COMPARISON OF AN ANAEROBIC BAFFLED REACTOR AND A COMPLETELY MIXED REACTOR

START-UP AND ORGANIC LOADING TESTS

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Abstract

The laboratory type of the conventional completely mixed anaerobic reactor (CMAR) is a 20 *l* glass bottle with a stirrer coming in through the neck. The CMAR was maintained in a waterbath at 37^{o}C. Two CMARs were operated, namely, the Test reactor and the Control reactor. All the tests for the CMAR where carried out in the Test reactor and the Control reactor was maintained at steady state. A second type of reactor, anaerobic baffled reactor (ABR) was also operated. The ABR is a rectangular perspex box with internal vertical baffles alternately hanging and standing. The baffles divide the reