuable chemicals from ubiquitous and renewable substrates by biotechnological products. Biorefining appears to be one of the most attractive options. This process is based on the acidogenic fermentation of MSW, followed by VFA extraction from the fermented broth and esterification (also known as electrolytic oxidation) to obtain valuable chemicals (e.g. octane-improving additives for gasoline) (Beccari et al., 1992).

Many years of research related to the production of octane enhancers from renewable sources, has been carried out in Italy. Processes such as the alcoholic and acetone-butanolic fermentation, were investigated. However, the admixture of fermentation products in unleaded gasoline is not financially advantageous regarding today's oil prices. This lower competitive position of the digestion by-products to oil, was mainly due: i) to high costs of valuable carbohydrate sources (i.e. corn, sorghum, etc.); and/or ii) to the complexity of processes based on bioconversion of alternative substrates, like lignocellulosic wastes. Biological operations could be a convenient alternative, if integrated in processes aimed to solve other environmental problems. Although the enzymatic saccharification of the organic fraction of MSW seems a possibility, the anaerobic

conversion of MSW into short chain organic acids (VFA) appears more promising. The reason is that VFA can be extracted and converted into octane enhancers with more attractive potential than alcohols. This is especially reflected into the corresponding C_2-C_6 methyl or ethyl esters from VFA (e.g. Motor Octane Number for methyl acetate, propionate and butyrate equal to 108, 107 and 104 respectively, compared to 97 for ethanol) (D'Addario et al., 1992).

D'Addario et al. (1992) emphasized that little research has been carried out to investigate anaerobic processes aimed to produce acids, and further studies are necessary for the following: i) establish operating conditions in the digester for VFA production; ii) obtain effluent able to undergo viable VFA recovery processes; iii) determine appropriate procedures to convert dilute VFA solutions into octane enhancers, like esters; and iv) achieve efficient and valid waste treatment. Conversion of biodegradable volatile solids into VFA is limited by hydrolysis of substrates. This approach is limited by two assumptions: i) complete conversion of substrates into VFA, without taking into account conversion into biogas and bacterial cells; and ii) negligible effects of microbial concentration. From the existing information they stated that neither could proper acidogenic reactors be selected nor operating conditions be planned. Their lab-scale data, partly presented in previous parts of this literature review, proved the potential of using acidification for the recovery of VFA from MSW.

3.9 Engineering Considerations for Two-Phase Applications

A number of engineering considerations found in the literature, relative to the development and practice of two-phase anaerobic digestion are listed in this section. The purpose was to use these examples, in order to provide some basic understanding on the overall necessity for the application of two-phase process. A few of these examples are relative to high concentrations of VFA. The purpose of the latter is to establish an argument for the existing degree of acidification (20-40%) suggested by Lettinga and Hulshoff (1991), in order to avoid detrimental effects in the operation of UASB reactors. Apparently, this degree of acidification has been considered generally as the maximum for pre-acidified wastewaters, even when UASB reactor designs are not used for treatment.

Since methanogens have a lower growth and metabolic rate than acidogens, a kinetic imbalance between the rates of production and utilization of VFA can be applied for phase separation. Also the conditions promoting optimum conversion of substrates to VFA are not conducive to stable and efficient conversion of VFA to CH_4. This natural tendency was observed by Ghosh and Henry (1981) when they attempted higher OLR and shorter HRT on a conventional one-phase digester. They concluded that it was only reasonable to promote this phenomena, optimizing high rate and stable anaerobic digestion, in a two-phase process.

Myburg and Britz (1993) studied the influence of OLR on the efficiency of a hybrid reactor (i.e. UASB and AF) treating landfill leachates. They applied HRT of 1 d, adjusted pH to neutral, added macro- and micro-nutrients and used mesophilic temperatures (35°C) for the 4 stages of

their experiments. OLR studied were from 21.7 to 29.0 kg COD/m^3·d made up as dilutions of a mixture of three different samples of landfill leachates. The initial acidified COD of the influent ranged from 57.6 to 93.1%, with VFA concentrations ranging from 10.0 to 14.9 g/l. n-Butyric was the main acid (44% of VFA), with Acetic second (37% of VFA) and Propionic third (15% of VFA). None of the given VFA concentrations was considered as inhibitory for the reactor; on the contrary the system performed with a high COD removal, even at the maximum OLR examined (around 84%) . Also at the maximum OLR indications of possible failure, if further OLR increase was attempted, were a decrease in CH_4 yield and COD removal with an increase of Propionic acid in the effluent. Therefore in their study, overloading of system in relation to active biomass would be the reason for digester failure, instead of acidified COD or high VFA concentrations causing an inhibition to methanogenesis.

They also concluded that the increase in effluent VFA, particularly Propionic acid, was an indication of digester overloading. This VFA increase impended digestion failure due to a kinetic phase separation, which allowed substrate conversion to VFA but precluded methanogenic activities. Larger methanogenic populations in fully acclimatized digesters could sufficiently degrade Propionic acid (the least favourable acid for methanogens), avoiding accumulation of this acid while digester overloading increases VFA concentrations. Furthermore with their observations on acclimatization through accumulation patterns of Propionic acid, they stated that Propionic acid should be the main parameter to indicate potential reactor problems, or to establish steady state digestion. Therefore Propionic concentration levels in steady state were to be considered as the threshold limit of digester loading capacity to maintain stability between the two phases.

Anderson and Saw (1992) reported an influent with very high concentrations of VFA being treated successfully in a UASB reactor. The reactor was used as the methanogenic-phase in a leach-bed two-phase process treating MSW. It was operated at 35°C, HRT 2 d and OLR 11 kg COD/m^3·d, achieving COD removal of 95% and yield 0.31 m^3 CH_4/kg COD removed. The two leach-bed reactors used for the study have reached maximum VFA concentrations of 20 and 26.2 g/l (Butyric was the major acid, followed by Acetic and Valeric), which appear to be 97% and 87% of the reported TS values respectively. Although completely acidified wastewaters were entering the UASB, with very high VFA concentrations, no inhibition or unstable performance of the UASB reactor was reported, or could be traced in the presented results.

Furthermore McCarty and Vath (1962) stated that previous studies on the digestion of pure volatile acids alone, indicated that only relatively slow rates of fermentation were possible (for a conventional high rate digestion of 3.3 g COD/l·d). From their studies they observed Acetate utilisation rates as high as 21.9 g COD/l·d. They believed that low rates were not due to an inability of methanogens to achieve higher rates, but rather due to unsuitable environmental conditions. So they concluded that there is no practical limit of any possible VFA fermentation rate under proper process control. Speece and McCarty (1987) referring to the latter conclusions, their literature review and their own studies, related proper process control with nutrient supplement that could enhance Acetate utilisation and increase by many times the commonly practised loading rates for anaerobic processes.

Hayes and Isaacson (1987) suggested that the most likely reason that two-phase processes are reported to have a higher content of CH_4 in sludge digestion, is the absorption and desorption of CO_2 from liquid streams. This is also the reason why all reported CH_4 to CO_2 ratios in the literature are significantly higher than ratios predicted from stoichiometric calculations. The amount of CO_2 absorbed in the aqueous phase of a digester is influenced primarily by pH and partial gas pressures in the reactor, but also by temperature, influent stream CO_2, alkalinity and ionic strength. CO_2 has alkalinity potential when dissolved.

Furthermore it was reported (Anon., 1993) that soft-drink wastewaters from the "Coca Cola & Schweppes Beverages Ltd" plant in Wakefield, were pre-acidifing in the influent sump, as a result of higher ambient temperatures during summertime (April to October). This was noticed by low pH (below 6.5) measured in the influent of the anaerobic facilities, compared to pH around 7.0-8.5 in other months. Such a fact indicates that a noticeable degree of acidification can naturally occur in easily biodegradable wastewaters before reaching anaerobic facilities, even without controlled conditions.

In the last few years an alternative way to use VFA produced from waste and wastewater acidification has been described for their benefits on Biological Nutrient Removal (BNR) at municipal wastewater treatment plants (MWTP). BNR requires easily degradable carbon compounds for bacterial metabolism. The importance of the available carbon source and addition of VFA, methanol or ethanol in the influent is common guideline to enhance BNR. In order to propose economical BNR processes many studies define the exact amount of such compounds required. Influent carbon compounds range from 50 to 400 mg/l, depending on many aspects (i.e. if the process is designed for N and/or P- removal, loading rate, compound used, etc.). Most BNR plants operate costly addition of chemicals to provide such compounds. Cost reduction is achieved when using an option found in other areas of waste management (e.g. sludge handling, agro-industrial WTPs), which can act as a VFA-producer. Acidification of organic matter in balancing or other storage tanks under anaerobic/anoxic conditions produces high VFA concentrations (up to 20 g/l). Recently effluent from an equalisation tank for cheese factory wastewaters (Comeau et al., 1996) and pre-acidified nightsoil (Choi et al., 1996) were successfully used as carbon source for BNR.

The Lethabile WTP, Republic of South Africa, integrates anaerobic and aerobic ponds with trickling filters to achieve organic and nutrient removal. Acetate was added for BNR and to reduce costs the WTP operators decided to receive from a local brewery wastewaters which were only pre-treated in settling tanks. The high COD (2.5 g/l) pre-acidifies in the brewery's tanks and the sewers before it enters the WTP. After applying this modification the operators stopped Acetate additions, while effluent P is within the required limits (<1mg/l) (Louw, 1994). Pitman et al. (1991) presented a similar outcome with a yeast factory for the Bushkoppie WTP, Republic of South Africa. Severn Trent Water Ltd, UK, successfully operated a full-scale plant research project to assess various BNR processes using pre-acidified sewage sludges, collected as supernatant of sludge thickeners and storage tanks (Upton et al., 1996).

3.10 Full-Scale Two-Phase Applications

There are not many two-phase applications worldwide, as becomes apparent when contacting some of the leading companies in the design and construction of anaerobic plants (i.e. Biothane, Paques, Biotim, etc.).

Pipyn (1996), expressing the views on behalf of Biotim, stated that out of the 130 digestion plants designed by their company, in only one was the two-phase process initially considered. No operational data could be provided about the acidification reactor in this plant. Their process design philosophy regarding pre-acidification is that in most wastewaters it occurs naturally in the conditioning tank. Therefore, they consider that it is unnecessary to engineer the acidogenic process. On the other hand they consider that acidification can be detrimental for the granulation process, when a UASB is designed. The latter was not justified further. Finally, they believe that wastewaters should be categorised in terms of biodegradability and alkalinity. If the wastewater is of high-strength and acidification might be required, this would be expected to occur with a HRT of 2 to 6 hrs for mesophilic temperatures and 8 to 10 hrs for moderate temperatures.

Zoutberg (1996), expressing the views on behalf of Biothane, and Snoek (1996), on behalf of Grontmij (1996), suggested that their companies although leaders in anerobic digester design and construction do not consider the use of two-phase process in their designs. Furthermore Zoutberg assumed that a conditioning tank, included in all their designs, would provide the required level of pre-acidification.

A similar approach was expressed by Habets (1996), expressing the views on behalf of Paques, a leading company in UASB design. His company always includes a conditioning tank prior to the UASB, where pre-acidification is assumed to occur naturally at levels below 40% acidified COD. The latter degree of acidification is considered by Paques to be a detrimental threshold that was suggested by Lettinga and Hulshoff (1991) as a level with potential negative impacts for UASB reactor design. As no further literature could be found to establish the reasons behind this boundary on the degree of acidification Hulshoff was contacted (1996). He stated that this figure was a result of observations regarding the start-up of a full-scale plant in the Netherlands, treating malting-house wastewaters. The problems observed in the start-up were attributed to almost fully acidified influent in the UASB. After some modifications, the degree of acidification dropped below the 40% acidified COD figure, and start-up was successful. Although the problem was mainly connected with the effect of excess quantities of acidogenic biomass entering the UASB, it seems that it was blamed on the degree of acidification.

The most important observation emerging from this review of commercial two-phase process design, is that no company could provide data on what is actually occurring in their conditioning tanks. Furthermore they all requested copies of any publications from the present study, regarding effects of design parameters on acidification, to be forwarded to them for consideration. It was claimed (Habets, 1996) that there are no such data available in the literature, particularly for real agro-industrial wastewaters.

Finally, regarding two-phase applications for agro-industrial wastewaters, only one case has been reported (Bardiya, 1995). Bardiya on behalf of "Daurala Sugar Works", India, described the positive effect of two-phase anaerobic digestion in the treatment of cane molasses using a CSTR for acidogenesis and a hybrid reactor (incorporating a UASB and a packed-bed filter) for methanogenesis. The plant was commissioned in 1991. The system was developed since early 1986 by the R&D of DCM Shriram, an Indian company with around 250 distilleries, from which "Daurala Sugar Works" is one of the largest. Previous to 1986, about 150 of the company's distilleries were using single-phase anaerobic digestion for their wastewaters, based on imported know-how (Biotim, Sulzer, Degremont, etc.). Still, none of the supplied anaerobic technologies were achieving the claimed efficiencies, mainly because of inadequate knowledge and expertise in the treatment of wastewaters similar to the characteristics of the Indian cane molasses.

Information about the reactor design at "Daurala Sugar Works" in India, are presented in Table 3.1. The main characteristics and performance of this application are presented in Table 3.2. All information are provided by Dr. M.C.Bardiya. Unfortunately, no information regarding the performance of the acidogenic reactor could be provided.

Table 3.1: Reactor design of the two-phase plant at "Daurala Sugar Works", India

Features	Acidogenic Reactor	Methane Reactor
Working Volume (m^3)	1,050	7,700
HRT (days)	1.4–1.5	9–10
OLR (kg COD/m^3·d)	48–50	9–11
pH	4.5–4.7	7.3–7.7
Temperature (°C)	37±2	37±2

Table 3.2: Wastewaters characteristics and performance at the two-phase plant at "Daurala Sugar Works", India

I. DISTILLERY WASTEWATERS - MAIN CHARACTERISTICS	
BOD (g/l)	35–50
COD (g/l)	90–130
SO_4 (g/l)	6–8
Plant Design Wastewater Volume (m^3/d)	1,400–1,600
II. PERFORMANCE RESULTS	
BOD removal	83–85%
COD removal	68–70%
Biogas production (m^3/d)	47,600–49,000
Biogas yield (m^3/m^3 wastewater)	33–35
CH_4 composition	63–65%
H_2S composition	<0.5%

Furthermore, Hajipakkos (1992) reported the commission in 1988, of a coffee wastewater treat-

ment plant in Cyprus, designed by Biwater Treatment Ltd. The plant included a pre-acidification tank in tandem with a UASB. The pre-acidification tank had a working volume of 810 m^3, estimated IIRT around 14 hrs and estimated OLR of 5.7 kg COD/m^3·d. It was receiving wastewaters with the following design characteristics: COD 4 g/l, SS 1.5 g/l, TFM 0.1 g/l, SO_4 50mg/l, temperature 28-40°C and pH 5-10. Additional treatment requirements included chemical (alkali and acids) and macro-nutrient dosages, in order to maintain neutral pH and overcome nutrient deficiencies in the UASB. There is no other information relevant to acidogenic process control, apart from the fact that the tank was fully covered to promote odour control and treatment. No data could be found about the acidogenic activities of this unit. Although the unit was constructed for pre-acidification it seems that the use would mostly be for balancing/equalisation of the flow, for treatment in the UASB.

An acidification tank is used in tandem with Biothane-UASB reactors for the treatment of wastewaters from the "Coca Cola & Schweppes Beverages Ltd", in Wakefield, UK. The tank has a total volume of 520 m^3 (liquid volume of 440 m^3). It operated with the following conditions: temperature of 30-35°C; pH of 6.8-7.5, as NaOH was added to prepare the pH for the UASB; and COD:N:P of 350:5:1, with added macro-nutrients. The estimated RT is between 7 and 20 hours. Also effluent from the UASB reactor was recycled back to the acidification tank, providing a substantial amount of active acidogenic and methanogenic biomass. No parameters are recorded for this tank to enable assessment of the performance for acidification. Gas composition measurements in the headspace of the tank, showed that CH_4 content could be as high as 60%, confirming that methanogenesis occurred in the acidification tank. This fact was also proved by SMA activity tests in the bulk of the biomass in this tank. Therefore it seems that the tank was mainly designed for balancing/conditioning purposes, while acidogenic activities were assumed to occur naturally in these semi-controlled conditions. A laboratory scale project was commissioned by the company, to assess the problems of CH_4 losses faced in the existing anaerobic facilities. The results proved the adverse effects for the anaerobic process of an acidification tank, when its operations are not properly engineered. As became evident in this laboratory project, all missing CH_4 gas from mass balance equations was produced and released in the acidification tank, due to the uncontrolled phase separation applied. The study also proposed a more simplified and economical application, to achieve the positive effects of two-phase process (Anon., 1993).

With regards to two-phase applications for sludges, Ghosh and Buoy (1989) and Alexiou et al. (1994) have reported data on the performance of the first full-scale two-phase plant. It was designed for the digestion of waste activated sludge. The plant constructed in the '90's was the result of scale-up progress from research work of Prof. Ghosh, the pioneer in two-phase digestion in the '70's. The plant operates successfully in USA, resulting in a dramatic improvement of the overall digestion process, as foreseen by laboratory and pilot-scale studies comparing it with conventional digestion.

Also a digestion plant for MSW that uses the two-phase concept (BTA process) was completed in 1991 at Helsingor, Denmark. Hydrolysis/acidification takes place in the first reactor with

RT of 3 days for the total volume of MSW. Soluble leachates of the first reactor with high VFA-content, are digested in a separate methanogenic reactor. The system is producing 120 m^3 biogas per tonne of MSW, with 65% CH_4 (Anon., 1992).

Chapter 4

AGRO-INDUSTRIES

4.1 General

Agro-industries play a major role in the global economy. Also, they are major contributors to the worldwide industrial pollution problem. The amount of plants and annual production of goods, classify them as one of the most important industrial sectors. They involve some of the oldest industrialised activities, even prior to the Industrial Revolution. Nowadays, there are more than 60 different groups of industries, in terms of pollution characteristics, each one having a number of sub-categories.

With the high rate of technological development it is difficult to cope with wastes and wastewaters of ever increasing complexity generated by agro-industries. Almost all compounds found in such wastes are of organic nature, mostly basic types of biopolymers. Many of them are characterised as high strength (compared to domestic wastes) and/or with recalcitrant and inhibitory organic compounds for biological treatment. The latter compounds are both from the natural environment and organic chemical industries.

Concepts like waste characterisation and pre-treatment are still little understood in more than two thirds of the planet. A continuous need for additional research proves itself in many related areas, such as appropriate pre-treatment and treatment technologies for strong wastes, energy recovery and reuse systems, sludge stabilisation and disposal problems. More than other sectors in the field of environmental engineering, agro-industries require a dynamic and comprehensive approach for appropriate waste and wastewater management.

4.2 Instant Coffee Production

4.2.1 General

Coffee is a tropical tree or shrub, of the Rubiaceae family. Its fruits grows in bunches, with a size similar to cherries. These kernels, commonly known as "coffee beans", are ground and processed into a powder that is used as a daily drink. Coffee beans contain 8-15% oils, 2-3%

sugars, 11-13% nitrogenous compounds, 1-2% alkaloids, 4-5% tannic acid, smaller fractions of other oils and caffeol, responsible for the distinctive aroma of coffee (Kostenberg & Marchaim, 1992).

The coffee industry is global, due to popular demand of coffee as a hot drink. Although the main coffee-bean producing countries are in South America, Central Africa and the Carribean, large proportions of the coffee bean are processed in Europe and North America. In UK the industry produces around 50 to 60 thousand tonnes of instant coffee every year (Fernandez & Forster, 1994; McDougall, 1996).

4.2.2 Anaerobic Treatment

Lanting et al. (1989) used Biothane-UASB reactors at a pilot-scale plant, to treat coffee wastewater under thermophilic temperatures, in comparison to mesophilic. Although their mesophilic studies have not been conclusive, they concluded that addition of micro-nutrients appeared to improve reactor stability.

Hajipakkos (1992) reported the application of a full-scale UASB, preceded by an uncontrolled pre-acidification tank and post-treatment with a submerged aerated filter, for the treatment of coffee wastewaters in Cyprus. The UASB with OLR 5.2 kg COD/m^3·d and HRT 15 hrs achieved 55% and 75% COD and BOD removal respectively, CH_4 around 72% and methane yield of 0.33 m^3/kg COD removed.

Kostenberg and Marchaim (1992) used lab-scale CSTR and thermophilic digestion (55°C) to treat coffee slurries, with HRT 20 and 40 days. They concluded that anaerobic digestion is a feasible process for this waste. Also their experiments showed that digestion neither required addition of nitrogen to maintain the known essential C:N ratio of 30:1, nor prior griding of coffee waste solids. However pH control proved necessary. In their suggestions they also included a multi-stage process.

Kozuchowska (1992) studied temperature effects on acidification of synthetic instant coffee production wastewaters, without pH control, in batch laboratory scale fermenters. It was found that 45°C was better than 37, 55 and 60°C for VFA production. Also, it was reported that Acetic was the major acid produced in these short-term batch studies, with n-Butyric acid second in order. Propionic and n-Valeric were also present in smaller concentrations. Finally, the first major peak of VFA production occurred on average around 3 hours after starting the test, so it was assumed that such RT was required to degrade readily biodegradable compounds in the substrate.

Fernandez and Forster (1994) reported results on a comparison between a thermophilic and a mesophilic upflow AF, used for the treatment of a synthetic coffee waste. They stated that the performance of the thermophilic filter was not as good as that of the mesophilic.

McDougall (1996) reported results from a comparison of a two-phase process with a single-phase

one, for the treatment of synthetic instant coffee wastewaters in AF. He concluded that the two-phase system had more 10% improvement in terms of COD and BOD removal.

Recently, a 3-year study has been undertaken by five Universities in UK, funded by the Science and Engineering Research Council, to evaluate the potential of anaerobic digestion in the treatment of coffee wastewaters. The project aimed at finding the appropriate conditions required to digest the various recalcitrant compounds of this high-strength wastewater. In parallel to the laboratory studies, four pilot-scale anaerobic reactors, one of each main type of high-rate design, have been applied in the coffee production plant of Nescafé-Nestlé in Hayes, UK. A review of the research that has been undertaken can be found in the report by Hawkes and Hawkes (1995). Finally, the conclusions highlighted the potential of two-phase digestion compared to single-phase, to support the digestion of the recalcitrant compounds found in the composition of the coffee wastewaters.

4.3 Slaughterhouses

4.3.1 General

In the meat industry the cattle, calves, sheep and hogs are first detained for a few hours and then immobilised by chemical, electrical or mechanical means. Blood increases the BOD of the wastewaters by 72%, when not recovered. On an average the blood of a single animal slaughtered, has a BOD population equivalent of 50. Unfortunately, only large slaughterhouses (with at least 1,000 animals slaughtered per day) have blood recovery. Another very important pollution source in the liquid wastes, comes from paunch handling. All ruminants, such as cattle and sheep, have two stomachs. The first called the paunch, contains large amounts (30-45 kg) of undigested materials. Most slaughterhouse plants today practice wet dumping of the paunch contents, where pressurised water is used to flush them into a water stream. If adequate physical separation methods are applied, up to 95% of the BOD of these contents will be removed.

Overall the meat industry wastes are either solids or liquids. The solids comprise of manure from the livestock pens and the paunch contents, usually composted or landfilled. The wastewaters are mainly organic materials and a small fraction of washing liquids and detergents. All kinds of biological processes are used for treatment of the liquid wastes. Air pollution problems are not hazardous and relate to odour control arising from decaying meat, proteins or blood and from the manure and paunch storage and handling.

Most plants use the local municipal facilities for treatment of their wastewaters, unless they are large plants. Some medium size plants (500-1,000 animals slaughtered per day) have pre-treatment facilities, while others and most of the small size plants (less than 500 animals per day) do not have any kind of treatment, except screening to prevent sewer blockages. The desirability of pre-treatment depends upon many factors, including: the capacity of municipal facilities; regional legislation and discharge pricing or penalties; the markets for proteins, solids and grease recovery and possible residential complaints (Sell, 1992).

4.3.2 Anaerobic Treatment

Steiner et al. (1985) reported treating slaughterhouse waste in lab-scale CSTR. Their studies evaluated performance with changes in OLR, HRT and temperature. They were also checking the efficiency of disinfection. They concluded that performance was steady with OLR up to 8.75 g VS/l·d and HRT 7 to 12 days (waste composition up to COD 165 g/l and VS 105 g/l). Overloading and failure of the digester occurred at OLR 10.5 g VS/l·d. Additionally thermophilic treatment was far superior for removal of pathogens. Finally, treating all wastes and wastewaters anaerobically would provide, under the tested conditions, about 65% of the energy requirements in the slaughterhouse.

Sell (1992) described the treatment used in the "Packerland Packing Company", a large slaughterhouse in Wisconsin, USA, slaughtering on average 2,300 animals per day and producing around 3,650 m^3 of liquid wastes. The plant applies fat and protein recovery and the main characteristics of the wastewaters are on average: COD 7.5 g/l, BOD 3.4 g/l and SS 2.7 g/l. Since the beginning of the '80's they replaced a flotation system used for treatment, with an anaerobic contact reactor operating at 34-37°C. Preliminary treatment involved an existing skimmer and an equalisation tank that would provide stable flow and organic load to the anaerobic reactor, ensuring a uniform feed for the process. The treatment resulted in the following removals: COD 84%, BOD 93% and SS 75%. Unfortunately no information was presented about retention times, either in the anaerobic reactor or for the equalisation tank, that would enable assessment of acidification levels.

Eckenfelder (1989) presented a full-scale anaerobic contact process treating meat-packing wastewaters, operated at 30-35°C, OLR 2.5 kg COD/m^3·d, HRT 13.3 hrs and SRT 13.3 days, and achieved 90% COD removal. He also referred to other examples of full-scale anaerobic contact reactors treating meat-packing wastewaters operated at 3.2 kg BOD/m^3·d, HRT 12 h and 30°C, or at 2.5 kg BOD/m^3·d, HRT 13.3 h and 35°C, both achieving 95% removal. Also a contact reactor, operating at 3.5 kg BOD/m^3·d, HRT 12.7 h and 35°C, achieved 96% removal of slaughterhouse wastewaters.

Various studies can be found in the literature on anaerobic digestion of slaughterhouse wastewaters using AF (Anderson & Donnelly, 1977), CSTR (Hartmann, 1989) or UASB (Sayed, 1987). All results prove the efficiency and successful performance of anaerobic digestion for slaughterhouse wastewaters. Although this wastewater is considered of high-strength, no two-phase applications were found in the literature.

Chapter 5

SUMMARY OF LITERATURE - PROJECT OBJECTIVES

5.1 Summary of literature

The published literature in the area of the two-phase process generates some confusion over the description of the process used (what level of acidification have been achieved), the engineering aspects involved (i.e. design configurations and operating conditions) and the substrates used, especially when general conclusions about acidification are presented. The published data used to substantiate the reported performance of each process is often inadequate. These factors along with qualitative and quantitative differences of real wastewaters from industries world-wide, prevent a direct comparison of results from different studies found in literature.

Historically it becomes evident that acidogenic activities, as part of anaerobic digestion, have been known since the beginning of the century. Still it was the mid '60's when it was initially stated in the literature that engineered phase separation would increase stability in anaerobic reactors and possibly increase substrate digestion rates. Pioneering research in the early '70's with the first report on two-phase digestion of sludge came as practical proof of those past assumptions. Today phase separation is a proposed option to single-stage digestion, due to different steady-state kinetic rates in the two main bacterial groups of anaerobic digestion. Also the different kinetic rates generate subsequent differences in the acclimatisation of the two bacterial groups to changing conditions. More applications of the process can be found in literature on sludge and MSW, than in the treatment of agro-industrial wastewaters. The latter is mainly due to the fact that hydrolysis and acidification appear to be the rate-limiting steps in the digestion of high solid content waste.

In recent decades, a lot of research work created a positive image for two-phase applications when compared to single-stage anaerobic digestion. However until now many anaerobic consultants do not have sufficient knowledge to utilise fully the potential of the two-phase process. Leading companies in the field of anaerobic digestion will often design pre-acidification tanks without understanding the uncontrolled acidogenic activities taking place in them. Therefore design is

based on an empirical approach or lack of knowledge of the effects of reactor design parameters on acidogenesis.

5.2 Project objectives

The main objective of this research project was to provide information on various basic design parameters that could be considered in order to operate acidogenic reactors for high-strength agro-industrial wastewaters.

Therefore the research is focused on the complete range of data related to the effects of various reactor design parameters, namely: temperature (from ambient to thermophilic); pH (from 4.5 to 7.0); HRT (from 6 to 12 hrs, with and without variations in the OLR); addition of commercial micro-nutrients; and mixing the reactor contents. The wastewaters studied are slaughterhouse, collected fresh each week, and synthetic wastewaters similar to those from instant coffee production.

Results are based on analyses for: VFA concentration and composition (acetic to caproic), Tot. and Filt.COD, Tot.BOD, TS, VS, SS, VSS, TKN, ammonia-N, phosphate-P, gas composition and for slaughterhouse wastewaters TFM and protein concentrations. Assessment of the effects of design parameters on the performance of acidogenic biomass are based on: VFA production and composition; acidified COD; and overall effluent quality in relation to subsequent methanogenic treatment requirements.

An extensive volume of data from laboratory-scale studies on acidification will be used to establish most design requirements for acidogens to convert organic matter into simple carbon source (i.e. VFA), for the industrial wastewaters used in this project. Also a discussion of the benefits from pre-acidification application in wastewater treatment, is presented as a set of general guidelines for process engineers.

Acidification will be assessed as a process for low-cost high-rate pre-treatment potential, especially when applied for readily biodegradable wastewaters, but also when it will be used for advanced wastewater treatment.

Chapter 6

MATERIALS, METHODS AND START-UP

6.1 Materials

The operational set-up, flow diagram and the reactor design are presented in Figure 6.1. The position of inlet, outlet, stirrer and pH probe was based on the volume of the liquid in the reactor, as presented in the reactor design in Figure 6.1.

Reactors were simple CSTR designs, as acidogenic units should not be more complex for economic reasons. All reactors were made of glass and had a volume of 1.1 litres. As there was no level-controller available for this experimental set-up, the level was maintained at 1.0 litre with accurate and constant calibration of the influent and effluent pumps. Whenever variations of the volume occurred, due to unstable pump operation, they were in the range of 0.1 litre according to the reactor design configurations.

All glassware used was Quickfit. Reactors were hermetically sealed and sealed-surfaces filled with lubricants. Also they were mixed continuously at 80-85 rpm. Mixing was applied with motors operating a central metal axis with stirring blades at the bottom.

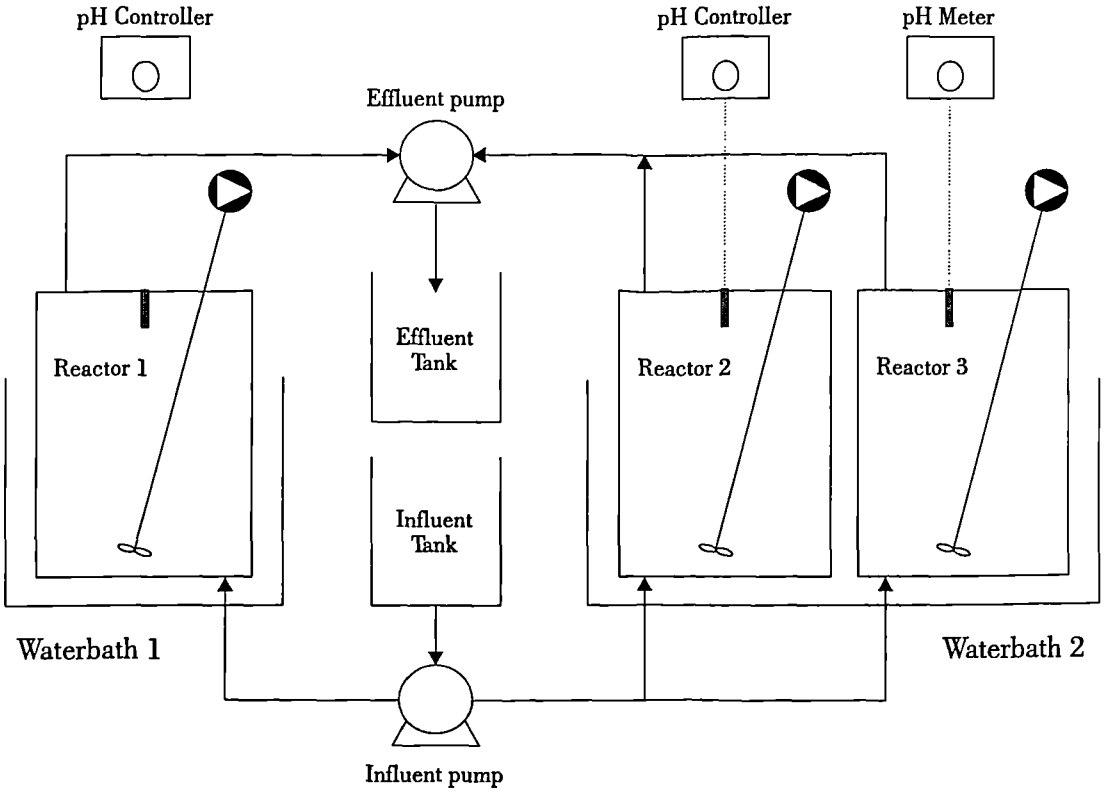
6.2 Methods

6.2.1 Operational & Analytical Methods

Six sets of experiments have been carried out, two on synthetic instant coffee wastewaters and four on real slaughterhouse wastewaters, collected weekly and stored at 4°C. To avoid high content of suspended solids a sieve, with a mesh size of 2 mm, was used in the collection of fresh slaughterhouse wastewaters at the plant. Between experiments the reactors were not operating for periods of up to 3 months.

All basic analyses was carried out according to Standard methods (1992). Samples were collected as grab or composite, depending whether they were for routine or additional analyses. GFA filter

(a) Operational Set-up and Flow Diagram



(b) Reactor Design

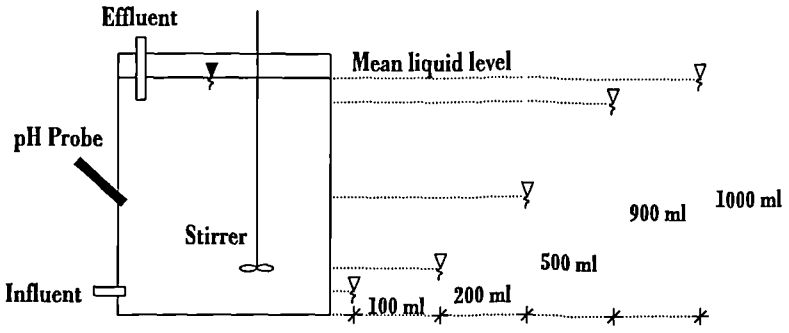


Figure 6.1: Operational set-up, flow diagram and reactor design

paper size 70 mm (Glass microfibre Whatman) was used for all filtered sample analyses.

The pH-controller used for Reactors 1 and 2 was E.I.L. 9140 Series. NaOH used for pH control in the coffee experiments and HCl used in the slaughterhouse experiments had normality 0.5 N. The pH-meter used for Reactor 3 was Corning Model 10.

VFA were analysed for 8 acids (namely Acetic, Propionic, n- and iso-Butyric, n- and iso-Valeric, n- and iso-Caproic). In Experiments 1 to 4, the Gas Liquid Chromatograph used was Pye Unicam Model 304, equipped with a flame ionization detector (FID), incorporated with PU 4700 Autoinjector and CDP4 Computing Integrator. Temperatures were: FID at 180°C, Column 165°C, Injector 180°C. Chromosorb column was 2 m by 2 mm packed with 10% AT-1000 on 80/100 Chromosorb W-AW. Carrier gas was N_2 at 20 ml/min. Injection volume was 1 microlitre and injection type was syringe-on column. Peak areas were measured and compared with a standard VFA volume and the units were calibrated on a daily basis. The GLC used in Experiments 5 and 6 was model Unicam 610 Series with autoinjector and PU 4811 computing integrator. All specifications of operation were the same with the previous GLC apart from the column temperature set at 140°C

Samples prepared for VFA analysis were filtered and immediately acidified with formic acid in Experiment 1 and phosphoric acid in Experiments 2 to 6, at 1 to 10 dilution. The samples were analysed on the same day, apart from the last two experiments (5 and especially 6) when samples had to be stored at 4°C, for up to a week sometimes, due to setting-up and extensive calibrations for the newly purchased unit.

The liquid displacement principle was to be used to measure volumetric gas production daily, connecting the reactors' gas outlet with 5 litres water containers via a Dreschel bottle. Unfortunately the minimal volumes of biogas produced during acidification made this method of measurement very unreliable. It was observed that volumes of displaced water were equally, or even more, affected by partial pressures generated from partial differences in the reactor's liquid level. The latter caused even by marginal differences between influent and effluent pump rates.

During the operation of the first experiment an attempt was made to connect the reactor achieving the most of the fermentation, with a standard gas measuring apparatus. This apparatus was used for the measurement of biogas from lab-scale single-stage and/or methanogenic reactors, in the range of 10-50 litres working volume. As expected the range of gas production from the acidogenic reactors was not able to reach the minimal level of detection of such a gas measuring unit. After 2 weeks, with only a marginal change observed in the rotating drum of the gas meter, the unit was put back in operation with an AF used as methanogenic reactor for the treatment of coffee wastewaters. After the second experiment on coffee wastewaters the method of water displacement was abandoned. This was decided as volumetric gas production results were not considered of major significance, but also no better alternative could be supplied by the laboratory, for such small levels of volumetric gas production.

Gas from the headspace of the reactor would be sampled with a syringe from a glass T-piece. The T-piece was placed in between the rubber tube connecting the gas outlet on the top of the

reactor and the Dreschel bottle. After the Dreschel bottle biogas was released to a collection system which was used in the laboratory for all anaerobic digestion projects, in accordance with safety regulations.

Gas produced was analysed for CH_4, CO_2 and residual air content (i.e. N_2, H_2, H_2S, etc.) with a Gas Chromatograph (GC). The GC used was BECKER Model 403 with thermal conductivity detector (TCD). Carrier gas was Helium, at 50 ml/min. Packing was Poropak Q with metal column 2 m length by 4 mm bore. Column temperature was 55°C. Injection temperature was 60°C. Sample size was 1 ml. Each gas surface produced was multiplied for the calculation of percentage composition with the following constants: CO_2 1.75; CH_4 2.36; residual air 2.06.

Protein analysis in Experiment 6 was carried out according to the Lowry method "Protein Assay Kit", supplied by Sigma Diagnostics. The whole range of standard curves from 500 to 800 absorbance wavelength has been produced and is presented in Appendix A. The curves used to calculate proteins concentration were 500 and 550, chosen as best curves according to the method suggested by the supplier for the selection of the best curves (Anon. 1989).

6.2.2 Numerical & Statistical Methods

Various methods have been suggested for the assessment of acidification phenomena, often using different terminology. For example the liquefaction efficiency, defined as the ratio of the sum of the acidified substrates and biogas COD to the initial substrate COD, was used by Ghosh (1987) to describe the process. Additionally, Negri et al. (1992) reported VFA with an Acetic acid equivalent. The same was suggested by Hajipakkos (1987).

Furthermore, Shin et al. (1992) suggested that variations in gas production rate, in parallel to the ratio of soluble to total COD, was considered as a key parameter to evaluate solubilisation rates of organic particulates. Therefore they expressed the extent of solubilisation as a percentage of effluent soluble COD to influent total COD. Sanders et al. (1996) reported some relatively complicated formulae to assess acidogenic phenomena involving also concentrations of proteins and CH_4 gas volume, expressed as COD.

Originally Eastman and Ferguson (1981) suggested to express VFA as COD composition, a concept broadly accepted by most researchers and professionals involved in wastewater treatment. An alternative to the evaluation of COD for VFA concentrations, with TOC measurements and the carbon content of VFA, has been described by Alexiou et al. (1993) and Alexiou and Anderson (1994).

In this project it was decided to express VFA as volumetric concentrations, expressed as COD, and relate this value to the Filt.COD value in the effluent of the acidogenic reactor, according to the formula for Acidified COD presented below:

$$Percentage\ of\ Acidified\ COD = \frac{COD\ in\ VFA\ (mg/l)}{Filtered\ COD\ (mg/l)} \times 100\ (\%)$$

The COD equivalents of the measured VFA were analytically calculated and presented by Alexiou and Anderson (1994). A Table of the most important constants used for the conversion of the different acids to COD, TOC and Acetic acid equivalent is presented in Appendix B.

The data selected as representative of quasi steady-state for the various operational conditions applied during the experiments, were statistically assessed according to Green and Margerison (1978), Box et al. (1978) and Fragakis (1985). The following statistical formulae have been used to evaluate all statistically evaluated parameters:

$$\text{Mean: } \mu = \frac{\sum x_n}{n}$$

$$\text{Standard Deviation: } s_{n-1} = \sqrt{\frac{\sum x_n^2 - \frac{\left(\sum x_n\right)^2}{n}}{n-1}}$$

$$\text{Coefficient of Variation: } C.V. = \frac{s_{n-1}}{\mu} \times 100\ (\%)$$

$$\text{Mean Standard error: } s_\mu = \frac{s_{n-1}}{\sqrt{n}}$$

$$\text{Confidence Interval of Mean: } \left[\mu \pm t_{n-1}\left(1 - \frac{a}{2}\right) \cdot s_\mu\right]$$

where x_n are the data used to evaluate the statistical parameters; n is the number of these data; $t_{n-1}\left(1 - \frac{a}{2}\right)$is the t-Student constant for $\left(1 - \frac{a}{2}\right)$probability, assuming t-Student distribution for the data selected to evaluate the statistical parameters; and a is the level of probability for the confidence interval. The value of 95% has been selected for a, in order to evaluate the intervals presented in this thesis. For this probability value the t-Student constant can be obtained from a t-Student table, found in various Statistical text books (Box et al., 1978, Fragakis, 1985).

Evolutionary Operation (EVOP) optimisation was used for some of the most important results found for the acidification of coffee and slaughterhouse wastewaters. Although the operation of Experiments 1 to 6 (Chapters 7 to 12) has not been carried out according to the EVOP experimental operation in relation to different factorial designs, the evaluation process was suggested (Britz, 1997) to be used for an additional assessment of the findings of the present study in relation to optimisation of the main design parameters (i.e. temperature, pH, HRT).

The calculation of the effects of the simplified 2×2 factorial designs in the EVOP examples (presented in Chapter 13), was made according to the formulae presented by Box and Draper (1969) and Box et al. (1978). As the EVOP examples of the presented 2×2 factorial designs were assumed to have operated only 1 cycle according to the EVOP operation of experiments, no Standard Deviation (S.D.) value could be calculated. Therefore it was assumed to use as S.D. a value equal to 15% of the mean value of each factorial design (the mean value calculated according to Box and Draper, 1969).

The value of 15% was selected as a CV of below or around 10-15% was accepted in the present study as an indicator of relatively steady state during each period of different operational conditions, based on the statistical analyses of the results on VFA concentration and the main acids produced. The same CV value is used by the group of Prof. Britz in South Africa for the evalu-

ation of steady state periods in acclimatised operations after at least 3 HRT cycles of operation (Britz, 1997).

6.3 Start-up

The reactors were seeded using sludge from digesters at the sewage treatment plant in Cramlington, Northumberland.

A number of experiments have been carried out trying to develop a reproducible synthetic coffee wastewater, which would be representative of the real wastewaters. Instant soluble coffee gives reproducible COD but insufficient suspended solids and oil compared to the real wastewaters.

Start-up was initiated by feeding whey, progressively shifting to a mixture of whey and instant coffee powder and finally only instant coffee powder. Whey is a rich source of minerals, calcium, phosphorus, potassium, sodium, copper and iron. It contains carbon mostly in the form of lactose, but also as proteins and fats. Also, it is a source of vitamins of the B-complex group (Tyagi et al., 1991). These characteristics are the reason why it is most often used for anaerobic reactor lab-scale start-up.

The major problem encountered during start-up was the delay, for around 6 months, of the University consortium responsible for the Nescafé project to decide on a synthetic wastewater, which could be used by all laboratories involved. Two interim formulations were concluded unsuitable: i) because of the immiscibility of the coffee oil, considered to be an important component of the real wastewaters; and ii) the proposed elution with boiling water from filter pressings, also proved to be impractical for preparation of the large volumes required daily. Furthermore these formulations produced far lower COD than originally assumed, in order to reach the COD level of real wastewaters.

Other attempts to use high amounts of SS from filter pressings, proved equally unreliable for laboratory scale reactors with small volumes (below 5 litres, with small tube diameters). Also 3 different combinations of macro-nutrients were evaluated, before concluding the final method to produce synthetic instant coffee wastewaters. The final method included the following ingredients in 1 litre volume of tap water: 10 gr of instant coffee; 206.5 mg of urea ($CO(NH_2)_2$); and 0.1 ml of the commercial mixture of nutrients produced by OMEX Environmental Ltd (Anon. 1995).

Chapter 7

EXPERIMENT 1: COFFEE-TEMPERATURE

7.1 Introduction

7.1.1 General

The significantly high temperatures (>70°C) used for the production of instant coffee, initiated thoughts to apply thermophilic two-phase anaerobic digestion for treatment. So in Experiment 1 different thermophilic and the optimum mesophilic temperatures were to be applied to acidogenic reactors, in order to assess such a potential.

Additionally an initial study would be done to assess pH effects. These would be tested operating reactors under non-pH-controlled (pH=4.5) and pH-controlled (pH=5.0) conditions. The value of pH 5.0 was chosen with the intention of operating close to optimal pH ranges (5.5-6.0) for acidogenesis (Zoetemeyer et al., 1982a), but also below optimal and tending towards the low pH nature of this wastewater for reasons of economy.

At that stage there were no previous studies investigating HRT or nutrient requirements for acidogenic systems, treating a similar recalcitrant wastewater. So the HRT and COD:N:P ratio were selected on an empirical basis. Also a commercial micro-nutrient mixture was added on an empirical basis. The mixture was called Nutromex N & P, produced by OMEX Environmental Ltd (Anon., 1995).

The extent of acidification which the investigation might achieve, as a pre-requisite of the methanogenic phase was also unknown. Neither was it clear how to adequately assess the effects of acidogenic phenomena on the wastewater, under any set of applied conditions. Therefore a broad range of parameters was to be analysed throughout this initial experiment.

7.1.2 Objectives

The objectives of Experiment 1 were:

Major:

- **Study the effect of thermophilic temperatures on acidification of a synthetic instant coffee wastewater in comparison with the optimum mesophilic temperature.**
- **Compare the effect of minimal to no pH-control on acidification of synthetic instant coffee wastewater.**

Minor:

- Analyse a wide range of parameters, so as to gain an adequate understanding of data handling to express acidogenic phenomena.
- Obtain sufficient experience to operate acidogenic reactors, in order to establish a realistic routine for future experimental work.

7.2 Experimental Conditions

7.2.1 Operation

Experiment 1 started straight after the preliminary tests. The total duration was approximately 53 days. There were 5 periods of collection of data of primary importance (a total of 25 days), while the rest of the time was used for acclimatisation periods (5 days for the final synthetic wastewater feed at the beginning and 23 days on gradual temperature changes).

The conditions originally selected to operate the 3 acidogenic reactors, during Experiment 1, are presented in Table 7.1.

Table 7.1: Experiment 1: Theoretical Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s) tested (°C)	37	45,50,55 60,65	45,50,55 60,65
pH	5.0	5.0	4.5
HRT (hours)	12	12	12
COD:N:P	400:5:1	400:5:1	400:5:1
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

Temperature changes during transition periods in the water-bath of reactors 2 and 3 were 1°C/day, in order to achieve minor disturbances while acclimatising to a new temperature (Hajipakkos, 1987). Temperature changes during Experiment 1 are presented in Figure 7.1.

The main characteristics of the synthetic instant coffee wastewater are presented in Table 7.2. These values are averages of all measurements made on the feed of the 3 reactors both in

Experiments 1 and 2, which studied acidification of instant coffee wastewaters.

The theoretical HRT value of 12 hrs was originally calibrated for the influent pump of the 3 reactors, and checked in the end of the experiment. Periodical volumes of feed consumed and duration of this consumption, were used to calculate the applied HRTs for different parts of this experiment. Theoretical and applied values of HRT are presented in Figure 7.2.

Applied HRT values and their percentage differences in relation to the theoretical value, are presented in Table 7.3.

A difference greater than 10% was found for the applied HRT, in 3 out of 5 periods of collection of data of primary importance; but also for the mean HRT value during the experiment. Therefore it was necessary to assess the results of this experiment with different HRT values, selected closer to the ones applied. So 15, 14, 12, 13.5 and 12 hrs were the chosen HRT values for periods with temperature: 45, 50, 55, 60 and 65°C respectively. As for reactor 1, with a temperature of 37°C, each period has to be assessed with a different applied HRT, allowing for a HRT study in the operation of this reactor.

The addition of macro-nutrients was originally assumed to have a Tot.COD:N:P value of 400:5:1, but was found to have a practical value around 400:8.2:1, or roughly 250:5 for the ratio of available Nitrogen. This is calculated from the values of Table 7.2 . This mistake was due to experimental errors and miscalculations of the necessary urea to be added in order to achieve the targeted ratio. The applied ratio did not negatively affect the experiment. On the contrary it can be beneficial to use a higher nitrogen ratio while treating recalcitrant wastewaters. No further consideration will be given to this matter, other than to acknowledge the applied ratio.

Table 7.1 is converted into Table 7.4, according to the facts mentioned above on the applied HRT and the addition of urea. The conditions given in Table 7.4 will be the ones used for the evaluation of the data produced in Experiment 1.

OLR values were calculated with the mean value of the Filt.COD from Table 7.2 and the values of HRTs, as presented in Figure 7.2. These values are presented in Figure 7.3.

In Table 7.5 information is presented about the ingredients of the synthetic wastewater, in comparison to theoretical values presented in Materials and Methods (Chapter 6). During the experiment the feed was prepared 2 to 3 times daily, in volumes of 2 litres, in order to avoid acidification in the storage container. The values presented are an average of each preparation made. The total volume of feed prepared for the 3 reactors, is also in Table 7.5.

7.2.2 Analyses & Diary of Problems

The type and frequency of analyses varied, depending on whether the experiment was on a transition period of operational conditions or a steady-state one. Also while on a steady-state period some measurements were done on a daily basis and others as additional with a fixed time interval.

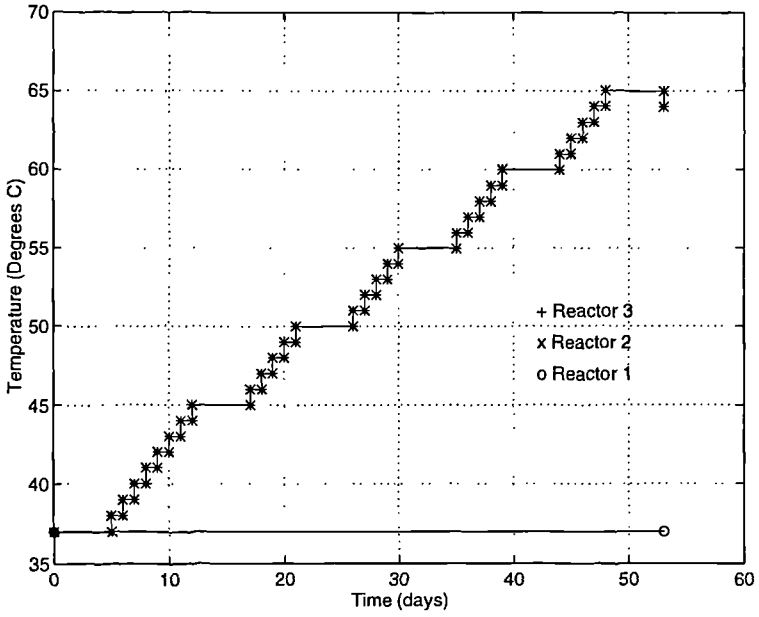


Figure 7.1: Changes in Temperature during Experiment 1

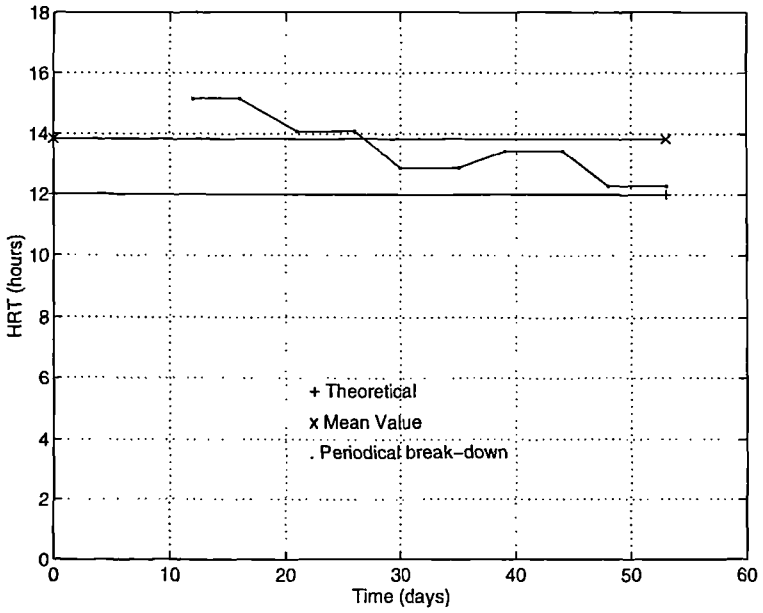


Figure 7.2: Applied and Theoretical Hydraulic Retention Time during Experiment 1

Table 7.2: Synthetic Instant Coffee Production Wastewater Characteristics

Parameter	Mean	Standard Deviation	Data
Total COD (mg/l)	10,459	528	6
Filtered COD (mg/l)	9,318	565	43
Total BOD (mg/l)	4,817	565	6
Total Solids (mg/l)	10,343	647	8
Volatile Total Solids (mg/l)	7,898	330	8
Suspended Solids (mg/l)	579	220	14
Volatile Suspended Solids (mg/l)	568	210	14
Total Kjeldahl Nitrogen (mg/l)	408	17	7
Ammonia-N (mg/l)	21	3.8	7
Phosphate-P (mg/l)	25	2.1	5
Volatile Fatty Acids (mg/l)	124.4	56.8	175
Acetic Acid (mg/l) (& % in VFA)	95.1 (76.5)	40.2	175
Propionic Acid (mg/l) (& % in VFA)	9.5 (7.6)	14.8	175
iso-Butyric Acid (mg/l) (& % in VFA)	3.9 (3.2)	4.9	175
n-Butyric Acid (mg/l) (& % in VFA)	5.2 (4.2)	5.8	175
iso-Valeric Acid (mg/l) (& % in VFA)	2.9 (2.4)	2.9	175
n-Valeric Acid (mg/l) (& % in VFA)	7.0 (5.6)	14.5	175
iso-Caproic Acid (mg/l) (& % in VFA)	0.4 (0.3)	1.9	175
n-Caproic Acid (mg/l) (& % in VFA)	0.3 (0.3)	1.7	175
COD in VFA (mg/l)	154	—	—
Acidified COD (%)	1.65	—	—
Ratio Tot.COD/Filt.COD	1.12	—	—
Ratio Tot.COD/Tot.BOD	2.17	—	—
Ratio Tot.COD/Tot.Solids	1.01	—	—
Percentage VTS in TS (%)	76.4	—	—
Percentage VSS in SS (%)	98.1	—	—
Percentage NH_3-N in TKN (%)	5.2	—	—
Ratio Tot.COD:N:P	400:8.2:1	—	—
pH	4.5	—	—
Temperature	15°-20°C	—	—

Table 7.3: Applied HRTs and percentage differences from the theoretical value of 12 hours

Period	HRT (hours)	Difference (%)
45°C	15.15	+26.27
50°C	14.08	+17.30
55°C	12.87	+7.21
60°C	13.43	+11.94
65°C	12.27	+2.26
Mean HRT	13.83	+15.22

Table 7.4: Operation of Reactors during Experiment 1

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s) tested (°C)	37	45,50,55 60,65	45,50,55 60,65
pH	5.0	5.0	4.5
HRT (hours)	12,13.5 14,15	12,13.5 14,15	12,13.5, 14,15
COD:N:P	400:8.2:1	400:8.2:1	400:8.2:1
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

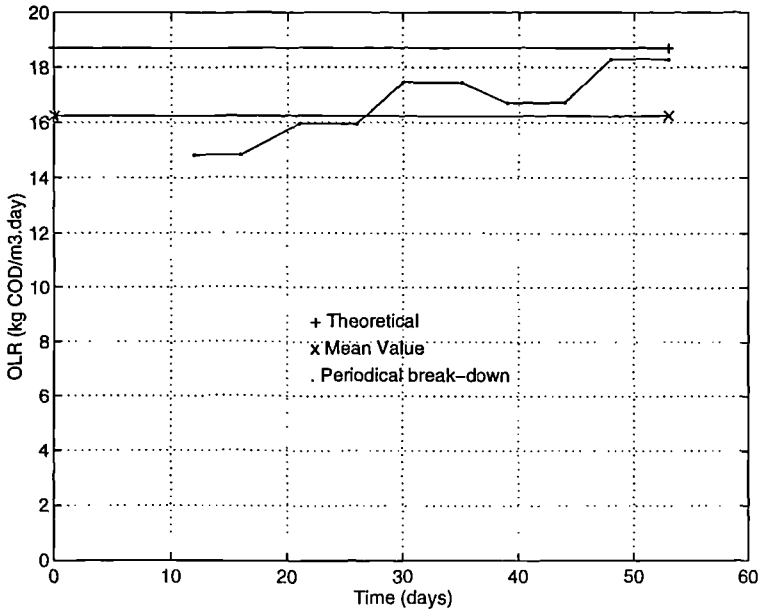


Figure 7.3: Organic Loading Rate during Experiment 1

While in a transition period, Reactors 2 and 3 were acclimatising gradually towards a new temperature. The new temperature would be applied in the water-bath of Reactors 2 and 3 some time after midday. Before the temperature change a gas sample would be analysed for gas composition. Also one sample of the effluent of the three reactors and the influent, would be analysed for VFA concentration and composition for 8 acids.

During the acclimatisation period for the final type of synthetic wastewater, the analyses carried out were similar to the one for temperature changes.

The steady-state periods were sets of 5 days. Every day analyses was done on two gas samples from each of the three reactors, the first around 8 o'clock in the morning and the second after about 12 hours, in the evening. Also on a daily basis three samples from the three effluents and the influent were collected, to analyse for VFA concentration and composition of 8 acids. These samples were collected on an average every 8 hours, starting around 8 o'clock in the morning.

Additional analyses was carried out for the effluent of the three reactors, on the third and the fifth day of a steady state period. A composite sample of each effluent was collected all through the night (for a period of approximately 8 hours), because the volumes required for all the expected analyses were far greater than the very small quantities produced even in one hour. These samples were analysed for Tot.COD, Filt.COD, Tot.BOD, TS, VTS, SS, VSS, TKN, NH_3-N and PO_4-P. Also at the beginning and the end of each set, the volume of NaOH in the pH-controllers used for Reactors 1 and 2 was measured, in order to calculate approximately the consumption rate of NaOH.

Gas and VFA analyses were always carried out as a single test. COD was a duplicate on two dilutions. All solids were single tests on two volumes. TKN, NH_3-N and PO_4-P were single tests on two dilutions. Finally, BOD was analysed in a single test and on one dilution only.

Problems that occurred during the operation of Experiment 1 and could have affected the steady state conditions were recorded. They are reported in Appendix C. The main problem appeared to have been the difficulty to maintain the expected HRT. This was also the reason why the practical HRT values were different from the theoretical one, as already mentioned. If necessary, further comments about any other of these problems that have proved to influence any reactor, will be mentioned while validating the data in the following paragraphs.

7.3 VFA

7.3.1 VFA as Total Concentration

In Figure 7.4 the Total VFA concentration is presented.

The extent of acidification is obvious comparing the concentration of the influent, to that of the effluents. Apparently, Reactor 1 is the one producing the most VFA and Reactor 3 producing the least acids. VFA concentrations were around 0.6 to 0.8 g/l.

Table 7.5: Ingredients of synthetic feed and total volume used during Experiment 1

Instant Coffee (g/2l feed)	Urea (g/2l feed)	OMEX (ml/2l feed)	Volume (l)
20.01	0.4130	0.2	139.0

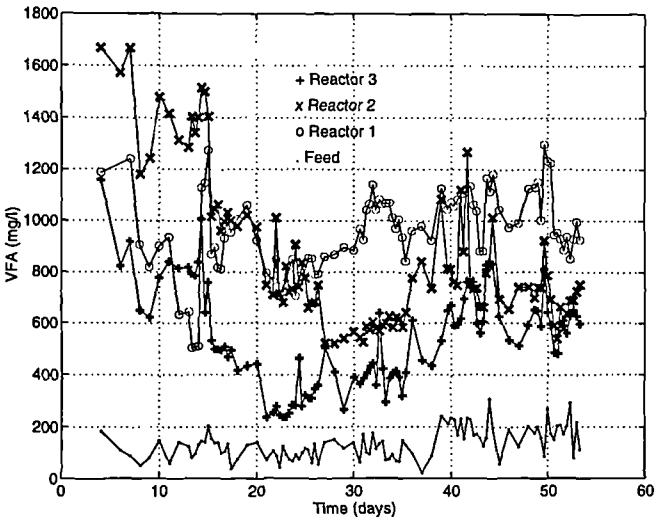


Figure 7.4: Total Volatile Fatty Acid Concentration during Experiment 1

7.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is given in Figure 7.5.

This graph can give a better indication of the extent of acidification in each reactor. It seems that less than 20% of the matter is acidified. Especially Reactor 1, which was the control reactor, appeared to have small variations in the production of VFA due to the HRT changes that occurred during Experiment 1.

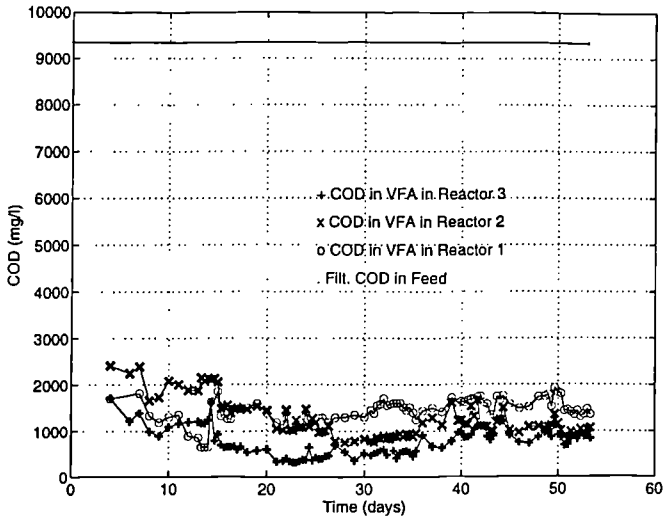


Figure 7.5: Feed Filt.COD & COD in VFA in Reactors during Experiment 1

7.4 Statistical Analyses

In Tables 7.6, 7.7 and 7.8 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 1. The confidence interval was calculated for 95% probability and t-Student distribution constants.

The data on VFA concentration and the different acids produced for Experiment 1 are presented in Appendix D (Tables D.1, D.2 and D.3).

Each of the presented sets of operational conditions was maintained for a duration equivalent to around 10 HRTs.

The main acids in Reactor 1 were Acetic and n-Butyric, which was a desirable composition for methanogenic digestion (Andrews & Pearson, 1965). In Reactor 2, it appeared that Acetic, n-Butyric and Propionic were the main acids, while the concentration of Propionic increased with the increase in temperature. As for Reactor 3 a similar pattern to Reactor 2 was observed for the main acids. The only difference was that the composition of Acetic was higher than that

of n-Butyric, while in the case of Reactor 2 they were more similar.

The percentage of each acid in the 3 reactors is presented in order to validate the major acids produced, but also in order to observe the variations in the acidogenic activities under the different operational conditions. Obviously Acetic, Propionic, n-Butyric and n-Valeric were the acids mainly produced under these conditions. Meanwhile Acetic in Reactors 1 and 2 was similar but slightly less to that for Reactor 3, particularly at temperatures above 55°C. Propionic was higher for Reactor 2 and n-Butyric was higher for Reactor 1. n-Butyric had similar concentrations both between Reactors 2 and 3. Finally all reactors produce 5 to 10% of n-Valeric, while Reactor 2 had slightly higher proportion than the two other reactors.

7.5 Additional Analyses

In Tables 7.9, 7.10 and 7.11 the results of additional analyses are presented. The results were produced from the 2 samples taken for additional analyses during each set of conditions. Only the results for gas were produced from daily measurements. All these results are presented only as an indication of the quality of the effluent from the 3 reactors, that would enter in commercial applications in a methanogenic reactor, and only a few comments could be made from them.

From the Filt.COD analysis it can be observed that almost no Filt.COD removal takes place in this experiment (values of less than 10% were the maximum found). Furthermore measurements were carried out on Total COD. These values had similar small changes as the ones presented for Filt.COD.

Similar observations as for Filt.COD appeared in the magnitude of TS and VTS. It was also interesting to observe for Reactors 2 and 3 (Tables 7.10 & 7.11) the decrease in SS and VSS with the increase in temperature and the slight decrease in HRT.

Nitrogen was measured as Total and Ammonia. The levels of TKN were quite stable and close to the magnitude predicted due to the miscalculations for the excess addition of urea. With respect to ammonia-N almost no differences appear between the reactors. Meanwhile these low ammonia-N values represented the low extent of acidification that took place, as well as the low content of easily degradable nitrogenous matter in the wastewater.

Only attempts were made to measure phosphate-P. The data obtained were not sufficient to be able to draw any conclusions or produce any presentable results about the fate of PO_4-P in the reactors. From the few data produced for Reactor 1 and 2 it appeared that the level of PO_4-P in the effluent of the reactors was similar to the influent.

Gas was only recorded as composition. Attempts were made to measure biogas volume produced, without success. The content of CO_2 in Reactors 2 and 3 appeared to be relatively low. While the CO_2 content in Reactor 1 although higher, oscillated to a great extent possibly indicating the strain of the system in the low pH values, and a high proportion of released CO_2 becoming soluble in the liquid.

Table 7.6: Statistical data of Reactor 1 in Experiment 1

T=37°C, pH=5.0, HRT=15.2hrs, n=7 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	901	76	8.5	29	831–972
Acetic	349	52	15.0	20	301–397
Propionic	52	7	12.8	3	46–58
iso-Butyric	7	2	24.7	<1	6–9
n-Butyric	446	19	4.3	7	428–464
iso-Valeric	2	<1	8.9	<1	<2–>2
n-Valeric	31	3	9.8	1	28–34
iso-Caproic	<1	<1	79.7	<1	<1–>1
n-Caproic	14	1	9.9	<1	12–14
T=37°C, pH=5.0, HRT=14.1hrs, n=11 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	824	33	4.0	10	802–847
Acetic	337	21	6.4	6	323–351
Propionic	56	11	19.0	3	49–63
iso-Butyric	11	5	41.9	1	8–14
n-Butyric	377	7	1.7	2	373–382
iso-Valeric	1	2	172.5	<1	0–2
n-Valeric	33	7	20.0	2	28–37
iso-Caproic	<1	<1	118.1	<1	<1–<1
n-Caproic	9	<1	7.2	<1	8–9
T=37°C, pH=5.0, HRT=12.9hrs, n=12 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,023	64	6.3	19	982–1,064
Acetic	437	30	6.8	9	419–456
Propionic	68	3	4.6	<1	66–70
iso-Butyric	7	4	53.7	1	5–10
n-Butyric	435	33	7.5	9	415–456
iso-Valeric	9	5	49.5	1	7–12
n-Valeric	52	5	9.1	1	49–55
iso-Caproic	1	<1	25.2	<1	1–2
n-Caproic	13	2	14.9	<1	11–14
T=37°C, pH=5.0, HRT=13.4hrs, n=10 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,084	34	3.1	11	1,059–1,108
Acetic	413	9	2.1	3	407–420
Propionic	84	4	4.3	1	82–87
iso-Butyric	20	1	5.4	<1	19–21
n-Butyric	486	30	6.1	9	465–507
iso-Valeric	5	5	113.8	2	<1–8
n-Valeric	59	4	7.2	1	56–62
iso-Caproic	2	<1	52.2	<1	1–2
n-Caproic	15	1	9.0	<1	14–16
T=37°C, pH=5.0, HRT=12.3hrs, n=14 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,033	145	14.0	39	950–1,117
Acetic	428	88	20.7	24	377–479
Propionic	71	16	21.8	4	62–80
iso-Butyric	17	7	40.9	2	13–22
n-Butyric	437	54	12.3	14	407–468
iso-Valeric	7	7	89.9	2	4–11
n-Valeric	54	17	30.8	4	45–64
iso-Caproic	2	<1	38.5	<1	<2–>2
n-Caproic	16	5	29.8	1	13–18

* units are (mg/l).
† units are (%).

Table 7.7: Statistical data of Reactor 2 in Experiment 1

T=45°C, pH=5.0, HRT=15.2hrs, n=7 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,017	34	3.4	13	985–1,049
Acetic	464	22	4.8	8	444–484
Propionic	117	14	12.3	5	104–130
iso-Butyric	12	8	70.0	3	4–19
n-Butyric	347	21	6.0	8	328–366
iso-Valeric	4	3	67.1	1	2–7
n-Valeric	39	11	27.7	4	29–48
iso-Caproic	<1	<1	16.8	<1	<1–<1
n-Caproic	34	8	24.7	3	26–42
T=50°C, pH=5.0, HRT=14.1hrs, n=12 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	734	57	7.7	16	698–770
Acetic	297	18	5.9	5	286–308
Propionic	103	19	18.1	5	91–115
iso-Butyric	12	4	29.3	1	10–14
n-Butyric	257	29	11.5	9	238–275
iso-Valeric	1	2	165.4	<1	0–2
n-Valeric	53	11	20.7	3	46–60
iso-Caproic	<1	<1	114.9	<1	<1–<1
n-Caproic	11	1	12.7	<1	10–12
T=55°C, pH=5.0, HRT=12.9hrs, n=11 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	603	23	3.8	7	587–618
Acetic	253	10	4.0	3	247–260
Propionic	98	5	5.6	2	94–101
iso-Butyric	16	2	13.3	<1	15–18
n-Butyric	171	19	10.9	6	159–184
iso-Valeric	5	4	73.2	1	3–8
n-Valeric	52	9	17.6	3	46–58
iso-Caproic	1	<1	16.3	<1	<1–>1
n-Caproic	6	1	19.7	<1	6–7
T=60°C, pH=5.0, HRT=13.4hrs, n=11 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	768	68	8.8	20	722–814
Acetic	310	33	10.7	10	288–333
Propionic	121	17	14.0	5	110–133
iso-Butyric	21	2	10.7	<1	20–23
n-Butyric	220	20	8.9	6	207–233
iso-Valeric	3	3	108.6	<1	<1–5
n-Valeric	77	12	15.3	4	69–85
iso-Caproic	4	4	109.4	1	<1–6
n-Caproic	12	6	45.9	2	8-16
T=65°C, pH=5.0, HRT=12.3hrs, n=11 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	668	75	11.2	23	617–718
Acetic	279	34	12.1	10	257–302
Propionic	100	16	15.6	5	90–111
iso-Butyric	19	4	20.4	1	16–21
n-Butyric	200	28	14.2	9	181–219
iso-Valeric	1	2	136.5	<1	<1–2
n-Valeric	59	11	19.0	3	52–67
iso-Caproic	2	1	56.1	<1	1–3
n-Caproic	7	1	20.0	<1	6–8

* units are (mg/l).
† units are (%).

Table 7.8: Statistical data of Reactor 3 in Experiment 1

T=45°C, pH=4.5, HRT=15.2hrs, n=7 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	500	19	3.7	7	482–517
Acetic	318	13	4.2	5	306–331
Propionic	46	4	9.7	2	42–50
iso-Butyric	15	4	27.6	2	11–18
n-Butyric	109	10	8.9	4	100–118
iso-Valeric	2	2	83.8	<1	<1–4
n-Valeric	7	2	32.4	<1	5–9
iso-Caproic	<1	<1	51.5	<1	<1–>1
n-Caproic	1	<1	37.3	<1	<1–2
T=50°C, pH=4.5, HRT=14.1hrs, n=8 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	313	30	9.7	11	288–339
Acetic	208	23	11.3	8	188–228
Propionic	26	4	14.7	1	23–29
iso-Butyric	11	3	23.2	<1	9–13
n-Butyric	62	9	14.8	3	54–69
iso-Valeric	2	3	159.7	<1	0–4
n-Valeric	5	1	31.3	<1	3–6
iso-Caproic	<1	<1	148.2	<1	0–<1
n-Caproic	<1	<1	197.7	<1	0–<1
T=55°C, pH=4.5, HRT=12.9hrs, n=10 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	399	36	8.9	11	378–425
Acetic	230	27	11.6	8	211–250
Propionic	30	5	17.4	2	27–34
iso-Butyric	15	3	23.5	1	12–17
n-Butyric	110	11	9.6	3	103–118
iso-Valeric	2	1	56.2	<1	1–3
n-Valeric	9	3	34.6	<1	7–11
iso-Caproic	1	<1	37.9	<1	<1–>1
n-Caproic	1	<1	18.7	<1	1–2
T=60°C, pH=4.5, HRT=13.4hrs, n=8 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	760	80	10.6	28	692–827
Acetic	337	45	13.3	16	299–374
Propionic	87	7	7.8	2	81–92
iso-Butyric	23	5	21.0	2	19–28
n-Butyric	236	30	12.8	11	211–262
iso-Valeric	8	6	78.4	2	3–14
n-Valeric	51	18	35.1	6	36–66
iso-Caproic	5	9	175.4	3	0–13
n-Caproic	12	9	79.9	3	4–20
T=65°C, pH=4.5, HRT=12.3hrs, n=11 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	583	54	9.3	16	547–619
Acetic	287	40	13.8	12	261–314
Propionic	59	17	29.7	5	47–70
iso-Butyric	20	6	28.5	2	16–24
n-Butyric	165	14	8.2	4	156–175
iso-Valeric	2	4	230.8	1	0–5
n-Valeric	44	20	46.7	6	30–57
iso-Caproic	2	1	77.1	<1	<1–2
n-Caproic	5	2	36.8	<1	3–6

* units are (mg/l).
† units are (%).

However similar findings for the percentage of CO_2 in biogas and the range that it was found to be, were reported by Zoetemeyer et al. (1982b). Also they reported a decrease in CO_2 content at thermophilic range. The residual proportion in biogas composition could be assumed to be H_2, as also presented by Zoetemeyer et al. (1982a, 1982b).

With respect to the amounts of methanogenic activity traced in Reactor 2 between temperatures of 43°C and 52°C, presented in Figure 7.6, it appeared that some species of methanogens could become active under those acidic conditions. Such species could be related to the hydrogen-oxidising methanogens, which have low doubling time (1 to 4 hrs) compared to other methanogens and thrive in conditions with high H_2 content (Eckenfelder, 1992). Also it indicated that methanogens present in the original inoculum of digested sludge were still able to survive although inactivated, after approximately 4 months operation in highly acidic environments.

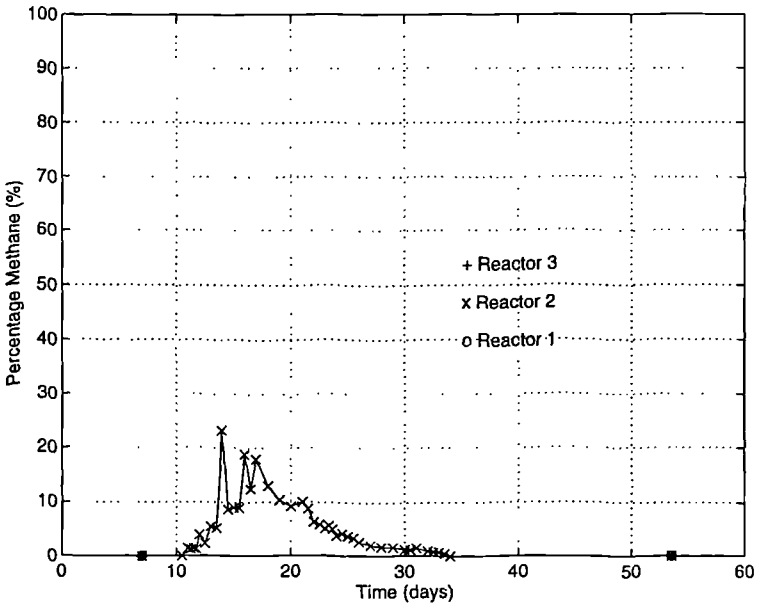


Figure 7.6: Percentage CH_4 in Total Gas Production during Experiment 1

7.5.1 NaOH Consumption

NaOH consumption is reported as ml consumed per hour of operation. The results are presented in Figure 7.7. The results presented are a good indication of the magnitude of alkali consumption. Also, they seem to present higher alkali consumptions for 45, 60 and 65°C, even though lower concentrations of acids were produced at these lower temperatures. The amounts consumed were within the range reported in the studies by Zoetemeyer et al. (1982a).

7.6 Key Points for Discussion

In Figures 7.8 and 7.9 the effect on VFA concentration and the percentage composition of acids for the control reactor 1 due to the HRT changes, is presented.

Table 7.9: Additional Analyses of Reactor 1 in Experiment 1

T=37°C, pH=5.0, HRT=15.2hrs	
Parameters	Mean Value
Total COD (mg/l)	9,880
Filtered COD (mg/l)	9,270
TS (mg/l)	8,340
VTS (mg/l) (% of TS)	6,360 (76.3)
SS (mg/l)	470
VSS (mg/l) (% of SS)	465 (98.9)
TKN (mg/l)	391
NH_3-N (mg/l) (% of TKN)	12 (3.1)
CO_2 in gas (%)	45.6
T=37°C, pH=5.0, HRT=14.1hrs	
Parameters	Mean Value
Total COD (mg/l)	10,280
Filtered COD (mg/l)	8,850
TS (mg/l)	8,580
VTS (mg/l) (% of TS)	6,910 (80.5)
SS (mg/l)	630
VSS (mg/l) (% of SS)	620 (98.4)
TKN (mg/l)	395
NH_3-N (mg/l) (% of TKN)	12 (3.0)
CO_2 in gas (%)	61.9
T=37°C, pH=5.0, HRT=12.9hrs	
Parameters	Mean Value
Total COD (mg/l)	9,975
Filtered COD (mg/l)	8,990
TS (mg/l)	7,615
VTS (mg/l) (% of TS)	6,410 (84.2)
SS (mg/l)	590
VSS (mg/l) (% of SS)	585 (99.2)
TKN (mg/l)	376
NH_3-N (mg/l) (% of TKN)	14 (3.7)
CO_2 in gas (%)	55.5
T=37°C, pH=5.0, HRT=13.4hrs	
Parameters	Mean Value
Total COD (mg/l)	9,860
Filtered COD (mg/l)	9,225
TS (mg/l)	8,240
VTS (mg/l) (% of TS)	6,805 (82.6)
SS (mg/l)	460
VSS (mg/l) (% of SS)	430 (93.5)
TKN (mg/l)	414
NH_3-N (mg/l) (% of TKN)	13 (3.1)
CO_2 in gas (%)	42.7
T=37°C, pH=5.0, HRT=12.3hrs	
Parameters	Mean Value
Total COD (mg/l)	10,760
Filtered COD (mg/l)	9,425
TS (mg/l)	8,470
VTS (mg/l) (% of TS)	7,140 (84.3)
SS (mg/l)	465
VSS (mg/l) (% of SS)	455 (98.9)
TKN (mg/l)	412
NH_3-N (mg/l) (% of TKN)	15 (3.6)
CO_2 in gas (%)	50.1

Table 7.10: Additional Analyses of Reactor 2 in Experiment 1

T=45°C, pH=5.0, HRT=15.2hrs	
Parameters	Mean Value
Total COD (mg/l)	10,310
Filtered COD (mg/l)	9,100
TS (mg/l)	8,380
VTS (mg/l) (% of TS)	7,020 (83.8)
SS (mg/l)	675
VSS (mg/l) (% of SS)	670 (99.3)
TKN (mg/l)	408
NH_3-N (mg/l) (% of TKN)	14 (3.4)
CO_2 in gas (%)	32.6
CH_4 in gas (%)	16.4
T=50°C, pH=5.0, HRT=14.1hrs	
Parameters	Mean Value
Total COD (mg/l)	9,650
Filtered COD (mg/l)	8,350
TS (mg/l)	8,590
VTS (mg/l) (% of TS)	6,785 (79.0)
SS (mg/l)	675
VSS (mg/l) (% of SS)	670 (99.3)
TKN (mg/l)	402
NH_3-N (mg/l) (% of TKN)	12 (3.0)
CO_2 in gas (%)	12.6
CH_4 in gas (%)	4.1
T=55°C, pH=5.0, HRT=12.9hrs	
Parameters	Mean Value
Total COD (mg/l)	9,985
Filtered COD (mg/l)	8,140
TS (mg/l)	8,630
VTS (mg/l) (% of TS)	7,170 (83.1)
SS (mg/l)	695
VSS (mg/l) (% of SS)	690 (99.3)
TKN (mg/l)	389
NH_3-N (mg/l) (% of TKN)	14 (3.6)
CO_2 in gas (%)	8.0
CH_4 in gas (%)	0.4
T=60°C, pH=5.0, HRT=13.4hrs	
Parameters	Mean Value
Total COD (mg/l)	9,300
Filtered COD (mg/l)	8,620
TS (mg/l)	8,610
VTS (mg/l) (% of TS)	7,080 (82.2)
SS (mg/l)	355
VSS (mg/l) (% of SS)	325 (91.5)
TKN (mg/l)	406
NH_3-N (mg/l) (% of TKN)	16 (3.9)
CO_2 in gas (%)	2.8
T=65°C, pH=5.0, HRT=12.3hrs	
Parameters	Mean Value
Total COD (mg/l)	9,850
Filtered COD (mg/l)	9,190
TS (mg/l)	9,070
VTS (mg/l) (% of TS)	7,520 (82.9)
SS (mg/l)	355
VSS (mg/l) (% of SS)	350 (98.6)
TKN (mg/l)	404
NH_3-N (mg/l) (% of TKN)	14 (3.5)
CO_2 in gas (%)	8.9

Table 7.11: Additional Analyses of Reactor 3 in Experiment 1

T=45°C, pH=4.5, HRT=15.2hrs	
Parameters	Mean Value
Total COD (mg/l)	10,220
Filtered COD (mg/l)	8,690
TS (mg/l)	9,070
VTS (mg/l) (% of TS)	7,990 (88.1)
SS (mg/l)	690
VSS (mg/l) (% of SS)	685 (99.3)
TKN (mg/l)	412
NH_3-N (mg/l) (% of TKN)	15 (3.6)
CO_2 in gas (%)	10.5
T=50°C, pH=4.5, HRT=14.1hrs	
Parameters	Mean Value
Total COD (mg/l)	9,850
Filtered COD (mg/l)	8,775
TS (mg/l)	9,170
VTS (mg/l) (% of TS)	7,590 (82.8)
SS (mg/l)	575
VSS (mg/l) (% of SS)	570 (99.1)
TKN (mg/l)	425
NH_3-N (mg/l) (% of TKN)	16 (3.8)
CO_2 in gas (%)	8.7
T=55°C, pH=4.5, HRT=12.9hrs	
Parameters	Mean Value
Total COD (mg/l)	10,085
Filtered COD (mg/l)	8,555
TS (mg/l)	9,030
VTS (mg/l) (% of TS)	7,660 (84.8)
SS (mg/l)	520
VSS (mg/l) (% of SS)	515 (99.0)
TKN (mg/l)	383
NH_3-N (mg/l) (% of TKN)	18 (4.7)
CO_2 in gas (%)	7.5
T=60°C, pH=4.5, HRT=13.4hrs	
Parameters	Mean Value
Total COD (mg/l)	10,020
Filtered COD (mg/l)	9,240
TS (mg/l)	8,680
VTS (mg/l) (% of TS)	7,420 (85.5)
SS (mg/l)	370
VSS (mg/l) (% of SS)	350 (94.6)
TKN (mg/l)	413
NH_3-N (mg/l) (% of TKN)	17 (4.1)
CO_2 in gas (%)	9.8
T=65°C, pH=4.5, HRT=12.3hrs	
Parameters	Mean Value
Total COD (mg/l)	10,295
Filtered COD (mg/l)	9,090
TS (mg/l)	9,375
VTS (mg/l) (% of TS)	7,790 (83.1)
SS (mg/l)	235
VSS (mg/l) (% of SS)	230 (97.9)
TKN (mg/l)	406
NH_3-N (mg/l) (% of TKN)	18 (4.4)
CO_2 in gas (%)	7.5

From the VFA concentration it appeared that there was a small difference between HRT 12-13 hrs and 14-15 hrs. Furthermore although the acetate and propionate-producing bacteria were not affected by the HRT change, butyrate-producing bacteria had a slight increase in their activity with the increase in HRT.

In order to assess temperature effects a direct comparison in VFA concentration and composition of major acids for the 3 reactors could not be made, as there was a change of HRT during the different operational conditions that were applied. However at each set of experimental conditions comparison could be made only between Reactors 2 and 3 with the control Reactor 1 at 37°C. Assessment of the effect of temperature on acidification in reactors 2 and 3 could only be made indirectly at this stage by comparison to the control reactor, until more information could evaluate in greater detail the effect of HRT in acidification of coffee wastewaters.

Overall in relation to temperature changes 37°C was better than all thermophilic temperatures studied, both in terms of concentration and VFA composition. Only at temperature 45°C and pH 5.0 the results were slightly higher, but the differences were not significant.

pH 5.0 was better than 4.5 for acidogenesis. This was apparent mainly at temperatures from 37 to 55°C, while at 60 and 65°C the two pH values had a smaller effect on the concentration of VFA produced and mostly had small differences in VFA composition.

In relation to HRT changes caused during the experiment, it appeared that no significant difference was caused either in VFA concentration or composition. Generally though, it seemed that the higher HRT values around 15 hrs, had lower VFA production. However further studies and comparisons would be required to establish the effect of HRT on acidification of coffee wastewater.

In relation to principle acids produced, Acetic was the primary acid with n-Butyric second and Propionic third. These 3 acids made up almost 90% of VFA composition. The differences in composition caused by the applied conditions should be compared with the desired range of acids for methanogens, as presented by Andrews and Pearson (1965).

Finally the range of parameters examined proved adequate to present results on acidification of wastewaters.

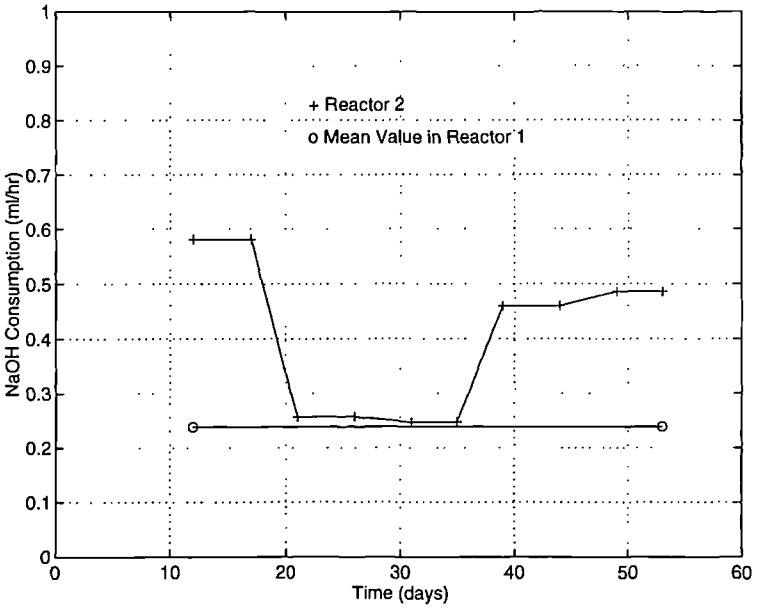


Figure 7.7: NaOH Consumption during Experiment 1

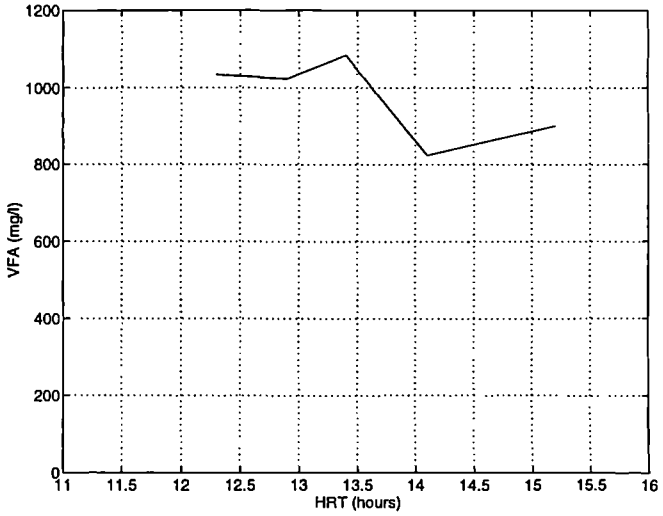


Figure 7.8: Effects on VFA concentration due to HRT changes in Reactor 1

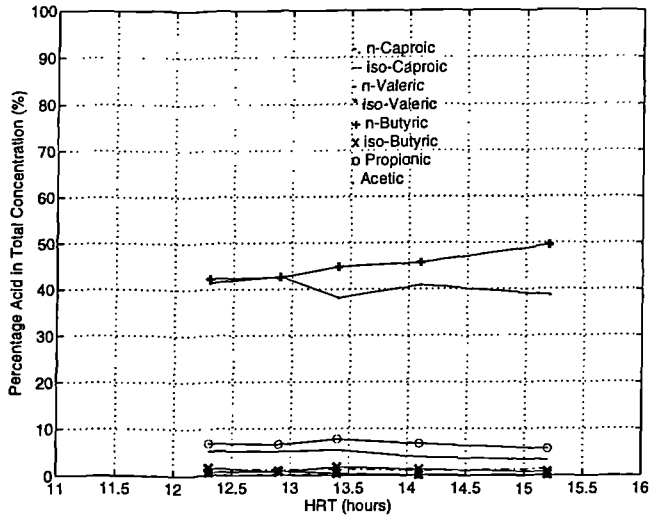


Figure 7.9: Effects on VFA composition due to HRT changes in Reactor 1

Chapter 8

EXPERIMENT 2: COFFEE-HRT

8.1 Introduction

8.1.1 General

In Experiment 2 different HRT values were to be applied to acidogenic reactors, in order to assess the potential of reduced HRT.

Additionally a study to assess pH effects on acidification of synthetic coffee wastewaters was carried out at the same time with the present study by another researcher, and found that pH 6.0 was better than 5.0 and 5.5, as pH 6.0 was producing the highest concentration of VFA (McDougall, 1996). This pH value would be tested operating reactors in comparison with a non-pH-controlled reactor. Temperature would be maintained at 37°C and compared to 45°C, as 45°C proved to be the only temperature to produce similar VFA results to 37°C from all the temperatures examined in Experiment 1 (Chapter 7).

The commercial nutrient mixture used from this Experiment onwards was Nutromex TEA or Nutromex Plus, which contained Nutromex N & P plus a range of trace metals (i.e. Co, Cu, Fe, Mn, Zn, etc.) carefully selected for anaerobic digestion plants (Anon., 1995). This was a new product by OMEX Environmental Ltd, supplied to Newcastle University Environmental Engineering laboratory when the previous supply of nutrient mixture finished by the end of Experiment 1.

8.1.2 Objectives

The objectives of Experiment 2 were:

Major:

- **Study the effect of HRT on acidification of a synthetic instant coffee wastewater.**

- **Compare the effect of optimal to no pH-control on acidification of synthetic instant coffee wastewater.**

  Minor:

- Assess the effects of the new type of commercial micro-nutrient applied.
- Assess further the potential of 45°C in comparison to 37°C.

8.2 Experimental Conditions

8.2.1 Operation

Experiment 2 started 4.5 months after the reactors were shut down in the end of Experiment 1. The total duration was approximately 87 days. There were 3 periods of collection of data of primary importance (a total of 18 days), while the rest of the time was used for acclimatisation (55 days for start-up at the beginning and 14 days on gradual HRT changes).

The conditions selected to operate the 3 acidogenic reactors during Experiment 2 are presented in Table 8.1.

Table 8.1: Experiment 2: Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature (°C)	45	37	37
pH	6.0	6.0	4.5
HRT tested (hrs)	12,9,6	12,9,6	12,9,6
COD:N:P	400:8.2:1	400:8.2:1	400:8.2:1
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

HRT changes in the reactors, during the experimental period are presented in Figure 8.1.

The characteristics of the synthetic instant coffee wastewater were already presented in Table 7.2 (Chapter 7).

The theoretical HRT value of 12, 9 and 6 hours, was originally calibrated for the influent pump of the 3 reactors and checked during the experiment. Volumes of feed consumed and duration of this consumption were used to calculate the applied HRTs for different stages of this experiment. Applied HRT values and their percentage differences in relation to the theoretical value, are presented in Table 8.2.

A difference greater than 10% was not observed for any of the applied HRT, so the studies can be based on the theoretical values.

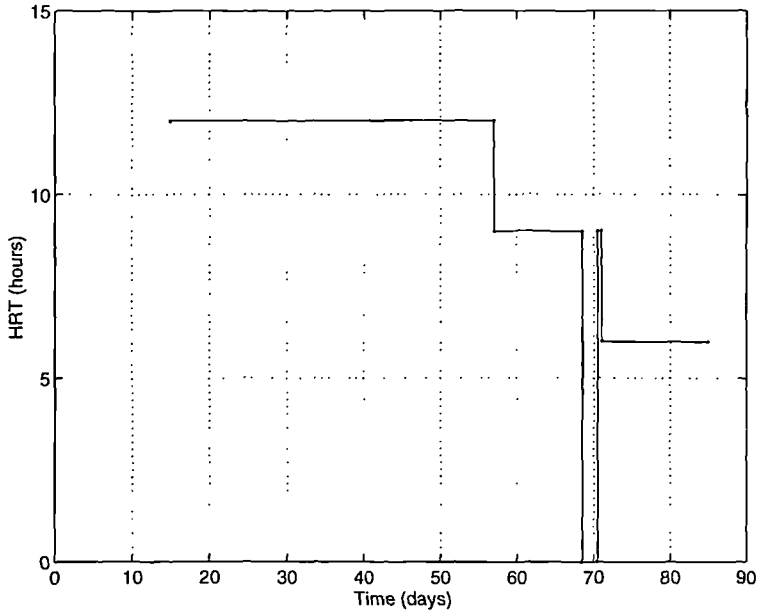


Figure 8.1: Changes in HRT during Experiment 2

Table 8.2: Applied HRT and per cent difference from the theoretical values of 12, 9 and 6 hrs

Period	HRT (hours)	Difference (%)
12 hrs	12.53	+4.42
9 hrs	8.90	-1.13
6 hrs	5.97	-0.55

OLR values were calculated with the mean value of the Filt.COD from Table 7.2 and the values of HRT presented in Figure 8.1. These values are presented in Figure 8.2.

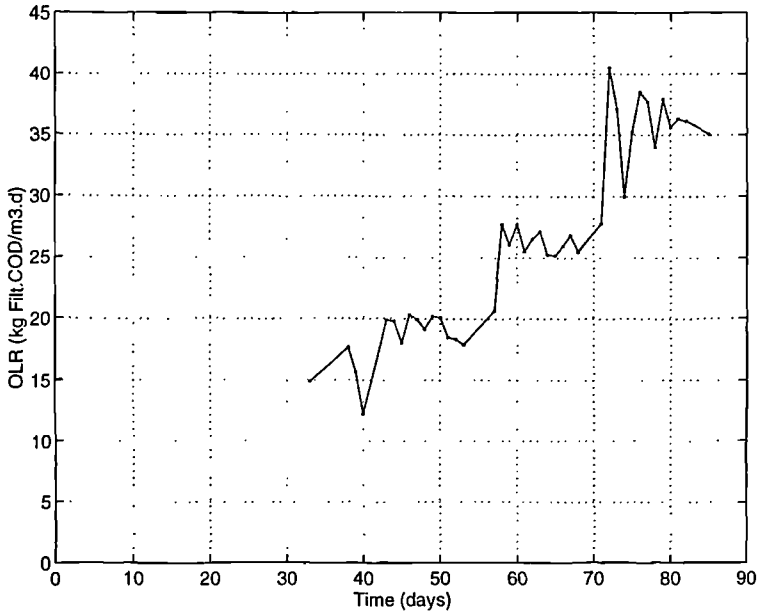


Figure 8.2: Organic Loading Rate during Experiment 2

In Table 8.3 information is presented about the ingredients of the synthetic wastewater, in comparison to theoretical values presented in Materials and Methods (Chapter 6). During this experiment the feed was prepared daily in the same way as in Experiment 1 (Chapter 7). The values presented are an average of each batch prepared. The total volume of feed prepared for the 3 reactors, is also presented.

Table 8.3: Ingredients of synthetic feed and total volume used during Experiment 2

Instant Coffee (gr/2l feed)	Urea (gr/2l feed)	OMEX (ml/2l feed)	Volume (l)
20.00	0.4133	0.2	506

8.2.2 Analyses & Diary of Problems

Type and frequency of analyses varied as in accordance to the practice in Experiment 1 (Chapter 7). During transition periods reactors 1, 2 and 3 were acclimatising gradually towards a new HRT value. The new HRT was applied to the system some time after midday. Before the HRT change a gas sample was analysed for gas composition. Also a sample of the effluent of the three reactors and the influent, were analysed for VFA concentration and composition of 8 acids.

The steady state periods were sets of 7 to 10 days. Every day analysis was done on two gas samples from each of the three reactors, the first around 8 o'clock in the morning and the second

12 hours later, in the evening. Also on a daily basis, two samples were collected from the three effluents and the influent, to analyse for VFA concentration and composition of 8 acids. These samples were collected on average every 10-12 hours, starting around 8 o'clock in the morning. In the first sample of the day Filt.COD analysis was carried out on a daily basis.

Additional analyses were carried out for the effluent of the three reactors, three days before the end and on the last day of a steady state period. A composite sample of each effluent was collected all through the night (for a period of approximately 10-12 hours). These samples would be analysed for Tot.COD, Tot.BOD, TS, VTS, SS, VSS, TKN, NH_3-N and PO_4-P. Also, at the beginning and end of each set, the volume of NaOH was measured in the pH-controllers used for reactors 1 and 2. All analyses were made with the same mode and number of dilutions, as in Experiment 1.

Problems that occurred during the operation of Experiment 2 and could have affected the steady state conditions were recorded. They were far less than the ones reported for Experiment 1. Also, they were of the type that did not give any noticeable effects on Experiment 2 (i.e. mainly disturbances from influent or effluent tubes being punctured, with changes of broken tubes lasting only small periods of time).

8.3 VFA

8.3.1 VFA as Total Concentration

In Figure 8.3 the Total VFA concentration is presented.

The concentrations of acids produced were higher than the ones produced in Experiment 1. VFA concentrations were around 1.0 to 2.0 g/l. This could be attributed to the better pH, but also the effect of the improved micro-nutrient mixture. Generally Reactor 2 appeared to be the one producing the most VFA, with Reactor 1 producing the least.

8.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is presented in Figure 8.4.

From the comparison of COD in VFA to influent COD it appeared that the extent of acidification was in the range of 20 to 40%. This was the desirable acidified range for pre-acidified wastewaters described in the literature (Lettinga & Hulshoff, 1991).

8.4 Statistical Analyses

In Tables 8.4, 8.5 and 8.6 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 2. All statistical values are prepared as

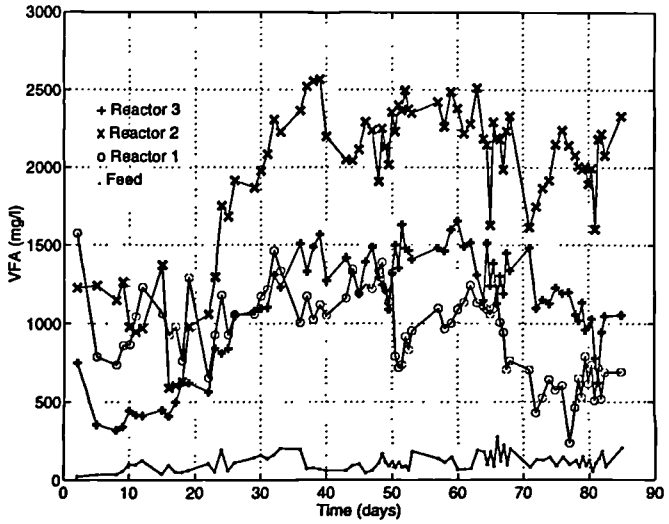


Figure 8.3: Total Volatile Fatty Acid Concentration during Experiment 2

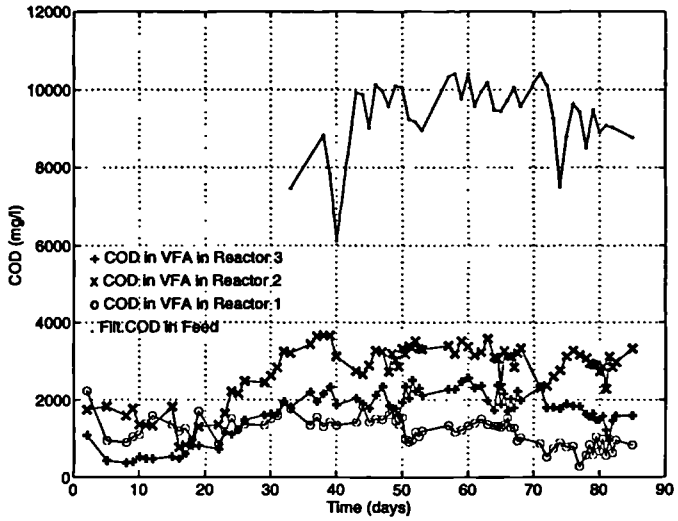


Figure 8.4: Feed Filt.COD & COD in VFA in Reactors during Experiment 2

already described in Materials and Methods (Chapter 6). The data for VFA concentration and composition for Experiment 2 can be found in Appendix D (Tables D.4, D.5 and D.6).

The duration that the three reactors were maintained under the operational conditions for HRT 12 hrs were equivalent to around 19 HRTs. While for the operational conditions at HRT 9 hrs this duration was equivalent to around 29 HRTs, and for HRT 6 hrs the duration was equivalent to around 55 HRTs.

The best VFA composition appeared to be the one for Reactor 1 with Acetic being significantly different from other acids. Meanwhile Reactor 2 had relatively similar percentage of Acetic and Propionic, although Acetic was mostly higher. Reactor 3 had a similar volume of Acetic and n-Butyric.

It was again obvious that Acetic, Propionic, n-Butyric and n-Valeric were the major components in the acidification of this type of wastewaters, while the other 4 acids appear only in minimal concentrations or they were not even detected. The changes in HRT do not seem to affect significantly the composition of VFA, apart from Reactor 1 at 45°C, that appeared to have a decrease in the activities of acetate-producing bacteria from 9 to 6 hrs.

8.5 Additional Analyses

In Tables 8.7, 8.8 and 8.9 the results of additional analyses are presented. The results were produced from the 2 samples taken for additional analyses during each set of conditions. Only the results on gas were based on daily measurements and the results on Filt.COD were from 5 data during each set of experimental conditions. These results were only an indication of the quality of the effluent from the 3 reactors.

From the presented values of Filt.COD, it appears that COD removal was up to 20%. Maximum Filt.COD removal appeared to be up to 17%, 22% and 19% for Reactors 1, 2 and 3 respectively. Measurements were carried out for Tot.COD and Tot.BOD, but these were found to have similar patterns to Filt.COD.

Similarly for TS and VTS, as for Filt.COD, these values only indicate the relative differences in the minimal removal of organic matter taking place in pre-acidification. Regarding SS and VSS values, it was observed that no major changes occurred in the biomass content due to HRT changes for Reactors 2 and 3. However Reactor 1 was most affected by the HRT changes from 12 to 9 hrs.

TKN values were of the same magnitude as in Experiment 1 (Chapter 7), without any noticeable changes due to HRT changes. Regarding NH_3-N it appeared that Reactor 2, which achieved 40% acidification, had the highest content of ammonia (up to 16% of TKN). This level of NH_3 showed a small decrease due to the reduction in HRT. Meanwhile, Reactor 1 and 3 had similar NH_3-N values to those in Experiment 1.

Table 8.4: Statistical data of Reactor 1 in Experiment 2

T=45°C, pH=6.0, HRT=12hrs, n=6 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	938	192	20.4	78	737–1,140
Acetic	663	177	26.6	72	478–849
Propionic	164	48	29.3	20	114–215
iso-Butyric	6	10	155.1	4	0–17
n-Butyric	80	33	41.4	14	45–115
iso-Valeric	9	14	155.2	6	0–24
n-Valeric	16	12	79.6	5	3–29
iso-Caproic	0	–	–	–	–
n-Caproic	0	–	–	–	–
T=45°C, pH=6.0, HRT=9hrs, n=6 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	930	159	17.1	65	763–1,097
Acetic	595	153	25.6	62	435–755
Propionic	197	42	21.5	17	153–242
iso-Butyric	0	–	–	–	–
n-Butyric	106	26	24.8	11	79–134
iso-Valeric	0	–	–	–	–
n-Valeric	31	14	45.2	6	16–46
iso-Caproic	0	–	–	–	–
n-Caproic	0	–	–	–	–
T=45°C, pH=6.0, HRT=6hrs, n=8 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	622	93	14.9	33	545–700
Acetic	484	69	14.4	25	426–542
Propionic	35	50	143.7	18	0–76
iso-Butyric	1	3	185.4	<1	0–4
n-Butyric	14	7	49.0	2	8–19
iso-Valeric	0	–	–	–	–
n-Valeric	89	88	99.2	31	15–163
iso-Caproic	0	–	–	–	–
n-Caproic	0	–	–	–	–

* units are (mg/l).

† units are (%).

Table 8.5: Statistical data of Reactor 2 in Experiment 2

T=37°C, pH=6.0, HRT=12hrs, n=9 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,291	148	6.5	49	2,177–2,405
Acetic	980	78	8.0	26	920–1,040
Propionic	761	52	6.8	17	721–801
iso-Butyric	<1	<1	300.0	<1	0–<1
n-Butyric	334	21	6.4	7	317–350
iso-Valeric	20	17	85.9	6	7–34
n-Valeric	178	15	8.2	5	167–189
iso-Caproic	0	–	–	–	–
n-Caproic	18	17	95.8	6	5–31
T=37°C, pH=6.0, HRT=9hrs, n=7 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,194	112	5.1	42	2,090–2,297
Acetic	930	46	5.0	18	887–973
Propionic	695	40	5.7	15	658–732
iso-Butyric	0	–	–	–	–
n-Butyric	319	18	5.6	7	302–335
iso-Valeric	8	<1	8.9	<1	7–9
n-Valeric	192	15	7.6	5	179–206
iso-Caproic	0	–	–	–	–
n-Caproic	50	4	8.9	2	46–54
T=37°C, pH=6.0, HRT=6hrs, n=8 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,018	84	4.2	30	1,948–2,088
Acetic	832	73	8.7	26	771–892
Propionic	640	38	6.0	14	608–672
iso-Butyric	0	–	–	–	–
n-Butyric	291	16	5.4	6	278–305
iso-Valeric	7	3	41.5	1	4–9
n-Valeric	205	15	7.5	5	192–218
iso-Caproic	0	–	–	–	–
n-Caproic	42	5	10.9	2	38–46

* units are (mg/l).

† units are (%).

Table 8.6: Statistical data of Reactor 3 in Experiment 2

T=37°C, pH=4.5, HRT=12hrs, n=9 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,386	138	9.9	46	1,281–1,492
Acetic	594	42	7.1	14	562–626
Propionic	53	4	8.2	1	49–56
iso-Butyric	0	–	–	–	–
n-Butyric	613	60	9.8	20	567–659
iso-Valeric	9	18	198.4	6	0–23
n-Valeric	95	87	92.3	29	28–162
iso-Caproic	<1	<1	300.0	<1	0–<1
n-Caproic	23	22	96.5	7	6–40
T=37°C, pH=4.5, HRT=9hrs, n=8 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,317	131	9.9	46	1,207–1,426
Acetic	523	49	9.3	17	483–564
Propionic	97	9	8.8	3	90–104
iso-Butyric	0	–	–	–	–
n-Butyric	504	20	3.9	7	488–521
iso-Valeric	0	–	–	–	–
n-Valeric	150	87	58.4	31	77–223
iso-Caproic	0	–	–	–	–
n-Caproic	42	2	4.0	<1	41–44
T=37°C, pH=4.5, HRT=6hrs, n=7 data					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	967	90	9.3	34	885–1,050
Acetic	383	42	11.1	16	344–423
Propionic	21	38	178.0	14	0–57
iso-Butyric	0	–	–	–	–
n-Butyric	514	41	8.0	16	475–552
iso-Valeric	0	–	–	–	–
n-Valeric	22	3	15.2	1	19–25
iso-Caproic	0	–	–	–	–
n-Caproic	27	3	9.3	<1	25–30

* units are (mg/l).

† units are (%).

The values presented for PO_4-P appeared similar to the influent ones. However it could be indicated that there was a small difference from the influent PO_4-P values for Reactors 1 and especially 2 (Table 8.7 & 8.8).

Gas was only recorded as composition. Attempts were made to measure volume produced, again without success, so the measurement was dropped in the middle of this experiment.

In terms of CO_2 composition it appeared that Reactor 2 had the highest content, and Reactor 1 had the least. Again after 22 days from the start-up of this experiment methanogenic activity appeared and established when conditions became steady in the operation of the experiment. After HRT of 6 hrs was applied the activity slowly diminished, giving an indication of the growth rate of the methanogens involved. As in Experiment 1 (Chapter 7) this methanogenic activity could be attributed to hydrogen-oxidising methanogens (Eckenfelder, 1992).

The values of CO_2 composition in biogas were within the range found by the studies of Zoetemeyer et al. (1982a, 1982b). As in Experiment 1 the residual unknown composition in the biogas could be assumed to be mostly H_2 (Popel, 1964).

The fact that at HRT 6 hrs methanogenic activities were minimised to the level of trace detection, demonstrated how HRT reduction could eventually preclude all methanogenic activities from an acidogenic reactor.

8.5.1 NaOH Consumption

NaOH consumption is reported as ml consumed per hour of operation. The results are presented in Figure 8.5.

The magnitude of the alkali consumption presented was up to 8 times higher than that recorded for Experiment 1 (Chapter 7). It is also worth pointing out that alkali consumption was more closely related to the high rate of VFA production, than to the actual VFA concentration. The latter is an interesting point for process design, especially when conditioning tanks operate both as pre-acidification units and for pH-adjustment for the methanogenic reactor. Also, it is interesting to point out the similarity in the consumption between reactors 1 and 2, although they had a different range of VFA production, as was also noticed in Experiment 1 (Chapter 7).

8.6 Key Points for Discussion

In Figures 8.6, 8.7, 8.8 and 8.9 the effect due to HRT changes in VFA concentration and the percentage of the 3 major acids for the 3 reactors is presented.

In relation to HRT it became obvious that none of the reactors had any significant changes in the production of VFA. A shift was mainly observed in composition of acids (less Acetic, more Propionic or n-Butyric, depending on the reactor). This observation is of great significance for process design as not only it reflected an obvious reduction (up to half size) in the volume of

Table 8.7: Additional Analyses of Reactor 1 in Experiment 2

T=45°C, pH=6.0, HRT=12hrs	
Parameters	Mean Value
Total COD (mg/l)	8,685
Filtered COD (mg/l)	7,980
Total BOD (mg/l)	3,960
TS (mg/l)	8,680
VTS (mg/l) (% of TS)	4,690 (54.0)
SS (mg/l)	440
VSS (mg/l) (% of SS)	420 (95.4)
TKN (mg/l)	355
NH_3-N (mg/l) (% of TKN)	19 (5.4)
PO_4-P (mg/l)	17
CO_2 in gas (%)	27.0
T=45°C, pH=6.0, HRT=9hrs	
Parameters	Mean Value
Total COD (mg/l)	9,530
Filtered COD (mg/l)	8,180
Total BOD (mg/l)	3,200
TS (mg/l)	9,450
VTS (mg/l) (% of TS)	5,940 (62.8)
SS (mg/l)	290
VSS (mg/l) (% of SS)	285 (99.1)
TKN (mg/l)	365
NH_3-N (mg/l) (% of TKN)	36 (9.8)
PO_4-P (mg/l)	17
CO_2 in gas (%)	20.5
T=45°C, pH=6.0, HRT=6hrs,	
Parameters	Mean Value
Total COD (mg/l)	9,485
Filtered COD (mg/l)	7,875
Total BOD (mg/l)	3,710
TS (mg/l)	9,430
VTS (mg/l) (% of TS)	6,220 (66.0)
SS (mg/l)	280
VSS (mg/l) (% of SS)	260 (92.3)
TKN (mg/l)	365
NH_3-N (mg/l) (% of TKN)	18 (4.9)
PO_4-P (mg/l)	18
CO_2 in gas (%)	8.4

Table 8.8: Additional Analyses of Reactor 2 in Experiment 2

T=37°C, pH=6.0, HRT=12hrs	
Parameters	Mean Value
Total COD (mg/l)	8,985
Filtered COD (mg/l)	7,735
Total BOD (mg/l)	4,325
TS (mg/l)	7,780
VTS (mg/l) (% of TS)	4,190 (53.9)
SS (mg/l)	780
VSS (mg/l) (% of SS)	740 (94.9)
TKN (mg/l)	358
NH_3-N (mg/l) (% of TKN)	58 (16.1)
PO_4-P (mg/l)	17
CO_2 in gas (%)	63.6
CH_4 in gas (%)	8.3
T=37°C, pH=6.0, HRT=9hrs	
Parameters	Mean Value
Total COD (mg/l)	9,990
Filtered COD (mg/l)	7,685
Total BOD (mg/l)	3,290
TS (mg/l)	8,980
VTS (mg/l) (% of TS)	5,680 (63.2)
SS (mg/l)	810
VSS (mg/l) (% of SS)	805 (99.2)
TKN (mg/l)	391
NH_3-N (mg/l) (% of TKN)	53 (13.4)
PO_4-P (mg/l)	15
CO_2 in gas (%)	55.0
CH_4 in gas (%)	9.2
T=37°C, pH=6.0, HRT=6hrs	
Parameters	Mean Value
Total COD (mg/l)	9,905
Filtered COD (mg/l)	7,035
Total BOD (mg/l)	3,860
TS (mg/l)	8,330
VTS (mg/l) (% of TS)	5,300 (63.6)
SS (mg/l)	695
VSS (mg/l) (% of SS)	660 (94.6)
TKN (mg/l)	383
NH_3-N (mg/l) (% of TKN)	49 (12.7)
PO_4-P (mg/l)	15
CO_2 in gas (%)	57.2
CH_4 in gas (%)	2.2

Table 8.9: Additional Analyses of Reactor 3 in Experiment 2

T=37°C, pH=4.5, HRT=12hrs	
Parameters	Mean Value
Total COD (mg/l)	9,840
Filtered COD (mg/l)	7,760
Total BOD (mg/l)	4,260
TS (mg/l)	7,060
VTS (mg/l) (% of TS)	5,900 (83.7)
SS (mg/l)	630
VSS (mg/l) (% of SS)	615 (97.2)
TKN (mg/l)	399
NH_3-N (mg/l) (% of TKN)	14 (3.5)
PO_4-P (mg/l)	22
CO_2 in gas (%)	40.1
T=37°C, pH=4.5, HRT=9hrs	
Parameters	Mean Value
Total COD (mg/l)	10,305
Filtered COD (mg/l)	8,020
Total BOD (mg/l)	3,240
TS (mg/l)	7,670
VTS (mg/l) (% of TS)	6,490 (84.6)
SS (mg/l)	555
VSS (mg/l) (% of SS)	550 (99.1)
TKN (mg/l)	402
NH_3-N (mg/l) (% of TKN)	12 (3.1)
PO_4-P (mg/l)	20
CO_2 in gas (%)	36.3
T=37°C, pH=4.5, HRT=6hrs	
Parameters	Mean Value
Total COD (mg/l)	10,660
Filtered COD (mg/l)	7,415
Total BOD (mg/l)	3,420
TS (mg/l)	7,590
VTS (mg/l) (% of TS)	6,710 (88.4)
SS (mg/l)	590
VSS (mg/l) (% of SS)	585 (99.6)
TKN (mg/l)	390
NH_3-N (mg/l) (% of TKN)	15 (3.9)
PO_4-P (mg/l)	20
CO_2 in gas (%)	32.4

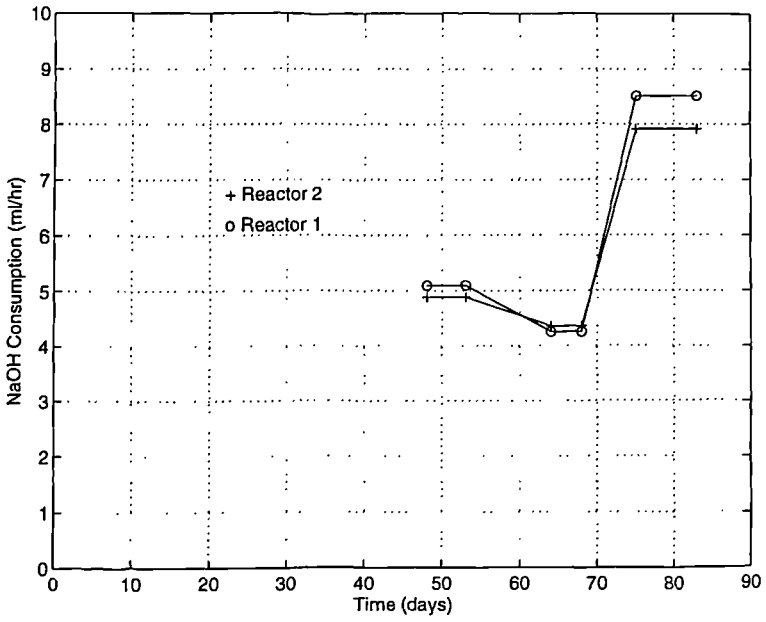


Figure 8.5: NaOH Consumption during Experiment 2

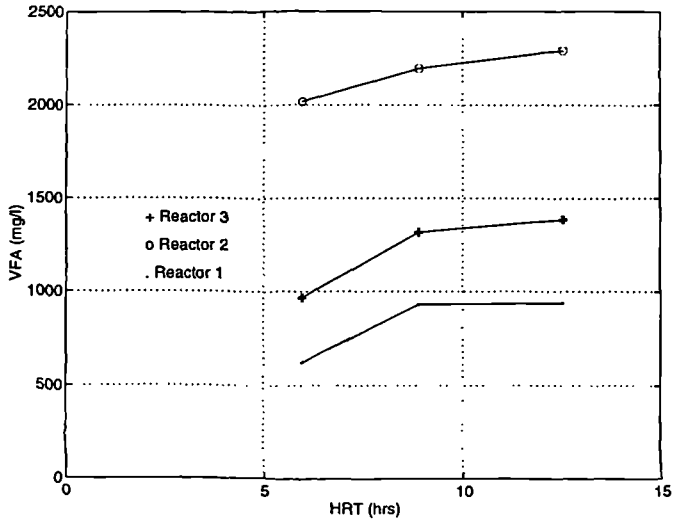
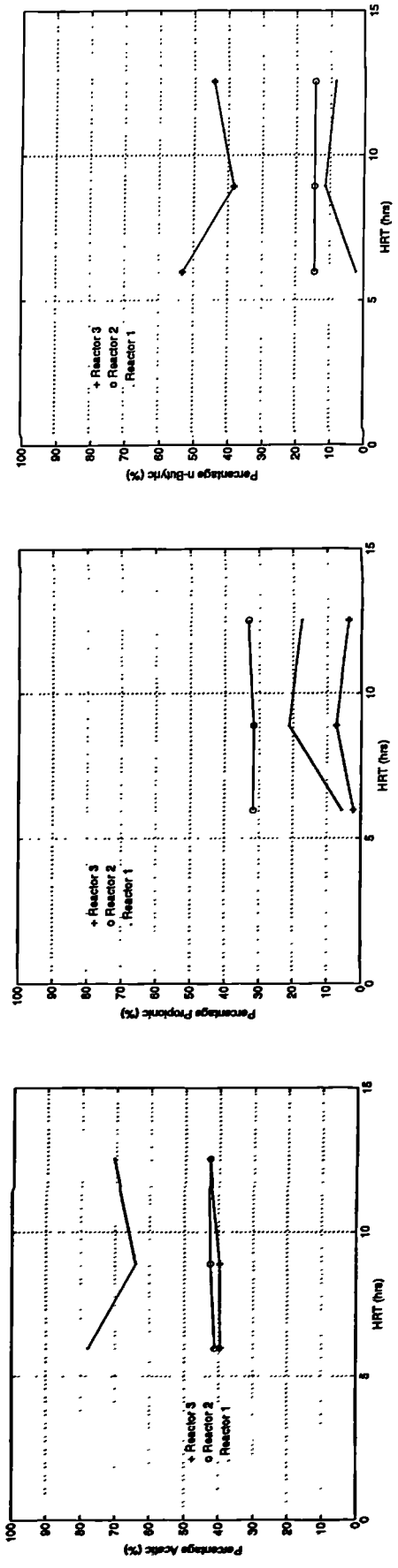


Figure 8.6: Effects on VFA concentration due to HRT changes



(a) Acetic acid (b) Propionic acid (c) n-Butyric acid

Figure 8.7: Effects of HRT on VFA composition with coffee wastewaters

the reactor, but it implied a significant increase in the VFA production rate.

pH 6.0 was better for acidogenesis of coffee wastewaters than 4.5 and the previously examined pH 5.0 (Experiment 1, Chapter 7) at the temperature of 37°C. However at 45°C, pH 6.0 appeared to be worse than pH 4.5 and 37°C. Furthermore in Experiment 1, pH 5.0 was better than pH 4.5 at 45°C. These observed differences related to different combinations of pH and temperatures indicated the significance in the role of these two design parameters, and proved that overall the effect on acidification was affected by both parameters and that both parameters were connected with an inter-relationship. Although such an inter-relationship became apparent the temperature effect appeared to be of greater importance that the one of pH. Overall 37°C was better than 45°C at all applied HRT values.

Furthermore, the magnitude of the VFA production had changed compared to the VFA concentrations produced in Experiment 1 for all reactors. This increased VFA production could be observed in Reactor 3 with pH 4.5 that was operating at the same pH during Experiment 1 although within different temperatures. Such an increase (almost double of the previous VFA yield and Acidified COD values for Reactor 3) indicated that the new commercial micro-nutrient mixture, richer in trace metals required by anaerobic bacteria, had a positive effect on acidification of nutrient deficient wastewaters such as coffee wastewaters. Similar observations about the effect of the micro-nutrient mixture were observed by McDougall (1996) with a decrease in the performance of the pre-acidification reactors at a period that he run out of mixture supplies for several days.

As in Experiment 1 the major acid produced was Acetic, with n-Butyric second and Propionic last for Reactors 1 and 3. As for Reactor 2 Propionic acid was the second best. These 3 acids made almost 85% of the VFA composition.

Two more interesting engineering points had emerged from Experiment 2. The first regarded alkali consumption and the increase observed with the decrease in HRT, which was not affected by different VFA production rates. The second was the apparent elimination of methanogenic activities at pH 6.0 with a decrease in HRT.

20-40% Acidified COD was achieved with the described conditions in Experiment 2. So it could be suggested that the most economic pre-acidification design to achieve almost 40% acidified COD for coffee wastewaters would be to apply 37°, pH 6.0, HRT around 6 to 9 hrs, and the use of a nutritional mixture for anaerobic digesters that includes trace metals. This would enable stable operation of a two-phase process for the treatment of this recalcitrant wastewater. Similar optimum operation for pre-acidification of coffee wastewaters was reported by McDougall (1996) suggesting HRT of 12 hrs.

Chapter 9

EXPERIMENT 3: SLAUGHTERHOUSE-TEMPERATURE

9.1 Introduction

9.1.1 General

The use of ambient to warm waters (15-30°C) to clean slaughterhouses initiated the thought to apply mesophilic two-phase anaerobic digestion for wastewater treatment. So in Experiment 3 different mesophilic and the optimum mesophilic temperature were to be applied in acidogenic reactors, in order to assess their potential to acidify slaughterhouse wastewaters.

Additionally an initial assessment would be done to evaluate pH effects. These would be tested operating reactors under non-pH-controlled (pH=7.0) and pH-controlled (pH=6.0) conditions. pH 6.0 was found to be the optimum pH by the present studies for acidification of coffee wastewaters (Chapters 7 & 8) and was also reported as optimum by Zoetemeyer et al. (1982a) for acidification of glucose. So the value of pH 6.0 was chosen with the intention to operate optimal pH ranges (5.5-6.0) for acidogenesis, but also tending towards the neutral pH-nature of this wastewater for economy.

At that stage there were no previous studies about HRT or nutrient requirements for acidogenic systems treating similar wastewaters. So HRT and COD:N:P ratio were selected on an empirical basis. Also a commercial micro-nutrient mixture, as stated in Chapter 8 (section 8.1.1), was added on an empirical basis.

9.1.2 Objectives

The objectives of Experiment 3 were:

Major:

- **Study the effect of mesophilic temperatures on acidification of a real slaughterhouse wastewater in comparison with the optimum mesophilic temperature (37°C).**
- **Compare the effect of "optimum" to no pH-control on acidification of real slaughterhouse wastewater.**

Minor:

- Analyse a wide range of parameters so as to gain adequate understanding of data handling for acidogenic phenomena in relation to real wastewaters.

9.2 Experimental Conditions

9.2.1 Operation

Experiment 3 started after 2 weeks break from the coffee experiments. The total duration was approximately 97 days. There were 5 periods of collection of data of primary importance (a total of 37 days), while the rest of the time was used for acclimatisation (12 days for the change of wastewater in the beginning and 48 days on gradual temperature changes).

The conditions the 3 acidogenic reactors operated during Experiment 3 are presented in Table 9.1.

Table 9.1: Experiment 3: Theoretical Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s) tested (°C)	37	25,30,35 40,45	25,30,35 40,45
pH	6.0	6.0	7.0
HRT (hours)	12	12	12
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

Temperature changes during transition periods in the water-bath of reactors 2 and 3 were 1°C/day in order to achieve minor disturbances while acclimatising to another temperature, as practiced in Experiment 1 (Chapter 7). Temperature changes during the experimental period are presented in Figure 9.1.

The main characteristics of the fresh slaughterhouse wastewater are presented in Table 9.2. These values are averages of all measurements made on the feed of the 3 reactors in Experiments 3, 4, 5 and 6 (Chapters 9-12), which studied acidification of slaughterhouse wastewaters. The extent of variation observed in the data of the collected samples on a weekly basis should be noted. The variations in Filt.COD during the day to day operation in the laboratory showed even greater variations.

It should be noted that slaughterhouse wastewater was quite similar in COD strength to the synthetic coffee wastewater. The main difference was that slaughterhouse is considered to be an easily biodegradable wastewater containing mainly proteins and lipids, while coffee wastewater is characterised as a recalcitrant type of wastewater containing easily biodegradable and complex carbohydrates and some fats.

The theoretical HRT value of 12 hrs was originally calibrated in the influent pump of the 3 reactors and checked during the experiment. The average HRT was 12.27 hrs which had a difference of +2.25% from the theoretical value. Therefore the results of this experiment could be assessed with the theoretical HRT value.

OLR values were calculated from the Filt.COD which was daily measured and the theoretical HRT. These values are presented in Figure 9.2. The great variation in OLR was the main reason that a direct comparison between applied temperatures would not be possible. However only a comparison could be made in relation to the control Reactor 1 at 37°C, until the effect of OLR on acidification of slaughterhouse wastewater would become better understood through further studies.

9.2.2 Analyses & Diary of Problems

Type and frequency of analyses varied according to the practices already mentioned for Experiment 1 and 2 (Chapters 7 & 8). The steady state periods were sets of 6 to 11 days, as more days were required to establish the balance in a steady OLR value. Daily analyses were done as in Experiment 2. Also samples were analysed as in Experiment 2. At the beginning and the end of each set the volume of HCl was measured for the pH-controllers used for Reactors 1 and 2.

No particular problems occurred during Experiment 3 that should be reported. As in Experiment 2 (Chapter 8) the problems recorded were all of minor effect to the performance of the reactors.

9.2.3 Filtered COD

Filtered COD analysis is presented in Figure 9.3. This graph shows the extent of variation experienced with the use of real wastewaters on a daily basis. It also showed the direct effect that the influent Filt.COD had in the effluent Filt.COD of the 3 reactors. Furthermore it became apparent that no significant COD removal took place (maximum values of around 30% for Filt.COD removal could be estimated, especially at low OLR values).

It appeared from the magnitude of the COD fluctuations that 40°C was the only temperature that could not be compared with other temperature results, as there was no satisfactory OLR stability during the collection of data at this operational condition.

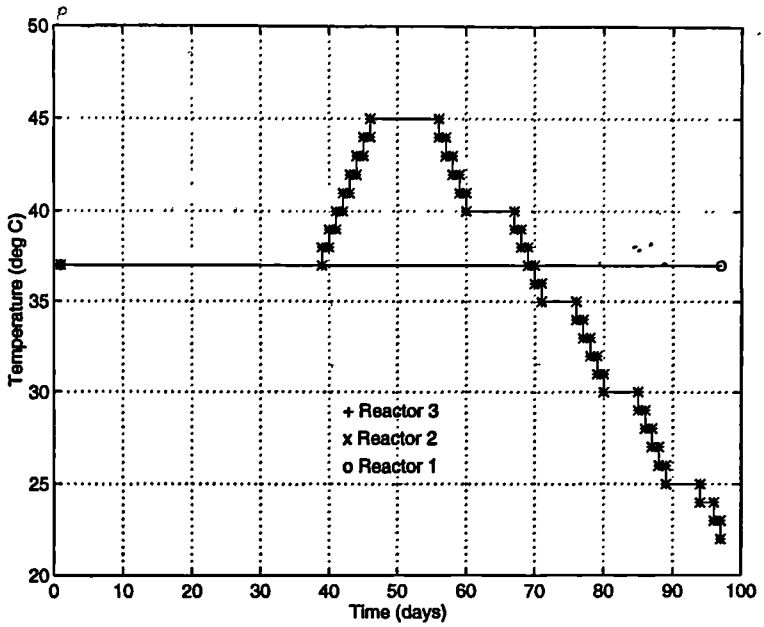


Figure 9.1: Changes in Temperature during Experiment 3

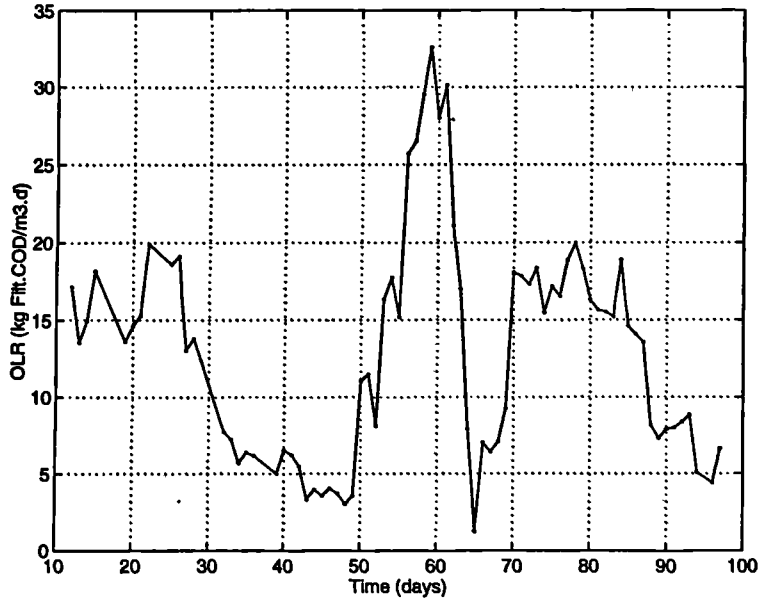


Figure 9.2: Organic Loading Rate during Experiment 3

Table 9.2: Real Slaughterhouse Wastewater Characteristics

Parameter	Mean	Minimum	Maximum	Standard Deviation
Total COD (mg/l)	9,066	2,725	13,325	4,196
Filtered COD (mg/l)	8,114	2,425	12,150	3,773
Total BOD (mg/l)	5,314	1,250	8,000	2,648
Total Solids (mg/l)	7,311	2,230	10,930	3,090
Volatile Total Solids (mg/l)	6,384	1,635	9,915	2,985
Suspended Solids (mg/l)	606	235	1,215	293
Volatile Suspended Solids (mg/l)	580	230	1,160	277
Total Kjeldahl Nitrogen (mg/l)	819	250	1,435	488
Ammonia-N (mg/l)	45	19	95	23
Phosphate-P (mg/l)	13	4	31	9.4
Fats (mg/l)	110	35	210	66
Volatile Fatty Acids (mg/l)	224	50	509	147
Acetic Acid (mg/l) (& % in VFA)	117 (52.2)	27	281	77
Propionic Acid (mg/l) (& % in VFA)	39 (17.4)	9	88	26
iso-Butyric Acid (mg/l) (& % in VFA)	12 (5.4)	2	34	11
n-Butyric Acid (mg/l) (& % in VFA)	27 (12.1)	5	57	20
iso-Valeric Acid (mg/l) (& % in VFA)	18 (8.0)	5	40	14
n-Valeric Acid (mg/l) (& % in VFA)	7 (3.1)	1	25	8
iso-Caproic Acid (mg/l) (& % in VFA)	3 (1.3)	0	7	3
n-Caproic Acid (mg/l) (& % in VFA)	1 (0.4)	0	3	1
COD in VFA (mg/l)	314	67	704	—
Acidified COD (%)	3.9	0.6	10.9	—
Ratio Tot.COD/Filt.COD	1.12	1.07	1.18	—
Ratio Tot.COD/Tot.BOD	1.71	1.57	2.18	—
Ratio Tot.COD/Tot.Solids	1.24	0.89	1.43	—
Percentage VTS in TS (%)	87.3	73.4	91.8	—
Percentage VSS in SS (%)	95.7	92.6	100	—
Percentage NH_3-N in TKN (%)	5.5	1.4	19.0	—
Ratio Tot.COD:N	400:19.1	400:4.9	400:25.4	—
Ratio Tot.COD:P	400:0.6	400:0.2	400:2.8	—
pH	7.2	6.7	7.3	—
Temperature	15°-20°C			—

9.3 VFA

9.3.1 VFA as Total Concentration

In Figure 9.4 the Total VFA concentration is presented. The magnitude of VFA produced was higher than that experienced with synthetic coffee wastewaters. VFA concentrations were ranging from 0.2 to 3.0 g/l, depending on operational conditions and the applied OLR.

Also it appeared that Reactor 1 at 37°C and pH 6.0 produced higher VFA concentrations than Reactor 2 at the same pH but with temperature changes. Reactor 3 with pH 7.0 produced far greater VFA concentrations at all temperatures, than Reactors 1 and 2. This was an obvious result of the effect of the different pH value applied rather than the effect of the temperature.

9.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is given in Figure 9.5.

This comparison provides information to assess the extent of acidified matter, under the given conditions. The degree of acidification ranged from 20 to 85% (on one occasion more than 90%). This degree was higher than the degree of acidification experienced with synthetic coffee wastewaters. It appeared that a higher degree of acidified matter was always produced from Reactor 3 with pH 7.0.

9.4 Statistical Analyses

In Tables 9.3, 9.4 and 9.5 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 3. The values in each set are presented with the mean Filt.COD in the feed, in order to be used for indirect comparisons with the control reactor (Reactor 1).

The data on VFA for Experiment 3 are presented in Appendix D (Tables D.7, D.8 and D.9). Also all statistical evaluations are based on what has been described in Materials and Methods (Chapter 6).

The first set of operational conditions that were calculated for Reactor 1 were collected for a duration equivalent to around 14 HRTs in order to be used for a comparison on the effects of the changing OLR on this control reactor. Unfortunately there was no steady state established in the two other reactors at the same duration for this specific OLR, as Reactors 2 and 3 were acclimatising on 45°C during the period that this OLR was applied.

The next set of data for Reactor 1 and the first set of data for Reactors 2 and 3 was applied for a duration that was equivalent to around 8 HRTs. So an adequate amount of data was

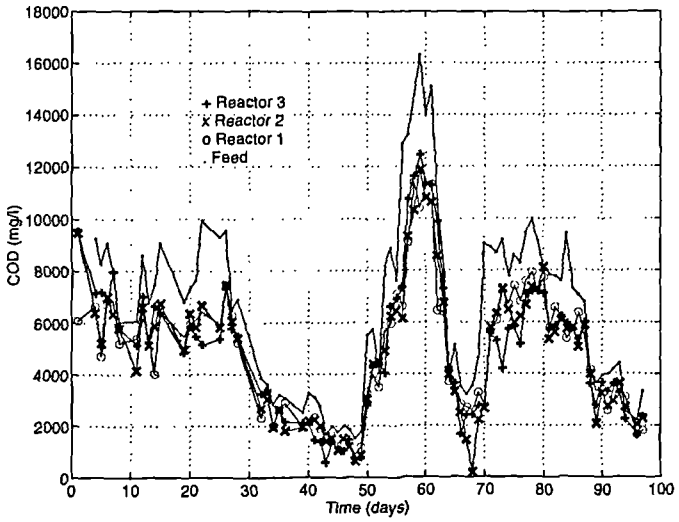


Figure 9.3: Filtered COD during Experiment 3

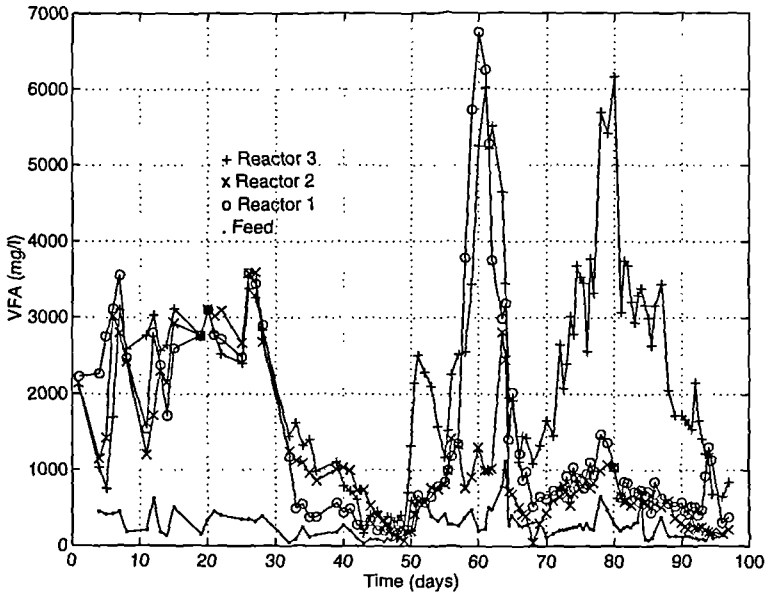


Figure 9.4: Total Volatile Fatty Acid Concentration during Experiment 3

collected in order to assess the effect of 45°C on acidification during this operational conditions. Unfortunately there was no stability in OLR during the time that the studies were carried out for 40°C, so as to be able to evaluate this period on a steady state operation.

However the third set of data, related to a new OLR, that was applied on Reactor 1 had a total duration equivalent to around 30 HRTs. This OLR was maintained through the second and third set of operational conditions evaluated for Reactors 2 and 3, with a duration equivalent to around 10 HRTs for the studies on temperatures 35 and 30°C.

Finally the fourth set of data for Reactor 1 was applied for a duration equivalent to around 12 HRTs. This last OLR and steady state was applied for a duration equivalent to around 10 HRTs for Reactors 2 and 3, when they were both operating at 25°C.

Acetic appeared to be the dominant acid for all 3 systems. Although Acetic was obviously the major produced acid for Reactor 1, occasionally in Reactor 2 and 3 Propionic was higher.

Among the main acids was n-Butyric which was produced in similar proportion to iso- Butyric, iso-Valeric and on occasions n-Valeric. Although iso-Butyric was produced in a high proportion (>5%) n-Butyric was always in a higher proportion. However iso- Valeric was more comparable to n-Butyric than iso-Butyric, and quite similar in proportion for all reactors.

It appeared for all reactors that the 4 main acids in VFA composition were Acetic, Propionic, n-Butyric and iso-Valeric. n-Valeric was a minor acid although occasionally it reached as much as 5%.

9.5 Additional Analyses

In Tables 9.6, 9.7 and 9.8 the results of additional analyses are presented. The results were produced from two samples taken for additional analyses during each set of conditions, that would be used for presentation only if they were taken at a period that stable OLR was applied. Otherwise just one sample would be used.

Gas composition results were produced from two daily measurements. All additional analyses are presented to give an indication of the quality of the effluent from the 3 reactors, and few general points could derive from them.

Total COD and BOD variations were according to those occurring in Filt.COD from the different OLR values. The magnitude of removal seemed of even less significance than with Filt.COD.

Similar observations were made with TS and VTS. The only interesting point is that at temperatures below 35°C, Reactor 3 with pH 7.0 appeared to have lower effluent values for both TS and VTS.

SS and VSS appeared to have a reducing trend after the temperature of 35°C was applied, which could possibly be an indication of changes in biomass or the decreasing values in the applied

Table 9.3: Statistical data of Reactor 1 in Experiment 3

T=37°C, pH=6.0, HRT=12hrs, n=8 data, Filt.COD$_f$=1,790 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	212	16	7.7	6	199-226
Acetic	105	8	7.5	3	99-112
Propionic	24	3	11.0	<1	22-26
iso-Butyric	20	1	7.1	<1	19-21
n-Butyric	18	3	14.5	<1	16-20
iso-Valeric	35	5	13.6	2	31-39
n-Valeric	6	1	22.3	<1	5-7
iso-Caproic	4	2	41.8	<1	2-5
n-Caproic	<1	<1	118.8	<1	<1-<1
T=37°C, pH=6.0, HRT=12hrs, n=4 data, Filt.COD$_f$=5,110 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	603	70	11.7	35	491-714
Acetic	254	29	11.5	15	208-301
Propionic	140	23	16.8	12	102-177
iso-Butyric	41	4	10.4	2	35-48
n-Butyric	72	9	12.1	4	58-85
iso-Valeric	68	11	16.0	5	51-85
n-Valeric	16	1	6.7	<1	14-17
iso-Caproic	11	2	17.6	<1	8-14
n-Caproic	<1	<1	83.0	<1	0-1
T=37°C, pH=6.0, HRT=12hrs, n=15 data, Filt.COD$_f$=8,290 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	783	107	13.7	28	724-842
Acetic	274	39	14.2	10	252-295
Propionic	168	34	20.0	9	149-186
iso-Butyric	76	12	16.4	3	69-83
n-Butyric	112	15	13.1	4	104-120
iso-Valeric	109	14	12.6	4	101-116
n-Valeric	30	10	32.1	2	25-35
iso-Caproic	15	6	38.9	1	12-18
n-Caproic	<1	<1	149.3	<1	<1-<1
T=37°C, pH=6.0, HRT=12hrs, n=8 data, Filt.COD$_f$=4,040 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	488	58	11.9	21	439-536
Acetic	174	17	10.0	6	159-188
Propionic	96	12	12.0	4	86-106
iso-Butyric	53	6	11.3	2	48-58
n-Butyric	61	7	11.3	2	55-66
iso-Valeric	87	21	23.9	7	70-105
n-Valeric	11	2	18.9	<1	9-13
iso-Caproic	6	1	22.9	<1	5-7
n-Caproic	0	–	–	–	–

* units are (mg/l).

† units are (%).

Table 9.4: Statistical data of Reactor 2 in Experiment 3

T=45°C, pH=6.0, HRT=12hrs, n=2 data, Filt.COD$_f$=5,110 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	584	10	1.7	7	493-676
Acetic	276	21	7.8	15	84-468
Propionic	120	6	4.7	4	69-171
iso-Butyric	39	1	2.7	<1	30-49
n-Butyric	60	9	15.0	6	0-141
iso-Valeric	68	3	3.9	2	44-93
n-Valeric	11	<1	4.7	<1	6-15
iso-Caproic	10	<1	4.5	<1	6-13
n-Caproic	<1	<1	55.5	<1	0-2
T=35°C, pH=6.0, HRT=12hrs, n=9 data, Filt.COD$_f$=8,290 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	757	108	14.3	36	674-840
Acetic	230	35	15.4	12	203-257
Propionic	233	74	31.8	25	176-290
iso-Butyric	48	40	84.2	13	17-79
n-Butyric	105	16	15.5	5	92-117
iso-Valeric	109	16	14.7	5	97-121
n-Valeric	19	6	33.6	2	14-24
iso-Caproic	13	4	27.6	1	10-15
n-Caproic	<1	<1	300.0	<1	0-<1
T=30°C, pH=6.0, HRT=12hrs, n=9 data, Filt.COD$_f$=8,290 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	606	51	8.5	17	567-646
Acetic	199	20	9.9	7	184-214
Propionic	130	10	7.9	3	122-138
iso-Butyric	64	7	10.3	2	59-69
n-Butyric	67	20	29.9	7	52-82
iso-Valeric	96	12	12.7	4	86-105
n-Valeric	44	13	29.3	4	34-53
iso-Caproic	7	2	24.3	<1	6-8
n-Caproic	<1	<1	158.4	<1	0-<1
T=25°C, pH=6.0, HRT=12hrs, n=6 data, Filt.COD$_f$=4,040 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	251	30	11.9	12	220-283
Acetic	105	14	13.2	6	90-119
Propionic	37	6	16.9	3	31-44
iso-Butyric	24	4	16.0	2	20-28
n-Butyric	30	5	16.4	2	24-35
iso-Valeric	45	7	15.4	3	38-52
n-Valeric	6	2	25.7	<1	4-8
iso-Caproic	5	<1	13.2	<1	4-6
n-Caproic	0	–	–	–	–

* units are (mg/l).

† units are (%).

Table 9.5: Statistical data of Reactor 3 in Experiment 3

T=45°C, pH=7.0, HRT=12hrs, n=4 data, Filt.COD$_f$=5,110 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,260	182	8.1	91	1,969-2,550
Acetic	729	49	6.7	24	652-807
Propionic	414	63	15.2	31	313-514
iso-Butyric	240	16	6.5	8	216-265
n-Butyric	401	54	13.4	27	315-487
iso-Valeric	361	42	11.7	21	294-428
n-Valeric	78	21	27.0	10	44-111
iso-Caproic	36	33	89.6	16	0-88
n-Caproic	<1	<1	95.1	<1	0-1
T=35°C, pH=7.0, HRT=12hrs, n=4 data, Filt.COD$_f$=8,290 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,912	385	13.2	192	2,301-3,524
Acetic	593	82	13.8	41	463-723
Propionic	939	109	11.6	54	766-1,111
iso-Butyric	324	62	19.3	31	225-424
n-Butyric	417	75	18.1	38	297-537
iso-Valeric	415	45	10.8	22	344-486
n-Valeric	125	32	26.0	16	73-176
iso-Caproic	99	15	14.8	7	76-122
n-Caproic	1	<1	85.7	<1	0-2
T=30°C, pH=7.0, HRT=12hrs, n=10 data, Filt.COD$_f$=8,290 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,091	253	8.2	96	2,857-3,325
Acetic	770	76	9.8	29	700-840
Propionic	781	50	6.4	19	734-827
iso-Butyric	353	31	8.7	12	325-381
n-Butyric	492	37	7.6	14	457-526
iso-Valeric	483	52	10.9	20	435-532
n-Valeric	142	13	9.3	5	130-154
iso-Caproic	70	11	16.4	4	59-80
n-Caproic	1	<1	74.3	<1	<1-2
T=25°C, pH=7.0, HRT=12hrs, n=6 data, Filt.COD$_f$=4,040 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,597	109	6.8	44	1,483-1,711
Acetic	385	27	7.0	11	357-413
Propionic	432	37	8.5	15	393-470
iso-Butyric	192	19	10.0	8	172-212
n-Butyric	232	22	9.3	9	209-254
iso-Valeric	274	27	9.9	11	246-303
n-Valeric	67	5	6.8	2	62-72
iso-Caproic	15	1	9.8	<1	14-17
n-Caproic	<1	<1	245.0	<1	0-<1

* units are (mg/l).

† units are (%).

OLR. But still these VSS trends were quite affected by the OLR changes and the fact that they were not statistically evaluated data.

No differences appeared between TKN in the 3 systems, although the values were changing according to OLR changes. Meanwhile, very high NH_3-N values were observed for Reactor 3 with the highest acidified effluent. These values were almost 85% of the respective TKN values.

Furthermore the two systems with lower degrees of acidification produced lower but still significant amounts of NH_3-N. The engineering design point that could be derived from the high NH_3-N values was that in the case of high COD values of the wastewaters (above 15 g/l) they could produce NH_3-N levels of up to 1.5 g/l, which are considered inhibitory for methanogenic activities (Owen, 1982).

The high content of NH_3-N could be considered responsible for the high buffer capacity of the process towards the increasing VFA concentrations (Speece & McCarty, 1964, Steiner et al., 1985).

The values reported for PO_4-P should be considered only as indicative, as no further comments could be made.

It appears from the results of TFM that the system at pH 7.0 could provide slightly increased removal for fatty matter, but the data were not statistically reliable.

Gas was only recorded as composition. It appeared that generally Reactor 1 had a higher content of CO_2 and CH_4 than the 2 other reactors. Also Reactor 3 has a high content of CH_4. Both of these systems occasionally achieved up to 50% CH_4. It was evident that it would be more difficult to preclude methanogenic activities under the applied conditions, as they appeared in all operational conditions even at trace detection levels. The rest of the biogas composition was assumed, as in Experiments 1 and 2 (Chapters 7 & 8), to be H_2. However H_2S was also expected to be present at small proportions (Popel, 1964). Unfortunately a more detailed analysis of gas composition was not available during the present study.

9.5.1 HCl Consumption

HCl consumption could not be presented in a graph as it was not recorded properly, due to work overload. From indicative results though during the different parts of Experiment 3, it appeared that volumes were of the same order of magnitude as NaOH consumption for Experiment 1.

The most reliable results on HCl consumption were produced during the part of the study on 30°C. During this part the HCl consumption recorded was 0.461 and 0.092 ml HCl/h for Reactor 1 (at 37°C) and Reactor 2 (at 30°C) respectively. These consumption rates were of similar magnitude to those recorded during Experiment 1 for NaOH consumption with coffee wastewater.

Table 9.6: Additional Analyses of Reactor 1 in Experiment 3

T=37°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=1,790 mg/l	
Parameters	Mean Value
Total COD (mg/l)	2,025
Filtered COD (mg/l)	1,260
Total BOD (mg/l)	885
TS (mg/l)	2,400
VTS (mg/l) (% of TS)	1,690 (70.4)
SS (mg/l)	480
VSS (mg/l) (% of SS)	480 (100)
TKN (mg/l)	231
NH_3-N (mg/l) (% of TKN)	84 (36.3)
PO_4-P (mg/l)	8
CO_2 in gas (%)	26.3
CH_4 in gas (%)	6.1
T=37°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=5,110 mg/l	
Parameters	Mean Value
Total COD (mg/l)	6,315
Filtered COD (mg/l)	3,595
Total BOD (mg/l)	2,815
TS (mg/l)	5,195
VTS (mg/l) (% of TS)	4,570 (88.0)
SS (mg/l)	1,115
VSS (mg/l) (% of SS)	1,065 (95.5)
TKN (mg/l)	670
NH_3-N (mg/l) (% of TKN)	122 (18.2)
PO_4-P (mg/l)	9
CO_2 in gas (%)	23.5
CH_4 in gas (%)	2.9
T=37°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=8,290 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,940
Filtered COD (mg/l)	6,750
Total BOD (mg/l)	5,370
TS (mg/l)	7,330
VTS (mg/l) (% of TS)	6,355 (86.7)
SS (mg/l)	1,145
VSS (mg/l) (% of SS)	1,105 (96.7)
TFM (mg/l)	90
TKN (mg/l)	1017
NH_3-N (mg/l) (% of TKN)	255 (25.1)
PO_4-P (mg/l)	12
CO_2 in gas (%)	38.9
CH_4 in gas (%)	14.7
T=37°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=4,040 mg/l	
Parameters	Mean Value
Total COD (mg/l)	4,455
Filtered COD (mg/l)	3,280
Total BOD (mg/l)	2,735
TS (mg/l)	3,270
VTS (mg/l) (% of TS)	2,740 (83.7)
SS (mg/l)	725
VSS (mg/l) (% of SS)	685 (94.6)
TFM (mg/l)	40
TKN (mg/l)	494
NH_3-N (mg/l) (% of TKN)	263 (53.3)
PO_4-P (mg/l)	9
CO_2 in gas (%)	33.1
CH_4 in gas (%)	21.7

Table 9.7: Additional Analyses of Reactor 2 in Experiment 3

T=45°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=5,110 mg/l	
Parameters	Mean Value
Total COD (mg/l)	6,340
Filtered COD (mg/l)	4,450
Total BOD (mg/l)	2,785
TS (mg/l)	5,030
VTS (mg/l) (% of TS)	4,280 (85.0)
SS (mg/l)	1,095
VSS (mg/l) (% of SS)	1,020 (93.1)
TKN (mg/l)	694
NH_3-N (mg/l) (% of TKN)	127 (18.2)
PO_4-P (mg/l)	9
CO_2 in gas (%)	24.5
CH_4 in gas (%)	0.3
T=35°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=8,290 mg/l	
Parameters	Mean Value
Total COD (mg/l)	9,195
Filtered COD (mg/l)	6,230
Total BOD (mg/l)	6,015
TS (mg/l)	7,320
VTS (mg/l) (% of TS)	6,450 (88.1)
SS (mg/l)	1,435
VSS (mg/l) (% of SS)	1,415 (98.5)
TFM (mg/l)	120
TKN (mg/l)	1,049
NH_3-N (mg/l) (% of TKN)	231 (22.0)
PO_4-P (mg/l)	8
CO_2 in gas (%)	33.1
CH_4 in gas (%)	1.3
T=30°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=8,290 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,650
Filtered COD (mg/l)	5,725
Total BOD (mg/l)	5,275
TS (mg/l)	6,825
VTS (mg/l) (% of TS)	6,195 (90.8)
SS (mg/l)	985
VSS (mg/l) (% of SS)	960 (97.5)
TFM (mg/l)	80
TKN (mg/l)	937
NH_3-N (mg/l) (% of TKN)	180 (19.2)
PO_4-P (mg/l)	9
CO_2 in gas (%)	23.2
CH_4 in gas (%)	1.4
T=25°C, pH=6.0, HRT=12hrs, Filt.COD$_f$=4,040 mg/l	
Parameters	Mean Value
Total COD (mg/l)	4,115
Filtered COD (mg/l)	3,280
Total BOD (mg/l)	2,240
TS (mg/l)	4,445
VTS (mg/l) (% of TS)	3,665 (82.5)
SS (mg/l)	630
VSS (mg/l) (% of SS)	610 (96.8)
TFM (mg/l)	50
TKN (mg/l)	522
NH_3-N (mg/l) (% of TKN)	92 (17.6)
PO_4-P (mg/l)	7
CO_2 in gas (%)	15.3
CH_4 in gas (%)	0.8

Table 9.8: Additional Analyses of Reactor 3 in Experiment 3

T=45°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=5,110 mg/l	
Parameters	Mean Value
Total COD (mg/l)	5,940
Filtered COD (mg/l)	3,870
Total BOD (mg/l)	2,900
TS (mg/l)	2,705
VTS (mg/l) (% of TS)	2,250 (83.2)
SS (mg/l)	535
VSS (mg/l) (% of SS)	450 (84.5)
TKN (mg/l)	711
NH_3-N (mg/l) (% of TKN)	509 (71.5)
PO_4-P (mg/l)	12
CO_2 in gas (%)	24.4
CH_4 in gas (%)	24.8
T=35°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=8,290 mg/l	
Parameters	Mean Value
Total COD (mg/l)	9,475
Filtered COD (mg/l)	5,580
Total BOD (mg/l)	6,790
TS (mg/l)	3,540
VTS (mg/l) (% of TS)	2,725 (77.0)
SS (mg/l)	1,305
VSS (mg/l) (% of SS)	1,255 (96.1)
TFM (mg/l)	95
TKN (mg/l)	1,108
NH_3-N (mg/l) (% of TKN)	883 (79.7)
PO_4-P (mg/l)	11
CO_2 in gas (%)	28.2
CH_4 in gas (%)	9.9
T=30°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=8,290 mg/l	
Parameters	Mean Value
Total COD (mg/l)	7,850
Filtered COD (mg/l)	5,930
Total BOD (mg/l)	5,910
TS (mg/l)	2,940
VTS (mg/l) (% of TS)	2,335 (79.2)
SS (mg/l)	925
VSS (mg/l) (% of SS)	855 (92.8)
TFM (mg/l)	95
TKN (mg/l)	960
NH_3-N (mg/l) (% of TKN)	809 (84.3)
PO_4-P (mg/l)	10
CO_2 in gas (%)	27.8
CH_4 in gas (%)	12.1
T=25°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=4,040 mg/l	
Parameters	Mean Value
Total COD (mg/l)	4,315
Filtered COD (mg/l)	3,470
Total BOD (mg/l)	2,785
TS (mg/l)	2,470
VTS (mg/l) (% of TS)	1,830 (74.0)
SS (mg/l)	575
VSS (mg/l) (% of SS)	540 (93.8)
TFM (mg/l)	45
TKN (mg/l)	490
NH_3-N (mg/l) (% of TKN)	363 (74.2)
PO_4-P (mg/l)	7
CO_2 in gas (%)	13.4
CH_4 in gas (%)	6.3

9.6 Key Points for Discussion

In Figures 9.6 and 9.7 the effect of VFA concentration and the percentage composition of acids for the control reactor 1 due to the OLR changes is presented.

Although a linear increase in VFA concentration was observed with the increase of OLR, smaller variations were observed for the proportion of the individual acids. The main difference of OLR changes was observed with Acetic and Propionic acids where the acetate and propionate-producing bacteria appeared to have a decrease and a small increase in their activity respectively.

A direct comparison of VFA concentration and composition of major acids for the 3 reactors related to the effects of temperature, could not be presented for Experiment 3. However an indirect comparison for the effects of temperature on acidification could only be made between Reactors 2 and 3 with the control Reactor 1 at 37°C, at each set of experimental conditions. This was due to the changing OLR during the different sets of operational conditions, and the need to obtain further data to evaluate the effect of OLR on acidification of slaughterhouse wastewater before any assumptions about OLR could be made.

Regarding the effect of temperature it appeared that 37°C was better than other temperatures at pH 6.0. Meanwhile for Reactors 2 and 3 there was similarity in the overall extent of acidification between 30 and 35°C, which for Reactor 2 was quite comparable and similar to 37°C. Furthermore 25°C was less favourable than higher temperatures, but Reactor 3 at pH 7.0 still produced up to 74% Acidified COD. However 45°C appeared to result in higher acidification than 25°C, but had more unstable and erratic results. The temperature of 40°C could not be properly assessed, due to great oscillations in OLR during the period of data collection for this operational condition.

Regarding the effect of pH, it became obvious that 7.0 was far better than 6.0, at all temperatures. While pH 6.0 had acidified matter ranging around 20%, pH 7.0 always had above 70% acidification.

Changes in OLR seemed to affect directly and very rapidly the VFA production. An indication of the OLR effects on acidification of slaughterhouse wastewaters can be observed by the control system at 37°C and pH 6.0. At this stage it could be assumed that OLR had a linear increase effect in the production of VFA and some small effects on the VFA composition particularly for the acetate-producing bacteria.

In relation to the degradation patterns it appeared that Acetic was the major acid, with Propionic being second. Also n-Butyric, iso-Butyric and iso-Valeric were all produced in similar proportions (between 10-20%). No significant changes could be observed in the composition of VFA with respect to any of the changes in Experiment 3.

VFA had reached levels as high as 6.0 g/l, with Acidified COD being as much as 90% and influent COD above 8.0 g/l. Furthermore it should be noticed that such high proportions of acidification can result in high ammonia-N concentrations (above 1.0 g/l).

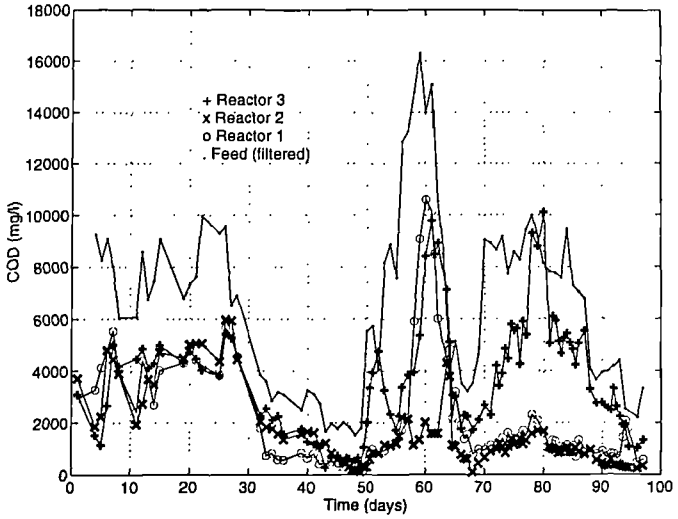


Figure 9.5: Feed Filt.COD & COD in VFA in Reactors during Experiment 3

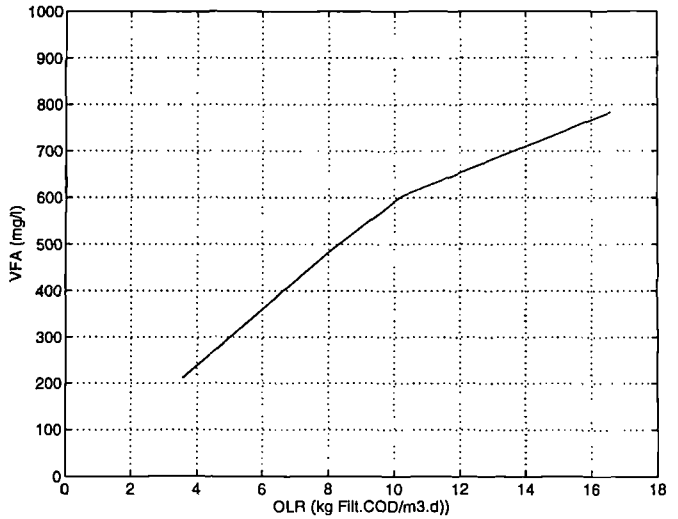


Figure 9.6: Effects on VFA concentration due to OLR changes in Reactor 1

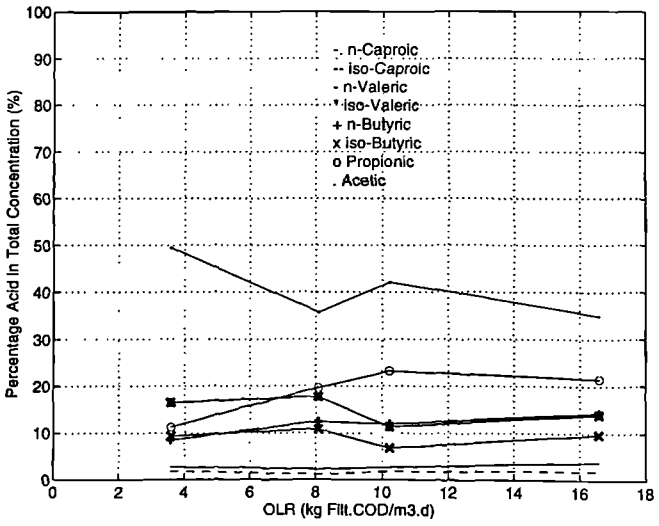


Figure 9.7: Effects on VFA composition due to OLR changes in Reactor 1

Chapter 10

EXPERIMENT 4: SLAUGHTERHOUSE-pH & HRT

10.1 Introduction

10.1.1 General

In Experiment 4 the effect of various pH values on slaughterhouse wastewaters were to be tested. In addition HRT values similar to those examined in Experiment 2 (Chapter 8) for synthetic coffee wastewaters were to be applied to acidogenic reactors, in order to assess their effect.

10.1.2 Objectives

The objectives of Experiment 4 were:

Major:

- **Study the effect of pH on acidification of real slaughterhouse wastewaters compared with a non-pH-controlled reactor.**
- **Evaluate the effect of HRT on acidification of real slaughterhouse wastewaters.**

Minor:

- Operate a system at 25°C in comparison to 37°C in order to assess the potential of low-cost pre-treatment.

10.2 Experimental Conditions

10.2.1 Operation

Experiment 4 started two months after the end of Experiment 3. The total duration was 54 days. There were 4 periods of collection of data of primary importance (a total of 20 days),

while the remaining time was used for acclimatisation (6 days at the beginning and 28 days on gradual pH and HRT changes).

The conditions selected to operate the 3 acidogenic reactors during Experiment 4, are presented in Table 10.1.

Table 10.1: Experiment 4: Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s)	25	37	37
pH	5.5, 6.0, 6.5	5.5, 6.0, 6.5	7.0
HRT (hours)	12, 9, 6	12, 9, 6	12, 9, 6
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

HRT and pH changes during the experimental period are presented in Figures 10.1 and 10.2 respectively.

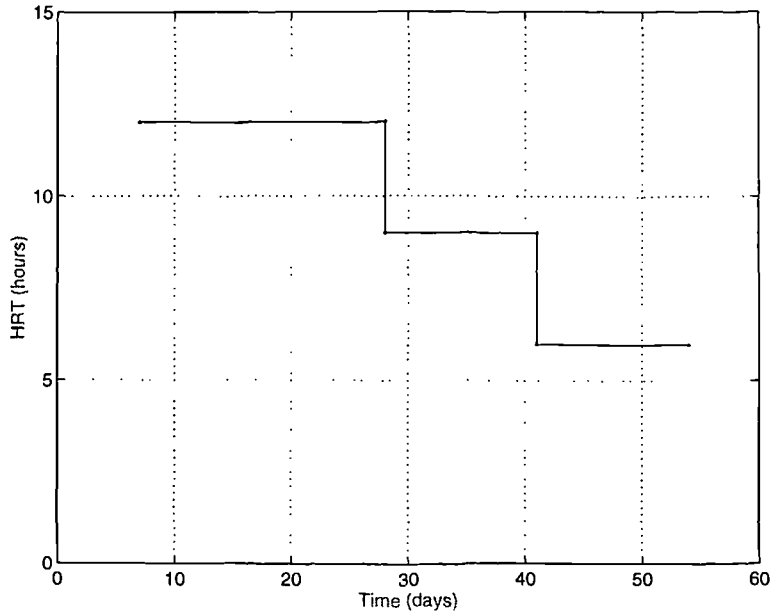


Figure 10.1: Changes in HRT during Experiment 4

The main characteristics of the fresh wastewater were presented in Table 9.2 (Chapter 9).

The theoretical HRT values were originally calibrated with the influent pump of the 3 reactors, and checked daily during the experiment. Periodic volumes and duration of feed consumed were used to calculate applied HRT for different periods of this experiment. Applied HRT values and their percentage differences in relation to the theoretical value, are presented in Table 10.2.

No difference greater than 10% was observed for the applied HRT, so the theoretical values apply for the assessment of the results.

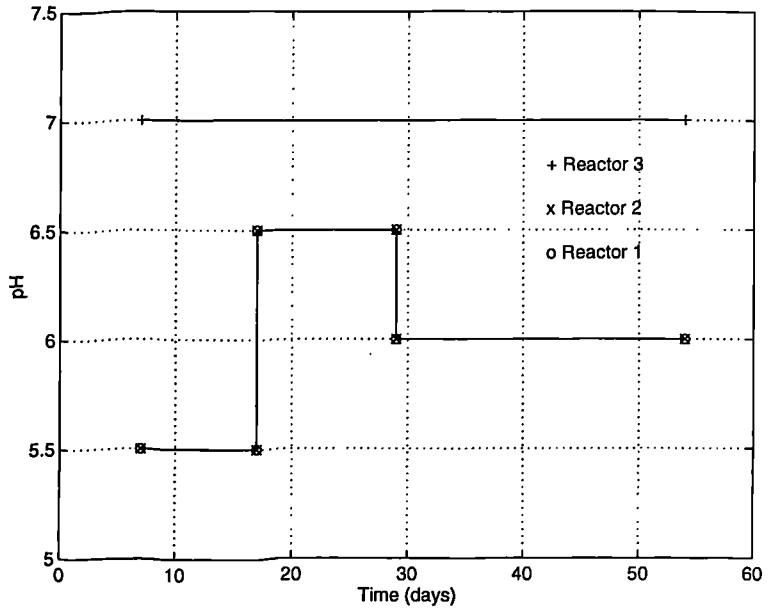


Figure 10.2: Changes in pH during Experiment 4

Table 10.2: Applied HRTs and percentage differences from the theoretical values

Period	HRT (hours)	Difference (%)
12 hrs	12.01	+0.08
9 hrs	8.71	-3.27
6 hrs	5.99	-0.17

OLR values were calculated from the Filt.COD, which was measured on a daily basis, and the values of HRT. These values are presented in Figure 10.3.

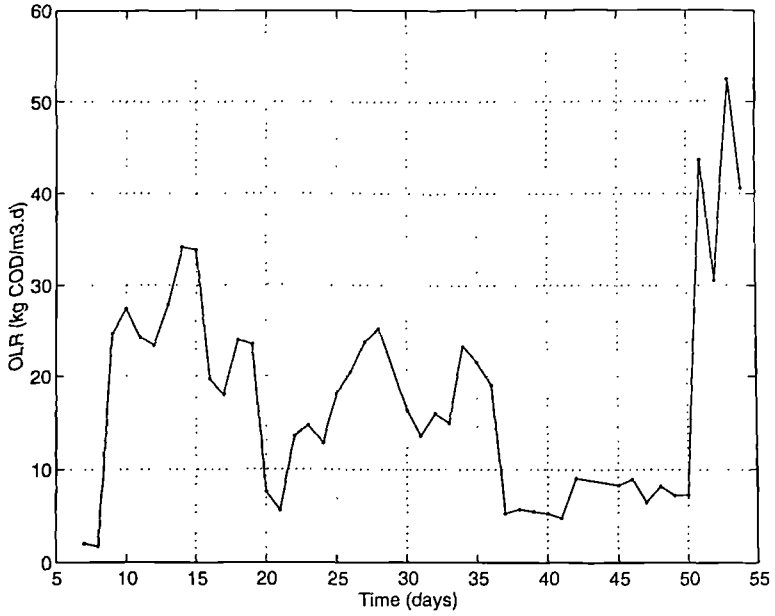


Figure 10.3: Organic Loading Rate during Experiment 4

10.2.2 Analyses & Diary of Problems

All aspects relevant to analyses were the same as those applied for Experiment 3 (Chapter 9).

No significant problems were recorded. The problems were similar to the minor ones observed in Experiments 2 and 3 (Chapters 8 & 9).

10.2.3 Filtered COD

Filtered COD analysis is presented in Figure 10.4. From the presented data, Filt.COD removal was generally around 20% but occasionally it reached up to 30-40%.

Apparently OLR changes during the study of HRT 9 hrs were quite different from those at HRT 6 hrs. The same occurred at HRT 12 hrs during the pH studies but with smaller variation. This made difficult a direct interpretation of the effect of pH and HRT on acidification of slaughterhouse wastewaters using only the results of Experiment 4.

10.3 VFA

10.3.1 VFA as Total Concentration

In Figure 10.5 the Total VFA concentration is presented. VFA production was of the same magnitude as in Experiment 3 (Chapter 9), with values up to 5.0 g/l.

Reactor 3 at pH 7.0 appeared to be superior to Reactors 1 and 2. When pH was above 6.0 Reactor 2 produced VFA concentrations similar to Reactor 3. Furthermore Reactor 1, with the lower temperature, always had the lowest VFA production.

10.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is given in Figure 10.6.

Considering the data presented in this comparison it was apparent that Acidified COD in the best acidogenic reactors (2 & 3) was above 50% when pH was 6.5 or 7.0 at all HRT and OLR values applied, while for Reactor 1 Acidified COD was in the range of 10 to 30%.

10.4 Statistical Analyses

In Tables 10.3, 10.4 and 10.5 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 4. All values in each set are presented with the average Filt.COD in the feed, so as to assess the changes in OLR that were occurring simultaneously with the other operational changes.

VFA data are presented in Appendix D (Tables D.10, D.11 and D.12).

The set of conditions that were applied for all three reactors to study the effect of pH on acidification had a duration equivalent to around 13 HRTs and 9 HRTs for the studies on pH 5.5 and 6.5 respectively. Furthermore for the HRT studies at 9 and 6 hrs all reactors operated under the same conditions for an equivalent period of around 9 HRTs and 28 HRTs respectively.

Reactor 1 produced mainly Acetic acid, as it was also found for the operation at these temperature and pH conditions in Experiment 3 (Chapter 9). Furthermore Propionic, n-Butyric , iso-Valeric and iso-Butyric were also acids produced at up to 10-20%. In Reactor 2 Acetic was the major acid, but the second acid, Propionic, dominated once. As with Reactor 1, n-Butyric, iso-Valeric and iso-Butyric were also present in Reactor 2 in considerable quantities. The VFA composition of Reactors 2 and 3 were similar, except that Propionic was less in proportion to Acetic in Reactor 3.

Overall from the present data Acetic proved the major acid in slaughterhouse wastewater acidification for Experiment 4, as was also found in the previous study on slaughterhouse wastewater

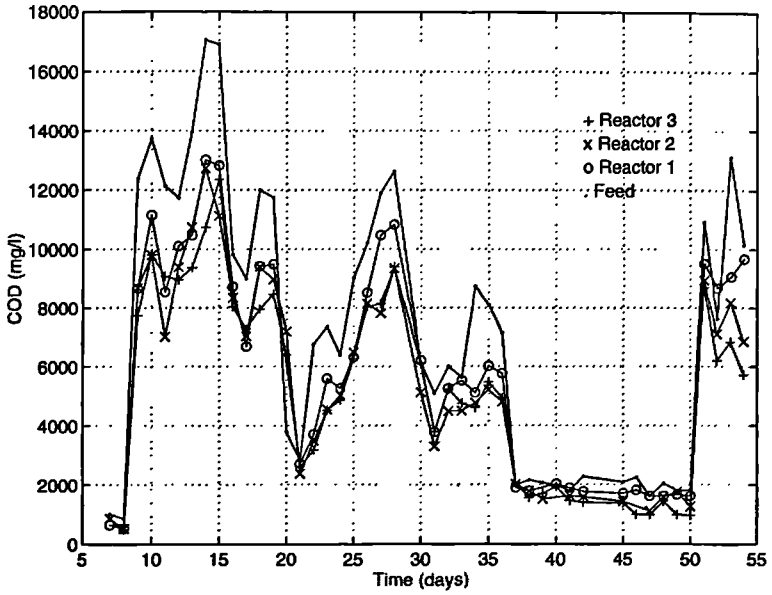


Figure 10.4: Filtered COD during Experiment 4

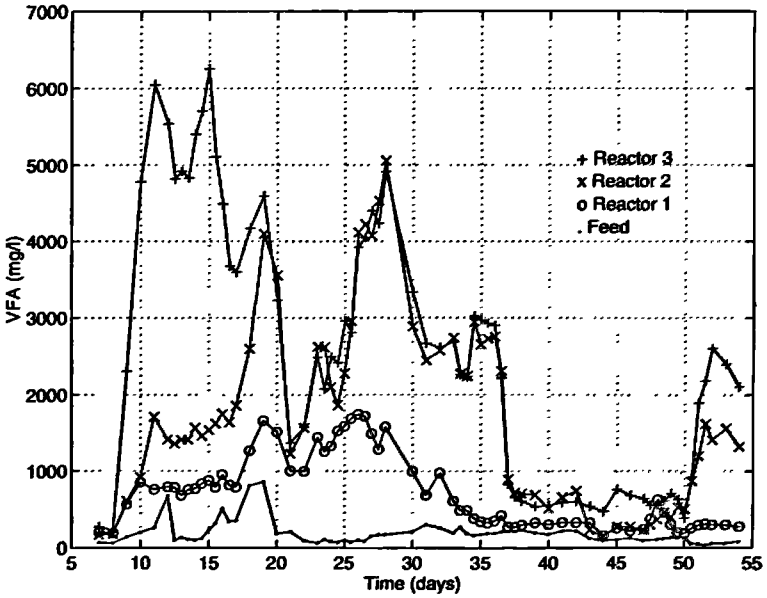


Figure 10.5: Total Volatile Fatty Acid Concentration during Experiment 4

(Chapter 9). Propionic acid came second, especially for Reactors 2 and 3. Other main acids were n-Butyric, iso-Valeric, iso-Butyric and occasionally n-Valeric. These results about the VFA pattern on the acidification of slaughterhouse wastewaters were similar to what was observed in Experiment 3 (Chapter 9).

10.5 Additional Analyses

In Tables 10.6, 10.7 and 10.8 the results of additional analyses are presented. The results were produced from one or two samples (depending on OLR stability during the sample collection) taken for additional analyses during each set of conditions. Gas composition was based on daily measurements. These results are presented only as indication of the effluent quality.

Total COD and BOD variations were according to those found in Filt.COD from the different OLR values.

Also TS and VTS were only indicative results as Total COD and BOD. Although the same applies for SS and VSS data, it was obvious that biomass levels in Reactor 1 were mostly less than those in Reactors 2 and 3.

No differences appeared between TKN in the 3 systems, although the values were changing according to OLR changes. TKN values were high and indicate the level of proteins in the wastewater. Furthermore NH_3-N reached again, as in Experiment 3 (Chapter 9), very high levels of around 1.2 g/l. As already stated, these high values should be considered only for their potential inhibitory effects on methanogens and their high buffering capacity for the acidogenic process (Owen, 1982; Steiner et al., 1985).

The results of PO_4-P should be considered only as indicative, as were the results for fatty matter.

Gas was recorded only as composition. CO_2 content was similar for Reactors 2 and 3. Meanwhile CH_4 content appeared to be far more in Reactor 3, due to pH 7.0. Also, Reactors 1 and 2 appeared to have similar low levels of CH_4 at HRT of 9 and 6 hrs. Finally it was evident that it was impossible to preclude methanogenic activities under the applied conditions, even at HRT 6 hrs. The residual gas composition was again assumed to be mostly H_2 as in the previous studies (Chapters 7-9).

10.5.1 HCl Consumption

HCl consumption was not carried out properly due to work overload. Indicative results produced were not sufficient for presentation partly because of the OLR changes, though they seemed similar to the magnitude reported for Experiment 3 (Chapter 9).

Table 10.3: Statistical data of Reactor 1 in Experiment 4

T=25°C, pH=5.5, HRT=12hrs, n=8 data, Filt.COD$_f$=13,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	787	57	7.3	20	739-835
Acetic	326	23	7.1	8	307-345
Propionic	124	8	6.3	3	117-131
iso-Butyric	78	5	6.4	2	73-82
n-Butyric	109	8	7.1	3	102-115
iso-Valeric	119	11	9.6	4	110-129
n-Valeric	22	5	23.6	2	17-26
iso-Caproic	10	2	18.5	<1	8-11
n-Caproic	<1	<1	165.1	<1	0-<1
T=25°C, pH=6.5, HRT=12hrs, n=6 data, Filt.COD$_f$=10,940 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,628	106	6.5	43	1,517-1,739
Acetic	635	25	4.0	10	609-662
Propionic	239	27	11.3	11	211-268
iso-Butyric	174	12	7.1	5	161-187
n-Butyric	259	25	9.6	10	233-285
iso-Valeric	253	34	13.5	14	217-289
n-Valeric	52	5	10.4	2	47-58
iso-Caproic	14	4	26.8	2	10-18
n-Caproic	<1	<1	150.3	<1	0-<1
T=25°C, pH=6.0, HRT=9hrs, n=5 data, Filt.COD$_f$=7,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	360	38	10.5	17	314-407
Acetic	160	19	11.9	9	136-184
Propionic	57	10	17.0	4	45-69
iso-Butyric	33	3	9.4	1	29-37
n-Butyric	41	6	13.9	3	34-48
iso-Valeric	49	5	10.4	2	43-56
n-Valeric	16	7	42.5	3	8-25
iso-Caproic	4	<1	21.2	<1	3-5
n-Caproic	<1	<1	223.6	<1	0-<1
T=25°C, pH=6.0, HRT=6hrs, n=6 data, Filt.COD$_f$=1,990 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	235	42	17.7	17	192-279
Acetic	122	14	11.8	6	107-138
Propionic	31	8	24.7	3	23-39
iso-Butyric	16	5	31.8	2	11-22
n-Butyric	22	6	26.8	2	16-29
iso-Valeric	28	7	24.9	3	21-36
n-Valeric	12	13	105.0	5	0-26
iso-Caproic	2	1	68.0	<1	<1-3
n-Caproic	<1	<1	105.9	<1	0-<1

* units are (mg/l).

† units are (%).

Table 10.4: Statistical data of Reactor 2 in Experiment 4

T=37°C, pH=5.5, HRT=12hrs, n=8 data, Filt.COD$_f$=13,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	1,474	94	6.4	33	1,396-1,552
Acetic	259	14	5.5	5	247-271
Propionic	384	19	4.9	7	369-400
iso-Butyric	179	9	5.2	3	171-187
n-Butyric	187	17	9.1	6	173-201
iso-Valeric	253	26	10.5	9	231-275
n-Valeric	204	26	12.9	9	182-226
iso-Caproic	6	1	22.0	<1	5-8
n-Caproic	2	1	67.9	<1	<1-2
T=37°C, pH=6.5, HRT=12hrs, n=4 data, Filt.COD$_f$=10,940 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,238	208	4.9	104	3,908-4,568
Acetic	941	46	4.9	23	867-1,015
Propionic	780	189	24.3	95	479-1,081
iso-Butyric	470	35	7.5	18	415-526
n-Butyric	982	163	16.6	82	722-1,242
iso-Valeric	732	109	14.9	55	558-905
n-Valeric	240	8	3.5	4	226-253
iso-Caproic	85	15	17.3	7	62-109
n-Caproic	8	2	29.8	1	4-11
T=37°C, pH=6.0, HRT=9hrs, n=5 data, Filt.COD$_f$=7,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,678	234	8.8	105	2,387-2,969
Acetic	716	63	8.8	28	638-794
Propionic	387	36	9.4	16	342-432
iso-Butyric	305	30	9.8	13	268-342
n-Butyric	645	58	9.0	26	573-717
iso-Valeric	396	39	9.9	17	347-444
n-Valeric	169	14	8.3	6	151-186
iso-Caproic	55	8	14.7	4	45-64
n-Caproic	5	<1	13.8	<1	4-6
T=37°C, pH=6.0, HRT=6hrs, n=5 data, Filt.COD$_f$=1,990 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	452	68	15.1	31	367-537
Acetic	142	16	11.6	7	122-162
Propionic	124	23	18.2	10	96-152
iso-Butyric	38	7	18.8	3	29-47
n-Butyric	58	17	29.5	8	37-79
iso-Valeric	66	13	19.9	6	50-83
n-Valeric	19	6	30.0	3	12-26
iso-Caproic	3	3	89.1	1	0-6
n-Caproic	1	1	82.4	<1	0-3

* units are (mg/l).

† units are (%).

Table 10.5: Statistical data of Reactor 3 in Experiment 4

T=37°C, pH=7.0, HRT=12hrs, n=4 data, Filt.COD_f=13,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,919	134	2.7	67	4,705-5,132
Acetic	1,329	131	9.9	65	1,120-1,537
Propionic	952	38	4.0	19	892-1,013
iso-Butyric	595	48	8.1	24	518-671
n-Butyric	978	109	11.1	54	805-1,151
iso-Valeric	780	88	11.3	44	639-920
n-Valeric	173	11	6.6	6	155-191
iso-Caproic	109	26	24.3	13	67-151
n-Caproic	4	3	65.6	1	0-8
T=37°C, pH=7.0, HRT=12hrs, n=4 data, Filt.COD_f=10,940 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,154	212	5.1	106	3,817-4,491
Acetic	995	52	5.2	26	913-1,078
Propionic	795	25	3.2	13	755-835
iso-Butyric	589	163	27.7	82	330-849
n-Butyric	806	63	7.8	32	706-906
iso-Valeric	770	71	9.2	36	657-883
n-Valeric	155	7	4.6	4	144-167
iso-Caproic	40	3	7.4	1	35-45
n-Caproic	3	<1	17.6	<1	2-4
T=37°C, pH=7.0, HRT=9hrs, n=4 data, Filt.COD_f=7,980 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,956	54	1.8	27	2,870-3,042
Acetic	798	13	1.6	6	777-818
Propionic	605	21	3.5	11	571-639
iso-Butyric	371	9	2.4	5	356-385
n-Butyric	523	11	2.2	6	505-541
iso-Valeric	459	22	4.7	11	425-494
n-Valeric	133	9	6.7	4	119-147
iso-Caproic	64	4	6.8	2	57-71
n-Caproic	4	<1	11.7	<1	3-5
T=37°C, pH=7.0, HRT=6hrs, n=6 data, Filt.COD_f=1,990 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	617	56	9.1	23	558-676
Acetic	167	45	26.7	18	120-214
Propionic	181	14	7.8	6	167-196
iso-Butyric	72	9	12.4	4	62-81
n-Butyric	62	8	13.3	3	53-71
iso-Valeric	112	6	5.2	2	106-118
n-Valeric	21	1	6.5	<1	19-22
iso-Caproic	2	2	91.8	<1	<1-3
n-Caproic	<1	<1	96.8	<1	0-<1

* units are (mg/l).

† units are (%).

Table 10.6: Additional Analyses of Reactor 1 in Experiment 4

T=25°C, pH=5.5, HRT=12hrs, Filt.COD$_f$=13,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	15,850
Filtered COD (mg/l)	11,600
Total BOD (mg/l)	10,200
TS (mg/l)	12,435
VTS (mg/l) (% of TS)	11,290 (90.8)
SS (mg/l)	1,385
VSS (mg/l) (% of SS)	1,350 (97.4)
TKN (mg/l)	1,813
NH_3-N (mg/l) (% of TKN)	250 (13.8)
PO_4-P (mg/l)	9
CO_2 in gas (%)	31.7
CH_4 in gas (%)	2.8
T=25°C, pH=6.5, HRT=12hrs, Filt.COD$_f$=10,940 mg/l	
Parameters	Mean Value
Total COD (mg/l)	12,475
Filtered COD (mg/l)	9,035
Total BOD (mg/l)	6,800
TS (mg/l)	8,020
VTS (mg/l) (% of TS)	6,905 (86.1)
SS (mg/l)	870
VSS (mg/l) (% of SS)	850 (97.7)
TFM (mg/l)	60
TKN (mg/l)	1,452
NH_3-N (mg/l) (% of TKN)	565 (38.9)
PO_4-P (mg/l)	14
CO_2 in gas (%)	20.1
CH_4 in gas (%)	8.0
T=25°C, pH=6.0, HRT=9hrs, Filt.COD$_f$=7,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,625
Filtered COD (mg/l)	5,630
Total BOD (mg/l)	5,100
TS (mg/l)	6,960
VTS (mg/l) (% of TS)	5,910 (84.9)
SS (mg/l)	870
VSS (mg/l) (% of SS)	855 (98.7)
TKN (mg/l)	1,024
NH_3-N (mg/l) (% of TKN)	131 (12.8)
PO_4-P (mg/l)	12
CO_2 in gas (%)	15.3
CH_4 in gas (%)	6.7
T=25°C, pH=6.0, HRT=6hrs, Filt.COD$_f$=1,990 mg/l	
Parameters	Mean Value
Total COD (mg/l)	3,070
Filtered COD (mg/l)	1,640
Total BOD (mg/l)	1,375
TS (mg/l)	2,435
VTS (mg/l) (% of TS)	1,470 (60.4)
SS (mg/l)	720
VSS (mg/l) (% of SS)	660 (91.8)
TFM (mg/l)	240
TKN (mg/l)	284
NH_3-N (mg/l) (% of TKN)	124 (43.6)
PO_4-P (mg/l)	15
CO_2 in gas (%)	13.3
CH_4 in gas (%)	7.6

Table 10.7: Additional Analyses of Reactor 2 in Experiment 4

T=37°C, pH=5.5, HRT=12hrs, Filt.COD_f=13,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	15,500
Filtered COD (mg/l)	10,995
Total BOD (mg/l)	10,500
TS (mg/l)	11,470
VTS (mg/l) (% of TS)	10,295 (89.8)
SS (mg/l)	1,075
VSS (mg/l) (% of SS)	1,045 (97.2)
TKN (mg/l)	1,812
NH_3-N (mg/l) (% of TKN)	474 (26.2)
PO_4-P (mg/l)	8
CO_2 in gas (%)	60.7
CH_4 in gas (%)	1.7
T=37°C, pH=6.5, HRT=12hrs, Filt.COD_f=10,940 mg/l	
Parameters	Mean Value
Total COD (mg/l)	11,925
Filtered COD (mg/l)	7,945
Total BOD (mg/l)	8,200
TS (mg/l)	4,195
VTS (mg/l) (% of TS)	3,310 (78.8)
SS (mg/l)	1,795
VSS (mg/l) (% of SS)	1,710 (95.4)
TFM (mg/l)	180
TKN (mg/l)	1,486
NH_3-N (mg/l) (% of TKN)	1,194 (80.3)
PO_4-P (mg/l)	13
CO_2 in gas (%)	52.3
CH_4 in gas (%)	17.5
T=37°C, pH=6.0, HRT=9hrs, Filt.COD_f=7,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,525
Filtered COD (mg/l)	4,930
Total BOD (mg/l)	5,000
TS (mg/l)	2,910
VTS (mg/l) (% of TS)	2,020 (69.4)
SS (mg/l)	1,290
VSS (mg/l) (% of SS)	1,220 (94.6)
TKN (mg/l)	1,009
NH_3-N (mg/l) (% of TKN)	803 (79.6)
PO_4-P (mg/l)	17
CO_2 in gas (%)	31.6
CH_4 in gas (%)	5.5
T=37°C, pH=6.0, HRT=6hrs, Filt.COD_f=1,990 mg/l	
Parameters	Mean Value
Total COD (mg/l)	3,305
Filtered COD (mg/l)	1,445
Total BOD (mg/l)	1,425
TS (mg/l)	2,045
VTS (mg/l) (% of TS)	1,590 (77.6)
SS (mg/l)	535
VSS (mg/l) (% of SS)	485 (90.6)
TFM (mg/l)	240
TKN (mg/l)	269
NH_3-N (mg/l) (% of TKN)	131 (48.7)
PO_4-P (mg/l)	15
CO_2 in gas (%)	19.2
CH_4 in gas (%)	0.6

Table 10.8: Additional Analyses of Reactor 3 in Experiment 4

T=37°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=13,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	15,450
Filtered COD (mg/l)	10,350
Total BOD (mg/l)	11,700
TS (mg/l)	4,740
VTS (mg/l) (% of TS)	3,585 (75.6)
SS (mg/l)	1,460
VSS (mg/l) (% of SS)	1,345 (92.1)
TKN (mg/l)	1,909
NH_3-N (mg/l) (% of TKN)	1,397 (73.2)
PO_4-P (mg/l)	11
CO_2 in gas (%)	24.1
CH_4 in gas (%)	53.7
T=37°C, pH=7.0, HRT=12hrs, Filt.COD$_f$=10,940 mg/l	
Parameters	Mean Value
Total COD (mg/l)	14,750
Filtered COD (mg/l)	7,960
Total BOD (mg/l)	8,600
TS (mg/l)	4,025
VTS (mg/l) (% of TS)	3,090 (76.8)
SS (mg/l)	1,340
VSS (mg/l) (% of SS)	1,275 (95.1)
TFM (mg/l)	95
TKN (mg/l)	1,485
NH_3-N (mg/l) (% of TKN)	1,227 (82.6)
PO_4-P (mg/l)	18
CO_2 in gas (%)	43.5
CH_4 in gas (%)	36.8
T=37°C, pH=7.0, HRT=9hrs, Filt.COD$_f$=7,980 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,375
Filtered COD (mg/l)	5,010
Total BOD (mg/l)	5,300
TS (mg/l)	2,975
VTS (mg/l) (% of TS)	2,220 (74.6)
SS (mg/l)	975
VSS (mg/l) (% of SS)	945 (96.9)
TKN (mg/l)	1,060
NH_3-N (mg/l) (% of TKN)	875 (82.5)
PO_4-P (mg/l)	10
CO_2 in gas (%)	36.8
CH_4 in gas (%)	27.1
T=37°C, pH=7.0, HRT=6hrs, Filt.COD$_f$=1,990 mg/l	
Parameters	Mean Value
Total COD (mg/l)	3,615
Filtered COD (mg/l)	1,105
Total BOD (mg/l)	1,600
TS (mg/l)	2,045
VTS (mg/l) (% of TS)	1,380 (67.6)
SS (mg/l)	710
VSS (mg/l) (% of SS)	640 (90.0)
TFM (mg/l)	65
TKN (mg/l)	280
NH_3-N (mg/l) (% of TKN)	205 (73.7)
PO_4-P (mg/l)	16
CO_2 in gas (%)	14.2
CH_4 in gas (%)	28.8

10.6 Key Points for Discussion

Considering the effect of pH, it was apparent that 7.0 was better than 5.5 and 6.0, while the results produced were similar to pH 6.5. For the system at 37°C, pH 6.5 and 7.0 resulted in Acidified COD as high as 95%. Furthermore, as pointed out with the results of Experiment 3 (Chapter 9), 25°C only produced acidified matter of up to 27% at pH around 6.5.

For HRT no direct assessment could be made due to the various OLR values that were applied. However it seemed that the HRT change did not significantly affect either the reactors at 37°C or the one at 25°C. Possibly a change occurred with the decrease from 9 to 6 hrs for all reactors based on the assumption that the effect of OLR was linear, which still needed to be further clarified. A further study on the effect of HRT would be required but not for pH as the optimum value had already been found to be pH around 6.5 to 7.0 for both temperatures.

Changes in OLR seemed to affect directly and very rapidly (within 1 to 2 HRT durations) the VFA production, as was also observed in Experiment 3 (Chapter 9). The magnitude of changes on acidification due to the OLR effect appeared to be similar for the 3 reactors, during the whole range of operational conditions that were studied.

Similar VFA composition patterns to those reported for Experiment 3 (Chapter 9) were observed for the 3 reactors during this study. Neither the effect of pH nor of HRT on VFA composition appeared significant during the operational conditions studied. Only small variations in the percentage content of each acid in VFA concentration was observed.

As in Experiment 3 (Chapter 9), VFA reached levels as high as 5.0 g/l, with Acidified COD being as much as 95% with influent COD above 10 g/l. Finally, similar high levels of ammonia-N were observed (above 1.2 g/l) as in Experiment 3.

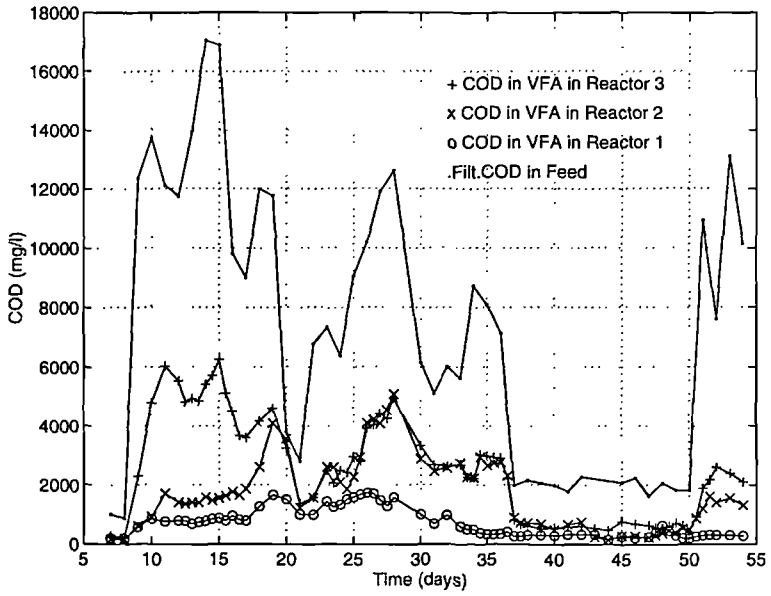


Figure 10.6: Feed Filt.COD & COD in VFA in Reactors during Experiment 4

Chapter 11

EXPERIMENT 5: SLAUGHTERHOUSE-MICRO-NUTRIENTS

11.1 Introduction

11.1.1 General

In this study it was decided to operate only non-pH-controlled reactors. In terms of temperature the reactors were operated as in Experiment 4 (Chapter 10). The main aim would be to observe any differences in acidogenic activities when the micro- nutrient addition stopped. This study should indicate whether the high nutritional content of slaughterhouse wastewaters could maintain the same performance for acidogenic bacteria, without any specialist micro-nutrient mixture.

11.1.2 Objectives

The objectives of Experiment 5 were:

Major:

- **Study the effect of a specialist micro-nutrient mixture on acidification of fresh slaughterhouse wastewaters, compared with the prescribed added concentration of 0.1 ml OMEX per litre of wastewater.**

Minor:

- Evaluate further the potential of acidification of fresh slaughterhouse wastewaters under low-cost operation.

11.2 Experimental Conditions

11.2.1 Operation

This experiment started 3 months after the end of Experiment 4. The total duration was 22 days. There were 2 periods of collection of data of primary importance (a total of 10 days), while the rest of the time was used for acclimatisation (6 days at the beginning and 6 days between the two periods).

The conditions selected to operate the 3 acidogenic reactors during Experiment 5, are presented in Table 11.1.

Table 11.1: Experiment 5: Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s)	25	37	37
pH	7.0	7.0	7.0
HRT (hours)	12	12	12
Micro-nutrients (ml OMEX/l feed)	0.1, 0.0	0.1, 0.0	0.1, 0.0

Changes in the addition of micro-nutrients during the experimental period are presented in Figure 11.1.

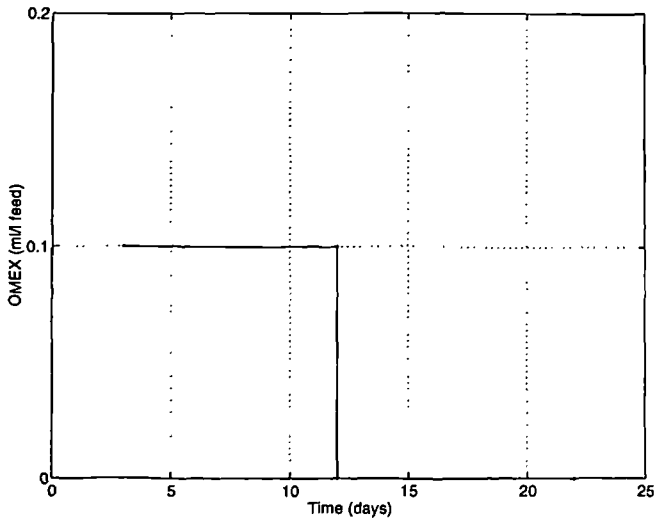


Figure 11.1: Changes in micro-nutrients during Experiment 5

The main characteristics of the fresh slaughterhouse wastewaters were presented in Table 9.2.

The influent pump for the 3 reactors was originally calibrated to give the theoretical HRT value

of 12 hrs, and checked daily during the experiment. The applied HRT value was calculated by the periodic volumes and times of feed consumed and it was found to be 12.13 hrs. The applied HRT value was +1.08% different from the theoretical value.

OLR values were calculated from the daily applied Filt.COD and the theoretical value for HRT. These values are presented in Figure 11.2.

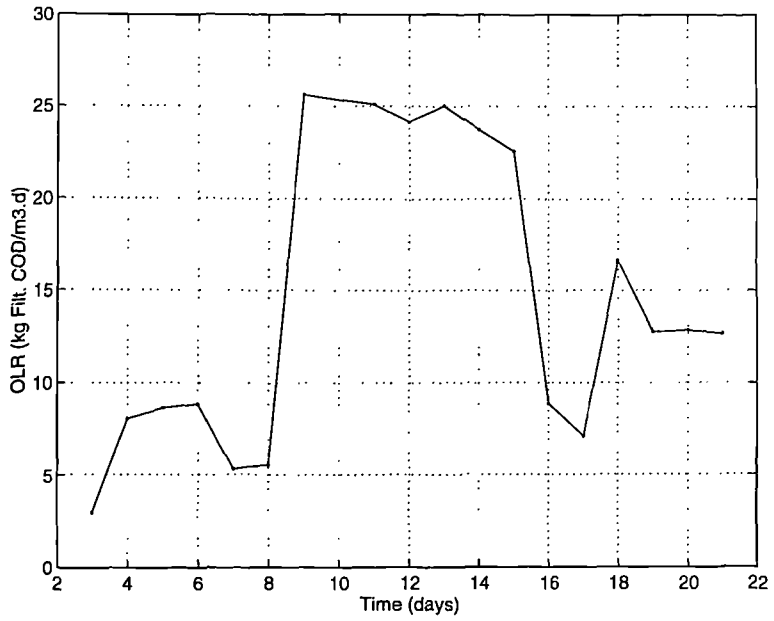


Figure 11.2: Organic Loading Rate during Experiment 5

11.2.2 Analyses & Diary of Problems

Type and frequency of analyses was very similar to that already described for the previous experiments (Chapters 9 & 10). The main change was that all additional analyses carried out previously for Tot.COD, BOD, TKN, NH_3-N, PO_4-P and TFM while on a steady state period, were no longer done. This decision was taken as the overload of additional analyses was considered unnecessary to continue to produce only indicative data.

Furthermore TS, VTS, SS and VSS became routine analyses. This came into effect as solids analyses appeared to be vital for an additional assessment of acidification by using VFA concentrations as a percentage of VTS to evaluate the quality of the effluent as acidified matter; but also to use more VSS data to assess the effect on the biomass of the operational conditions applied in the reactors. This decision was taken to obtain further understanding of the variations occurring in VFA production, because of the constantly changing strength in the COD of the slaughterhouse wastewaters during each study.

Problems that occurred during the operation of Experiment 5 were very similar to those in Experiments 2, 3 and 4 (Chapters 8, 9 & 10) and did not affect the overall performance of the reactors.

11.2.3 Filtered COD

The Filtered COD analysis is presented in Figure 11.3. Values of Filt.COD removal were around 15-20%.

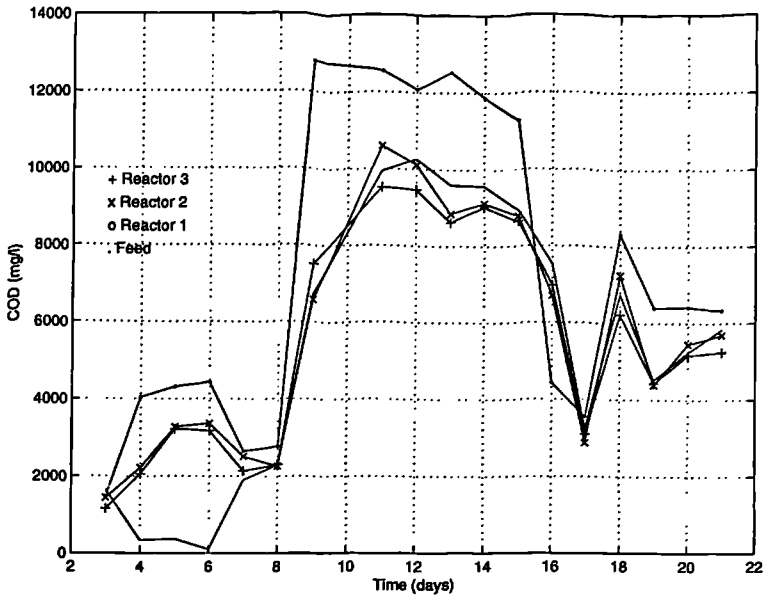


Figure 11.3: Filtered COD during Experiment 5

11.3 VFA

11.3.1 VFA as Total Concentration

In Figure 11.4 the Total VFA concentration is presented. VFA concentrations appeared to be quite similar for all systems, before and after stopping the addition of micro-nutrients.

The concentrations became as high as 5.0 g/l as in previous experiments on slaughterhouse wastewater (Chapter 9 & 10). Also around the time that micro-nutrients additions stopped and the maximum OLR for this study was applied, the highest values of VFA concentrations appeared in all 3 reactors.

11.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is given in Figure 11.5.

From the presented data it appeared that maximum Acidified COD was achieved by Reactor 2 (around 90%) after the addition of OMEX stopped. While Reactor 3, operating under the same conditions, did not achieve the same level of Acidified COD but only 77%.

Furthermore Reactor 3 achieved 84% Acidified COD before the micro-nutrient addition was terminated, and Reactor 2 reached only 70% under the same operational conditions.

This difference in the performance of the two reactors under the same operational conditions pointed out that these two reactors could have been operated with two slightly different bacterial contents.

Meanwhile, Reactor 1 with reduced temperature and no pH-control achieved above 50% Acidified COD, both with and without the addition of micro- nutrients.

11.4 Statistical Analyses

In Tables 11.2, 11.3 and 11.4 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 5. The first set of operational conditions had a duration equivalent to around 8 HRTs, while the second set was operated for around 9 HRTs.

VFA data are presented in Appendix D (Tables D.13, D.14 & D.15).

Acetic appeared to be the major acid produced, especially for Reactor 1. As in Experiments 3 and 4 (Chapters 9 & 10) similar observations were made for the proportion of the various acids and their respective ranges. For Reactors 2 and 3 Propionic, n-Butyric and iso-Valeric were produced in concentrations as high as Acetic.

For Reactor 1 some change was observed in the proportion of Acetic acid, which could be attributed to the change in the addition of micro-nutrients, although the effect of OLR on acidification had not been fully established yet. Overall no other significant changes could be observed, corresponding to the change in micro-nutrients.

11.5 Additional Analyses

In Tables 11.5, 11.6 and 11.7 the results of additional analyses are presented. The results were produced from daily samples taken for additional analyses during each set of conditions.

TS and VTS were only indicative results and as with Filt.COD no significant observations emerged. The results for VTS were used with the VFA concentrations to obtain the percentage of acidified matter and so derive values comparable to the acidified COD values. The VFA percentage on VTS ranged from 50 to 100% (with Reactors 2 and 3 exceeding 100% VFA content in VTS, at their highest Acidified COD values).

Furthermore results for VSS proved that no major changes became apparent for the biomass after the addition of micro-nutrients stopped. VSS values were higher for all reactors after the OMEX mixture was stopped, which was probably the effect of an increase in VSS reflecting a decrease in OLR. Generally Reactor 3 had higher VSS content than Reactor 2.

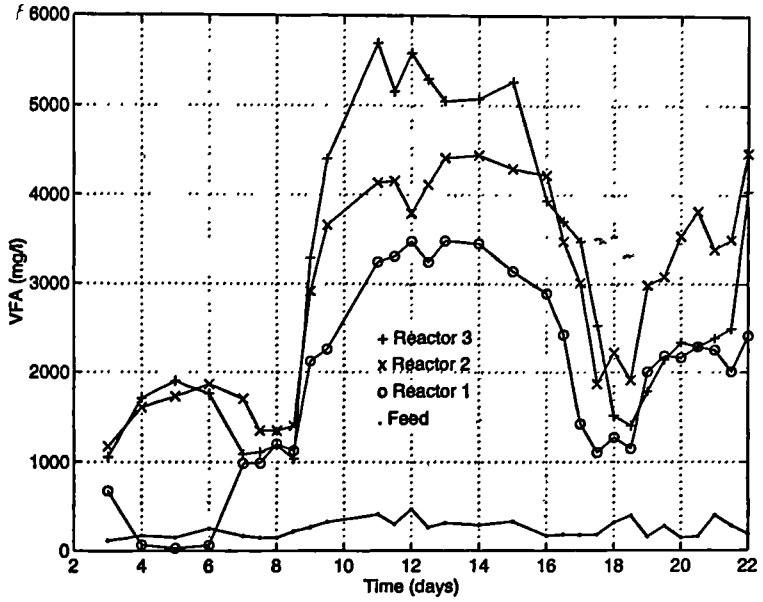


Figure 11.4: Total Volatile Fatty Acid Concentration during Experiment 5

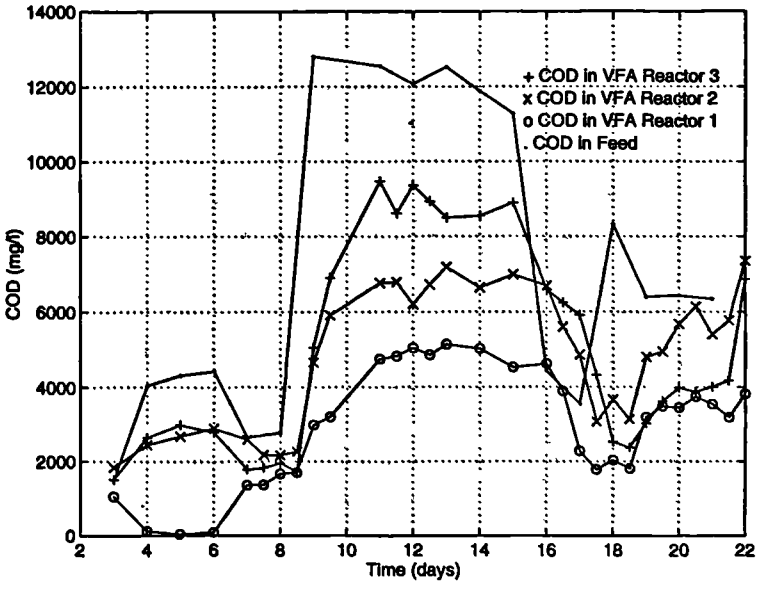


Figure 11.5: Feed Filt.COD & COD in VFA in Reactors during Experiment 5

Table 11.2: Statistical data of Reactor 1 in Experiment 5

T=25°C, pH=7.0, HRT=12hrs, with OMEX, n=3 data, Filt.COD$_f$=12,180 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,427	100	2.9	58	3,178-3,677
Acetic	1,624	71	4.4	41	1,448-1,801
Propionic	472	1	0.2	<1	469-475
iso-Butyric	288	7	2.6	4	269-306
n-Butyric	456	23	5.0	13	399-513
iso-Valeric	435	32	7.4	19	355-516
n-Valeric	91	14	14.9	8	57-125
iso-Caproic	60	4	7.4	3	49-71
n-Caproic	1	<1	37.0	<1	0-2
T=25°C, pH=7.0, HRT=12hrs, no OMEX, n=7 data, Filt.COD$_f$=6,390 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,187	147	6.7	56	2,051-2,323
Acetic	689	53	7.7	20	640-738
Propionic	341	38	11.2	14	306-377
iso-Butyric	223	28	12.6	11	197-250
n-Butyric	324	32	10.0	12	294-354
iso-Valeric	530	39	7.3	15	494-530
n-Valeric	57	8	14.5	3	49-65
iso-Caproic	22	5	21.5	2	17-26
n-Caproic	1	1	86.4	<1	<1-3

* units are (mg/l).

† units are (%).

Table 11.3: Statistical data of Reactor 2 in Experiment 5

T=37°C, pH=7.0, HRT=12hrs, with OMEX, n=3 data, Filt.COD$_f$=12,180 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,205	142	3.4	71	3,979-4,430
Acetic	1,029	100	9.8	50	869-1,188
Propionic	874	62	7.1	31	775-973
iso-Butyric	481	21	4.4	11	447-515
n-Butyric	629	35	5.6	18	573-685
iso-Valeric	1,010	66	6.5	33	905-11156
n-Valeric	160	9	5.5	4	146-174
iso-Caproic	19	6	33.6	3	9-29
n-Caproic	2	2	70.4	<1	0-5
T=37°C, pH=7.0, HRT=12hrs, no OMEX, n=3data, Filt.COD$_f$=6,390 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,152	210	6.7	121	2,630-3,674
Acetic	1,008	68	6.8	39	839-1,178
Propionic	438	76	17.3	44	250-627
iso-Butyric	277	18	6.4	10	233-321
n-Butyric	473	27	5.8	16	405-541
iso-Valeric	867	11	1.3	6	839-895
n-Valeric	68	11	16.1	6	41-96
iso-Caproic	19	2	12.7	1	13-25
n-Caproic	1	1	93.5	<1	0-4

* units are (mg/l).

† units are (%).

Table 11.4: Statistical data of Reactor 3 in Experiment 5

T=37°C, pH=7.0, HRT=12hrs, with OMEX, n=3 data, Filt.COD$_f$=12,180 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,871	407	8.4	235	3,859-5,883
Acetic	1,064	192	18.0	111	588-1,541
Propionic	1,037	80	7.7	46	837-1,236
iso-Butyric	613	115	18.8	67	327-899
n-Butyric	976	276	28.3	159	290-1,662
iso-Valeric	1,001	272	27.2	157	324-1,677
n-Valeric	72	9	12.7	5	49-94
iso-Caproic	107	45	42.6	26	0-219
n-Caproic	2	1	51.4	<1	0-5
T=37°C, pH=7.0, HRT=12hrs, no OMEX, n=4 data, Filt.COD$_f$=6,390 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	2,374	87	3.7	43	2,236-2,512
Acetic	410	17	4.2	9	382-437
Propionic	517	63	12.3	32	416-618
iso-Butyric	247	10	4.1	5	231-264
n-Butyric	489	26	5.2	13	449-530
iso-Valeric	602	62	10.4	31	503-702
n-Valeric	92	12	12.6	6	74-111
iso-Caproic	14	9	64.9	5	0-29
n-Caproic	1	<1	47.0	<1	<1-2

* units are (mg/l).

† units are (%).

Results for CO_2 and CH_4 composition were similar to those observed in Experiments 3 and 4 (Chapters 9 & 10). CO_2 and CH_4 contents were similar for Reactors 2 and 3, while Reactor 1 had lower levels for both gases. Finally, methanogenic activities were not precluded under the applied conditions and the residual gas composition was assumed to be H_2.

Table 11.5: Additional Analyses of Reactor 1 in Experiment 5

T=25°C, pH=7.0, HRT=12hrs, with OMEX, Filt.COD$_f$=12,180 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	10,115
TS (mg/l)	7,670
VTS (mg/l) (% of TS)	6,445 (84.1)
SS (mg/l)	825
VSS (mg/l) (% of SS)	785 (95.2)
CO_2 in gas (%)	34.0
CH_4 in gas (%)	20.8
T=25°C, pH=7.0, HRT=12hrs, no OMEX, Filt.COD$_f$=6,390 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	5,540
TS (mg/l)	5,470
VTS (mg/l) (% of TS)	4,590 (84.0)
SS (mg/l)	1,730
VSS (mg/l) (% of SS)	1,590 (92.1)
CO_2 in gas (%)	20.3
CH_4 in gas (%)	15.3

11.6 Key Points for Discussion

The addition of micro-nutrients did not appear to have any significant effect on the degree of acidification for slaughterhouse wastewaters. Although due to OLR changes a direct comparison of the two conditions could not be made to assess directly the effect of micro-nutrient addition. The composition of acids and maximum Acidified COD gave similar values to those obtained by Experiments 3 and 4 (Chapters 9 & 10).

The difference between Reactors 2 and 3, that became obvious when they were operated under the same conditions, could only be explained by the fact that the two acidogenic reactors had a different history of operations which could have developed two similar but not the same bacterial contents in them. The latter could be observed with the difference in acetate-producing bacteria when OMEX addition stopped, the different VSS contents and the different CH_4 content when OMEX was not added.

Although Experiment 5 made the comparison of the two reactors obvious, it was also made obvious that the reactors were quite similar when considering their performance, but not with exactly similar bacterial content. So it should be considered when future work is carried out

Table 11.6: Additional Analyses of Reactor 2 in Experiment 5

T=37°C, pH=7.0, HRT=12hrs, with OMEX, Filt.COD$_f$=12,180 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	10,365
TS (mg/l)	6,950
VTS (mg/l) (% of TS)	5,670 (81.6)
SS (mg/l)	445
VSS (mg/l) (% of SS)	425 (95.7)
CO_2 in gas (%)	41.9
CH_4 in gas (%)	46.4
T=37°C, pH=7.0, HRT=12hrs, no OMEX, Filt.COD$_f$=6,390 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	5,570
TS (mg/l)	3,620
VTS (mg/l) (% of TS)	2,755 (76.1)
SS (mg/l)	530
VSS (mg/l) (% of SS)	500 (93.9)
CO_2 in gas (%)	45.5
CH_4 in gas (%)	12.2

Table 11.7: Additional Analyses of Reactor 3 in Experiment 5

T=37°C, pH=7.0, HRT=12hrs, with OMEX, Filt.COD$_f$=12,180 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	9,500
TS (mg/l)	4,575
VTS (mg/l) (% of TS)	3,355 (73.4)
SS (mg/l)	1,690
VSS (mg/l) (% of SS)	1,605 (95.0)
CO_2 in gas (%)	57.1
CH_4 in gas (%)	35.9
T=37°C, pH=7.0, HRT=12hrs, no OMEX, Filt.COD$_f$=6,390 mg/l	
Parameters	Mean Value
Filtered COD (mg/l)	5,200
TS (mg/l)	4,175
VTS (mg/l) (% of TS)	3,225 (77.3)
SS (mg/l)	1,955
VSS (mg/l) (% of SS)	1,790 (91.7)
CO_2 in gas (%)	43.6
CH_4 in gas (%)	24.9

to adequately mix all the reactor contents and re-start the reactors with the same biomass in the beginning of each new experiment. This would enable the research to be carried out with the same biomass that has already been acclimatised in the wastewater, but not with "similar" biomass due to the different operational history of each reactor. Still as most performance values were similar between the two reactors it was assumed for the discussion of the present study (Chapter 13) to use average values from both reactors as data for the applied operational conditions at each set.

One interesting point arises from the use of acidification as low-cost pre-treatment. It has been observed that Reactor 1 with minimal control could successfully achieve above 50% Acidified COD, consisting mainly of Acetic acid. Furthermore this degree of acidification was achieved without any operational instability due to OLR changes, which would be often experienced when a pre-acidification reactor is also used as a balancing tank.

Chapter 12

EXPERIMENT 6: SLAUGHTERHOUSE-HRT & MIXING

12.1 Introduction

12.1.1 General

In this experiment it was decided to operate the reactors with and without mixing. Also, it was decided to compare the performance with previous results under the same conditions used in Experiment 5 (Chapter 11), using micro-nutrients. All this information was to be collected operating the same HRT conditions, as in Experiment 4 (Chapter 10). Finally, it was considered necessary to evaluate VFA results in parallel with protein concentrations.

12.1.2 Objectives

The objectives of Experiment 6 were:

Major:

- **Study the effect of mixing on acidification of real slaughterhouse wastewaters.**
- **Repeat the operational changes on HRT that have been applied for Experiment 4 (Chapter 10).**

Minor:

- Analyse samples for proteins in order to assess the potential of more specialist biochemical analysis for the evaluation of acidogenic phenomena.

12.2 Experimental Conditions

12.2.1 Operation

Experiment 6 started 3 weeks after the end of Experiment 5. The total duration was 48 days. There were 3 periods of collection of data of primary importance (a total of 15 days), while the remaining time was used for acclimatisation (12 days at the beginning and 21 days on gradual HRT changes).

The conditions in the 3 acidogenic reactors during Experiment 6 are presented in Table 12.1.

Table 12.1: Experiment 6: Reactor set-up

Conditions	Reactor 1	Reactor 2	Reactor 3
Temperature(s)	25	37	37
Mixing (rpm)	80	80	80, 0
pH	7.0	7.0	7.0
HRT (hours)	12, 9, 6	12, 9, 6	12, 9, 6
Micro-nutrients (ml OMEX/l feed)	0.1	0.1	0.1

HRT and mixing changes during the experimental period are presented in Figures 12.1 and 12.2 respectively.

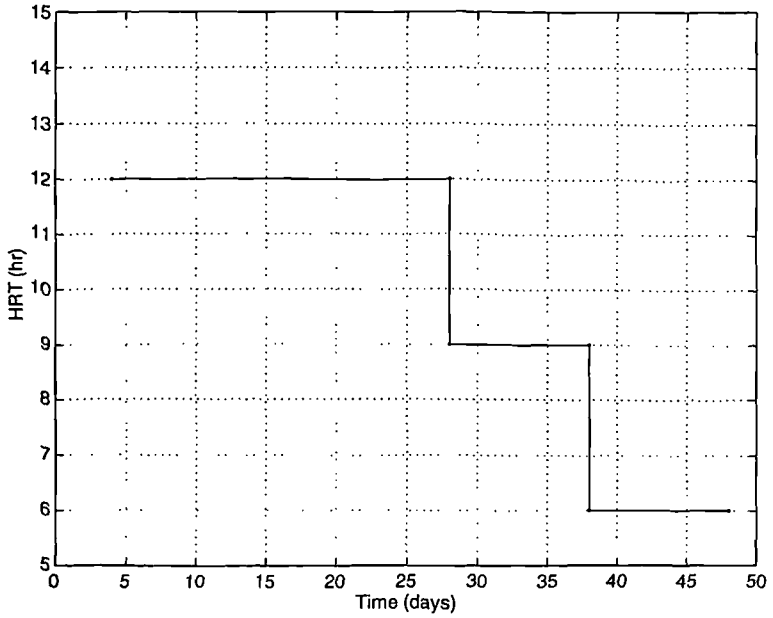


Figure 12.1: Changes in HRT during Experiment 6

The main characteristics of the wastewaters were presented in Table 9.2 (Chapter 9).

Theoretical HRT values were originally calibrated for the influent pump of the 3 reactors, and

checked daily during the experiment. Periodic volumes and duration of feed consumed were used to calculate the applied HRT for different periods. Applied HRT values and their percentage differences in relation to the theoretical value, are presented in Table 12.2.

Table 12.2: Applied HRTs and percentage differences from the theoretical values

Period	HRT (hours)	Difference (%)
12 hrs	12.15	+1.21
9 hrs	9.24	+2.68
6 hrs	6.00	+0.07

A difference greater than 10% was not observed for the applied HRT, so the theoretical values were used for the assessment of the results.

OLR values were calculated with the daily Filt.COD value and the theoretical HRT values. These values are presented in Figure 12.3.

12.2.2 Analyses & Diary of Problems

Type and frequency of analyses varied as in Experiment 5 (Chapter 11). The only change was that fats and proteins were analysed as described in Experiments 3 and 4 (Chapters 9 & 10) for all the additional analyses.

Only small operational malfunctions occurred which did not appear to affect the performance of the reactors.

12.2.3 Filtered COD

Filtered COD analysis is presented in Figure 12.4.

As in the previous experiments Filt.COD removal was below or around 20%. OLR changes became most significant during the study of HRT 9 hrs. However most of the early results for this period were collected for OLR close to that applied in the HRT period of 6 and 12 hrs, and therefore could enable for a direct comparison when the OLR effect on acidification would be evaluated.

12.3 VFA

12.3.1 VFA as Total Concentration

In Figure 12.5 the Total VFA concentration is presented.

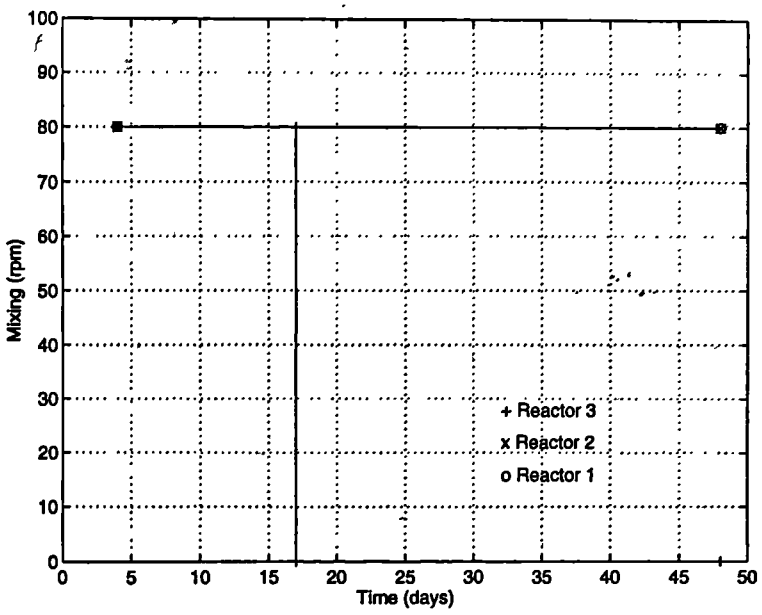


Figure 12.2: Changes in mixing during Experiment 6

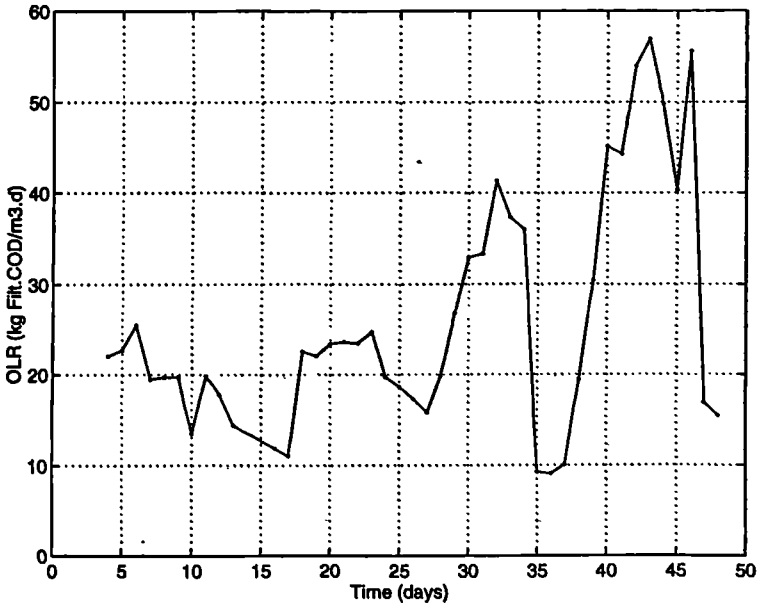


Figure 12.3: Organic Loading Rate during Experiment 6

VFA production was again up to a level of 5.0 g/l, with no major differences between Reactors 2 and 3. Reactor 1 had results lower than those for the two other reactors.

12.3.2 COD in VFA compared to Filt.COD in Feed

A comparison of the COD in VFA in the reactors to the Filt.COD value in the feed is given in Figure 12.6.

From this comparison it appeared that the degree of acidification was similar to those obtained in the previous experiments for these conditions (see sections 9.3.2, 10.3.2 & 11.3.2). Also, there was no apparent difference from Reactor 2 after the mixing in Reactor 3 stopped.

12.4 Statistical Analyses

In Tables 12.3, 12.4 and 12.5 the statistical results of the 3 reactors are presented in relation to the operational conditions applied in Experiment 6. VFA data are presented in Appendix D (Tables D.16, D.17 and D.18).

The first set of data for HRT 12 hrs was applied for a duration equivalent of around 14 HRTs, while the second was applied for around 8 HRTs. Also the set of data for HRT 9hrs was applied for a duration equivalent of around 8 HRTs. However the set of data for HRT 6 hrs was applied for around 18 HRTs.

VFA compositions appeared similar to those observed in previous experiments (see Chapters 9, 10 & 11), with the same order of magnitude in the composition for the main acids. However no apparent difference was observed for the reactors with and without mixing, apart from a difference in Acetic acid when the high OLR value was applied at HRT 12 hrs.

12.5 Additional Analyses

In Tables 12.6, 12.7 and 12.8 the results of additional analyses are presented. The results for Tot.COD, solids and gas were based on daily measurements, while the results for proteins and TFM were produced from 1 or 2 samples taken for additional analyses during each set of conditions when OLR was stable. These results are presented only as an indication of the effluent quality from the 3 reactors.

Tot.COD values indicated that there was no significant difference between the performance of the systems. Their variations were similar to those occurring in Filt.COD for the different OLR values.

The ratio of VFA composition to VTS was used, as in Experiment 5 (Chapter 11), as an additional indicator to the acidified COD values, in order to identify more easily the periods of stable performance affected by the great variations of OLR. VFA was in a range between 40 to

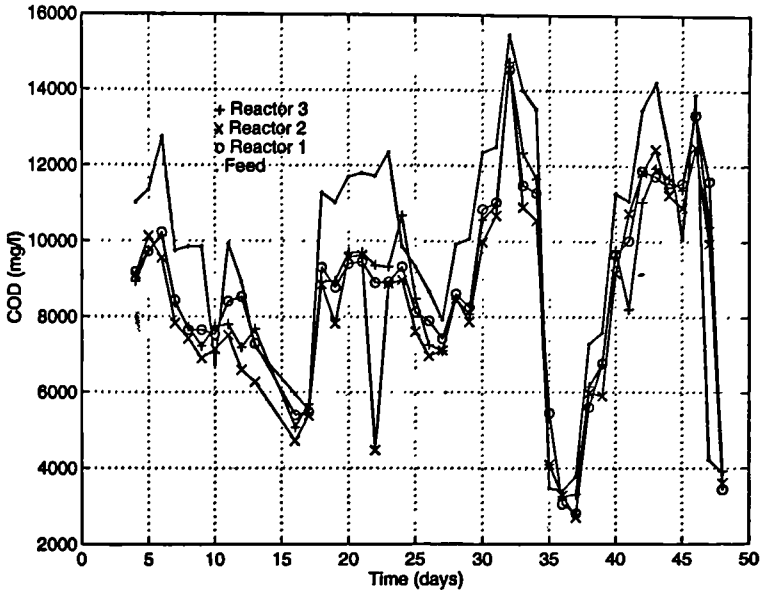


Figure 12.4: Filtered COD during Experiment 6

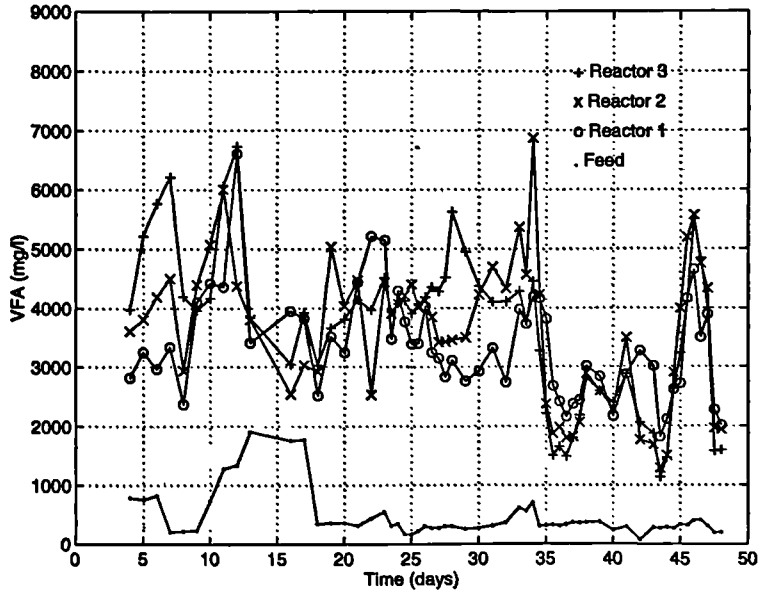


Figure 12.5: Total Volatile Fatty Acid Concentration during Experiment 6

Table 12.3: Statistical data of Reactor 1 in Experiment 6

T=25°C, pH=7.0, HRT=12hrs, with mixing, n=4 data, Filt.COD$_f$=11,395 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,634	460	12.7	230	2,903-4,366
Acetic	884	125	14.2	63	685-1,084
Propionic	548	99	18.1	50	390-706
iso-Butyric	415	81	19.6	41	286-544
n-Butyric	621	142	22.9	71	394-848
iso-Valeric	936	160	17.1	80	682-1,190
n-Valeric	147	47	31.8	23	73-221
iso-Caproic	79	37	47.2	19	20-139
n-Caproic	4	1	37.7	<1	1-6
T=25°C, pH=7.0, HRT=12hrs, with mixing, n=6 data, Filt.COD$_f$=8,965 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,189	211	6.6	86	2,967-3,411
Acetic	648	74	11.4	30	571-725
Propionic	549	51	9.4	21	495-603
iso-Butyric	386	30	7.7	12	355-417
n-Butyric	596	75	12.6	31	517-674
iso-Valeric	837	106	12.6	43	726-948
n-Valeric	114	38	33.3	15	74-154
iso-Caproic	54	29	54.4	11.9	23-84
n-Caproic	6	4	65.0	2	2-10
T=25°C, pH=7.0, HRT=9hrs, with mixing, n=5 data, Filt.COD$_f$=13,750 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	3,978	207	5.2	92	3,721-4,234
Acetic	1,078	49	4.6	22	1,017-1,138
Propionic	709	51	7.2	23	646-772
iso-Butyric	442	29	6.6	13	406-478
n-Butyric	720	52	7.2	23	656-784
iso-Valeric	771	27	3.5	12	737-804
n-Valeric	177	10	5.5	4	164-189
iso-Caproic	76	19	25.4	9	52-99
n-Caproic	6	2	34.0	<1	4-9
T=25°C, pH=7.0, HRT=6hrs, with mixing, n=4 data, Filt.COD$_f$=12,830 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,056	483	11.9	242	3,287-4,825
Acetic	921	91	9.8	45	777-1,065
Propionic	846	114	13.4	57	665-1,027
iso-Butyric	475	59	12.4	29	381-569
n-Butyric	725	105	14.5	53	558-892
iso-Valeric	885	120	13.5	60	694-1,075
n-Valeric	168	19	11.1	9	138-197
iso-Caproic	33	4	10.8	2	28-39
n-Caproic	3	2	58.4	<1	<1-6

* units are (mg/l).

† units are (%).

Table 12.4: Statistical data of Reactor 2 in Experiment 6

T=37°C, pH=7.0, HRT=12hrs, with mixing, n=4 data, Filt.COD$_f$=11,395 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,227	281	6.7	141	3,780-4,675
Acetic	1,112	220	19.8	110	761-1,463
Propionic	585	69	16.0	47	436-734
iso-Butyric	516	25	4.9	13	476-556
n-Butyric	724	63	8.7	31	624-825
iso-Valeric	1,094	48	4.4	24	1,017-1,171
n-Valeric	145	2	1.7	1	141-149
iso-Caproic	47	26	55.9	13	5-89
n-Caproic	3	2	56.5	<1	<1-7
T=37°C, pH=7.0, HRT=12hrs, with mixing, n=5 data, Filt.COD$_f$=8,965 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,124	211	5.1	94	3,863-4,386
Acetic	850	108	12.7	48	716-983
Propionic	638	23	3.6	10	610-666
iso-Butyric	548	28	5.1	13	514-583
n-Butyric	797	74	9.3	33	705-889
iso-Valeric	1,117	141	12.6	63	943-1,292
n-Valeric	150	36	24.1	16	105-194
iso-Caproic	21	14	66.8	6	4-39
n-Caproic	3	2	59.9	<1	<1-5
T=37°C, pH=7.0, HRT=9hrs, with mixing, n=3 data, Filt.COD$_f$=13,750 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,384	173	3.9	100	3,955-4,813
Acetic	959	45	4.7	26	849-1,070
Propionic	973	39	4.0	23	876-1,071
iso-Butyric	521	34	6.6	20	436-606
n-Butyric	733	58	7.9	33	589-877
iso-Valeric	957	42	4.4	24	852-1,061
n-Valeric	192	18	9.6	11	146-238
iso-Caproic	45	4	9.2	2	35-55
n-Caproic	4	1	30.7	<1	<1-6
T=37°C, pH=7.0, HRT=6hrs, with mixing, n=5 data, Filt.COD$_f$=12,830 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,771	632	13.2	283	3,987-5,556
Acetic	1,119	125	11.2	56	964-1,274
Propionic	1,007	125	12.5	56	851-1,162
iso-Butyric	590	89	15.0	40	481-700
n-Butyric	708	96	13.6	43	589-827
iso-Valeric	912	179	19.6	80	589-1,134
n-Valeric	215	53	24.4	23	150-280
iso-Caproic	208	53	25.4	24	143-274
n-Caproic	12	5	41.5	2	6-18

* units are (mg/l).

† units are (%).

Table 12.5: Statistical data of Reactor 3 in Experiment 6

T=37°C, pH=7.0, HRT=12hrs, no mixing, n=5 data, Filt.COD$_f$=11,395 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,129	233	5.6	104	3,840-4,419
Acetic	1,463	190	13.0	85	1,228-1,698
Propionic	372	58	15.7	26	300-445
iso-Butyric	382	21	5.5	9	356-408
n-Butyric	734	60	8.2	27	659-808
iso-Valeric	986	164	16.6	73	783-1,189
n-Valeric	161	23	14.6	10	132-190
iso-Caproic	29	5	18.1	2	23-36
n-Caproic	2	<1	26.0	<1	2-3
T=37°C, pH=7.0, HRT=12hrs, no mixing, n=5 data, Filt.COD$_f$=8,965 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,115	141	3.4	63	3,940-4,291
Acetic	975	326	33.4	146	570-1,380
Propionic	596	162	27.1	72	395-797
iso-Butyric	490	81	16.5	36	389-590
n-Butyric	807	72	8.9	32	717-896
iso-Valeric	1,063	189	17.8	85	828-1,298
n-Valeric	160	35	21.8	16	117-203
iso-Caproic	23	4	17.8	2	18-28
n-Caproic	2	1	54.7	<1	<1-3
T=37°C, pH=7.0, HRT=9hrs, no mixing, n=4 data, Filt.COD$_f$=13,750 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,160	295	7.1	147	3,691-4,682
Acetic	973	80	8.2	40	845-1,100
Propionic	900	70	7.8	35	789-1,011
iso-Butyric	464	41	8.9	21	399-529
n-Butyric	762	61	8.0	31	665-860
iso-Valeric	837	49	5.8	24	760-915
n-Valeric	180	11	6.0	5	163-197
iso-Caproic	39	14	36.3	7	16-61
n-Caproic	5	4	94.8	2	0-11
T=37°C, pH=7.0, HRT=6hrs, no mixing, n=4 data, Filt.COD$_f$=12,830 mg/l					
Parameters	Mean Value*	Standard Deviation*	Coefficient of Variation†	Standard Error*	Confidence Interval*
VFA	4,616	704	15.3	352	3,496-5,736
Acetic	1,138	157	13.8	79	888-1,388
Propionic	808	124	15.3	62	611-1,005
iso-Butyric	566	86	15.2	43	429-702
n-Butyric	856	145	17.0	73	625-1,088
iso-Valeric	971	158	16.3	79	720-1,223
n-Valeric	192	32	16.8	16	141-244
iso-Caproic	75	7	9.8	4	63-87
n-Caproic	10	4	36.9	2	4-15

* units are (mg/l).

† units are (%).

100% of the VTS (with values around or even exceeding 100% at the highest values of Acidified COD).

Neither the TS and VTS data, nor those presented for SS and VSS proved any significant differences between the reactors with and without mixing. However smaller VSS values observed in Reactor 3 compared to Reactor 2 could be because more settling of biomass could have been occurring.

TFM results were only indicative, as in Experiments 3 and 4 (Chapters 9 & 10). The results for proteins are presented in Figure 12.7. Protein results appeared to give a very similar pattern to those for Total COD or TS. The difference noted was that removal of organic matter was more obvious using the protein concentration. Protein removal ranged from 50 to 95% with values increasing at higher Acidified COD values. Therefore protein concentration and its removal may be a more useful parameter that COD and its removal to help understand the effects of acidification under various conditions for slaughterhouse wastewaters.

Finally results on the content of CO_2 and CH_4 were similar to the range that was presented in previous experiments (Chapters 9-11), while it was impossible to preclude methanogenic activities under all applied conditions during Experiment 6.

12.6 Key Points for Discussion

Regarding the effects of mixing it became apparent that there were no obvious differences in reactor performance with and without mechanical stirring, although no direct comparison could be made due to OLR changes. This confirmed the findings of Ghosh (1987), who advocated upflow operation of acidogenic reactors to avoid mechanical mixing.

Furthermore no major differences were observed between HRT of 12, 9 and 6 hrs, confirming the findings of Experiment 4 (Chapter 10), when it is assumed that OLR had a small and linear effect on acidification for the applied conditions. Similar observations to those in Experiment 4 were made on the performance of Reactor 1 (at 25°C) due to HRT changes, although in this study the pH was 7.0 while in Experiment 4 the pH was 6.0.

Using low-cost operation for Reactor 1, it was shown that this system could achieve above 55% Acidified COD with minimal control in operation. This is of considerable benefit for process design of low-cost pre-treatment technologies for agro-industrial wastewaters.

Observations on VFA concentrations, composition of different acids and the magnitude of the main acids produced were found to be similar to Experiments 3, 4 and 5 (Chapters 9, 10 & 11).

Finally, protein concentration was found to be a more representative parameter to assess removal and conversion of organic matter to VFA during pre-acidification of slaughterhouse wastewaters than COD, BOD and TS data.

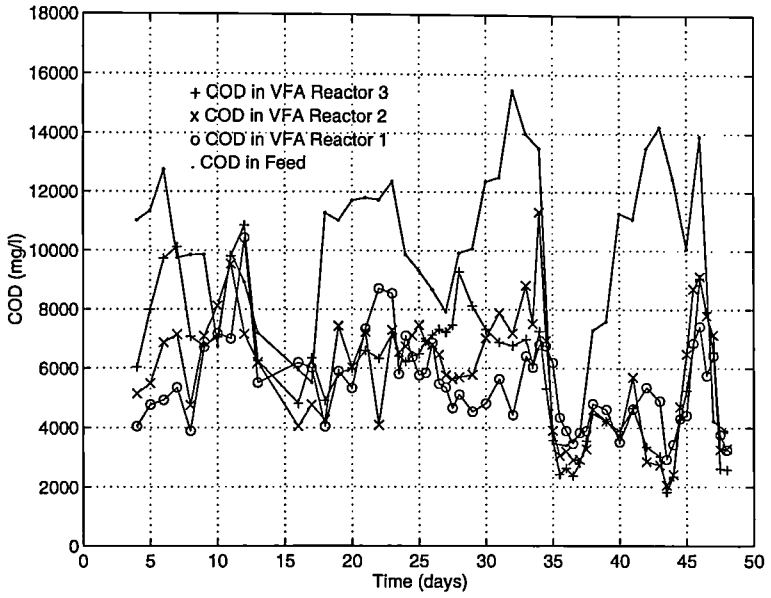


Figure 12.6: Feed Filt.COD & COD in VFA in Reactors during Experiment 6

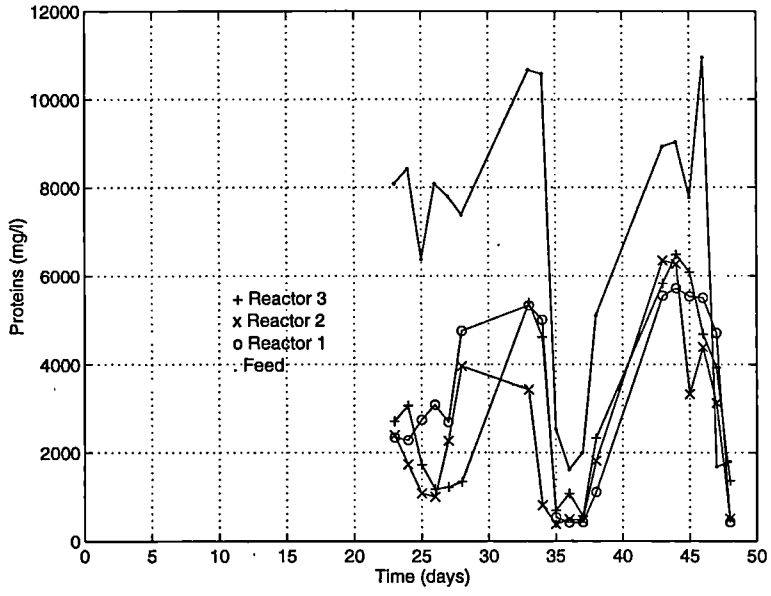


Figure 12.7: Proteins during Experiment 6

Table 12.6: Additional Analyses of Reactor 1 in Experiment 6

T=25°C, pH=7.0, HRT=12hrs, with mixing, Filt.COD$_f$=11,395 mg/l	
Parameters	Mean Value
Total COD (mg/l)	11,655
Filtered COD (mg/l)	9,155
TS (mg/l)	7,715
VTS (mg/l) (% of TS)	6,555 (85.0)
SS (mg/l)	1,100
VSS (mg/l) (% of SS)	1,095 (99.4)
Proteins (mg/l)	2,315
CO_2 in gas (%)	41.1
CH_4 in gas (%)	33.5
T=25°C, pH=7.0, HRT=12hrs, with mixing, Filt.COD$_f$=8,965 mg/l	
Parameters	Mean Value
Total COD (mg/l)	9,525
Filtered COD (mg/l)	8,015
TS (mg/l)	5,960
VTS (mg/l) (% of TS)	5,140 (86.2)
SS (mg/l)	725
VSS (mg/l) (% of SS)	705 (96.8)
Proteins (mg/l)	3,320
TFM (mg/l)	105
CO_2 in gas (%)	33.1
CH_4 in gas (%)	47.4
T=25°C, pH=7.0, HRT=9hrs, with mixing, Filt.COD$_f$=13,750 mg/l	
Parameters	Mean Value
Total COD (mg/l)	15,200
Filtered COD (mg/l)	11,375
TS (mg/l)	10,965
VTS (mg/l) (% of TS)	9,765 (89.1)
SS (mg/l)	2,075
VSS (mg/l) (% of SS)	1,950 (93.9)
Proteins (mg/l)	5,165
CO_2 in gas (%)	41.1
CH_4 in gas (%)	46.6
T=25°C, pH=7.0, HRT=6hrs, with mixing, Filt.COD$_f$=12,830 mg/l	
Parameters	Mean Value
Total COD (mg/l)	15,100
Filtered COD (mg/l)	11,985
TS (mg/l)	9,555
VTS (mg/l) (% of TS)	8,565 (89.7)
SS (mg/l)	1,020
VSS (mg/l) (% of SS)	975 (95.4)
Proteins (mg/l)	5,575
TFM (mg/l)	130
CO_2 in gas (%)	36.5
CH_4 in gas (%)	26.7

Table 12.7: Additional Analyses of Reactor 2 in Experiment 6

T=37°C, pH=7.0, HRT=12hrs, with mixing, Filt.COD$_f$=11,395 mg/l	
Parameters	Mean Value
Total COD (mg/l)	11,275
Filtered COD (mg/l)	8,955
TS (mg/l)	6,605
VTS (mg/l) (% of TS)	5,520 (83.6)
SS (mg/l)	935
VSS (mg/l) (% of SS)	885 (94.5)
Proteins (mg/l)	2.070
CO_2 in gas (%)	44.7
CH_4 in gas (%)	38.2
T=37°C, pH=7.0, HRT=12hrs, with mixing, Filt.COD$_f$=8,965 mg/l	
Parameters	Mean Value
Total COD (mg/l)	8,775
Filtered COD (mg/l)	7,550
TS (mg/l)	4,355
VTS (mg/l) (% of TS)	3,565 (81.9)
SS (mg/l)	675
VSS (mg/l) (% of SS)	640 (94.8)
Proteins (mg/l)	2,075
TFM (mg/l)	85
CO_2 in gas (%)	41.9
CH_4 in gas (%)	43.9
T=37°C, pH=7.0, HRT=9hrs, with mixing, Filt.COD$_f$=13,750 mg/l	
Parameters	Mean Value
Total COD (mg/l)	13,750
Filtered COD (mg/l)	10,725
TS (mg/l)	6,565
VTS (mg/l) (% of TS)	5,405 (82.3)
SS (mg/l)	1,470
VSS (mg/l) (% of SS)	1,400 (95.2)
Proteins (mg/l)	2,120
CO_2 in gas (%)	46.9
CH_4 in gas (%)	42.3
T=37°C, pH=7.0, HRT=6hrs, with mixing, Filt.COD$_f$=12,830 mg/l	
Parameters	Mean Value
Total COD (mg/l)	14,285
Filtered COD (mg/l)	11,785
TS (mg/l)	8,670
VTS (mg/l) (% of TS)	7,685 (88.6)
SS (mg/l)	865
VSS (mg/l) (% of SS)	830 (95.5)
Proteins (mg/l)	5,085
TFM (mg/l)	140
CO_2 in gas (%)	46.5
CH_4 in gas (%)	46.4

Table 12.8: Additional Analyses of Reactor 3 in Experiment 6

T=37°C, pH=7.0, HRT=12hrs, no mixing, Filt.COD$_f$=11,395 mg/l	
Parameters	Mean Value
Total COD (mg/l)	11,415
Filtered COD (mg/l)	9,520
TS (mg/l)	7,130
VTS (mg/l) (% of TS)	6,065 (85.1)
SS (mg/l)	605
VSS (mg/l) (% of SS)	590 (98.2)
Proteins (mg/l)	2,890
CO_2 in gas (%)	55.0
CH_4 in gas (%)	31.8
T=37°C, pH=7.0, HRT=12hrs, no mixing, Filt.COD$_f$=8,965 mg/l	
Parameters	Mean Value
Total COD (mg/l)	9,225
Filtered COD (mg/l)	7,835
TS (mg/l)	3,830
VTS (mg/l) (% of TS)	3,015 (78.8)
SS (mg/l)	580
VSS (mg/l) (% of SS)	570 (97.9)
Proteins (mg/l)	1,360
TFM (mg/l)	85
CO_2 in gas (%)	56.7
CH_4 in gas (%)	33.4
T=37°C, pH=7.0, HRT=9hrs, no mixing, Filt.COD$_f$=13,750 mg/l	
Parameters	Mean Value
Total COD (mg/l)	14,775
Filtered COD (mg/l)	12,000
TS (mg/l)	9,750
VTS (mg/l) (% of TS)	8,520 (87.4)
SS (mg/l)	875
VSS (mg/l) (% of SS)	845 (96.3)
Proteins (mg/l)	5,005
CO_2 in gas (%)	50.8
CH_4 in gas (%)	40.0
T=37°C, pH=7.0, HRT=6hrs, no mixing, Filt.COD$_f$=12,830 mg/l	
Parameters	Mean Value
Total COD (mg/l)	14,235
Filtered COD (mg/l)	11,900
TS (mg/l)	8,975
VTS (mg/l) (% of TS)	7,985 (89.0)
SS (mg/l)	670
VSS (mg/l) (% of SS)	645 (96.8)
Proteins (mg/l)	5,760
TFM (mg/l)	90
CO_2 in gas (%)	49.2
CH_4 in gas (%)	37.9

Chapter 13

SUMMARY AND DISCUSSION

13.1 Coffee wastewaters

During the studies on synthetic coffee wastewaters 24 different operational conditions were examined. For all of them a wide range of data was presented in Chapters 7 and 8 for the assessment of acidogenic phenomena, but also as indicatory data for subsequent treatment. The most important results related to the performance of the acidogenic reactors and the composition of the main acids produced for each one of the operational conditions with coffee wastewaters are presented in Table 13.1.

Acidified COD was between 5 and 42%. VFA concentrations were in the range of 0.3 to 2.3 g/l, with VFA yields in relation to Filt.COD in the substrate from 0.03 to 0.25 kg VFA/kg F.COD$_f$. The range of Filt.COD removal was 0-22%.

Acetic was always found to be the major acid (mostly >40%). Propionic and n-Butyric were also present in high proportions. n-Butyric was mostly the second acid and on a few cases it was also higher or equal to Acetic acid. Propionic was always the third acid apart from when the pH was 6.0 (Experiment 2, Chapter 8) and it became higher than n-Butyric acid for the reactors that operated at 37 and 45°C.

The total concentration of VFA consisted of up to 85% of these three acids. Additionally, n-Valeric was also produced in proportions of around 5-10%, with the exception of two cases when it reached up to 14 and 17%.

iso-Butyric, iso-Valeric, iso-Caproic and n-Caproic acids were produced in small quantities. Generally iso-Butyric and n-Caproic acids were fifth and sixth in order of magnitude in the VFA composition for Experiment 1 (Chapter 7), while iso-Valeric and iso-Caproic acids were seventh and eighth. However in Experiment 2 (Chapter 8) n- Caproic and iso-Valeric acids were fifth and sixth in order of magnitude or occasionally they were not detected. Mostly iso-Butyric and iso-Caproic acids were not detected at all.

Table 13.1: Operation and performance with coffee wastewaters

Temp. (°C)	pH	HRT (hrs)	OLR (kg F.COD/m^3·d)	VFA (mg/l)	Acid.COD (%)	VFA yield (kg VFA/kg F.COD_f)	F.$COD_{rem.}$ (%)	Acet. (% in VFA)	Prop. (% in VFA)	n-But. (% in VFA)	n-Val. (% in VFA)
37	5	15	14.8	900	15	0.10	1	38.7	5.8	49.5	3.4
37	5	14	15.9	825	14	0.09	5	40.9	6.8	45.8	4.0
37	5	13	17.3	1,025	17	0.11	4	42.7	6.6	42.5	5.1
37	5	13	16.7	1,085	18	0.12	1	38.1	7.7	44.8	5.4
37	5	12	18.2	1,035	16	0.11	0	41.4	6.9	42.3	5.2
45	5	15	14.8	1,015	16	0.11	2	45.6	11.5	34.1	3.8
50	5	14	15.9	735	13	0.08	10	40.5	14.0	35.0	7.2
55	5	13	17.3	605	11	0.06	13	42.0	16.3	28.4	8.6
60	5	13	16.7	770	13	0.08	7	40.4	15.8	28.6	10.0
65	5	12	18.2	670	11	0.07	1	41.8	15.0	29.9	8.8
45	4.5	15	14.8	500	7	0.05	7	63.6	9.2	21.8	1.4
50	4.5	14	15.9	315	5	0.03	6	66.5	8.3	19.8	1.6
55	4.5	13	17.3	400	6	0.04	8	57.6	7.5	27.6	2.3
60	4.5	13	16.7	760	12	0.08	1	44.3	11.4	31.1	6.7
65	4.5	12	18.2	585	9	0.06	2	49.2	10.1	28.3	7.5
45	6	12	18.6	940	15	0.10	17	70.7	17.5	8.5	17.1
45	6	9	24.8	930	15	0.10	15	64.0	21.2	11.4	3.3
45	6	6	37.3	620	10	0.07	12	77.8	5.6	2.3	14.3
37	6	12	18.6	2,290	42	0.25	20	42.8	33.2	14.6	7.8
37	6	9	24.8	2,195	41	0.24	20	42.4	31.7	14.5	8.8
37	6	6	37.3	2,020	41	0.22	22	41.2	31.7	14.4	10.2
37	4.5	12	18.6	1,385	27	0.15	19	42.9	3.8	44.2	6.9
37	4.5	9	24.8	1,315	25	0.14	17	39.7	7.4	38.3	11.4
37	4.5	6	37.3	965	20	0.10	18	39.6	2.2	53.2	2.3

13.1.1 Temperature Effects

In Figure 13.1 the effects of temperature on Acidified COD are presented. The data used were from Experiment 1, assuming that there was no significant changes due to the HRT changes that occurred during the study for any of the reactors at any temperature. This assumption has already been demonstrated graphically for the control reactor at 37°C in paragraph 7.8.2 (Chapter 7). The data used for 37°C is an average of the 5 data that were assessed for the control reactor in each of the 5 sets of experimental conditions applied in Experiment 1 (Chapter 7).

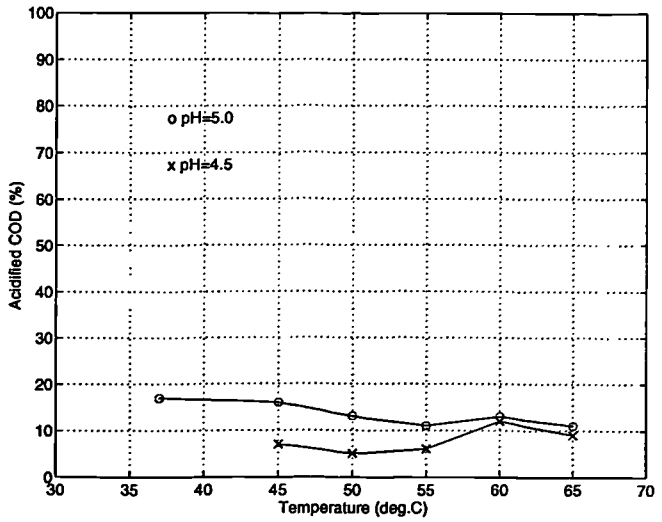


Figure 13.1: Effects of Temperature on Acidified COD with coffee wastewaters

Temperature studies were carried out to compare the optimum mesophilic (37°C) to various thermophilic temperatures. It was found in both experiments (Chapters 7 and 8) that 37°C was better, with improvement that ranged from 7 to 75% (based on acidified COD), over the examined thermophilic temperatures (45- 65°C).

Only in the case of pH 5.0 in Experiment 1 (Chapter 7), 45°C was slightly above 37°C, if a comparison is made with the value at HRT 15 hrs, but the difference appeared to be minor and insignificant. However the Acidified COD values between 37 and 45°C at pH 5.0 were the same, if the average of all values in Experiment 1 was used for the control reactor at 37°C.

Overall at pH 5.0 45°C appeared to be the best temperature in terms of acidified COD with a difference of 31% from the worst at 55°C. For pH 5.0 the order from the best to the worst temperature based on Acidified COD was: 45>60>50>65>55.

However at pH 4.5 60°C was the best temperature with 58% difference from 50°C which was the worst. The order for pH 4.5 was: 60>65>45>55>50. This order could be quite significant for process engineers in the event that a balancing tank is operated for pre-acidification in the treatment of coffee wastewaters. It appeared with uncontrolled operation at this low pH and with minimal cooling of the high temperatures (>70°C) of the wastewaters (normally occurring

by the time that the effluent reaches the wastewater treatment plant facilities) Acidified COD could reach values of 10%, but this value could be halved if operated at 50-55°C.

Between the two pH values that were applied 5.0 was always better than 4.5, with maximum difference of 62% at 50°C. The difference was above 45% at temperatures between 45-55°C but became almost negligible at 60°C and around 18% at 65°C. This indicated that in the thermophilic range and for low pH values the effect of pH on pre-acidification of coffee wastewaters was less above 55°C and more below 55°C towards the mesophilic range.

Figure 13.2 presents the effects of temperature on Acetic, Propionic and n-Butyric acids, which were the acids mainly produced during the acidification studies on coffee wastewaters.

From Figure 13.2.a it appears that the performance of acetate-producing bacteria was affected more by the temperature increase from 45 to 65°C in the reactor operating without pH control. Almost a third of the activity was reduced between the maximum at 50°C and the minimum at 60°C. However the effect of temperature on these bacteria between 37 and 65°C appeared to be very small at pH 5.0, with the variations being negligible above 50°C.

Furthermore although no significant changes appeared in the activities of propionate- producing bacteria at all applied conditions (Figure 13.2.b), the temperature changes affected their activities especially at pH 5.0 where a gradual but stable increase of about 40% occurred between 45 to 60°C. Also their activity more than doubled in the thermophilic range compared to 37°C. However at pH 4.5 very small and insignificant variations appeared due to the effects of the thermophilic temperatures for the activity of the propionate-producers.

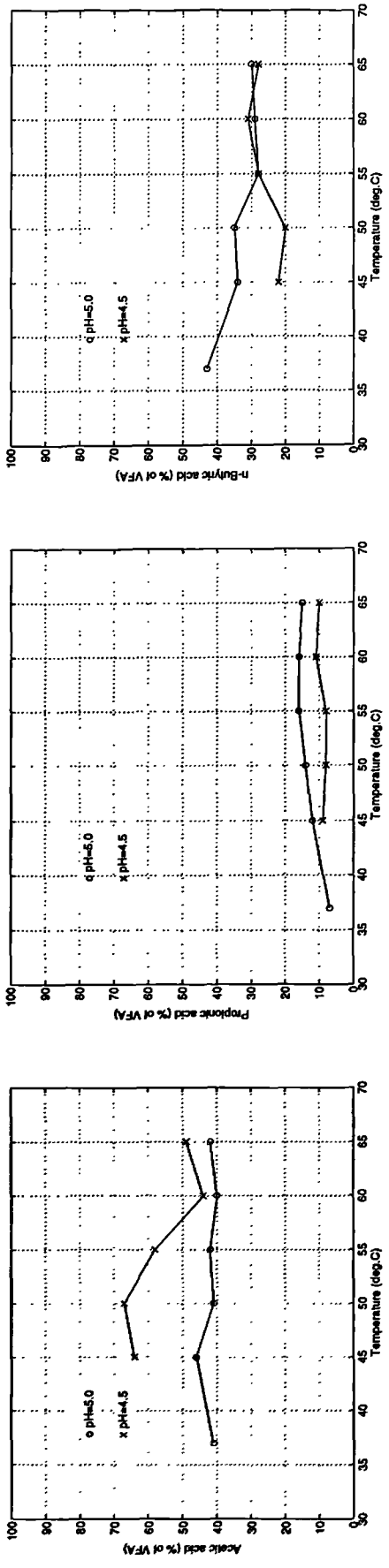
The effect of temperature was also obvious with the butyrate-producing bacteria from 37 to 55°C (Figure 13.2.c) for both pH values. Although for pH 5.0 the trend was decreasing by a third from 37 to 55°C and for pH 4.5 the trend was increasing to a similar extent between 45 and 55°C. However above 55°C the activities of the butyrate-producing bacteria were not changed neither for the non-pH-controlled reactor nor the reactor at pH 5.0.

13.1.2 HRT Effects

Figure 13.3 presents the effects of HRT on Acidified COD during Experiments 1 and 2 (Chapters 7 & 8) on coffee wastewaters.

HRT studies provided a very important engineering consideration. As was observed for the best performing acidogenic reactor, the decrease in HRT resulted in insignificant decrease in VFA concentration and acidified COD. Also there was no change observed in the composition of acids indicating the stability of the biological activities in this reactor.

Meanwhile for the two other reactors, with reduced efficiency, the HRT decrease resulted in reductions of 26% for the reactor with pH 4.5 and 37°C, and 33% for the one with pH 6.0 at 45°C. For both reactors the decrease began after the reduction of HRT from 9 to 6 hrs.



(a) Acetic acid (b) Propionic acid (c) n-Butyric acid

Figure 13.2: Effects of Temperature on VFA composition with coffee wastewaters

Also the results from Experiment 1 (Chapter 7) on HRT changes from 12 to 15 hrs appeared to be quite stable in terms of acidified COD, with marginal differences between 12 and 13 hrs and a small decrease observed towards 14 and 15 hrs. This point of relative stability in the performance of the reactor at 37°C during the HRT changes from 12 to 15 hrs, made more valid the assumption used in the previous paragraph on temperature effects (Section 13.1.1) to compare data of the three reactors produced in Experiment 1, as if they were data produced under the same HRT.

Overall the OLR values applied were in the range of 15 to 37 kg F.COD/m^3·d, which proved pre-acidification reactors to be high-rate systems capable of producing above 40% Acidified COD in their effluent in HRT as low as 6 hrs, with a recalcitrant high strength wastewater like coffee.

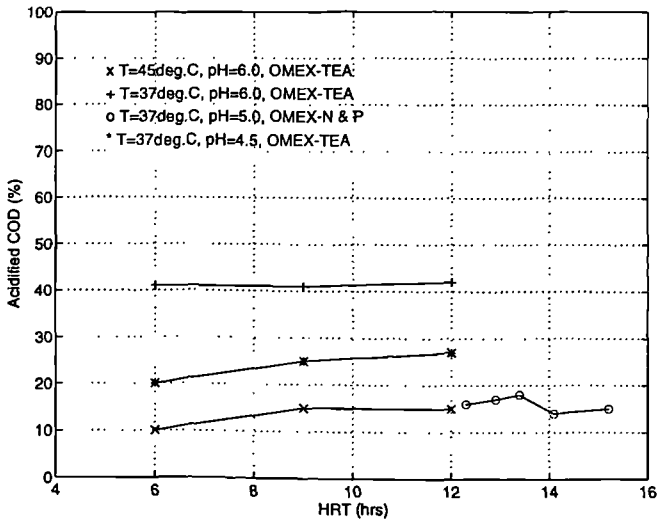


Figure 13.3: Effects of HRT on Acidified COD with coffee wastewaters

Furthermore from the pH comparisons it appeared that pH 6.0 was better than 4.5 at 37°C with differences from 36 up to 50% while the HRT decreased from 12 to 6 hrs. Also in comparison to pH 5.0 at HRT 12 hrs, pH 6.0 is 60% better.

As the results from the reactor with pH 4.5 at 12 hrs were 37% better than those at pH 5.0, it could be assumed that this fact related to the improved micro-nutrient additive from OMEX Ltd, which was rich in various trace metals for anaerobic digesters. Therefore the use of trace metals must have almost doubled the performance of the biomass, compared to the studies carried out in Experiment 1 (Chapter 7).

However the fact that in Experiment 2 (Chapter 8), pH 4.5 and 37°C resulted in up to 50% better Acidified COD than pH 6.0 and 45°C, indicated the close inter-relationship of pH and temperature as key design parameters for acidification. So it is important that results describing acidogenesis should always report these two significant environmental parameters to allow for any reactor process design.

Regarding the temperature differences between the reactors during Experiment 2 with a pH 6.0, the reactor at 37°C was from 63 to 75% better than the reactor at 45°C with the difference increasing with the decrease in HRT from 9 to 6 hrs.

In Figure 13.4 the effects of HRT on the main acids produced from coffee wastewater acidification are presented. All 3 graphs combine results both from Experiments 1 and 2 (Chapters 7 & 8).

From Figure 13.4.a on Acetic acid it appears that acetate-producing bacteria are not affected to any significant extent by the change of HRT either from 6 to 12 hours or from 12 to 15 hours, regardless their pH, as long as they operate at 37°C. But at 45°C variations in their activities occurred, with a 9% decrease (as a percentage of VFA) from 12 to 9 hrs and a subsequent increase of 22% from 9 to 6 hrs.

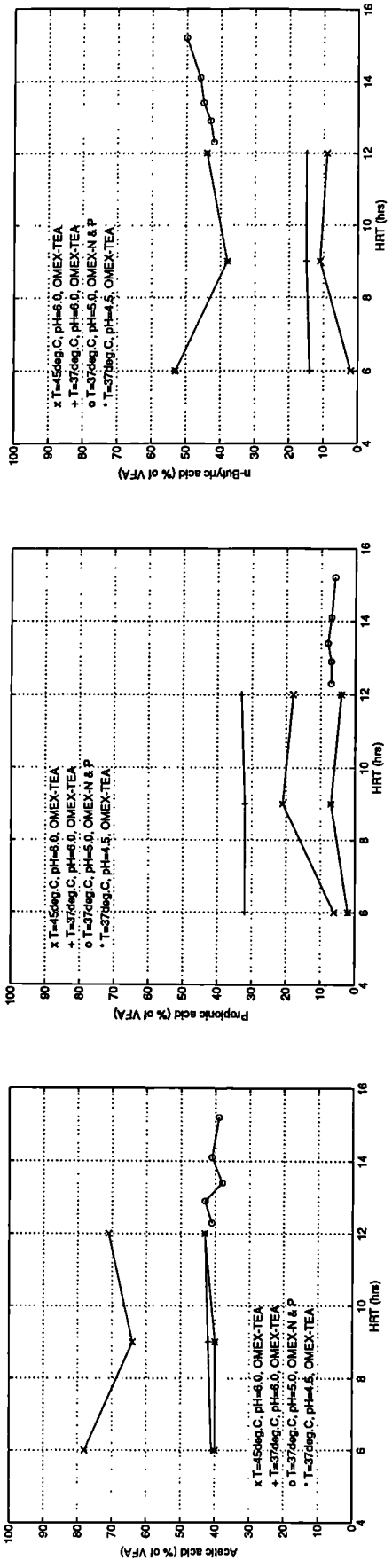
Similar observations to those about the acetate-producing bacteria were made regarding the activities of propionate-producing bacteria (Figure 13.4.b). No changes appeared with the changes from 12 to 6 hrs and from 15 to 12 hrs for the reactors at 37°C operating at pH 6.0 and pH 5.0 respectively. Furthermore an increase was observed between 12 and 9 hrs and a decrease between 9 and 6 hrs for the reactor at 37°C and pH 4.5. However the content of propionic was below 10% of the VFA composition so little could be deduced from these variations about the propionate-producing bacteria. Finally more significant variations were only observed at 45°C and pH 6.0, with a small increase from 12 to 9 hrs but a 74% decrease from 9 to 6 hrs.

In relation to butyrate-producing bacteria, as observed in Figure 13.4.c, they appeared mostly tolerant to HRT changes at 37°C and pH 6.0. However a small and gradual decrease of 14% in activity occurred at pH 5.0 and with HRT changes from 15 to 12 hrs. Relatively greater changes occurred with the non-pH-controlled reactor where their activities had a small decrease from 12 to 9 hrs and a 39% increase from 9 to 6 hrs. Finally for the reactor at 45°C similar changes occurred as with the other bacterial groups, starting with a small increase of activity from 12 to 9 hrs and an 80% decrease with a change from 9 to 6 hrs.

13.1.3 EVOP Examples

The use of the Evolutionary Operation methodology as it has been described by Box and Draper (1969) was not applied for the operation of Experiments 1 and 2 (Chapters 7 & 8). The present studies were not run in the operational cycles that are required in order to implement the EVOP statistical assessment of the effects of each variable design parameter on the performance of the acidogenic reactors treating coffee wastewaters. During the present studies three reactors were used for the collection of comparative data instead of one operating for several cycles in the cyclical mode between the design parameters that their effect were to be assessed, as would be required by an EVOP experiment.

For example considering Experiment 2 (Chapter 8) and the study of the effects of pH (from 4.5 to 6.0) and HRT (from 12 to 9 hrs), that was operated in this study using 2 reactors at two different pH values; after assessment was made for HRT 12 hrs then the same two reactors



(a) Acetic acid (b) Propionic acid (c) n-Butyric acid

Figure 13.4: Effects of HRT on VFA composition with coffee wastewaters

were acclimatised to HRT 9 hrs and the assessment was carried out for this new HRT. The total duration for these present studies was around 30 days (including the acclimatisation periods) and each reactor operated for more than 30 HRTs in order to obtain a valid and acclimatised number of data.

If the same study was made using the EVOP process then one reactor would need to operate in the following mode: 1) pH 4.5, HRT 9 hrs, 2) pH 6.0, HRT 12 hrs, 3) pH 4.5, HRT 12 hrs, and 4) pH 6.0, HRT 9 hrs. Operating the units from conditions 1 to 4 would be considered as 1 EVOP cycle for the assessment of the effects of these design parameters on acidification. In order to obtain a good number of valid data and start being able to statistically assess the effects using the EVOP methodology at least 3 cycles are required and a range of 3 to 6 cycles is suggested by Box and Draper (1969) depending on the required level of standard deviation. The total duration of such a study assuming an operation of 10 HRTs to obtain acclimatisation in each new set of operational conditions and to have a few valid data for an adequate statistical assessment of the mean values, would require at least around 18 days for each complete cycle. Therefore 54 days would be the minimal time requirement for the assessment of these four parameters if the minimal operation of 3 EVOP cycles was applied.

If the EVOP operation was carried out assessing each operational set for up to 30 HRTs, as in the studies of Experiment 2, then at least 158 days would be required to obtain, according to the EVOP methodology, an initial statistical assessment of the effect of pH and HRT on acidification of coffee wastewaters.

This long duration required for a proper assessment of a 2×2 factorial design according to the EVOP methodology, could be very easily extended if one considered possible mechanical and other operational problems that occur during most laboratory experiments. Even more when considering the use of EVOP for most anaerobic digestion studies where HRT is equal to several days or even weeks (i.e. sludge and solid waste treatment), then the use of the method even for an application of 6 HRTs (3 HRTs for acclimatization and at least 3 HRTs to obtain an adequate set of data for statistical analysis) for each operational condition, would require experiments operating from several months to few years in order to appropriately use the EVOP methodology for the optimisation of few design parameters. Therefore EVOP could only be applied for high rate digestion systems and only for wastewaters, if an adequate amount of time is available for such lengthy studies.

According to Britz (Britz, 1997) the EVOP process can be used for high rate anaerobic digestion experiments even in the event that one or two EVOP cycles have been operated, assuming that standard deviations are relatively small compared to the effects that some design parameters will have on anaerobic digestion phenomena. Therefore the process can indicate adequately the optimisation of the phenomena studied, as long as results are collected from adequately acclimatised operations of anaerobic digesters, where mean value variations have a CV that is below 10-15%. Also the process can clearly indicate which design parameters can have a greater effect than others in the specific anaerobic digestion phenomena studied.

In this study although the process has not been operated according to the EVOP mode, an improvised approach was suggested to be applied (Britz, 1997). Based on this approach the data collected from the two reactors could be assumed to have been operating under the same or similar microbiological content and therefore the results could be collectively used for a 2×2 factorial design approach. So in the present study one could use the EVOP methodology for results made out of 1 cycle.

The fact that no standard deviation could be calculated as with the EVOP methodology could be compromised by the use of an average standard deviation equal to the average of the 15% of the mean values used for each assessment. With this assumption if the mean values produced in each set of operational conditions has a CV in the range of 0-15% then the produced effect, which is a differential result of these mean values, will also have a similar or smaller average standard deviation.

Based on this improvised approach on EVOP, Table 13.2 presents as an example 5 2×2 factorial designs, all based on results from Experiment 2 (Chapter 8), in order to assess the effects of HRT, pH and temperature on the performance as 'Acidified COD' of the acidogenic reactors treating coffee wastewaters. All calculations for the effects and the mean value are based on the formulae described by Box and Draper (1969) for a 2×2 factorial design with no control operation and for 1 cycle, while the standard deviation is 15% of the average of the 4 values used for each of the operational conditions as presented in Table 13.1 for Acidified COD.

Table 13.2: EVOP Examples of 2×2 factorial designs for coffee wastewaters

	Example 1[1)]	Example 2[1)]	Example 3[2)]	Example 4[2)]	Example 5[3)]
Parameter A	HRT: 9, 12 hrs	HRT: 6, 9 hrs	HRT: 9, 12 hrs	HRT: 6, 9 hrs	T: 37, 45°C
Parameter B	pH: 4.5, 6.0	pH: 4.5, 6.0	T: 37, 45°C	T: 37, 45°C	pH: 4.5, 6.0
Effect A[4)]	1.5 ±5.1	2.5±4.8	0.5±4.2	2.5±4.0	- 23.5±3.4
Effect B[4)]	15.5±5.1	18.5±4.8	-26.5±4.2	-28.5±4.0	11.5±3.4
Effect AxB[4)]	-0.5±5.1	-2.5±4.8	-0.5±4.2	2.5±4.0	- 3.5±3.4
S.D. [5)]	5.1	4.8	4.2	4.0	3.4

[1)] conditions applied at 37°C.

[2)] conditions applied at pH 6.0.

[3)] conditions applied at Experiments 1 & 2 for HRT around 12 hrs.

[4)] effect calculated as difference on Acidified COD (%).

[5)] Standard Deviation assumed 15% of Mean Value.

As has already been concluded previously (section 13.1.2) and is also presented in Table 13.2 the effect of HRT is negligible, while both temperature and pH appear to have a more significant effect. Overall it is proved with the EVOP values on the effects of these three design parameters that HRT had no effect, while pH change from 4.5 to 6.0 had an effect of 15.5% of Acidified COD for HRT change from 12 to 9 hrs and an effect of 18.5% of Acidified COD for HRT change from 9 to 6 hrs. The effect of temperature from 37 to 45°C though was greater with -26.5%

of Acidified COD for HRT from 12 to 9 hrs and an effect of -28.5% of Acidified COD for HRT change from 9 to 6 hrs. From example 5 it became obvious that temperature had a greater effect than pH for coffee wastewater acidification.

So overall for the design parameters and ranges that were examined the following in terms of importance on the effect of each parameter on acidification of coffee wastewaters, was found by the EVOP examples:

$$Temperature > pH > HRT$$

Therefore with using the EVOP methodology it was indicated that optimum pH is 6.0 and optimum temperature is 37°C with HRT as low as 6 hrs. Also the effect of temperature is expected to be greater than that of pH in the ranges examined. The same conclusions about optimum conditions of acidogenic digester operation were also deduced from the previous assessments (Sections 13.1.1 & 13.1.2) on the effects of pH, temperature and HRT on Acidified COD during Experiment 2.

13.2 Slaughterhouse wastewaters

In the experiments on real slaughterhouse wastewaters 40 operational conditions were examined (including the various OLR changes in addition to the planned operational conditions). For all these conditions the main performance results and the main acids produced during all the applied conditions are presented in Table 13.3, to assess acidogenic phenomena.

VFA concentrations rapidly responded to OLR changes and were in the range of 0.8 to 4.9 g/l. Acidified COD was from 9 to 95% and in most operational conditions examined it could be considered that Acidified COD was reaching levels of complete acidification (>75%). VFA yields ranged from 0.05 to 0.46 kg VFA/kg F.COD$_f$, while Filt.COD removal was between 7-44%.

In most experiments Acetic was the major acid (on average above 28%). Propionic, n-Butyric and iso-Valeric were also produced acids in reasonable abundance. Occasionally iso-Valeric and sometimes Propionic were produced at proportions as high as Acetic acid. Also n-Butyric was mainly the third or the fourth main acid but occasionally it would be second.

The total concentration of VFA consisted of at least 85% from these four acids. Additionally, iso-Butyric and n-Valeric were also produced at times in proportions of up to 15% and 10% respectively. iso-Butyric was the fifth major acid with proportions sometimes similar to those of n-Butyric. n-Valeric was mainly produced at around 5-10% but few values were above 15%. n- and iso-Caproic were produced at very small proportions (<2%) but were most of the times detected.

Table 13.3: Operation and performance with slaughterhouse wastewaters

Temp. (°C)	pH	HRT (hrs)	OLR [1]	Filt.COD$_f$ (g/l)	VFA (mg/l)	Acid.COD (%)	VFA yield [2]	F.COD$_{rem.}$ (%)	Acet. (%VFA)	Prop. (%VFA)	iso-But. (%VFA)	n-But. (%VFA)	iso-Val. (%VFA)	n-Val. (%VFA)
37	6	12	3.6	1.8	210	25	0.12	30	49.5	11.3	9.4	8.5	16.5	2.8
37	6	12	10.2	5.1	605	25	0.12	30	42.1	23.2	6.8	11.9	11.3	2.7
37	6	12	16.6	8.3	785	18	0.09	19	35.0	21.5	9.7	14.3	13.9	3.8
37	6	12	8.1	4.0	490	23	0.12	19	35.7	19.7	10.9	12.5	17.8	2.3
45	6	12	10.2	5.1	585	19	0.11	13	47.3	20.6	6.7	10.3	11.6	1.9
35	6	12	16.6	8.3	755	19	0.09	25	30.4	30.8	6.3	13.9	14.4	2.5
30	6	12	16.6	8.3	605	17	0.07	31	32.8	21.5	10.6	11.1	15.8	7.3
25	6	12	8.1	4.0	250	12	0.06	19	41.8	14.7	9.6	12.0	17.9	2.4
45	7	12	10.2	5.1	2,260	92	0.44	24	32.3	18.3	10.6	17.7	16.0	3.5
35	7	12	16.6	8.3	2,910	85	0.35	33	20.4	32.2	11.1	14.3	14.2	4.3
30	7	12	16.6	8.3	3,090	84	0.37	28	23.9	24.9	11.4	15.9	15.6	4.6
25	7	12	8.1	4.0	1,595	74	0.40	14	24.1	27.1	12.0	14.5	17.2	4.2
25	5.5	12	28.0	14.0	785	10	0.06	17	41.4	15.8	9.9	13.9	15.1	2.8
25	6.5	12	21.9	10.9	1,630	27	0.15	17	39.0	14.7	10.7	15.9	15.5	3.2
25	6	9	21.3	8.0	360	9	0.05	29	44.4	15.8	9.2	11.4	13.6	4.4
25	6	6	8.0	2.0	235	20	0.13	18	51.9	13.2	6.8	9.4	11.9	5.1
37	5.5	12	28.0	14.0	1,475	22	0.11	21	17.6	26.1	12.1	12.7	17.2	13.8
37	6.5	12	21.9	10.9	4,240	88	0.39	27	22.2	18.4	11.1	23.2	17.3	5.7
37	6	9	21.3	8.0	2,680	88	0.34	38	26.7	14.5	11.4	24.1	14.8	6.3
37	6	6	8.0	2.0	450	48	0.23	27	31.4	27.4	8.4	12.8	14.6	4.2
37	7	12	28.0	14.0	4,920	76	0.35	26	27.0	19.4	12.1	19.9	15.9	3.5
37	7	12	21.9	10.9	4,155	85	0.38	27	24.0	19.1	14.2	19.4	18.5	3.7
37	7	9	21.3	8.0	2,955	95	0.37	37	27.0	20.5	12.6	17.7	15.5	4.5
37	7	6	8.0	2.0	615	88	0.31	44	27.1	29.3	11.7	10.1	18.2	3.4

(continued)

Table 13.3 *(continued)*

Temp. (°C)	pH	HRT (hrs)	OLR [1]	Filt.COD$_f$ (g/l)	VFA (mg/l)	Acid.COD (%)	VFA yield [2]	F.COD$_{rem.}$ (%)	Acet. (%VFA)	Prop. (%VFA)	iso-But. (%VFA)	n-But. (%VFA)	iso-Val. (%VFA)	n-Val. (%VFA)
25	7	12	24.4	12.2	3,425	49	0.28	17	47.4	13.8	8.4	13.3	12.7	2.7
25*	7*	12*	12.8	6.4	2,185	63	0.34	13	31.5	15.6	10.2	14.8	24.2	2.6
37	7	12	24.4	12.2	4,540	77	0.38	21	23.1	21.1	12.1	17.7	22.2	2.6
37*	7*	12*	12.8	6.4	2,765	84	0.43	16	25.7	17.3	9.5	17.4	26.6	2.9
25	7	12	22.8	11.4	3,635	66	0.32	20	24.3	15.1	11.4	17.1	25.8	4.0
25	7	12	17.9	9.0	3,190	67	0.36	11	19.8	17.2	12.2	19.1	26.1	3.8
25	7	9	36.7	13.8	3,980	57	0.29	17	27.1	17.8	11.1	18.1	19.4	4.5
25	7	6	51.3	12.8	4,055	56	0.32	7	22.7	20.9	11.7	17.9	21.8	4.1
37	7	12	22.8	11.4	4,225	78	0.37	21	26.3	13.8	12.2	17.1	25.9	3.4
37	7	12	17.9	9.0	4,125	92	0.46	16	20.6	15.5	13.3	19.3	27.1	3.6
37	7	9	36.7	13.8	4,385	67	0.32	22	21.9	22.2	11.9	16.7	21.8	4.4
37	7	6	51.3	12.8	4,770	67	0.37	8	23.5	21.1	12.4	14.8	19.1	4.5
37†	7†	12†	22.8	11.4	4,130	69	0.36	16	36.0	8.5	9.3	17.7	24.1	3.8
37†	7†	12†	17.9	9.0	4,115	87	0.46	13	23.7	14.5	11.9	19.6	25.8	3.9
37†	7†	9†	36.7	13.8	4,160	57	0.30	13	23.4	21.6	11.2	18.3	20.1	4.3
37†	7†	6†	51.3	12.8	4,615	64	0.36	7	24.7	17.5	12.3	18.5	21.0	4.2

[1] OLR units: kg Filt.COD/m^3·d.

[2] VFA yield units: kg VFA/kg F.COD$_f$.

* conditions applied without OMEX-TEA.

† conditions applied without mixing.

13.2.1 OLR Effects

In Figure 13.5 the effects of the OLR variations on Acidified COD are presented using results from all slaughterhouse experiments (Chapters 9-12).

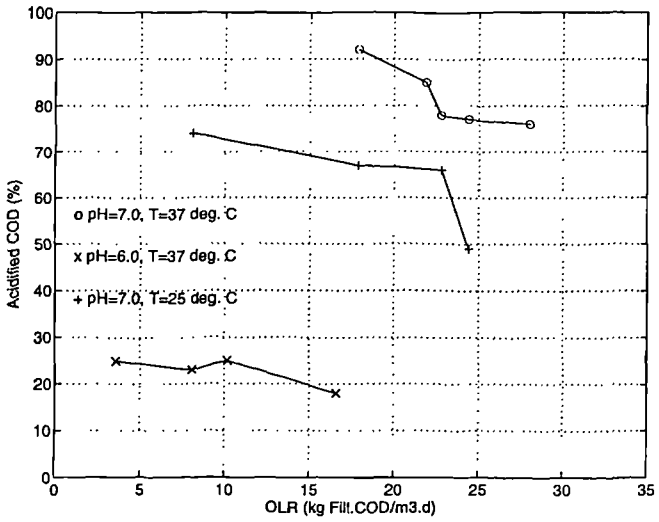


Figure 13.5: Effects of OLR on Acidified COD with slaughterhouse wastewaters

OLR values applied were in the range of 2 to 51 kg F.COD/m^3·d. Most OLR values were equivalent to high-rate acidogenic activities. Various combinations of OLR with other operational parameters have been examined, mainly due to the unpredicted characteristics of the freshly collected slaughterhouse wastewater. The latter provided a more realistic approach in this study, since normally pre-acidification tanks are considered as flow balancing tanks.

Overall at HRT 12 hrs, pH 7.0 and 37°C the effluent appeared to be completely acidified (values as high as 90% Acidified COD) with OLR around 18 kg Filt.COD/m^3·d. With higher OLR values there was a gradual decrease observed in Acidified COD for these operational conditions.

However at HRT 12 hrs, pH 7.0 and 25°C the system had relatively stable operation with high Acidified COD (>70%) until OLR around 23 kg Filt.COD/m^3·d when the system has a drop of about 26%, due to overloading.

Furthermore at HRT 12 hrs, pH 6.0 and 37°C the system not only had almost 3.5 times worse performance in Acidified COD from that observed at pH 7.0 and 25°C, but also had started to decrease in the performance at OLR 17 kg Filt.COD/m^3·d.

Overall with the presented OLR effects it was obvious that the most tolerant reactors with gradual decrease at OLR increase, were those at 37°C at either pH; while the one at 25°C would suddenly respond with a decrease in performance to digester overload.

Also it appeared that operations at pH 7.0 (no pH control) were far better than at pH 6.0 which was found optimum in the studies on coffee wastewater. pH was a parameter that had a greater

effect on acidification than temperature.

In Figure 13.6 the effects of OLR on the main acids produced are presented.

Overall it appears that acetate-producing bacteria were those who faced wider changes when operating at either 25°C or at pH 6.0. The effect on these type of bacteria from OLR was negligible at pH 7.0 and 37°C (Figure 13.6.a).

Small variations were observed for the propionate-producing bacteria at the optimum pH and temperature (Figure 13.6.b). However a small increase in activity was observed for the reactor at pH 6.0 with an increase in OLR up to 10 kg Filt.COD/m^3·d, and with relative stability above this OLR value. 25°C and pH 7.0 resulted in a stable decrease in the activity of the same type of bacteria.

Even smaller but quite similar trends were observed for the butyrate-producing bacteria with the reactors at 37°C with the OLR changes, but a gradual increase with the OLR increase and a small with the digester overload appeared for 25°C and pH 7.0 (Figure 13.6.c).

Finally the hydrolytic biomass related to the production of iso-Valeric had more erratic function with the reactors at 37°C, and a steady increase with the OLR increase followed by a decrease at the OLR when the overload occurred for the reactor operating at 25°C (Figure 13.6.d).

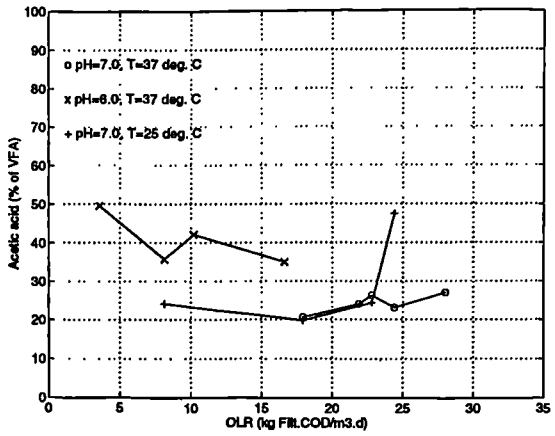
As it appeared from the VFA composition although the production of VFA by the bacteria was mostly affected by pH, their behaviour towards the OLR changes was mostly affected by temperature.

13.2.2 Temperature Effects

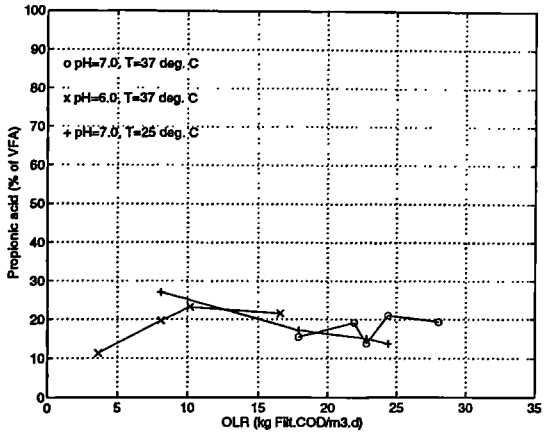
Temperature studies in Experiment 3 (Chapter 9) compared the optimum mesophilic (37°C) to the whole mesophilic range (25-45°C). 37°C was found to be better than all other temperatures. Apparently the difference appeared to be insignificant (less than 5%, in terms of Acidified COD) when compared to 30 and 35°C. Furthermore, 25°C was selected as an alternative, although its performance was at least worse in terms of Acidified COD. As in Experiment 3 there were significant OLR variations that were observed to have an effect on digester performance, particularly at low pH values, the comparison to assess the effect of temperature used results from the other Experiments on slaughterhouse wastewaters but with similar OLR values and at HRT 12 hrs.

Figure 13.7 presents the effects of temperature on Acidified COD during the slaughterhouse experiments. With this graph the effects of a range from 25 to 37°C could be evaluated. While for the result on 45°C and pH 6.0 generated in Experiment 3 it could be concluded by a direct comparison with the value of the control reactor at 37°C, but at similar operational conditions, that 45°C was 24% worse in Acidified COD than 37°C at pH 6.0.

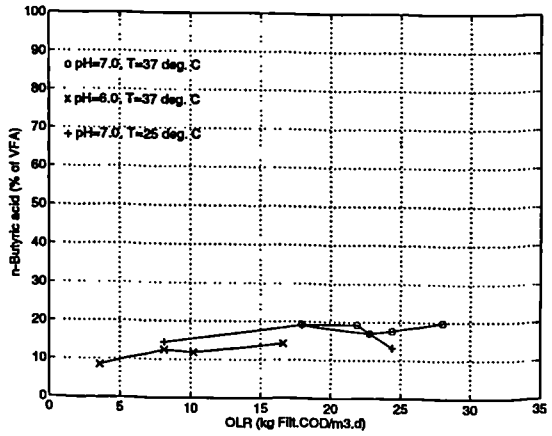
From Figure 13.7 the same conclusion could obviously derive about the effect of pH as was also found out with the assessment of the OLR effects. Furthermore the magnitude of the pH



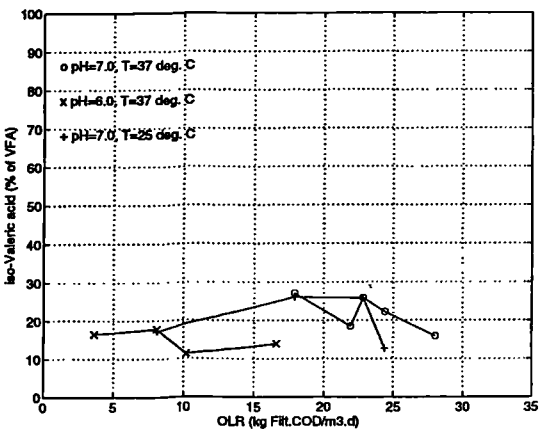
(a) Acetic acid



(b) Propionic acid



(c) n-Butyric acid



(d) iso-Valeric acid

Figure 13.6: Effects of OLR on VFA composition with slaughterhouse wastewaters

effect appeared to be far more significant than that for temperature which did not have major variations between 30 and 37°C, but had about 27% decrease in Acidified COD from 37 to 25 °C.

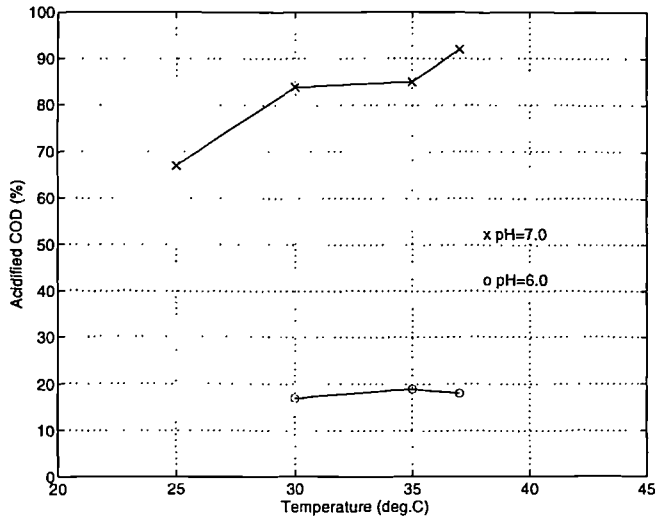


Figure 13.7: Effects of Temperature on Acidified COD with slaughterhouse wastewaters

Figure 13.8 present the variations of the activities of the main acidogenic bacteria due to the temperature changes.

Small variations were observed for the acetate and the butyrate-producing bacteria within the examined range of temperatures for both pH values (Figures 13.8.a & 13.8.c). However at both pH values the propionate-producing bacteria had a maximum at 35°C (Figure 13.8.b).

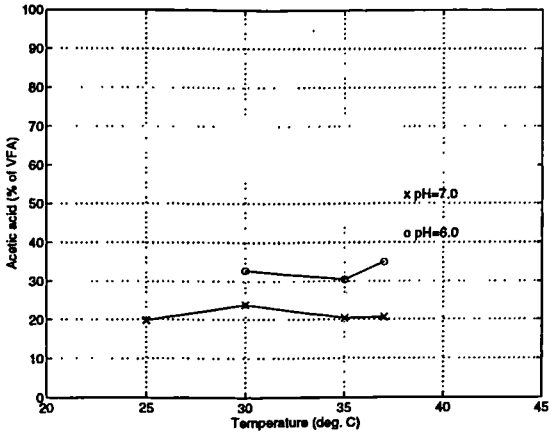
The results related to the activities of the hydrolytic bacteria that produce iso-Valeric acid were quite stable between 30 to 37°C for pH 6.0 with similar proportions to those between 30 to 35°C for pH 7.0 (Figure 13.8.d). However results were more erratic for 25 and 37°C and pH 7.0.

13.2.3 pH Effects

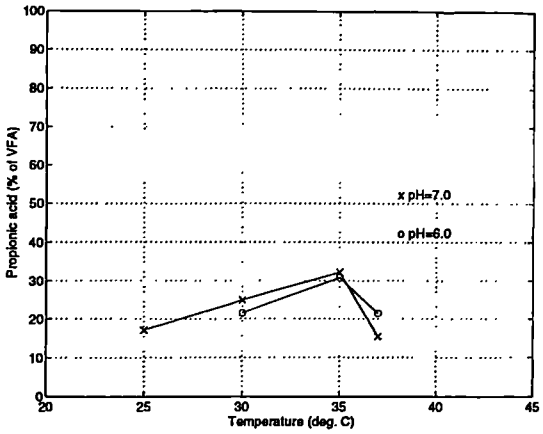
In Figure 13.9 the effects of pH on Acidified COD are presented.

As it has already been stated (Sections 13.2.1 and 13.2.2) pH 7.0 was better that the lower pH values and the optimum for 37°C was observed around 6.5 to 7.0, but for 25° the optimum pH was 7.0. An indirect comparison with the results produced in Experiment 4 (Chapter 10) between 5.5 and 7.0 at 37°C, proved that pH 7.0 was up to 4 times better than pH 5.5 in Acidified COD.

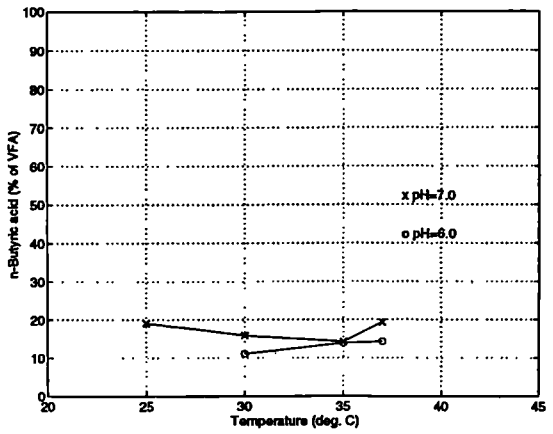
In Figure 13.10 the variations due to pH changes of the main acidogenic bacteria are presented, along with the observed variations in the composition of the main acids.



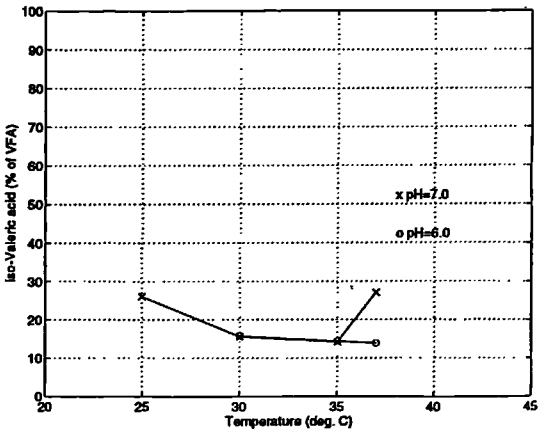
(a) Acetic acid



(b) Propionic acid



(c) n-Butyric acid



(d) iso-Valeric acid

Figure 13.8: Effects of Temperature on VFA composition with slaughterhouse wastewaters

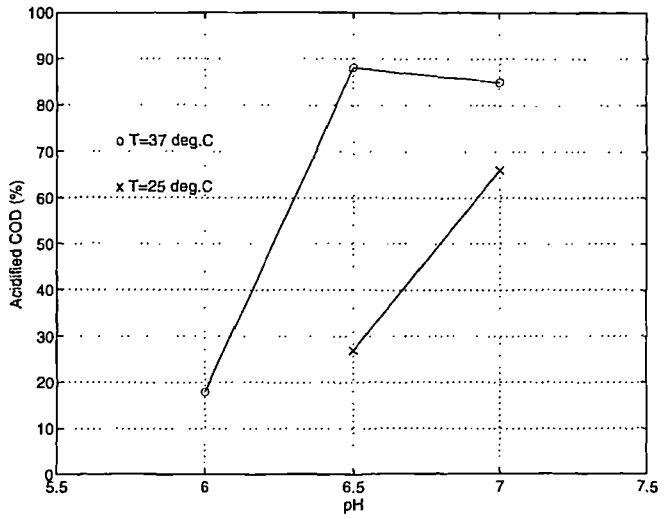


Figure 13.9: Effects of pH on Acidified COD with slaughterhouse wastewaters

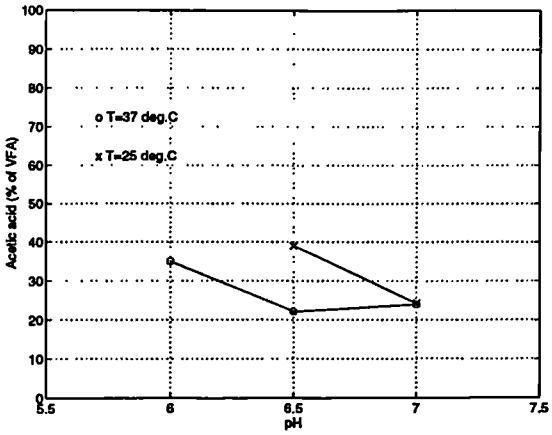
Acetate-producing bacteria appeared to have a decrease in their activity with a pH increase from 6.0 to 6.5 for 37°C and from 6.5 to 7.0 for 25°C (Figure 13.8.a). However propionate-producing bacteria were not affected by the pH change at any temperature (Figure 13.8.b).

Butyrate-producing bacteria were slightly affected at 37°C with the change from pH 6.0 to 6.5 but were not affected with the change from 6.5 to 7.0 for any temperature (Figure 13.8.c). While an increase in activities of the hydrolytic bacteria producing iso-Valeric acid was observed with the increase of pH for both temperatures (Figure 13.8.d).

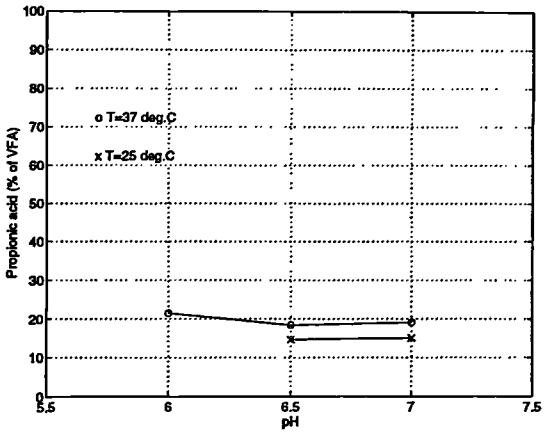
13.2.4 HRT Effects

Figure 13.11 presents the effects of HRT on acidified COD during the studies on slaughterhouse wastewaters. This graph was prepared using the results from Experiment 6 (Chapter 12), based on the assumption that the strength in the COD was quite similar and there was no significant effect due to the change in the Filt.COD from 11.4 to 13.8 g/l. This assumption could be accepted for 37°C and pH 7.0 that the reactor tolerated such high COD levels. However the graph for 25°C is only presented as an indication because around 12 to 13 g/l was found to be the digester overload OLR for this operation from Figure 13.5 (Section 13.2.1).

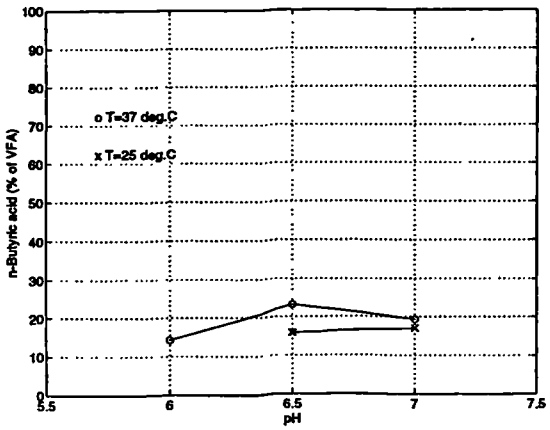
Overall the comparison of HRT values was quite similar to what was already observed for coffee wastewaters (Section 13.1.2). No significant change was observed between 6 and 9 hrs but a small decrease which was quite similar for the two temperatures appeared from 12 to 9 hrs. For both temperatures this decrease from 12 to 9 hrs was around 14% in Acidified COD. It should be pointed out though that the Acidified COD that was produced at HRT 6 hrs was as high as 56 and 67% for 25 and 37°C respectively



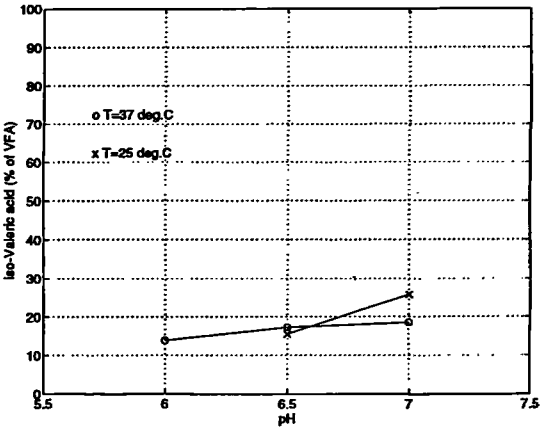
(a) Acetic acid



(b) Propionic acid



(c) n-Butyric acid



(d) iso-Valeric acid

Figure 13.10: Effects of pH on VFA composition with slaughterhouse wastewaters

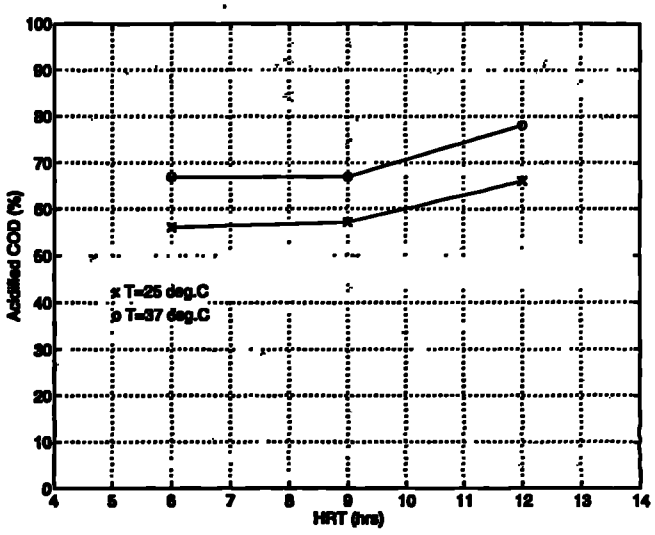


Figure 13.11: Effects of HRT on Acidified COD with slaughterhouse wastewaters

The HRT results provide similar beneficial engineering considerations to those described for synthetic instant coffee wastewaters, in relation to the size of the digester required to achieve above 40% Acidified COD.

In Figure 13.12 the effects of HRT on the main acids produced are presented.

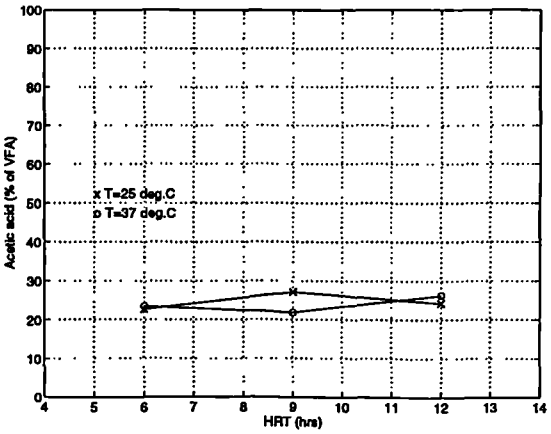
As it was obvious no significant change appeared for any type of bacteria involved in the production of the main acids, neither due to the HRT change nor due to the different temperature (Figures 13.8.a-13.8.d).

13.2.5 EVOP Examples

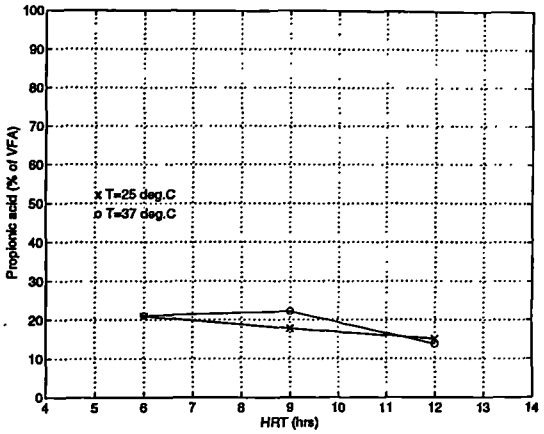
As for Experiments 1 and 2 for coffee wastewater, neither Experiments 3 to 6 (Chapters 9-12) for slaughterhouse wastewater were carried out according to the EVOP experimental operation. However some of the results were used to enable the evaluation of EVOP optimisation as for coffee wastewater (Section 13.1.3).

So Table 13.4 presents as an example 4 2×2 factorial designs, all based on various results from Experiment 3 to 6 (Chapters 9 to 12), in order to assess the effects of HRT, pH, temperature, OLR and mixing on the performance as 'Acidified COD' of the acidogenic reactors treating slaughterhouse wastewaters. All calculations for the effects and the mean value are based on the formulae described by Box and Draper (1969) for a 2×2 factorial design with no control operation and for 1 cycle, while the standard deviation is 15% of the average of the 4 values used for each of the operational conditions as presented in Table 13.3 for Acidified COD.

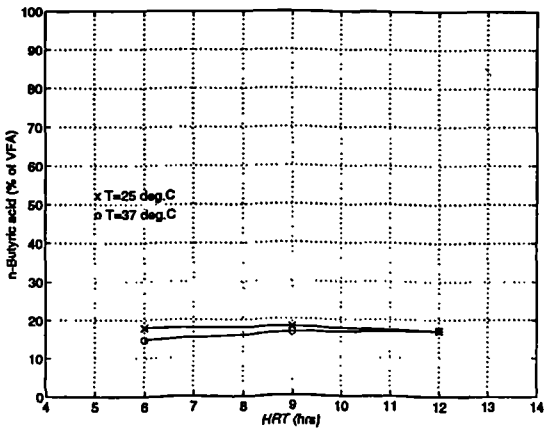
From example 1 it appeared that pH had the most important effect on acidification of slaughterhouse wastewaters, as it had already been established by the results of the present study in



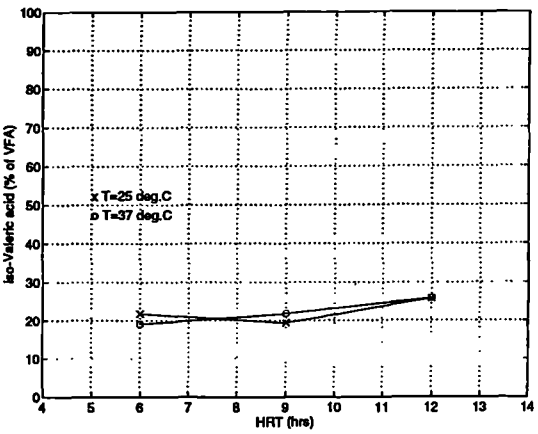
(a) Acetic acid



(b) Propionic acid



(c) n-Butyric acid



(d) iso-Valeric acid

Figure 13.12: Effects of HRT on VFA composition with slaughterhouse wastewaters

Table 13.4: EVOP Examples of 2×2 factorial designs for slaughterhouse wastewaters

	Example 1[1)]	Example 2[2)]	Example 3[3)]	Example 4[4)]
Parameter A	pH: 6, 7	T: 25, 37°C	HRT: 9, 12 hrs	OLR[7)]: 9.0, 11.4
Parameter B	T: 30, 35°C	OLR[7)]: 9.0, 11.4	OLR[7)]: 8.5, 13.9	Mixing: No, Yes
Effect A[5)]	66.5 ±7.7	18.5±11.4	3.0±12.4	- 16.0±12.2
Effect B[5)]	1.5±7.7	-7.5±11.4	-22.0±12.4	7.0±12.2
Effect AxB[5)]	-0.5±7.7	-6.5±11.4	6.0±12.4	2.0±12.2
S.D. [6)]	7.7	11.4	12.4	12.2

[1)] conditions applied at HRT 12 hrs and $F.COD_f$ 8.3g/l

[2)] conditions applied at pH 7.0 and HRT 12 hrs.

[3)] conditions applied at pH 7.0 and 37°C.

[4)] conditions applied at pH 7.0, 37°C and HRT 12 hrs.

[5)] effect calculated as difference on Acidified COD (%).

[6)] Standard Deviation assumed 15% of Mean Value.

[7)] OLR units: kg F.COD/ m^3·d .

Sections 13.2.1-13.2.3.

From example 2 it was observed that OLR had a smaller effect compared to temperature, and from example 3 it was observed that OLR had a greater effect than HRT. Also from example 4 it was also found that mixing had a smaller effect than OLR, but the small effect of mixing appeared to be higher that that for HRT from an indirect comparison between examples 3 and 4. The same findings about temperature, OLR, HRT and mixing were already established in Sections 13.2.1-13.2.4 and Chapter 6 about mixing.

So overall for the design parameters and ranges that were examined the following in terms of importance for the effect of each parameter on acidification of slaughterhouse wastewater, was found by the EVOP examples:

$$pH > Temperature > OLR > Mixing > HRT$$

13.3 Concepts related to the design of acidogenic reactors

13.3.1 Design parameters

The most important point of the present studies was that acidification and the operational conditions in order to optimise the performance of this process was mainly dependent on the type of wastewater that was acidified. This was concluded particularly when the same conditions were applied for the two wastewaters (i.e. pH 6.0, 37°C, HRT 12 hrs and OLR around 18.6 and 16.6 kg F.COD/ m^3·d for coffee and slaughterhouse wastewaters respectively); and the reactor operating with coffee wastewaters reached its optimum performance with Acidified COD around 42%, while the one operating on slaughterhouse wastewaters had one of its lowest performances

and achieved only 18% Acidified COD. This example came as practical proof that although slaughterhouse is more easily biodegradable than coffee wastewaters the appropriate conditions for acidification of this particular type of wastewater need to be identified before any optimisation could be achieved.

This conclusion made it obvious that all concluded optimum operational conditions from various researchers related mainly to the type of substrate that was used in their studies, and were primarily based on the biochemical content of their substrate.

Also another important point of the present study was that the two different types of wastewaters used, resulted in two different compositions of VFA which were related to the particular degradation pathway of each wastewater before it became simplified acids (i.e. Acetic, Propionic and n-Butyric). So for coffee wastewaters the principles acids were Acetic, Propionic and n-Butyric within a range of 38-78%, 2- 33% and 2-53% respectively. However for slaughterhouse wastewater the principle acids were Acetic, Propionic, n-Butyric and iso-Valeric within a range of 18-52%, 9- 32%, 9-24% and 11-26% respectively.

Overall 20-40% of acidification was achieved with several of the described conditions applied on coffee wastewaters. This degree of acidification was recommended by Lettinga and Hulshoff (1991) in order to sustain balanced anaerobic activities in UASB reactors. Optimum results in terms of Acidified COD and VFA composition, were obtained with the following conditions: 37°; pH 6.0; HRT 6 to 9 hrs; OLR 25-37 kg F.COD/ m^3·d and the use of urea and micro-nutrients, rich in trace metals. Such an application would benefit the treatment of a recalcitrant high-strength wastewater, as in instant coffee production.

Also 20-95% of acidification was achieved on most of the described conditions for slaughterhouse wastewaters. Optimum results for these wastewaters in terms of Acidified COD and VFA composition, could be obtained with the following low-cost conditions: 25°; pH 7.0; HRT 6 hrs; OLR up to 51 kg F.COD/ m^3·d; no micro-nutrients; and no mixing. Such an application would fulfil the pre-treatment of an easily biodegradable high-strength wastewater, such as that from slaughterhouses. In the event that the aim is to completely acidify slaughterhouse wastewaters (Acidified COD>75%) then the previous conditions are recommended with a temperature of 37°C.

McDougall et al. (1993) and McDougall (1996) suggested similar conditions to optimise the acidification of coffee wastewaters with HRT 12-24 hrs and achieved 40-50% Acidified COD. However it was recommended to use pH 5.5, 35°C and HRT 12 hrs to optimise dairy wastewaters with COD around 4 g/l, and pH 6.0, 20°C and 12 hrs to achieve 40% Acidified COD for brewery wastewaters with COD 3.5 g/l (McDougall et al., 1993).

Dinsdale et al. (1997) reported similar results for coffee wastewater acidification with a thermophilic application at 55°C, no pH control (pH 4.8 to 5.8) and HRT from 12 to 24 hrs, and achieved 32-37% Acidified COD. During their HRT studies they observed changes between 12 and 24 hrs, with HRT 24 hrs producing almost double acidified COD than HRT 12 hrs. Also they concluded that pH control at pH 6.0 did not result in better acidification than operating

at no pH control (pH around 5.0).

Several studies (Kozuchowska, 1992; McDougall, 1996; Dinsdale et al., 1997) have reported that the major acids produced by acidification of coffee wastewaters were Acetic with Propionic and n-Butyric being second or third depending on the operational conditions that were applied. Also n-Valeric acid was produced at smaller quantities. The same VFA composition was reported by the present study.

Zoetemeyer et al. (1982a) reported their studies on pH along with HRT changes at 30°C for a synthetic substrate containing mainly glucose as carbon source. pH 6.0 was found to result in a maximum growth rate for acidogens with HRT as low as 4 hrs. As in the present study, they observed no changes in the composition of Acetic acid with the changes in HRT, but they reported major decrease to Propionic acid until it was not detected in the studies below HRT 4 hrs. However although a reduction was also observed in n-Butyric acid due to the decrease of HRT from 9 to 3 hrs, its proportion was always higher than that for Acetic.

Further studies to establish the optimum temperature within the mesophilic and thermophilic ranges for glucose acidification by Zoetemeyer et al. (1982b), reported that 37°C was the optimum for mesophilic and 52°C the optimum for thermophilic. Although in the present study the same optimum has been found for the mesophilic range for all applied pH and HRT values, 60°C was found optimum at pH 5.0 for coffee wastewaters. This difference in findings might be because of the two different types of substrates but also because the studies of Zoetemeyer et al.(1982b) were carried out at pH 6.0.

Dinopoulou and Lester (1989) found no statistically significant differences in the acidification of a synthetic wastewater based on meat extract at 37°C and no pH control (pH around 6.0), due to HRT changes from 3.5 to 13.5 hrs and with OLR values from 5 to 10 kg F.COD/ m^3·d. The main acids observed in their studies were Acetic, Propionic, n-Butyric and n-Valeric in similar proportions to those found in the present study on coffee wastewaters. Also their Acidified COD values can be estimated to range between 45-65%.

Various other researchers report their studies on acidification of various substrates examining a few operational conditions while assessing the benefits of two-phase digestion over the single phase (Tanaka & Matsuo, 1986; Hanaki et al., 1987; Hajipakkos, 1987; van den Merwe et al., 1994). Overall most researchers optimise to a small proportion their studies but most times fail to suggest what were the values of Acidified COD and VFA composition achieved or does not always report all other main operational parameters.

The present studies for both types of wastewaters have examined in detail all the main operational conditions applied on digesters and assessed the option of acidification both as a pretreatment process as well as a process that could be used for the provision of VFA to a methane reactor. So low-cost options were also defined in order to enable process engineers to apply the same unit as a balancing tank if necessary.

With the use of EVOP optimisation it has been established that for coffee wastewaters temper-

ature was the most important design parameter from all the ones examined, with pH the second in importance design parameter. While for slaughterhouse wastewaters pH was established by EVOP optimisation to be the most important parameter, with temperature second in importance design parameter. For both wastewaters HRT changes from 6 to 12 hrs appeared to have negligible effect. Also for slaughterhouse wastewaters the effect of OLR and mixing although small was found to be more important than HRT for the design of acidogenic reactors.

Few more points of engineering importance derived from the present studies on acidification. The first in relation to coffee wastewaters was the magnitude of alkali consumption, especially regarding the comments in Experiment 2 (Chapter 8), and the fact that similar amounts of NaOH were consumed by reactors with different values of Acidified COD. Similar magnitude of NaOH consumption had been reported by Zoetemeyer et al. (1982a).

While, the second related to coffee wastewater reflected an obvious elimination of methanogenic activities using reduced HRT values, which was not achieved at any stage of the slaughterhouse studies even when acidified COD was as low as 15%.

Also in relation to slaughterhouse wastewaters the first point was related to the magnitude of NH_3-N produced, especially with levels as high as 1.2 g/l, regarding the potentially inhibitory levels at 1.5 g/l (Owen, 1982); but also the high buffering capacity generated with such high NH_3-N content for the acidogenic process (Speece & McCarty, 1964; Steiner et al., 1985).

While another point from slaughterhouse wastewaters related to the potential of the pre-acidification process as low-cost high-rate pre-treatment.

Also in Experiment 5 (Chapter 11), it was found that there was no apparent change when the addition of micro-nutrients stopped. This observation indicated that slaughterhouse wastewaters have adequate nutrient concentrations, without requirements even for specialist trace metals. However the same did not apply for the coffee wastewater, based on the findings related to the two different types of micro- nutrient mixture applied in Experiments 1 and 2 (Chapters 7 & 8).

Finally mixing appeared not to be required for acidogenic reactors, when operating in an upflow mode, as it was also reported by Ghosh (1987).

Overall with relation to the VFA products from the two wastewaters that were acidified several points have already been established about the effects of the design parameters on the biomass active in the acidogenic reactor (Section 13.1 and 13.2). For both treatment processes acetate, propionate and butyrate-producing bacteria were the main acidogenic groups that were involved in the process, along with the hydrolytic bacteria producing iso-Valeric in slaughterhouse acidification. Any changes in the design parameters should be aimed at controlling the group which has the most desirable end products as described by Andrews and Pearson (1965).

Finally in relation to gas composition the range of CO_2 found in those studies was related with the proportions that were presented by Zoetemeyer et al. (1982a, 1982b). CH_4 was present in all applied conditions in relation to slaughterhouse wastewaters acidification, and in several of the conditions applied for coffee wastewaters. It was assumed that it was generated by hydrogen-

oxidising methanogens who thrive on H_2 and are more tolerant than the other methanogens as they have a comparatively high doubling time (Eckenfelder, 1992). Similar levels of CH_4 content have been reported by Dinsdale et al. (1997). The residual VFA content in the biogas composition which at times is as high as 80% was assumed to be primarily H_2 and other gases (i.e. N_2, H_2S, etc.) (Popel, 1964), as it was also reported in the case of Zoetemeyer et al. (1982a, 1982b).

13.3.2 Guidelines for process engineers

The task of a process engineer who designs a pre-acidification process is to ensure the provision of an adequate degree of acidification along with a VFA composition that reflects the preferable range of VFA for methanogens, as described by Andrews and Pearson (1965).

Primarily it should be emphasised that there is no best value for Acidified COD, but there is a targeted value of Acidified COD dependent on treatment requirements (whether complete or partly acidification is needed) and process economy. So the selection of the most appropriate degree of acidification is based on each specific treatment application.

For example VFA concentrations of up to 20 g/l have been used successfully as substrate for high rate methanogenesis in various types of anaerobic digester designs, especially in the treatment of MSW with a two-phase leaching process.

The high content of VFA concentration in relation to potential inhibitory levels of acidogenic products (i.e. free butyric acid as an inhibitor of acidification) for the acidogenic reactor is the only problem that could be experienced when such high concentrations of VFA are targeted.

Furthermore it should be clarified that such a VFA concentration (i.e. 20 g/l) could be the result of a partly acidified process (i.e. 20% of the total organic matter of MSW) or the result of a completely acidified process (i.e. 90% acidified leachate wastewaters). In most types of agro-industrial wastewaters even with complete acidification of the substrate (Acidified COD >75%) such high VFA concentrations are not experienced (generally VFA concentration values of completely acidified high strength agro-industrial wastewaters would have maximum of 5 to 10 g/l).

Overall a specific degree of acidification (i.e. 20 to 40% as Acidified COD), that a wastewater has achieved in an acidogenic reactor should and could not be a threshold target. In relation to inhibition either of the acidogenic or the methanogenic reactor the Acidified COD value does not provide any information. As inhibition of acidification by its own end products is often related to undissociated concentrations of main acids produced (i.e. free butyric acid) and inhibition of methanogenic activities by high concentrations of VFA is mostly related to OLR overload, which is a design parameter fully dependent on the OLR capacity of the methanogenic reactor (in most cases an engineered high-rate methanogenic reactor would tolerate more easily a high OLR of VFA than a similar OLR of another substrate that would require to be hydrolysed and acidified in the same reactor at the same high OLR value).

Based on the above facts on the level of Acidified COD and the current assumption that 20 to 40% is a threshold for process design, above which inhibition factors could occur, it is recommended that for each specific process design the level of Acidified COD should be carefully considered in relation to overall treatment process requirements and economy. For example in some cases (i.e. MSW) Acidified COD below 20% could be the maximum economically obtainable and an adequate degree of acidification for a sustainable digestion process, while in other cases (i.e. high strength biodegradable agro-industrial wastewaters, as slaughterhouse) Acidified COD above 90% could be what a high rate digestion process requires.

From the results produced in this project, the literature on two-phase processes and the pre-treatment requirements of agro-industrial wastewaters, the following simplified guidelines are proposed for process engineers to benefit from the use of acidogenic phenomena:

- acidification is strongly related to the type of substrate that is to be acidified and the use of optimum conditions found from other types of wastewaters could be occasionally very misleading. Even a short and simple lab-scale study carried out on the acidification of the particular type of wastewater prior to the design of the process, might prove very beneficial;
- depending on the type of wastewater a characteristic type of VFA composition and other acidification products are expected, based on the hydrolytic and acidogenic metabolic pathway related to this type of wastewater. From the literature and the present study it appeared that in the acidification of wastewaters rich in carbohydrates, the main acids expected to be present in the acidified effluent are: Acetic, Propionic and n-Butyric acid, with a possible considerable proportion of n-Valeric. While for slaughterhouse and similar types of protein-rich wastewaters the main acids produced are: Acetic, Propionic, n-Butyric and iso-Valeric, with possible considerable amounts from iso-Butyric and n-Valeric;
- pH and temperature appear to be quite significant design parameters, particularly for high strength wastewaters (COD>10 g/l), and they are strongly inter-related. Choice of one value for one of these parameters would need to be optimised for the other value. Also variation of these two parameters could achieve high Acidified COD levels and also more desirable VFA compositions, with the effects that they will also have on the activities of the hydrolytic/acidogenic biomass;
- HRT of the pre-acidification reactor should be determined by taking into consideration both requirements for VFA production and for flow balancing. However, it should not produce such a level of acidification that might cause adverse effects in the operation of the methanogenic reactor, especially in the case of a UASB. Since the degree of acidification is a function of both environmental conditions and the characteristics of the wastewater to be treated, if no information is available for the desired HRT, a study should be carried out on site;
- no settling, recycle of biomass or mixing is required for the performance of the process as a low-cost pre-treatment application. However such design configurations, especially mixing,

are not found to affect significantly the degree of acidification in order to economically justify their use especially when easily biodegradable wastewaters are to be treated. Mixing can be adequately achieved by operating the acidogenic reactor in an upflow mode;

- the reactor or tank used for pre-acidification can and should be as simple as possible, so as to avoid high capital and operational costs. If a balancing tank is used for this purpose it should be covered, in order to maintain anaerobic conditions and prevent odours spreading;

- it should be considered in the case that a two-phase process is to be applied instead of a single-phase that the volume of the acidogenic reactor will not be added to the total volume of digester requirement, but it should be subtracted from the total required digestion volume, since part or half of the anaerobic activities will be achieved in the engineered first-phase. This in most cases will result in an overall capital and operational cost decrease for the digester requirements;

- the use of a pre-acidification reactor in the case where a balancing tank might not be required, should be justified both by the improvement in the overall wastewater treatment, but also by estimating capital and operational cost benefits or losses. This consideration is essential for all cases where biological pre-treatment or treatment is evaluated;

- the acidogenic end products are mainly simple fatty acids, which can provide a simple carbon source for other types of bacteria apart from methanogens. For example tertiary biological nutrient removal processes utilise to a great extent commercially purchased and added chemicals in the form of acetate. Also, aerobic bacteria can be recipients of acidogenic end products with quite a few benefits to consider (Kollatsch, as cited in Hausler, 1969). Or even biological pre-treatment of agro-industrial wastewaters can be used before discharge to a local sewer;

- the presence of methanogenic bacteria in a pre-acidification tank is possible and their elimination in acidogenic reactors difficult to achieve in most full-scale processes, where the application of pure acidogenic cultures or strict phase separation are not practical. Therefore, acidogenic biogas should be removed safely due to the potential explosive characteristics of CH_4, at a content between 5-15%;

- as VFA are increasingly considered to be recyclable bioproducts, this potential should be considered if VFA production is expected to be above 10 g/l.

Chapter 14

CONCLUSIONS

The project derived the following conclusions:

- An extensive amount of data has been provided to design and operate high-rate acidogenic reactors for two high-strength agro-industrial wastewaters. Overall 24 sets of operational conditions have been examined for synthetic instant coffee wastewaters and 40 for real slaughterhouse wastewaters.
- The data provided a number of additional design considerations for each individual wastewater. Also an extensive list of guidelines for the design of acidogenic reactors has been recommended for process engineers.
- The biochemical acidification pathway of synthetic instant coffee wastewaters consists of Acetic, Propionic, n-Butyric as principal acids and small proportions of n-Valeric acid.
- Meanwhile the biochemical acidification pathway of slaughterhouse wastewaters consists of Acetic, Propionic, n-Butyric and iso-Valeric as principal acids and small proportions of iso-Butyric and n-Valeric acids.
- Also, it became apparent, that different wastewater characteristics not only acidify differently but also respond differently to operational conditions that could be proposed optimum for another type of wastewater.
- Apparently, 37°C proved the temperature that both wastewaters produced the highest degree of acidification. But in terms of pH and HRT, the result was dependant on wastewater characteristics.
- In relation to pH values, pH 6.0 was found optimum for pre-acidification of coffee wastewater at 37°C. However pH 7.0 was found optimum for pre-acidification of slaughterhouse wastewater at the best temperature. But it was also found that optimum pH is closely related to the temperature that was applied.
- HRT values from 6 to 12 hrs had small effects on the Acidified COD for both wastewaters.

- Addition of micro-nutrient mixtures and mixing were also found to have a small effect on the acidification of slaughterhouse wastewaters
- Pre-acidification appeared to be a significant tool for high-rate low-cost pre- treatment of biodegradable agro-industrial wastewaters, as slaughterhouse.
- The following operations has been proposed for acidification (up to 40% acidified COD) of Synthetic Instant Coffee Wastewaters: 37°C; pH 6.0; HRT 6 to 9 hrs; and addition of urea and micro-nutrients, rich in trace metals.
- Also the following operations has been proposed for low-cost acidification (above 40% acidified COD), of Slaughterhouse Wastewaters: 25°C; pH 7.0; HRT 6 hrs; no added micro-nutrients; and no mixing.
- However the following operations has been proposed for complete acidification (above 75% acidified COD), of Slaughterhouse Wastewaters: 37°C; pH 7.0; HRT 6 hrs; no added micro-nutrients; and no mixing.
- According to the results and the EVOP optimisation the order of importance on the effect of each design parameter on Acidified COD, as evaluated on acidification of coffee wastewaters, was found to be:

$$Temperature > pH > HRT$$

- Furthermore the order of importance on the effect of each design parameter evaluated on Acidified COD, as evaluated on acidification of slaughterhouse wastewaters, was found to be:

$$pH > Temperature > OLR > Mixing > HRT$$

- The applied conditions for coffee wastewater precluded occasionally methanogenic activities, but they were not eliminated at any of the applied conditions on slaughterhouse wastewaters.

Chapter 15

FUTURE WORK

The following are suggested for future work:

- Continue the same type of research project, so as to obtain results for other types of industrial wastewaters.
- Proceed further with the data presented in order to define for each condition the respective VFA volumetric production rate and specific VFA yield so as to proceed into kinetic studies and modelling of the presented data. (Modelling of the data under the described conditions is further discussed with Dr Vavillin in Russia and N.Bozinis in the Imperial College)
- Establish in detail the microbiological and biochemical aspects of the described operational conditions.
- Establish more detailed results about the complete range of gases generated, both as composition as well as volumes.
- operate the same experiments using reactors that always start each experiment with the same biomass (i.e. before setting the applied conditions of each study, mix adequately the contents of all three reactors and separate them into the three digesters), so as to ensure that during the progress of the full range of experiments the reactors do not end up operating with similar biomass but not exactly the same, as it was found for Reactors 2 and 3 in Experiment 5 (Chapter 11).
- Connect the project results with the applications of commercial companies, agro-industries or even the BMB Initiative of the DTI; in order to promote full-scale applications of the presented results.
- Examine the presented data for acidogenic reactors using a methanogenic reactor so as to obtain data for the whole two-phase process.

Chapter 16

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Appendix A

Standard Curves

In Figures A.1, A.2 and A.3 the range of standard curves, the curve of 500 wavelength and the curve of 550 wavelength, are presented respectively.

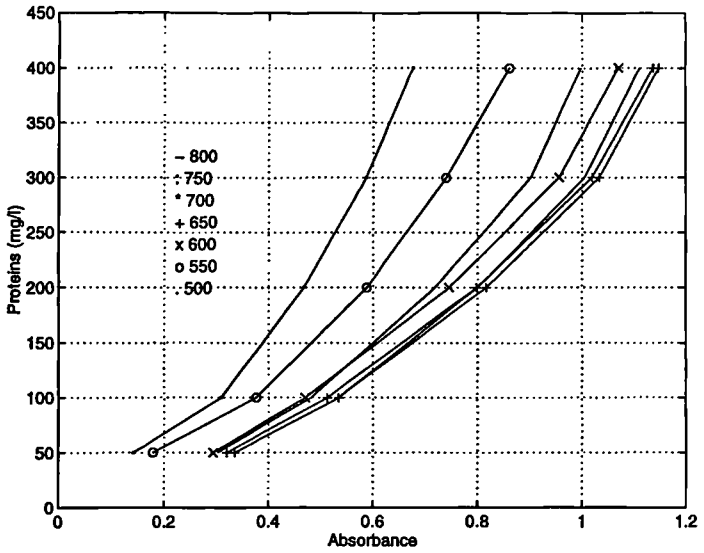

Figure A.1: Standard curves for the calculation of proteins at all wavelengths

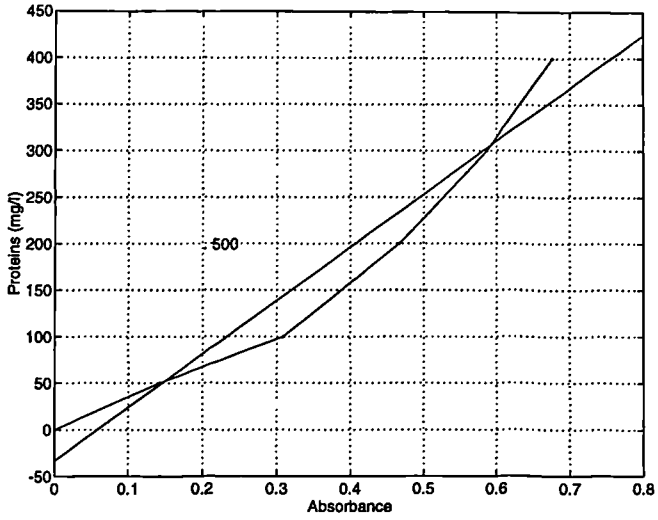

Figure A.2: Standard curve for the calculation of proteins at 500 wavelength

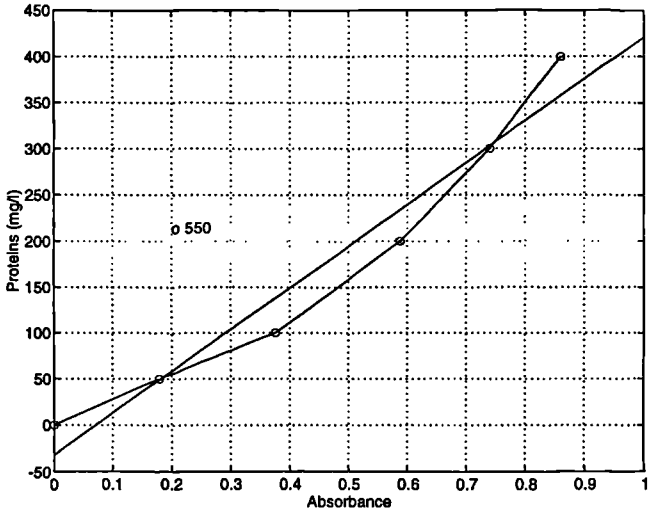

Figure A.3: Standard curve for the calculation of proteins at 550 wavelength

Appendix B

Conversion Factors for VFA

In Table B.1 conversion factors in relation to COD, Carbon and Acetic Acid for the most often evaluated VFA are presented.

Table B.1: Conversion Factors for VFA

VFA	COD equivalent (constant)	Carbon (%)	Acetic acid equivalent (constant)
Acetic	1.066	40.00	1.00
Propionic	1.512	48.64	1.75
Butyric	1.816	54.53	2.50
Valeric	2.036	58.80	3.25
Caproic	2.204	62.04	4.00

Appendix C

Operational Problems

In Table C.1 the diary of operational problems in Experiment 1 is presented.

Table C.1: Operational problems during Experiment 1

Day	Time	Problem/Comment/Observation
0	17:05	The COD:N:P ratio in the synthetic wastewater changes from 400:6.8:4.4 to 400:8.2:1.
1	17:45	pH(1)=5.5. Slow increase for the last 2 days. HCl added manually.
3	00:05	Change pump tube INF(2). It was punctured.
3	14:20	Change, with an 1.5mm diameter instead of the 2.0mm used until now, pump tube INF(3). It was punctured.
4	12:10	Change, back to 2.0mm diameter instead of the 1.5mm used yesterday, pump tube INF(3). It was affecting the HRT.
4	12:55	Stopped INF(1) for 35 minutes, because reactor 1 was overflowing. It was overfilling since this morning. I try to assist manually emptying it from the effluent tube.
4	15:15	Stopped INF(1) for 10 minutes, because reactor 1 was overflowing. I try to assist manually emptying it a bit more from the effluent tube.
4	16:50	For 5 minutes, I use the effluent of reactor 1 as influent, for its calibration.
5	12:25	pH(1)=5.5. Due to NaOH leak, from the overflow yesterday. HCl added manually.
6	17:00	Air leaked reactor 2 for less than 3.5 hours. Pump tube INF(2) disconnected.
9	—	In the morning I install a gas-meter in reactor 2, in order to try its potential to measure the very small gas volumes produced. After 3 days it is removed, unsuccessfully.
13	14:30	Air leaked reactor 3 for less than 1 minute. Tube disconnected while cleaning it.
13	14:35	Air leaked reactor 3 for less than 1 minute. Tube broke twice while cleaning it.
17	08:15	I found pump tube INF(2)&(3) clogged. The whole system appears to have used half of the influent expected since yesterday night. Both reactors can not give out any effluent for samples. Air leaks in reactor 2, as all the water has been sucked from the Dressler bottle. It happened after 23:50 yesterday night and probably more than half of the night.
17	13:55	Pumps closed for 15 minutes. Run out of feed less than 15 minutes ago.

(continued)

Table C.1 (continued)

21	09:30	Change pump tube EFL(3). It was punctured.
21	09:40	Change pump tube INF(1). It looked ready to puncture.
22	23:45	Change pump tube INF(2). It was punctured.
23	15:00	Because I observe flow problems, for 15 minutes I disturb the operation of reactor 1 adjusting pump tube INF(1).
23	15:30	Change pump tube INF(2). It was getting overfilled.
24	—	All day I observe wrong consumption of wastewater (prepared fresh wastewater only once instead of the three times expected).
26	—	Reactor 1 is observed since yesterday evening to overfill.
27	—	All day calibrating reactor 1 and it seems to operate properly.
28	09:05	Pumps closed for 10 minutes. Run out of feed less than 30 minutes ago.
28	17:55	Pumps closed for 15 minutes. Just run out of feed.
28	18:00	Today I observe reactor 2 with HRT problems. It was overfilling in the morning and in the afternoon I measured HRT=8-10 hours, as it was emptying fast.
30	—	Morning observation shows stability in influent/effluent consumption for the whole system, compared to the last few days.
30	14:25	Air leaked reactor 2 for less than 1 hour. Pump tube EFL(2) disconnected.
33	11:45	Pumps closed for 10 minutes. Run out of feed less than 30 minutes ago.
34	13:00	Pumps closed for 15 minutes. Just run out of feed.
37	11:15	Change pump tube INF(3). It was overfilling since yesterday and now overflowed.
37	15:00	Pumps closed for 10 minutes. Just run out of feed.
38	21:45	Change pump tube EFL(2). It was punctured.
38	21:50	pH(2)=8.5. Sudden, less than 30 minutes ago. HCl added manually. Dropped to 2.7 and adjusted to 5.5.
38	21:55	Change pump tube INF(3). It was still overfilling since yesterday.
39	09:15	pH(2)=5.6. From last night's problem. HCl added manually. Dropped to 5.0.
39	10:10	Change pump tube INF(1). It was punctured.
39	10:10	Air leaked reactor 1 for less than 1 minute. While changing pump tube INF(1).
39	10:15	Because I observe flow problems, for 5 minutes I disturb the operation of reactor 1 adjusting pump tube INF(1).
39	10:25	pH(1)=5.4. Started earlier this morning. HCl added manually. Dropped to 5.2.
45	15:50	Pumps closed for 5 minutes. Run out of feed less than 30 minutes ago.
46	10:55	Change pump tube INF(2). It was punctured.
49	09:10	Change pump tube EFL(3). It was punctured.
49	13:30	Pumps closed for 10 minutes. Run out of feed about 30 minutes ago.
50	09:05	Change pump tube EFL(1). It was punctured.
52	22:45	Pumps closed for 5 minutes. Run out of feed less than 30 minutes ago.
52	22:50	Run out of micro-nutrient fertiliser (OMEX). I add tap water in the container and use the crystallised residues.
53	21:45	I stop all pumps. The experiment is finished.

Appendix D

Experimental Data

The data of VFA concentration and the percentage of the different acids produced during Experiments 1 to 6, are presented in the following tables.

Table D.1: Experiment 1, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	1187.128	50.27	9.13	29.95	0.19
7	1242.704	47.28	7.27	36.25	0.00
8	908.544	44.88	8.31	40.52	0.00
9	820.704	48.85	5.92	38.30	0.47
10	902.612	49.50	6.34	36.34	0.15
11	936.886	49.94	5.33	38.78	0.16
12	633.986	55.67	2.97	38.44	0.01
13	644.316	63.56	7.19	25.82	0.07
13.33	505.032	70.66	3.67	22.15	0.32
13.67	509.260	71.99	4.08	20.64	0.31
14	512.130	73.40	4.03	19.15	0.26
14.33	1128.856	49.88	3.60	41.12	0.23
14.67	1147.826	49.37	5.24	39.90	0.23
15	1273.542	48.01	4.98	41.39	0.23
15.33	869.762	37.92	6.09	50.03	0.20
15.67	895.728	38.39	6.07	49.53	0.22
16	817.366	35.64	5.09	53.00	0.25
16.33	812.694	34.67	5.30	52.85	0.25
16.67	932.378	41.32	6.05	47.05	0.21
17	1023.086	41.19	5.69	47.31	0.23
17.33	956.788	40.60	5.81	47.89	0.21
18	1007.456	42.06	6.57	45.67	0.21
19	1060.782	41.44	6.74	45.77	0.17
20	923.836	40.53	6.33	47.19	0.15
21	796.516	43.22	3.81	47.84	0.16
21.67	765.448	43.86	3.74	46.62	0.14
22	848.594	43.19	3.60	47.61	0.14
22.33	703.828	43.10	3.74	48.03	0.14
22.67	744.316	46.09	3.54	44.84	0.14
23	758.552	41.56	3.66	49.37	0.13
23.33	812.202	41.33	6.17	46.01	0.14
23.67	851.000	40.87	7.12	45.53	0.81
24	707.470	17.84	9.73	63.34	0.20
24.33	784.322	39.35	7.59	47.01	0.00
24.67	792.242	38.90	8.06	47.45	0.00

(continued)

Table D.1 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
25	806.756	38.61	8.14	47.31	0.00
25.33	855.328	42.38	6.91	43.87	0.13
25.67	854.688	40.77	7.01	45.23	0.13
26	791.470	41.31	4.57	47.81	0.13
26.33	791.850	42.22	4.64	46.88	0.00
27	860.950	41.39	6.93	44.36	0.15
28	868.018	42.32	7.67	42.52	0.01
29	895.962	40.62	8.20	43.26	0.25
30	884.952	46.12	4.74	43.18	0.20
30.67	968.382	42.96	7.39	41.34	0.22
31	925.628	43.00	6.71	42.79	0.24
31.33	1041.526	43.32	6.50	43.25	0.24
31.67	1064.562	43.75	6.38	42.29	0.86
32	1140.604	43.32	5.80	43.63	0.75
32.33	1042.232	44.28	6.70	39.81	1.50
32.67	1083.954	47.76	3.90	42.68	0.30
33	1067.836	42.84	5.99	42.76	0.84
33.33	1070.708	40.71	5.79	41.22	1.32
33.67	1069.537	40.26	6.22	41.24	1.19
34	1011.508	39.68	6.32	40.37	1.19
34.33	970.313	39.73	6.42	39.37	1.14
34.67	1006.256	39.26	6.78	38.64	1.10
35	937.157	38.93	6.79	38.49	1.17
35.33	843.612	43.18	8.61	42.07	0.19
36	962.308	41.39	9.49	41.90	0.89
37	980.252	36.99	9.82	43.36	0.15
38	924.740	36.96	9.25	43.90	0.17
39	1126.106	38.91	9.26	41.96	0.16
39.67	1045.064	38.05	8.14	44.16	0.14
40	1071.108	38.33	7.88	44.82	0.13
40.33	1057.462	38.29	7.77	44.63	0.14
40.67	1083.160	38.07	7.51	45.26	0.15
41	1105.656	38.19	7.46	45.40	0.08
41.33	1107.394	37.82	7.40	45.85	0.15
41.67	1126.232	37.46	7.36	46.08	0.84
42	1135.586	37.07	7.11	46.83	0.15
42.33	1064.068	39.48	8.65	43.03	0.97
42.67	1039.492	38.99	8.53	42.05	1.48
43	882.914	38.17	8.60	43.67	0.18
43.33	884.314	38.89	9.15	42.44	0.77
43.67	1166.516	42.93	8.45	37.66	0.88
44	1116.348	43.11	8.19	38.18	1.25
44.33	1181.714	43.21	8.85	38.08	0.74
45	1044.528	39.25	9.32	42.02	0.99
46	974.894	37.98	9.87	42.52	0.93
47	990.972	36.97	8.79	44.38	1.01
48	1125.864	36.48	8.36	42.62	1.85
48.67	1131.196	37.13	8.20	42.25	1.87
49	1151.296	36.94	8.10	41.69	1.75
49.33	1003.286	39.06	8.87	41.23	0.99
49.67	1297.428	45.43	6.10	39.13	0.98
50	1233.960	44.37	5.71	41.50	0.21
50.33	1225.988	47.74	4.00	41.40	0.22
50.67	944.932	40.09	7.10	45.45	0.22
51	953.800	39.14	6.60	47.45	0.20
51.33	928.160	39.73	8.20	44.29	0.23
51.67	885.626	38.87	7.93	43.21	0.23
52	937.434	38.12	7.07	45.81	0.23
52.33	850.970	36.70	9.75	42.01	0.76
52.67	928.102	47.66	5.11	38.52	0.75
53	995.106	46.13	4.44	40.87	0.98
53.33	927.212	47.31	5.81	38.42	0.91

Table D.2: Experiment 1, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	1669.264	49.50	8.21	32.66	0.65
6	1572.564	50.65	10.59	31.32	0.55
7	1667.832	51.95	7.10	31.50	0.32
8	1181.268	55.82	6.16	29.43	0.59
9	1243.820	55.59	10.62	24.81	0.16
10	1478.286	55.23	8.34	27.11	0.26
11	1414.584	54.43	6.77	28.90	0.21
12	1312.892	53.34	5.99	30.12	0.70
13	1285.308	50.03	6.61	32.17	0.79
13.33	1404.084	42.96	6.43	28.97	0.63
13.67	1341.882	41.11	6.27	30.45	0.28
14	1399.376	41.91	9.12	25.61	1.01
14.33	1512.674	56.81	6.19	28.33	0.33
14.67	1497.892	52.76	8.39	28.97	0.32
15	1403.154	48.90	7.66	28.13	0.51
15.33	1015.762	46.31	10.57	34.08	0.28
15.67	1047.012	46.10	11.61	32.20	0.29
16	1064.250	45.52	11.45	31.04	0.26
16.33	960.754	47.45	10.25	34.17	0.29
16.67	998.378	44.32	10.28	37.43	0.76
17	1032.812	46.78	12.86	32.26	0.96
17.33	1001.360	42.83	13.41	37.69	0.25
18	980.084	40.29	10.87	39.15	0.66
19	1022.660	40.92	11.71	38.16	0.79
20	975.522	42.63	11.04	37.96	0.61
21	748.528	50.30	10.94	32.88	0.12
21.67	711.104	47.89	10.98	32.96	0.11
22	1011.606	44.95	19.28	26.99	0.58
22.33	716.152	46.01	11.44	35.33	0.14
22.67	682.206	41.24	12.30	36.87	0.17
23	823.608	39.17	14.40	35.43	0.84
23.33	723.058	39.35	14.24	34.33	0.19
23.67	732.542	40.85	13.58	35.92	0.00
24	908.248	16.78	31.12	38.46	1.15
24.33	742.082	40.04	15.40	35.26	0.00
24.67	836.906	34.90	17.49	36.81	0.00
25	777.714	34.73	14.77	37.90	0.01
25.33	660.304	43.25	12.48	34.32	0.13
25.67	677.444	44.20	13.73	32.06	0.07
26	682.048	42.15	13.76	32.09	0.12
26.33	748.384	41.94	13.56	32.53	0.14
27	523.306	41.69	13.82	30.97	0.21
28	520.736	48.38	11.41	30.09	0.44
29	540.172	47.58	12.62	29.60	1.18
30	565.846	46.00	12.82	30.39	1.02
30.67	544 552	46.80	13.50	28.54	1.01
31	525.504	47.65	14.54	27.02	0.25
31.33	587.946	46.35	13.57	29.03	0.99
31.67	574.174	47.30	13.99	27.94	0.88
32	605.116	45.03	15.20	27.15	0.88
32.33	580.028	45.53	15.58	27.88	0.90
32.67	570.086	44.65	16.95	26.32	0.98
33	592.560	42.50	16.58	26.08	1.50
33.33	600.844	42.39	17.77	25.29	1.35
33.67	627.868	40.68	16.47	26.84	1.94
34	584.016	41.61	15.96	28.17	0.16
34.33	624.813	39.11	15.61	30.42	1.12

(continued)

Table D.2 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
34.67	617.570	40.26	15.94	30.71	0.18
35	585.848	39.94	15.76	30.10	0.17
35.33	642.922	40.46	16.26	32.33	0.19
36	777.610	36.48	17.04	32.71	0.84
37	841.684	34.09	18.53	32.25	0.14
38	738.078	34.90	17.86	29.77	1.68
39	1083.736	39.70	20.90	27.66	0.69
39.67	814.372	38.86	17.45	31.00	0.14
40	811.702	37.48	17.02	29.80	0.12
40.33	761.134	39.41	16.90	28.07	0.13
40.67	749.126	37.91	16.41	29.20	0.12
41	1120.874	54.46	21.13	14.29	0.58
41.33	881.996	41.14	17.25	28.33	0.15
41.67	1267.450	61.19	19.92	10.65	0.69
42	765.666	45.77	17.11	23.46	0.14
42.33	736.556	40.54	15.48	28.97	1.12
42.67	738.284	41.64	15.38	28.28	0.15
43	664.016	40.84	16.76	28.95	0.20
43.33	662.608	39.89	15.04	31.21	0.16
43.67	799.060	43.53	12.68	25.68	0.99
44	830.004	42.93	13.44	26.05	0.95
44.33	1015.478	43.42	14.52	27.98	0.18
45	694.226	43.76	12.97	31.24	0.15
46	653.828	42.21	14.73	31.35	0.49
47	738.950	41.26	16.72	29.98	0.60
48	740.826	40.93	15.93	29.71	1.22
48.67	697.734	42.13	15.93	30.49	0.00
49	737.758	40.87	17.19	30.41	0.75
49.33	736.190	37.41	15.38	31.98	0.13
49.67	924.390	45.38	12.98	29.04	0.14
50	785.416	44.74	12.75	31.02	0.18
50.33	691.742	45.62	12.89	29.87	0.00
50.67	590.852	42.85	14.44	29.99	0.10
51	539.950	43.59	14.35	28.45	0.00
51.33	662.512	42.09	15.70	28.17	0.16
51.67	578.844	43.17	14.11	28.41	0.18
52	637.928	40.59	15.30	30.48	0.16
52.33	686.048	37.86	16.60	29.33	0.13
52.67	690.028	52.79	8.93	27.75	0.13
53	723.472	50.89	9.76	27.71	0.12
53.33	750.782	51.48	9.90	28.22	0.11

Table D.3: Experiment 1, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	1158.87	45.34	5.38	41.12	0.73
6	822.788	43.93	5.63	44.29	0.19
7	920.328	41.28	5.85	43.38	0.18
8	650.328	40.04	4.11	46.50	0.62
9	623.268	51.47	4.88	36.32	0.00
10	777.626	54.06	5.97	35.07	0.00
11	840.216	56.07	4.70	35.68	0.00
12	814.568	46.87	5.95	41.02	0.22
13	818.224	46.25	6.01	41.76	0.24
13.33	793.462	45.12	5.69	43.10	0.24
13.67	786.072	44.32	5.69	43.28	0.21
14	837.864	45.44	4.26	43.82	0.23
14.33	1008.79	46.90	3.30	10.56	0.17
14.67	642.066	76.40	5.09	16.10	0.41
15	758.428	76.46	6.21	14.79	0.69
15.33	532.200	64.17	8.67	19.61	0.90
15.67	497.344	63.48	10.03	22.06	0.35
16	497.398	62.79	8.48	22.89	0.33
16.33	495.516	62.02	9.29	23.93	0.12
16.67	509.472	64.08	8.96	22.19	0.49
17	469.652	68.72	8.37	19.14	0.00
17.33	495.952	60.86	10.64	23.45	1.03
18	417.552	65.99	9.16	20.73	0.01
19	433.500	63.44	8.96	23.24	0.00
20	440.736	56.70	9.40	27.27	0.54
21	239.256	56.11	5.46	34.36	0.35
21.67	256.438	58.50	5.39	28.41	0.19
22	281.004	55.89	5.58	31.04	0.22
22.33	246.060	58.18	6.20	30.86	0.02
22.67	239.058	65.41	5.57	21.07	0.45
23	238.254	64.98	5.61	23.16	0.19
23.33	252.988	63.59	5.14	24.66	1.12
23.67	284.420	64.16	7.24	23.64	0.03
24	284.124	62.81	7.30	24.49	0.51
24.33	464.010	54.07	8.26	24.74	5.32
24.67	280.240	67.64	8.82	17.31	0.00
25	321.582	62.82	9.02	19.88	2.43
25.33	313.468	71.55	8.03	15.93	0.00
25.67	313.180	68.43	8.46	17.86	0.17
26	348.544	66.56	8.31	19.50	0.44
26.33	362.402	66.45	8.54	19.81	0.44
27	503.059	56.76	8.33	25.26	0.00
28	412.046	62.23	8.40	19.32	1.67
29	270.318	66.91	5.38	19.88	0.00
30	392.092	64.38	8.72	20.07	0.61
30.67	367.892	67.89	8.73	20.39	0.01
31	389.208	63.33	7.50	22.05	0.00
31.33	409.314	63.29	7.42	22.12	0.00
31.67	431.468	61.45	7.81	23.20	0.56
32	443.690	59.05	7.97	26.09	0.93
32.33	363.224	61.69	7.34	24.41	0.28
32.67	638.890	55.95	8.51	29.42	0.19
33	421.498	58.04	7.77	27.17	0.88
33.33	298.026	58.11	5.67	29.91	0.23
33.67	389.376	57.70	7.91	27.57	0.52
34	406.304	57.86	7.82	27.33	0.61
34.33	413.742	56.73	7.73	28.19	0.54

(continued)

Table D.3 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
34.67	394.290	56.37	7.67	28.87	0.50
35	320.584	52.74	5.28	33.69	0.26
35.33	408.944	54.40	8.15	31.31	0.08
36	612.312	41.93	8.27	41.61	1.09
37	456.876	48.82	8.31	27.38	0.00
38	436.546	47.45	8.19	29.29	0.00
39	533.728	46.77	12.04	35.15	0.23
39.67	648.912	42.69	10.04	37.76	0.22
40	668.034	43.50	10.07	36.77	0.91
40.33	591.460	45.60	10.19	34.43	0.19
40.67	594.788	45.07	10.66	34.45	0.18
41	618.078	44.52	11.33	35.68	0.20
41.33	696.480	43.83	12.04	34.91	1.12
41.67	763.410	41.80	12.35	36.14	0.89
42	742.222	41.14	12.23	36.61	0.78
42.33	763.216	44.61	12.16	32.30	1.02
42.67	598.118	46.87	10.78	28.70	0.15
43	560.740	47.01	12.43	29.66	0.19
43.33	605.896	44.51	12.21	31.14	0.17
43.67	827.882	45.43	9.79	24.52	2.32
44	844.160	44.98	10.34	27.86	1.92
44.33	833.178	47.81	10.73	27.23	0.16
45	626.102	47.50	11.26	25.99	0.14
46	532.902	50.23	12.59	28.39	0.00
47	511.028	47.95	12.81	28.53	0.00
48	591.824	43.73	13.73	31.33	0.00
48.67	651.758	41.36	14.14	33.16	0.54
49	647.212	41.16	13.52	34.57	0.13
49.33	587.264	44.90	11.72	26.20	0.36
49.67	810.420	57.90	9.65	23.23	0.14
50	641.730	53.19	8.55	26.72	0.01
50.33	594.668	51.47	8.68	28.96	0.00
50.67	485.000	50.97	9.59	29.77	0.00
51	481.604	48.93	9.64	31.21	0.00
51.33	599.092	47.56	12.81	28.42	0.15
51.67	610.076	48.99	10.86	27.72	2.42
52	560.690	46.21	10.34	31.66	0.19
52.33	626.856	39.52	15.46	26.39	0.15
52.67	650.700	62.30	6.30	23.63	0.13
53	628.576	53.76	6.53	30.34	0.13
53.33	598.392	56.55	6.40	25.85	0.00

Table D.4: Experiment 2, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
2	1572.984	48.91	19.37	21.61	0.66
5	790.988	78.72	11.69	8.15	0.00
8	738.970	77.39	12.11	9.19	0.00
9	862.036	76.80	13.16	8.59	0.00
10	868.042	68.59	15.55	14.30	0.00
11	1044.622	62.00	13.30	22.29	0.00
12	1229.120	66.24	11.13	20.31	0.00
15	1063.418	67.72	12.87	17.10	0.00
16	929.414	68.45	12.97	16.35	0.00
17	980.008	67.52	12.54	17.25	0.00
18	766.236	66.72	12.16	18.37	0.00
19	1289.863	61.96	14.47	18.39	0.00
22	654.326	58.53	23.05	14.62	0.00
23	929.734	66.67	16.73	12.96	0.00
24	1179.054	65.27	15.99	14.94	0.00
25	928.714	58.91	18.99	18.61	0.00
26	1056.916	64.76	16.60	16.16	0.00
29	1060.560	70.07	12.45	14.51	0.65
30	1171.622	66.54	14.50	14.78	0.74
31	1216.542	62.27	19.90	12.29	0.39
32	1461.464	60.00	23.05	13.10	0.23
33	1333.414	57.26	23.22	17.45	0.17
36	1006.398	63.26	5.66	28.03	0.00
37	1171.622	64.77	4.64	28.02	0.24
38	1026.248	70.09	7.20	20.75	0.00
39	1116.964	68.77	8.16	21.01	0.00
40	1053.412	69.26	9.19	18.62	0.84
43	1161.304	73.45	15.44	9.74	0.54
44	1344.760	60.37	15.57	5.58	0.26
45	1192.612	74.68	19.66	5.22	0.44
46	1246.096	73.29	20.51	3.33	0.41
47	1220.044	72.21	20.41	4.09	0.35
48	1333.020	73.47	19.35	2.46	4.72
48.5	1386.740	71.77	19.32	2.35	6.56
49	1211.220	75.39	19.38	2.87	2.35
49.5	1132.428	75.06	18.79	3.85	2.30
50	1319.140	73.07	18.55	5.64	2.28
50.5	793.048	70.91	15.95	10.74	0.00
51	720.028	65.82	20.74	10.95	0.00
51.5	739.792	63.97	16.12	14.01	0.00
52	918.472	67.45	15.15	12.36	0.00
52.5	835.536	67.10	18.19	12.02	0.00
53	954.664	70.95	12.64	14.53	0.00
57	1095.084	74.91	12.50	10.81	0.00
58	969.056	76.76	12.60	8.86	0.00
59	1001.644	75.56	12.61	8.89	0.00
60	1087.568	71.56	19.29	7.40	0.00
61	1133.556	70.83	19.10	8.11	0.00
62	1241.056	73.83	17.47	7.10	0.00
63	1131.976	75.66	15.98	6.97	0.00
64	1110.648	76.08	17.24	5.82	0.00
64.5	1090.484	73.04	20.22	5.71	0.00
65	1058.952	71.99	20.14	6.54	0.00
65.5	1095.724	69.58	20.71	8.06	0.00
66	1250.132	69.60	22.97	6.02	0.00
66.5	1008.388	65.04	22.21	10.16	0.00
67	944.740	54.95	24.71	15.44	0.00

(continued)

Table D.4 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
67.5	708.824	59.51	19.57	15.84	0.00
68	765.428	58.86	19.38	15.58	0.00
71	706.168	75.62	10.96	7.37	0.00
72	431.268	74.57	13.26	10.12	0.00
73	525.900	63.65	0.00	5.33	0.00
74	644.604	60.38	7.63	11.78	0.00
75	575.016	57.59	23.61	0.00	0.00
76	605.524	73.81	0.00	2.27	0.00
77	235.512	89.07	0.00	0.00	0.00
78	464.104	82.49	0.00	0.00	0.00
78.5	652.328	77.21	0.00	0.00	0.00
79	531.152	97.60	0.00	1.36	0.00
79.5	794.856	74.28	0.00	1.97	0.00
80	617.704	96.87	0.00	3.13	0.00
80.5	738.740	71.40	0.00	2.51	0.00
81	510.572	96.67	0.00	3.33	0.00
81.5	720.452	57.55	16.92	1.95	0.00
82	518.728	76.56	13.97	3.31	0.00
82.5	689.364	60.23	12.07	2.24	0.00
85	692.956	75.12	18.98	5.90	0.00

Table D.5: Experiment 2, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
2	1225.398	52.47	7.71	32.19	0.47
5	1239.580	43.64	15.10	29.64	0.00
8	1146.908	54.69	14.44	23.59	0.27
9	1260.258	53.02	13.39	24.98	0.00
10	978.742	55.03	12.39	26.33	0.00
11	948.894	51.38	12.47	29.31	0.00
12	970.112	56.44	12.93	24.30	0.30
15	1372.404	60.76	15.60	18.65	0.00
16	590.486	64.33	12.92	19.33	0.00
17	606.898	58.69	8.58	26.60	0.00
18	631.764	53.11	12.23	28.51	0.00
19	980.984	63.06	11.42	19.36	0.00
22	1060.258	66.68	13.49	17.86	0.00
23	1295.946	68.53	10.75	17.91	0.00
24	1750.310	69.38	12.60	13.49	0.00
25	1680.912	62.98	20.19	13.66	0.42
26	1915.342	61.10	23.75	11.45	0.70
29	1867.264	59.86	22.47	12.91	0.77
30	1977.106	57.76	22.88	13.69	0.94
31	2084.302	52.22	25.94	15.48	0.85
32	2305.218	44.46	30.63	16.59	0.98
33	2228.110	39.31	34.58	16.49	0.95
36	2364.574	38.13	32.89	17.95	1.25
37	2521.162	39.43	33.03	17.20	1.23
38	2555.016	40.91	32.19	16.69	1.04
39	2566.452	42.44	31.23	16.80	0.82
40	2196.220	42.65	30.67	16.78	1.02
43	2048.812	59.41	15.90	16.73	4.26
44	2043.568	60.30	22.70	13.09	0.52
45	2113.780	48.45	32.09	13.18	1.51
46	2290.124	40.79	34.23	15.59	1.09
47	2238.196	38.92	33.44	16.67	1.05
48	1908.916	40.68	33.99	15.85	1.07
48.5	2249.004	42.49	32.37	15.55	1.02
49	2131.656	42.27	32.50	16.04	1.38
49.5	2016.620	41.96	33.71	14.67	2.32
50	2353.424	42.33	34.08	14.30	2.01
50.5	2230.608	41.59	33.53	14.68	0.39
51	2401.464	42.96	33.16	14.84	0.00
51.5	2363.196	43.05	33.20	14.09	0.35
52	2494.900	43.34	33.15	14.29	0.37
52.5	2375.916	44.67	33.29	12.88	0.43
53	2349.180	43.71	33.62	13.61	0.33
57	2420.064	44.79	32.35	13.24	0.33
58	2261.028	43.92	32.66	13.52	0.35
59	2484.808	43.21	34.02	13.30	0.34
60	2379.024	42.64	33.46	13.63	0.33
61	2217.652	42.65	33.22	13.66	0.37
62	2277.756	42.74	33.27	13.74	0.35
63	2512.444	42.40	33.29	13.93	0.34
64	2184.648	42.68	33.01	14.13	0.33
64.5	2145.172	42.32	32.99	14.13	0.36
65	1625.512	41.90	32.94	14.43	0.00
65.5	2289.796	42.52	31.91	14.87	0.32
66	2191.028	42.12	31.72	14.80	0.41
66.5	2179.084	42.46	31.32	14.74	0.40
67	1986.452	42.68	30.93	14.66	0.40

(continued)

Table D.5 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
67.5	2234.396	42.20	31.43	14.02	0.32
68	2330.088	42.47	31.40	14.52	0.34
71	1611.508	44.53	25.42	17.50	0.45
72	1744.240	47.92	36.99	9.89	0.00
73	1865.548	43.41	37.35	11.72	0.33
74	1916.724	38.63	36.53	13.92	0.41
75	2149.676	37.53	36.85	13.93	0.39
76	2238.788	36.16	38.00	13.82	0.35
77	2143.156	35.43	36.71	14.82	0.33
78	2079.848	34.48	37.59	14.46	0.38
78.5	2008.876	36.74	34.91	14.75	0.37
79	1988.600	38.33	32.39	14.91	0.46
79.5	2006.112	40.65	32.33	14.54	0.37
80	1893.336	41.32	31.96	14.18	0.44
80.5	1995.580	41.85	32.78	13.78	0.35
81	1599.412	43.04	30.62	14.62	0.00
81.5	2183.020	43.21	30.86	14.02	0.36
82	2216.146	43.31	29.84	14.21	0.37
82.5	2079.696	44.11	28.86	15.16	0.00
85	2329.048	43.90	29.96	13.92	0.46

Table D.6: Experiment 2, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
2	751.380	46.16	17.75	29.38	1.05
5	353.588	75.88	15.43	6.64	0.00
8	319.298	84.83	11.90	3.27	0.00
9	340.532	85.57	11.48	2.94	0.00
10	444.136	82.02	11.76	6.22	0.00
11	416.402	86.52	9.91	3.57	0.00
12	412.328	84.79	10.32	4.89	0.00
15	449.340	80.56	10.73	7.62	0.00
16	410.022	80.28	10.00	8.42	0.53
17	499.720	79.34	8.41	7.08	0.00
18	624.588	65.97	12.83	18.94	0.00
19	623.244	67.87	7.43	14.49	1.62
22	564.154	64.37	13.52	20.00	0.00
23	843.324	57.56	8.82	21.79	0.00
24	811.620	58.16	9.22	23.60	0.00
25	841.974	48.60	8.99	34.84	0.64
26	1047.860	52.78	7.44	36.46	0.00
29	1079.700	43.35	5.91	46.06	0.63
30	1096.532	42.72	5.54	46.35	0.00
31	1104.246	41.40	6.54	47.30	0.00
32	1306.776	41.87	6.88	45.84	0.00
33	1229.526	44.11	7.09	44.78	0.00
36	1507.172	46.83	6.14	40.72	0.00
37	1330.022	45.75	5.45	44.07	0.00
38	1485.908	46.30	5.75	45.26	0.00
39	1563.964	43.49	5.61	42.27	0.00
40	1270.748	44.43	5.86	44.91	0.20
43	1416.988	49.13	5.64	41.26	0.00
44	1321.944	49.39	4.65	42.21	0.00
45	1187.372	42.27	4.37	46.12	0.00
46	1391.252	40.80	4.26	40.62	0.00
47	1484.428	36.92	3.43	38.94	0.00
48	1290.952	49.96	4.18	42.98	0.00
48.5	1244.668	48.24	4.04	45.29	0.00
49	1209.120	45.93	3.84	44.67	3.47
49.5	1088.372	45.96	3.79	44.33	4.15
50	1316.036	46.45	3.75	44.36	3.17
50.5	1498.072	38.00	3.26	41.32	0.00
51	1351.120	41.31	3.83	47.87	0.00
51.5	1627.752	39.77	3.58	43.42	0.00
52	1476.760	42.46	3.73	47.10	0.00
52.5	1462.888	36.37	4.03	41.24	0.00
53	1406.424	41.64	5.03	45.74	0.00
57	1479.488	36.89	11.21	38.95	0.00
58	1459.732	35.93	10.85	34.39	0.00
59	1596.624	36.68	12.43	32.67	0.00
60	1651.928	36.99	11.11	32.72	0.00
61	1489.860	37.54	10.22	33.07	0.00
62	1514.788	37.24	8.37	34.95	0.00
63	1307.916	40.74	8.50	39.76	0.00
64	1145.060	40.74	7.87	41.94	0.00
64.5	1512.548	36.79	6.98	35.28	0.00
65	1238.512	40.90	7.62	41.41	0.00
65.5	1383.768	37.45	6.99	37.34	0.00
66	1126.488	40.10	7.52	42.95	0.00
66.5	1301.192	37.49	6.84	37.13	0.00
67	1187.836	41.60	7.71	41.88	0.00

(continued)

Table D.6 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
67.5	1447.336	40.44	7.15	33.55	0.00
68	1336.024	43.97	8.15	39.09	0.00
71	1481.492	33.47	11.03	33.33	0.00
72	1098.140	30.28	0.00	51.54	0.00
73	1148.184	34.67	4.98	48.67	0.00
74	1126.064	34.03	5.12	52.33	0.00
75	1226.268	37.42	5.80	49.78	0.00
76	1190.552	39.37	0.00	52.88	0.00
77	1197.340	42.00	0.00	51.20	0.00
78	1058.744	40.01	0.00	54.26	0.00
78.5	1019.524	40.24	0.00	54.32	0.00
79	1130.900	50.27	0.00	45.28	0.00
79.5	962.096	39.94	0.00	55.21	0.00
80	984.140	40.94	5.71	49.01	0.00
80.5	1029.568	40.22	0.00	54.35	0.00
81	781.952	37.42	0.00	56.61	0.00
81.5	623.756	38.37	0.00	55.54	0.00
82	946.688	39.53	0.00	55.14	0.00
82.5	1047.452	38.65	8.91	48.02	0.00
85	1054.384	43.03	0.00	51.80	0.00

Table D.7: Experiment 3, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
1	2220.94	62.69	17.18	0.00	12.77	1.53
4	2263.416	44.83	21.32	5.67	14.61	8.70
5	2752.174	37.69	23.72	7.80	14.56	10.99
6	3116.744	34.15	22.46	8.44	16.12	12.09
7	3558.764	33.79	19.59	9.88	16.45	11.88
8	2467.736	26.04	20.89	10.46	18.98	15.57
11	1539.9	34.90	14.12	10.46	17.27	14.64
12	2804.28	33.61	15.97	10.86	17.98	15.45
13	2377.66	33.90	16.50	10.72	17.76	16.40
14	1716.3	33.39	17.45	11.45	16.34	15.86
15	2595.888	35.36	16.86	10.53	17.21	13.97
19	2764.122	32.91	18.92	11.87	16.56	15.28
20	3101.776	35.52	14.75	8.68	11.82	10.96
21	2775.688	31.40	16.96	11.86	16.30	14.67
22	2717.916	32.91	18.50	12.54	17.62	15.43
25	2477.408	34.82	17.95	11.53	16.46	13.89
26	3579.192	31.00	18.25	11.15	17.09	14.51
27	3450.516	33.24	18.73	11.09	15.99	14.86
28	2908.868	33.64	16.83	11.43	16.20	14.44
32	1167.632	38.53	16.20	12.52	14.23	14.87
33	491.2	39.93	16.19	10.14	14.55	14.78
34	553.252	42.45	15.26	10.91	12.83	13.41
35	380.316	39.38	14.00	9.07	14.86	18.18
36	382.416	45.28	15.22	9.14	11.15	13.79
39	574.128	44.04	17.61	9.34	10.85	12.84
40	440.548	45.55	14.92	6.97	10.60	10.40
41	493.48	29.72	11.00	5.78	8.76	39.48
42	281.448	45.97	13.96	9.66	10.15	14.21
43	268.34	35.62	16.79	11.92	11.83	18.63
44	334.076	40.18	17.95	11.14	10.35	15.91
45	204.492	48.82	12.62	9.70	9.18	15.27
46	206.288	49.11	12.72	9.60	8.97	15.74
46.5	233.772	47.83	11.03	8.41	9.52	18.02
47	234.144	47.61	10.83	8.84	9.13	17.67
47.5	189.228	49.10	10.86	9.42	8.05	17.68
48	216.9	46.34	12.08	10.04	8.40	17.64
48.5	195.968	54.89	10.17	8.98	7.54	15.05
49	216.928	53.64	10.39	9.30	7.82	15.04
49.5	217.352	57.54	1.46	1.98	12.59	16.89
50	654.88	47.72	19.76	8.74	9.86	10.42
50.5	516.74	43.75	20.64	7.11	11.58	11.33
51	673.344	42.03	22.36	6.89	11.81	12.39
52	575.628	40.39	24.57	6.81	12.23	11.36
53	644.428	42.87	24.93	6.76	11.85	9.91
54	789.748	36.50	29.48	9.52	12.36	9.16
55	846.9	35.54	34.06	9.47	8.39	6.14
55.5	998.624	36.33	40.09	4.70	8.52	6.51
56	1175.816	35.32	40.06	6.67	8.72	6.92
57	1341.948	28.79	31.69	11.61	11.52	13.80
58	3788.504	26.24	32.38	11.27	12.77	15.05
59	5726.268	25.14	30.85	12.22	11.22	13.61
60	6752.172	22.15	44.36	0.00	12.89	10.64
61	6254.676	25.01	21.97	12.89	17.17	13.10
61.5	5281.905	26.21	21.71	12.70	17.66	14.44
62	3755.865	27.54	21.04	11.85	18.15	15.62
63.5	2988.465	45.76	13.72	8.84	13.30	11.20
64	3192.59	41.42	13.94	10.20	13.97	11.75

(continued)

Table D.7 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
64.5	1403.348	33.09	20.61	10.37	15.24	14.39
65	2022.988	27.78	24.81	10.69	15.78	15.35
66	1217.128	27.26	25.40	10.05	15.52	16.61
66.5	870.2	30.85	20.48	9.62	15.87	18.85
67	974.232	30.83	21.01	10.40	16.22	17.56
68	517.676	41.12	17.62	9.44	11.62	14.27
69	653.776	40.63	19.65	10.30	10.32	14.50
70	624.088	33.09	25.14	9.59	13.02	14.07
71	729.448	33.42	23.83	8.96	14.56	14.44
72	723.328	33.81	24.38	8.88	14.60	13.92
72.5	760.932	35.29	23.18	8.53	14.22	13.83
73	922.7	36.22	24.05	8.23	13.67	12.41
73.5	740.832	33.74	25.08	8.72	13.80	13.41
74	1035.664	35.66	24.90	9.31	13.63	12.81
74.5	899.896	36.87	19.37	10.61	13.25	11.40
75	862.68	36.37	22.87	8.86	13.18	11.91
75.5	760.044	35.91	20.03	9.89	13.19	12.64
76	953.508	35.28	22.45	10.34	13.44	13.93
76.5	1109.524	36.78	19.51	11.74	13.44	13.69
77	947.336	32.75	23.36	10.61	14.77	13.79
78	1468.096	30.42	21.65	11.38	16.35	15.38
79	1357.716	35.92	20.17	10.43	15.00	14.18
80	1039.392	34.15	19.95	10.39	15.95	15.15
81	646.48	33.13	19.06	10.34	17.06	16.04
81.5	849.632	32.96	19.39	10.46	14.89	14.37
82	832.416	35.25	18.75	10.37	14.71	14.05
82.5	605.644	34.40	20.47	9.85	14.13	13.72
83	711.788	36.77	19.98	9.88	14.03	14.23
83.5	699.368	37.64	19.71	10.06	13.21	13.83
84	740.032	35.05	18.52	10.02	15.41	16.17
84.5	676.128	37.13	18.98	10.73	13.72	14.85
85	724.36	35.86	17.68	10.98	14.99	15.63
85.5	440.928	34.27	18.20	14.77	8.93	16.80
86	847.148	33.23	13.80	15.06	16.43	18.79
87	622.88	31.02	12.97	13.85	15.30	20.29
88	567.044	36.71	18.01	11.97	12.23	16.71
89	525.94	37.08	18.80	11.62	11.97	16.53
90	578.164	33.58	18.98	9.69	11.37	23.45
90.5	458.336	36.41	20.14	10.97	12.29	16.23
91	426.404	36.21	20.14	11.10	12.64	16.55
91.5	522.08	34.25	21.13	10.95	13.10	16.87
92	518.236	34.85	19.65	11.15	13.36	17.20
92.5	404.672	36.16	18.98	10.86	12.73	17.88
93	468.624	36.98	19.75	10.59	12.07	17.36
93.5	921.552	32.46	22.98	10.99	13.69	15.55
94	1307.7	26.77	25.92	11.86	15.00	16.96
94.5	1125.424	26.25	27.83	11.58	15.23	16.13
96	312.096	36.74	18.37	12.13	10.03	18.77
97	380.452	37.95	17.17	12.67	9.96	18.16

Table D.8: Experiment 3, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
1	2135.512	2.41	40.00	5.72	24.31	12.22
4	1145.85	25.27	23.52	7.38	17.89	13.54
5	1420.752	29.85	22.29	8.12	16.07	13.40
6	3015.14	26.02	27.56	9.51	12.63	14.97
7	2795.512	27.00	25.47	9.28	14.43	14.90
8	2419.672	24.68	24.60	10.57	15.92	16.18
11	1202.66	26.53	21.94	9.97	16.27	16.07
12	1723.848	27.87	23.55	10.03	15.61	16.53
13	2301.36	24.00	28.79	10.88	13.69	15.96
14	2149.148	21.53	29.81	11.40	13.21	15.59
15	2927.908	22.31	29.58	10.27	14.75	14.98
19	2762.088	21.79	26.93	11.36	15.60	14.29
20	3114.576	26.18	23.56	9.71	12.78	12.28
21	3022.096	15.98	27.60	12.23	16.99	18.07
22	3089.496	20.44	26.50	12.26	16.93	15.72
25	2664.484	19.62	27.21	12.31	17.61	16.92
26	3581.58	14.54	31.13	10.92	19.48	15.98
27	3599.244	19.15	26.05	11.66	18.95	16.94
28	2685.952	16.92	28.66	11.01	19.18	16.26
32	1239.712	19.48	23.88	14.18	18.47	18.40
33	1120.552	18.28	29.46	11.93	17.11	17.80
34	1097.984	25.56	22.33	12.17	14.86	19.68
35	965.416	20.56	28.48	10.88	14.93	19.64
36	859.084	26.56	28.45	12.02	11.65	16.92
39	1010.944	28.58	30.16	10.50	11.70	15.34
40	1031.148	22.61	28.77	14.98	13.29	14.51
41	994.248	21.08	31.22	12.70	11.86	18.51
42	738.788	22.75	31.05	14.38	9.58	17.44
43	745.652	17.89	37.24	13.19	7.58	18.08
44	531.98	26.28	34.69	15.04	3.57	16.00
45	382.72	31.84	31.47	11.88	3.19	17.62
46	343.692	34.02	29.52	10.96	4.22	17.39
46.5	286.068	38.86	24.29	8.11	4.68	16.59
47	196.708	46.70	15.57	8.47	6.54	18.08
47.5	138.944	51.26	11.18	9.29	6.40	17.23
48	125.452	48.29	8.16	12.10	4.46	22.99
48.5	107.452	58.73	5.87	10.20	3.21	18.17
49	67.808	41.91	3.04	17.23	0.00	32.14
49.5	184.688	55.56	11.96	7.04	6.06	12.40
50	196.56	46.25	15.50	9.33	5.66	16.16
50.5	420.612	50.70	16.40	7.48	7.16	14.36
51	591.576	49.23	19.64	6.77	9.04	11.88
52	577.16	45.22	21.52	6.68	11.47	11.52
53	768.236	40.41	22.25	7.88	13.79	11.97
54	747.608	36.82	24.17	6.86	18.38	9.57
55	815.012	32.79	25.91	5.14	20.97	7.94
55.5	988.6	30.27	32.96	4.84	19.09	7.80
56	1408.384	26.63	28.01	8.61	15.86	14.87
57	1335.12	27.70	23.50	10.21	12.86	19.65
58	766.036	43.38	15.64	9.00	11.46	15.77
59	908.248	44.04	19.23	8.53	10.39	12.52
60	1292.96	29.64	30.82	0.00	16.93	16.80
61	993.768	31.92	15.93	8.26	15.76	12.54
61.5	996.432	32.39	17.40	8.86	16.80	18.82
62	1016.168	33.25	19.78	8.54	16.24	16.82
63.5	2807.656	39.33	14.76	8.06	13.82	11.08
64	2449.736	37.79	15.20	9.02	14.64	12.60

(continued)

Table D.8 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
64.5	720.492	29.19	22.56	9.74	15.93	17.23
65	690.18	30.49	22.72	9.48	15.52	16.68
66	510.792	36.34	21.32	8.60	14.47	15.18
66.5	419.22	40.00	15.73	9.52	13.62	18.08
67	390.636	37.68	15.81	10.57	15.09	17.65
68	44.096	0.00	0.00	8.78	0.00	44.88
69	294.064	47.35	12.64	4.13	11.30	12.34
70	435.024	40.40	16.65	8.08	8.79	13.26
71	599.864	41.67	18.64	8.45	9.70	13.98
72	643.236	38.27	18.98	9.16	12.54	14.97
72.5	788.968	37.01	21.13	8.73	15.28	14.41
73	685.928	33.01	24.77	8.21	14.16	13.97
73.5	540.456	30.55	26.65	8.65	14.24	14.80
74	695.852	31.10	28.63	8.63	13.87	14.23
74.5	899.176	28.84	39.47	0.00	13.73	13.17
75	758.88	28.96	39.36	0.00	13.05	13.30
75.5	793.688	27.96	26.90	12.01	14.68	14.98
76	878.592	28.91	26.78	12.00	13.83	14.95
76.5	771.476	28.02	40.90	0.00	11.97	15.76
77	838.056	28.39	27.15	10.97	13.72	15.23
78	991.152	25.14	28.14	11.50	14.38	16.41
79	1069.092	26.86	27.84	11.16	13.50	15.07
80	1038.432	24.58	26.36	11.27	13.65	16.01
81	656.832	26.29	21.76	11.02	14.34	17.72
81.5	583.612	15.79	23.14	13.70	13.61	19.70
82	606.408	33.23	19.73	10.82	9.67	15.19
82.5	540.044	33.88	22.38	10.29	8.62	15.38
83	568.372	34.71	22.74	10.11	8.11	14.93
83.5	625.304	33.16	22.66	10.64	10.37	15.35
84	665.936	31.45	21.86	10.81	10.76	15.57
84.5	566.92	34.33	21.78	10.40	9.86	15.44
85	553.892	33.30	22.14	10.47	10.92	15.66
85.5	673.54	35.69	18.70	10.27	15.40	16.55
86	553.044	28.04	22.18	11.56	9.98	16.96
87	510.268	34.57	20.15	12.48	7.19	17.28
88	594.888	30.50	20.51	11.31	7.58	25.00
89	366.5	38.49	17.19	10.57	10.86	17.83
90	307.416	41.61	15.79	9.93	11.49	16.45
90.5	253.608	43.80	15.51	9.79	10.45	16.03
91	218.196	43.65	15.79	10.03	10.30	16.02
91.5	403.324	44.18	17.87	10.27	9.53	14.23
92	242.584	40.00	15.17	10.38	11.63	17.87
92.5	239.488	37.53	12.90	8.53	14.27	22.57
93	247.476	43.33	13.36	8.35	12.24	19.16
93.5	202.016	42.37	13.15	8.95	10.51	17.17
94	183.652	42.94	13.96	9.52	10.35	17.56
94.5	175.764	45.34	13.69	9.23	8.71	17.02
96	163.332	43.85	12.91	10.72	7.81	19.69
97	216.228	37.34	11.73	11.28	9.59	18.77

Table D.9: Experiment 3, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
1	2094.892	40.82	22.14	2.54	21.39	2.63
4	1029.942	41.57	21.13	3.56	18.52	4.92
5	758.864	36.96	24.07	5.61	16.75	7.36
6	1694.164	28.85	25.19	8.28	15.33	13.09
7	3101.696	25.08	21.85	9.90	18.40	15.38
8	2586.52	24.94	20.48	10.55	19.03	16.57
11	2764.832	24.33	24.89	10.49	16.44	16.36
12	3034.796	25.54	24.83	10.03	17.03	16.02
13	2568.968	24.62	25.73	10.37	16.98	15.80
14	2642.936	21.19	27.18	11.01	17.89	16.45
15	3116.508	24.72	24.78	11.91	17.65	15.38
19	2760.996	31.54	18.78	11.80	17.87	15.71
20	3112.42	37.58	17.48	10.26	15.09	12.14
21	2799.924	29.21	17.26	12.08	18.50	17.23
22	2524.284	29.43	17.38	11.52	17.11	17.42
25	2397.596	29.93	16.70	12.24	18.97	17.89
26	3382.836	27.40	18.12	11.29	21.55	16.65
27	3269.824	26.96	17.60	12.10	19.91	18.29
28	2841.292	27.99	17.34	12.24	20.32	17.10
32	1438.568	31.21	16.89	13.01	17.16	18.26
33	1615.22	30.08	19.37	11.54	17.67	17.04
34	1320.836	29.14	18.84	12.08	15.59	19.62
35	1400.012	24.64	25.52	10.80	14.80	19.97
36	975.164	25.63	27.17	12.66	11.35	18.57
39	1112.024	27.81	25.49	12.17	12.50	17.74
40	791.256	25.26	24.55	14.74	12.37	18.22
41	722.4	18.89	29.42	15.80	13.22	19.04
42	617.836	16.17	28.70	14.55	14.63	21.56
43	174.388	34.47	2.42	18.82	9.73	29.91
44	407.924	22.49	24.48	15.31	12.22	23.12
45	452.512	18.13	28.94	15.39	11.96	22.94
46	310.112	17.52	26.26	16.15	12.30	24.62
46.5	387.492	19.54	22.45	14.71	13.87	26.25
47	373.244	20.49	19.62	15.05	14.32	27.34
47.5	309.192	24.99	16.93	15.55	12.84	26.74
48	336.636	25.39	15.52	16.52	11.31	29.02
48.5	402.724	31.76	16.33	16.20	9.85	22.72
49	260.872	32.65	14.30	17.18	7.56	24.65
49.5	701.724	41.61	19.43	9.97	11.04	15.18
50	1317.66	39.20	18.07	10.60	12.20	16.22
50.5	2144.044	34.14	16.87	10.47	16.29	14.32
51	2508.08	31.52	18.63	10.22	18.02	16.00
52	2282.648	29.39	20.53	10.07	19.40	16.92
53	2103.976	34.43	16.92	11.93	17.10	16.57
54	1569.052	40.50	19.56	10.21	14.65	12.22
55	1168.244	39.31	25.70	7.61	14.15	10.45
55.5	1517.536	34.64	32.19	6.27	13.81	8.99
56	2251.396	35.19	27.10	8.20	14.46	10.39
57	2518.232	33.84	22.71	9.35	16.07	13.11
58	2554.412	34.04	21.28	9.90	15.33	14.30
59	3438.196	31.66	21.60	10.48	15.94	14.49
60	5251.884	26.54	21.76	11.38	18.54	16.12
61	6017.58	22.41	23.63	11.20	20.74	16.76
61.5	5216.624	22.34	23.29	12.27	20.44	16.72
62	5511.076	23.38	22.84	11.88	20.30	16.62
63.5	4647.032	33.12	23.28	10.63	15.87	13.25
64	3452.56	41.60	19.12	10.32	14.42	11.77

(continued)

Table D.9 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
64.5	1952.652	30.15	22.12	10.97	16.84	16.06
65	1919.292	29.03	23.20	10.89	16.96	16.16
66	1107.556	27.59	22.33	10.75	16.33	19.41
66.5	1446.576	28.37	25.09	10.20	15.22	17.73
67	1416.712	27.33	27.79	10.47	13.84	16.89
68	1082.748	26.08	29.46	11.24	11.28	17.10
69	1322.68	23.60	31.08	11.38	11.00	16.26
70	1650.992	20.17	31.96	11.53	13.14	15.16
71	1448.956	26.24	27.01	11.62	13.62	15.25
72	2647.628	25.97	28.92	11.21	11.84	15.54
72.5	2076.76	19.69	27.95	14.28	10.10	19.23
73	2395.132	19.94	31.43	10.33	14.41	16.43
73.5	3015.68	20.74	33.58	11.00	13.90	14.04
74	2781.884	20.43	34.96	10.77	13.08	12.96
74.5	3681.132	20.08	46.56	0.00	12.52	12.56
75	3533.56	20.57	45.78	0.00	13.60	11.98
75.5	3456.644	20.28	29.40	12.13	15.58	13.94
76	2561.384	18.26	27.72	15.02	11.24	18.43
76.5	3778.096	23.03	42.34	0.00	14.40	14.61
77	3322.948	20.73	28.54	12.09	17.38	15.74
78	5690.852	19.95	26.66	12.47	18.36	16.97
79	5420.312	21.19	27.49	12.15	17.22	16.17
80	6166.532	19.06	27.68	12.01	18.81	17.03
81	3070.38	18.69	26.34	11.84	19.09	16.99
81.5	3747.336	21.94	26.31	11.02	16.57	15.33
82	3681.224	24.24	24.78	11.97	16.11	15.32
82.5	3207.84	24.59	26.06	11.40	15.69	15.13
83	2937.716	25.88	26.02	11.15	14.78	15.02
83.5	3292.156	25.52	24.63	11.55	15.81	15.62
84	3387.212	25.58	23.81	11.40	15.68	16.86
84.5	3170.176	24.54	25.33	11.49	16.00	15.54
85	3002.244	23.70	25.25	11.53	16.67	15.64
85.5	2639.268	24.36	25.96	11.38	16.81	15.43
86	3162.76	24.88	24.94	11.26	17.39	15.73
87	3439.944	23.80	25.14	11.13	17.64	16.77
88	2057.28	24.30	25.00	11.91	16.89	16.51
89	1720.532	24.03	26.49	12.13	15.30	16.67
90	1718.576	23.06	25.85	11.99	15.49	18.63
90.5	1666.916	23.43	27.51	12.75	14.23	16.97
91	1595.416	23.25	27.95	12.70	13.91	16.92
91.5	1540.148	22.96	29.22	11.90	13.62	17.19
92	2147.772	24.40	43.04	0.00	12.14	15.14
92.5	1648.22	26.08	26.28	11.36	14.78	16.38
93	1413.06	26.06	25.39	11.32	14.99	16.79
93.5	1213.496	27.30	22.82	11.15	16.00	17.64
94	1215.608	29.17	20.88	11.09	16.40	17.74
94.5	678.316	30.46	18.63	10.82	15.88	19.67
96	663.344	34.63	14.65	12.94	13.99	19.52
97	846.416	34.33	14.35	13.65	13.54	19.90

Table D.10: Experiment 4, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
7	202.154	43.13	10.21	6.48	9.52	13.17
8	188.914	45.26	9.67	7.32	8.17	22.72
9	567.596	46.74	12.86	8.79	10.94	12.87
10	852.92	43.37	15.68	9.04	12.97	13.91
11	761.352	43.11	14.76	9.14	12.96	14.85
12	795.372	41.22	15.04	9.96	13.48	15.82
12.5	786.932	40.92	16.03	10.42	13.77	15.10
13	684.84	42.02	16.36	9.93	13.48	14.70
13.5	754.824	41.37	16.16	10.08	14.26	14.43
14	770.28	42.03	15.76	10.10	14.24	14.22
14.5	838.3	41.41	15.49	9.23	13.59	15.37
15	878.324	41.63	15.78	9.62	13.50	15.03
15.5	787.364	40.74	15.48	9.57	14.34	16.26
16	952.008	45.39	16.47	8.02	13.05	13.50
16.5	818.532	47.29	15.42	7.40	13.29	12.09
17	788.76	47.49	15.86	7.87	12.88	12.14
18	1270.9	47.49	16.62	7.16	14.22	10.71
19	1662.76	48.61	16.37	7.96	14.18	9.50
20	1514.296	46.58	14.41	8.99	14.85	11.10
21	1002.52	40.84	12.25	9.54	17.45	14.86
22	995.332	40.60	12.14	10.37	17.05	15.54
23	1444.688	42.82	12.40	11.22	15.64	14.56
23.5	1255.324	43.40	12.21	10.10	15.57	14.77
24	1334.216	41.95	12.59	10.29	16.15	15.01
24.5	1525.676	40.92	13.20	10.62	16.44	15.01
25	1590.948	40.34	13.82	10.67	16.47	15.17
25.5	1693.74	37.05	13.93	10.61	15.91	18.53
26	1745.176	38.25	15.22	10.73	16.41	15.03
26.5	1719.392	38.08	15.84	10.83	15.83	15.00
27	1494.376	39.90	16.09	10.60	14.36	14.47
27.5	1286.52	39.30	17.43	11.42	12.98	14.49
28	1580.256	38.10	18.21	11.28	13.42	14.61
30	995.004	37.99	18.63	10.56	14.05	13.67
31	681.908	37.46	18.86	9.13	12.76	13.55
32	975.504	41.43	19.27	9.87	12.32	12.71
33	606.836	37.36	16.88	8.35	12.26	13.84
33.5	483.592	40.22	14.36	6.58	11.50	12.82
34	483.54	44.18	16.99	8.01	12.88	13.16
34.5	381.404	44.90	16.45	9.27	11.64	13.34
35	339.032	43.92	14.51	10.09	11.32	15.55
35.5	321.764	42.79	14.60	8.93	10.66	13.02
36	344.06	45.22	16.04	9.25	10.99	13.45
36.5	415.512	44.82	16.92	8.81	11.64	13.11
37	268.832	52.33	14.74	7.25	9.83	11.83
37.5	264.512	55.83	14.10	6.95	7.77	11.56
38	297.288	58.36	14.06	5.96	8.32	9.98
39	324.996	50.97	11.64	5.72	7.53	10.79
40	302.012	54.13	16.35	6.05	9.42	9.88
41	329.98	50.43	15.02	5.60	7.60	10.04
42	328.144	56.81	14.88	6.02	8.37	10.09
43	326.764	60.91	14.41	5.20	7.31	8.50
44	161.088	59.48	10.57	6.87	6.73	13.79
45	254.26	48.14	9.88	6.25	7.10	12.57
46	218.616	56.42	12.24	7.51	9.05	11.29
47	243.56	53.21	13.96	8.37	8.47	12.53
47.5	381.76	45.06	9.62	7.32	11.83	13.78
48	631.336	43.46	12.51	9.24	15.27	15.07

(continued)

Table D.10 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
48.5	461.512	45.82	11.69	8.31	13.99	13.86
49	303.18	47.79	15.12	7.58	11.29	13.01
49.5	204.628	52.77	14.08	5.89	10.97	11.89
50	186.552	56.66	14.63	4.86	10.27	10.42
50.5	254.988	52.16	14.01	6.14	10.50	13.16
51	293.488	50.17	15.00	7.14	10.66	13.51
51.5	313.432	48.56	14.66	8.57	10.04	14.48
52	305.608	47.79	13.99	8.93	9.50	15.12
53	303.448	48.36	15.61	8.85	9.13	14.56
54	277.408	45.30	16.20	9.36	9.89	15.67

Table D.11: Experiment 4, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
7	165.574	44.11	14.66	9.13	10.48	17.59
8	176.87	35.93	20.95	8.34	8.45	17.43
9	611.3	40.18	19.62	9.33	11.69	14.71
10	917.776	34.40	23.29	9.47	12.94	15.97
11	1714.728	22.60	27.15	11.47	13.13	17.13
12	1414.208	19.21	26.95	12.22	11.39	18.26
12.5	1360.024	17.39	26.83	12.67	12.71	16.79
13	1409.216	18.10	26.54	12.10	13.12	15.91
13.5	1409.5	17.23	26.21	12.25	13.06	16.08
14	1570.88	17.67	26.00	12.19	13.70	15.69
14.5	1464.616	17.57	25.30	11.97	12.64	17.71
15	1539.928	16.74	25.35	11.81	12.24	18.45
15.5	1624.632	16.67	25.57	11.95	12.64	18.10
16	1753.672	19.64	25.83	11.57	13.07	16.18
16.5	1642.9	21.40	25.27	10.49	14.07	15.22
17	1860.676	21.90	25.81	10.35	14.37	14.93
18	2593.74	25.53	25.23	10.36	15.39	13.96
19	4096.044	28.23	21.85	10.34	16.71	15.60
20	3550.688	28.01	21.73	10.16	16.39	17.05
21	1225.584	25.61	23.67	12.14	16.14	16.15
22	1569.304	21.68	23.09	12.62	16.16	21.50
23	2623.256	24.22	27.07	12.18	14.81	16.40
23.5	2616.264	22.75	28.69	10.93	14.84	17.08
24	2102.428	20.71	29.27	11.48	15.14	17.28
24.5	1865.268	19.21	30.82	12.36	14.46	17.04
25	2275.124	19.82	31.17	11.69	14.15	16.95
25.5	2957.948	22.01	30.03	10.76	14.69	16.02
26	4117.188	21.75	25.42	10.59	18.88	15.01
26.5	4226.904	21.94	18.44	11.05	22.10	18.50
27	4073.44	23.02	15.38	11.28	26.10	16.40
27.5	4533.756	22.17	14.71	11.45	25.43	18.94
28	5059.908	22.35	15.42	15.13	24.12	15.05
30	2888.124	27.03	19.31	11.02	21.01	14.48
31	2447.208	27.81	17.32	10.76	22.23	15.29
32	2575.036	28.24	18.52	10.41	21.05	15.64
33	2739.932	27.80	17.73	10.97	21.98	15.79
33.5	2272.288	28.97	16.83	9.74	22.69	15.09
34	2243.932	27.84	15.64	9.86	24.25	15.19
34.5	2948.912	27.60	14.39	11.37	22.99	14.92
35	2653.212	26.59	14.45	11.42	23.67	15.34
35.5	2727.912	25.25	15.01	11.57	25.06	14.63
36	2752.048	26.51	14.05	11.50	24.90	14.56
36.5	2306.804	27.90	14.27	11.10	23.85	14.42
37	891.096	30.20	17.12	9.80	20.12	14.22
37.5	718.504	33.55	19.81	9.93	15.38	14.17
38	702.144	32.92	22.84	9.65	13.70	13.94
39	684.488	28.93	25.70	10.50	13.80	14.76
40	506.208	32.05	26.33	8.09	12.63	12.35
41	657.596	25.20	30.53	9.65	13.38	14.06
42	739.504	28.47	25.65	9.73	15.42	13.63
43	231.52	62.04	14.22	3.91	8.21	8.18
44	149.924	46.40	8.17	4.86	7.63	13.92
45	275.724	47.97	18.12	8.94	9.29	11.35
46	274.62	36.90	16.53	6.31	7.50	12.07
47	232.848	42.46	24.21	9.24	9.20	11.88
47.5	300.06	37.51	26.10	8.56	10.11	13.29
48	368.728	31.73	26.25	7.69	9.80	14.02

(continued)

Table D.11 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
48.5	459.332	33.81	27.17	8.29	11.83	13.96
49	397.676	34.50	26.98	8.54	12.18	14.05
49.5	531.184	29.73	28.21	8.67	14.33	15.39
50	501.836	28.44	28.52	8.60	14.72	15.45
50.5	868.196	26.27	28.44	10.80	15.19	15.58
51	1196.932	24.93	29.37	10.67	15.34	15.43
51.5	1620.176	26.97	25.65	10.56	13.93	19.23
52	1411.5	25.51	27.72	11.79	14.66	15.68
53	1562.68	22.72	28.14	12.88	14.55	16.94
54	1321.76	23.06	29.57	13.39	13.87	15.15

Table D.12: Experiment 4, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
7	275.422	54.36	10.66	9.50	3.94	18.67
8	228.536	56.05	6.97	9.72	1.91	20.69
9	2297.736	43.42	14.66	11.04	12.59	12.40
10	4778.36	36.61	15.97	10.69	17.03	16.72
11	6045.528	32.51	19.52	10.38	16.31	15.82
12	5533.72	30.52	20.41	10.83	17.59	14.48
12.5	4818.896	30.19	19.53	11.64	17.80	14.90
13	4917.424	28.73	18.97	11.74	19.19	15.31
13.5	4830.108	26.49	19.17	11.90	20.50	15.29
14	5401	27.89	18.74	11.97	19.84	15.01
14.5	5703.544	26.29	19.22	12.12	20.49	16.27
15	6255.46	25.12	19.18	11.99	21.51	16.91
15.5	5108.948	22.85	19.74	13.03	21.89	17.82
16	4491.9	27.91	19.19	12.58	19.89	16.70
16.5	3672.192	29.74	19.42	11.26	19.51	16.31
17	3594.976	29.99	19.71	11.50	18.93	16.27
18	4170.764	31.72	19.45	11.65	18.09	15.61
19	4592.984	31.95	18.42	11.38	18.26	16.58
20	3227.76	32.26	18.12	11.53	17.75	16.71
21	1372.216	28.53	21.75	12.63	16.21	16.58
22	1580.704	27.89	23.51	13.26	14.62	16.82
23	2485.26	28.03	22.28	12.76	15.15	16.68
23.5	2079.496	24.74	22.59	12.60	16.43	17.48
24	2489.604	26.96	21.58	12.01	16.73	16.93
24.5	2414.088	26.56	21.60	11.94	16.93	17.60
25	2965.132	25.29	21.46	12.21	18.02	17.96
25.5	2808.468	25.16	21.13	12.32	18.47	18.06
26	3922.14	23.99	20.18	12.34	19.20	19.25
26.5	4050.832	24.53	19.65	12.31	19.67	18.88
27	4405.904	24.18	18.78	12.34	20.35	19.63
27.5	4236.728	23.15	18.08	19.61	18.35	16.36
28	4914.06	22.84	18.59	12.55	20.64	20.55
30	3335.892	28.62	18.52	11.61	19.42	15.88
31	2670.648	29.80	19.58	11.58	17.97	17.08
32	2611.396	29.87	19.90	12.01	16.96	17.51
33	2689.164	29.92	19.78	13.56	16.08	16.97
33.5	2263.188	30.23	19.60	11.14	17.50	17.09
34	2235.536	30.04	19.53	11.19	17.16	17.30
34.5	3024.116	26.79	20.74	12.62	17.62	15.78
35	2970.912	26.81	20.39	12.58	17.05	16.11
35.5	2929.34	26.63	20.89	12.52	17.97	15.05
36	2900.056	27.70	19.87	12.44	18.10	15.16
36.5	2220.12	28.42	19.68	12.34	17.74	15.41
37	823.148	25.23	23.69	13.31	15.95	16.55
37.5	657.548	28.58	23.86	11.94	13.73	17.21
38	605.932	26.59	26.04	12.17	12.99	17.05
39	531.82	24.28	26.22	13.30	13.36	18.25
40	547.9	24.42	30.40	11.22	13.18	16.38
41	587.832	27.40	29.70	10.76	12.11	15.54
42	598.808	20.00	30.12	12.72	14.12	18.48
43	535.104	17.27	35.45	13.61	10.44	19.27
44	470.956	13.86	31.98	13.92	8.01	22.30
45	762.724	23.42	29.16	13.83	10.29	18.18
46	684.544	25.49	26.96	14.59	8.90	20.06
47	639.448	21.00	32.71	13.89	9.48	19.28
47.5	557.552	23.55	30.71	12.22	9.69	19.91
48	556.216	24.95	30.72	12.01	9.31	19.39

(continued)

Table D.12 (continued)

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
48.5	612.552	27.30	28.90	11.66	10.52	17.88
49	705.368	35.11	25.41	10.17	9.72	15.73
49.5	630.28	29.12	28.76	10.09	11.57	17.11
50	389.972	22.14	24.99	15.35	6.19	27.25
50.5	917.348	28.56	26.03	12.36	12.59	17.34
51	1891.604	29.08	25.48	11.19	14.16	16.98
51.5	2177.852	28.80	24.25	12.77	14.09	16.13
52	2599.516	25.31	27.27	12.46	15.82	15.11
53	2391.576	25.98	27.02	12.16	15.55	15.45
54	2107.352	26.58	26.63	12.73	14.51	15.46

Table D.13: Experiment 5, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
3	675.36	36.81	15.98	11.33	6.46	25.52
4	64.188	3.68	1.99	26.18	8.53	50.03
5	24.98	35.77	6.05	7.19	9.50	38.02
6	59.82	54.44	9.59	8.44	3.64	21.26
7	985.604	59.08	9.65	7.31	7.87	11.86
7.5	989.188	59.14	8.98	7.04	7.60	12.08
8	1202.32	58.96	9.21	6.52	8.05	13.40
8.5	1127.086	43.11	12.44	9.89	10.65	21.05
9	2121.96	55.87	10.58	7.67	9.91	10.71
9.5	2259.248	53.49	11.32	7.83	10.75	11.55
11	3247.462	46.66	14.31	8.64	13.39	12.94
11.5	3311.228	47.27	14.25	8.54	13.23	12.25
12	3483.212	48.90	13.58	8.17	12.86	12.37
12.5	3245.476	41.58	16.42	9.36	15.35	13.14
13	3487.112	46.02	13.49	8.50	13.81	13.47
14	3457.684	49.24	11.80	8.11	13.74	12.02
15	3143.998	51.15	11.08	7.71	12.98	11.98
16	2890.956	32.55	13.49	9.15	15.84	24.82
16.5	2427.7	32.10	13.49	8.97	15.48	26.03
17	1427.876	31.13	16.77	9.01	15.31	20.32
17.5	1108.392	30.43	15.70	8.85	15.34	21.95
18	1276.472	31.79	16.23	8.99	15.13	20.28
18.5	1154.964	33.56	17.46	9.15	15.57	17.36
19	2004.848	33.02	14.83	8.68	14.09	26.14
19.5	2181.764	32.18	14.52	10.07	14.12	25.51
20	2166.204	32.29	15.04	10.30	14.39	24.67
20.5	2288.344	26.72	16.86	11.34	15.17	25.39
21	2251.344	33.47	15.91	10.11	15.30	21.57
21.5	2007.472	32.05	15.46	10.39	14.90	23.63
22	2410.256	31.07	16.36	10.43	15.55	22.92

Table D.14: Experiment 5, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
3	1173.734	36.75	14.87	9.66	9.87	16.20
4	1610.218	42.39	12.38	10.39	11.17	15.24
5	1726.22	33.55	20.08	11.22	12.78	17.48
6	1862.328	31.91	23.15	11.19	12.05	17.22
7	1702.478	37.07	21.55	10.35	10.11	15.70
7.5	1351.776	26.00	23.29	11.90	11.55	24.84
8	1350.036	26.52	24.86	11.40	10.68	24.15
8.5	1405.526	26.41	24.27	11.68	10.81	24.35
9	2915.462	26.45	24.18	12.00	13.57	20.87
9.5	3662.792	25.28	22.66	11.89	14.20	22.76
11	4134.316	23.24	22.32	12.16	14.70	23.25
11.5	4153.616	23.66	22.05	11.55	14.61	23.52
12	3793.344	23.85	21.42	11.07	14.86	23.88
12.5	4114.592	24.16	21.16	11.00	15.08	24.19
13	4415.476	26.67	17.83	11.08	15.42	25.06
14	4447.504	42.95	13.79	11.37	13.78	14.63
15	4293.048	27.88	13.88	11.53	18.45	24.74
16	4221.14	34.35	11.93	9.93	15.68	25.41
16.5	3481.12	31.72	11.76	8.90	16.73	28.44
17	3015.14	31.51	13.00	8.60	14.60	30.16
17.5	1867.804	27.14	14.64	8.62	15.39	26.16
18	2222.396	26.16	16.41	9.08	14.29	24.70
18.5	1912.012	27.95	14.81	8.21	13.89	25.06
19	2986.204	32.00	12.87	8.66	14.90	28.83
19.5	3081.62	31.95	13.17	9.01	15.36	27.91
20	3540.932	31.44	14.37	9.42	15.48	26.46
20.5	3815.104	31.18	13.90	9.20	14.81	28.50
21	3388.36	32.03	15.50	8.69	14.75	25.97
21.5	3498.38	23.32	19.00	10.79	16.82	26.17
22	4471.096	23.74	19.07	11.14	17.55	24.49

Table D.15: Experiment 5, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
3	1055.324	50.43	14.81	8.71	7.60	13.26
4	1712.332	39.12	15.94	11.07	9.41	15.34
5	1895.924	29.98	23.99	12.05	10.09	17.78
6	1757.296	27.09	25.41	12.31	11.14	18.78
7	1088.588	25.94	17.40	11.40	14.76	27.63
7.5	1110.794	24.17	19.50	12.05	13.49	28.22
8	1188.112	21.98	23.30	12.32	12.95	26.93
8.5	1036.946	20.22	24.23	12.48	12.35	27.84
9	3290.552	33.39	23.98	10.35	13.40	15.03
9.5	4404.818	29.06	25.60	10.91	14.92	15.77
11	5692.592	20.22	20.14	13.07	21.28	20.73
11.5	5157.176	19.37	19.53	13.35	22.23	21.18
12	5580.6	18.60	19.96	13.19	22.63	21.80
12.5	5291.792	17.85	19.30	13.10	22.58	23.69
13	5051.056	18.09	19.31	13.27	22.24	24.07
14	5075.412	18.32	18.53	12.17	23.37	24.30
15	5263.308	18.49	16.92	11.68	24.13	24.72
16	3937.312	22.17	14.56	11.62	21.25	26.21
16.5	3705.385	23.01	13.76	10.47	17.56	16.27
17	3482.536	22.29	12.80	10.59	15.03	37.15
17.5	2528.684	20.27	13.35	9.08	18.45	36.22
18	1518.608	22.10	18.01	10.19	19.41	22.15
18.5	1408.208	20.64	17.94	9.83	19.05	20.81
19	1783.688	19.04	18.30	9.87	19.95	21.58
19.5	2136.108	18.53	19.65	10.40	20.04	26.94
20	2335.008	16.93	19.61	10.15	20.01	29.65
20.5	2287.804	17.54	20.45	10.55	20.87	26.08
21	2382.212	17.11	23.49	10.57	20.44	23.71
21.5	2490.196	17.44	23.41	10.43	21.11	22.32
22	4037.032	15.07	22.68	10.70	21.93	24.86

Table D.16: Experiment 6, Reactor 1

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	2816.678	52.11	9.49	5.47	15.27	12.01
5	3247.624	47.15	11.51	5.92	17.08	12.91
6	2967.508	21.95	18.40	10.30	21.76	18.55
7	3333.176	27.38	18.12	11.07	20.18	15.55
8	2373.12	24.08	19.32	10.52	20.13	16.33
9	4110.84	28.63	14.28	9.51	14.23	30.47
10	4429.964	30.00	14.59	8.17	14.63	30.01
11	4362.876	29.88	17.11	8.10	15.36	25.87
12	6604.612	31.38	18.60	9.32	15.89	20.85
13	3410.308	25.79	21.43	9.77	17.07	20.55
16	3950.864	31.48	20.56	7.28	18.91	15.62
17	3826.46	33.25	17.70	6.43	16.12	22.08
18	2519.148	30.12	17.66	9.71	15.35	12.94
19	3517.148	24.41	13.85	9.12	14.79	32.94
20	3244.316	26.75	14.00	11.72	15.41	27.39
21	4442.296	24.40	15.87	11.60	17.53	24.51
22	5214.04	23.08	15.10	11.29	17.97	26.40
23	5147.036	23.48	15.88	11.52	19.44	22.51
23.5	3475.404	21.72	16.52	13.05	18.90	22.43
24	4299.928	24.56	15.73	11.76	18.78	21.34
24.5	3771.352	18.58	18.43	12.08	20.50	22.48
25	3388.356	17.65	18.26	12.01	20.68	23.17
25.5	3401.416	17.03	17.23	12.50	18.62	27.94
26	4032.592	18.53	15.74	12.64	20.03	28.59
26.5	3249.592	19.82	17.21	12.38	19.13	28.25
27	3152.372	18.94	16.95	11.73	18.83	29.05
27.5	2832.096	24.74	16.59	12.29	17.32	24.36
28	3110.6	24.70	16.98	11.72	17.16	24.48
29	2762.82	24.54	17.64	11.95	17.29	23.91
30	2929.352	22.93	20.50	12.26	17.29	18.86
31	3320.396	19.25	17.72	11.09	16.32	31.93
32	2742.648	26.91	19.84	9.98	16.17	16.14
33	3980.564	27.41	18.71	10.80	17.50	19.92
33.5	3731.648	27.26	18.29	10.85	17.58	19.73
34	4194.164	26.86	17.81	11.27	18.46	19.06
34.5	4168.368	26.81	17.72	11.29	18.53	18.58
35	3813.712	27.14	16.56	11.37	18.37	19.67
35.5	2673.864	27.66	15.97	11.20	17.22	21.79
36	2415.936	28.84	16.37	11.20	16.29	21.78
36.5	2157.204	29.83	15.92	11.25	15.21	22.77
37	2373.868	29.23	16.45	11.26	14.98	22.97
37.5	2439.816	29.65	18.50	11.29	14.87	20.03
38	3010.752	28.27	20.26	11.53	16.62	16.24
39	2834.548	25.26	19.60	11.40	16.75	21.18
40	2172.516	25.08	22.17	12.28	15.52	20.21
41	2872.952	25.40	21.66	12.21	16.92	18.92
42	3268.696	23.84	19.51	12.64	17.76	21.36
43	3007.652	24.49	20.40	12.05	16.65	21.63
43.5	1817.348	25.84	20.60	11.18	16.22	21.45
44	2117.38	24.74	21.68	11.12	16.75	16.57
44.5	2614.596	22.52	20.71	10.68	16.59	23.94
45	2717.344	23.17	22.75	11.41	18.44	19.08
45.5	4166.544	24.06	21.47	11.86	17.51	20.03
46	4655.148	21.34	20.75	11.69	18.60	22.74
46.5	3504.404	23.42	19.97	11.56	17.56	22.44
47	3898.248	22.30	21.12	11.71	17.68	22.07
47.5	2272.168	21.76	21.26	11.83	14.80	25.69
48	2015.096	25.60	22.48	12.49	13.77	20.60

Table D.17: Experiment 6, Reactor 2

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	3609.968	51.96	12.75	6.18	13.34	12.38
5	3809.274	50.14	12.87	6.27	13.91	12.75
6	4193.112	25.98	16.29	11.37	17.49	24.56
7	4508.488	34.62	11.87	9.29	15.28	25.71
8	2929.428	27.78	15.57	11.99	16.65	23.07
9	4401.544	31.12	13.51	9.57	15.56	26.32
10	5072.2	30.35	15.89	10.16	17.51	19.13
11	6004.636	31.63	16.08	9.83	16.77	20.04
12	4379.88	25.87	16.57	12.21	18.89	19.73
13	3806.928	25.90	18.03	11.43	18.46	18.95
16	2540.116	28.73	20.20	9.72	17.58	18.49
17	3029.364	31.19	19.67	8.96	16.59	18.34
18	2913.452	48.94	11.16	9.61	10.39	12.43
19	5039.652	48.17	9.60	10.22	11.33	13.51
20	4036.948	46.70	9.26	9.06	11.56	16.37
21	4478.772	31.42	10.55	11.96	16.81	24.77
22	2530.916	29.33	13.59	12.47	16.09	23.98
23	4448.74	25.76	15.75	11.79	16.96	25.78
23.5	3910.14	23.22	14.57	12.25	16.11	27.86
24	4071.092	24.23	14.65	12.91	18.67	25.33
24.5	4221.408	20.40	15.15	13.19	20.69	25.54
25	4408.94	19.40	15.34	13.33	19.79	27.46
25.5	4009.98	17.90	15.44	12.91	17.59	32.59
26	4129.072	24.53	15.22	13.42	18.91	23.07
26.5	3852.632	20.80	16.24	13.63	19.58	26.97
27	3422.02	20.29	16.91	13.13	17.72	28.89
27.5	3429.644	24.84	17.44	12.60	15.99	24.58
28	3458.004	23.60	18.72	12.62	15.96	24.70
29	3502.808	23.09	19.72	12.72	15.18	25.56
30	4232.464	20.22	22.27	12.81	16.57	22.77
31	4697.424	18.27	22.28	13.16	15.98	25.16
32	4341.124	21.06	21.94	12.51	15.92	23.00
33	5368.156	21.63	22.86	11.77	17.70	20.72
33.5	4573.696	21.94	22.28	11.75	17.47	20.94
34	6870.972	21.04	23.17	12.01	17.47	21.10
34.5	4236.528	22.68	22.41	11.36	16.74	21.58
35	2375.032	22.51	22.70	10.95	14.27	25.02
35.5	1875.504	25.11	21.36	10.76	12.73	25.83
36	1982.644	26.54	22.47	11.21	11.19	24.40
36.5	1800.644	26.46	22.09	11.14	10.07	26.15
37	1820.14	27.40	23.04	11.12	9.48	24.86
37.5	2063.024	28.37	23.35	12.08	10.94	19.84
38	2886.432	27.04	23.37	12.08	11.51	20.73
39	2593.284	23.11	24.95	12.56	10.86	23.32
40	2288.684	25.53	21.94	12.22	13.79	20.05
41	3495.26	24.65	21.17	12.69	14.04	20.52
42	1764.896	26.68	19.18	12.34	15.03	21.63
43	1689.604	27.72	16.89	11.33	16.81	21.57
43.5	1289.24	29.85	18.05	12.07	15.27	16.03
44	1500.38	27.91	18.13	11.62	15.10	21.04
44.5	2899.016	26.27	19.43	11.85	16.04	18.17
45	3995.192	25.20	22.19	12.21	14.72	16.29
45.5	5191.444	21.60	19.59	11.79	13.60	22.06
46	5566.276	23.43	21.79	12.96	15.31	17.59
46.5	4762.964	24.39	20.67	12.52	15.20	18.91
47	4339.848	23.07	21.50	12.31	15.44	20.34
47.5	1963.472	21.17	22.25	13.62	11.81	24.52
48	1939.432	21.40	21.04	13.00	10.93	28.22

Table D.18: Experiment 6, Reactor 3

Time (days)	VFA (mg/l)	Acetic (% VFA)	Propionic (% VFA)	iso-Butyric (% VFA)	n-Butyric (% VFA)	iso-Valeric (% VFA)
4	3980.782	38.36	19.81	6.85	15.36	12.71
5	5219.412	35.69	20.03	6.99	18.02	12.99
6	5765.536	15.76	23.84	11.24	21.92	22.00
7	6207.2	23.23	21.76	10.32	20.29	20.13
8	4199.08	15.53	24.12	11.72	23.30	18.03
9	3968.148	13.28	24.89	11.42	23.43	21.22
10	4171.44	14.48	25.53	11.20	23.52	17.30
11	6069.828	24.26	23.11	9.85	19.26	19.23
12	6729.204	25.76	21.79	9.40	17.68	21.30
13	3842.008	23.10	22.87	10.54	19.22	19.59
16	3042.172	29.10	21.90	7.56	18.19	17.30
17	3924.204	27.38	19.54	6.92	17.07	24.94
18	3022.116	26.43	18.19	12.38	15.35	20.87
19	3660.548	35.71	9.13	10.72	14.17	21.69
20	3814.828	38.93	5.46	9.41	17.18	25.11
21	4145.92	35.67	7.02	9.06	18.46	25.75
22	3970.852	34.47	9.10	9.24	18.27	24.58
23	4517.784	34.45	10.05	9.21	18.04	23.66
23.5	3933.344	30.64	9.36	9.27	17.95	28.21
24	4079.292	41.84	9.54	9.50	16.07	17.31
24.5	4127.012	37.06	11.14	10.12	17.96	18.42
25	3913.98	25.73	11.63	10.37	20.38	26.88
25.5	4057.916	20.11	13.32	11.99	18.89	31.38
26	4187.632	17.83	16.70	14.18	19.12	27.33
26.5	4356.532	19.05	18.11	12.89	21.36	25.66
27	4290.02	18.10	19.24	12.71	21.64	25.27
27.5	4524.976	22.49	18.50	12.61	19.21	23.22
28	5620.212	21.62	19.95	12.43	19.46	22.18
29	4953.676	22.72	21.12	12.60	17.68	23.26
30	4373.692	18.35	21.68	12.46	19.24	23.30
31	4105.436	18.03	22.11	12.27	19.11	23.40
32	4109.984	21.87	21.35	12.18	18.00	21.03
33	4289.388	23.57	21.99	10.93	18.44	19.75
33.5	3774.644	24.21	21.50	10.74	18.26	20.29
34	4464.368	23.91	21.67	10.79	18.58	19.53
34.5	3269.38	24.95	20.73	10.52	17.72	20.07
35	2213.696	27.52	19.27	10.34	14.91	22.65
35.5	1507.56	28.73	18.64	9.98	13.72	24.26
36	1650.3	30.26	19.31	9.95	12.42	22.98
36.5	1488.416	31.07	18.28	9.87	11.53	24.48
37	1798.86	33.77	18.94	10.59	11.57	17.59
37.5	2183.364	31.76	17.76	11.39	12.21	21.73
38	2806.86	29.28	20.02	11.51	13.07	20.82
39	2588.404	24.01	22.42	12.45	13.48	22.11
40	2398.144	26.39	19.79	11.90	15.80	20.66
41	2875.628	25.65	19.96	12.23	16.78	19.41
42	2057.444	26.49	17.13	12.05	15.52	23.75
43	1870.388	27.38	17.65	11.69	15.68	22.24
43.5	1140.372	30.89	16.86	11.08	15.48	15.78
44	1466.176	29.16	17.94	10.43	15.55	21.61
44.5	2628.008	26.91	16.99	11.21	17.52	21.04
45	3231.336	26.55	17.47	11.37	17.53	21.51
45.5	4110.596	24.88	17.25	12.38	17.71	21.68
46	5508.388	23.95	17.73	12.27	18.62	21.46
46.5	4847.296	25.13	17.03	12.18	19.17	20.51
47	3997.72	24.79	18.04	12.21	18.57	20.46
47.5	1576.888	24.78	15.83	11.58	13.34	30.04
48	1593.044	28.16	17.13	12.91	12.95	19.47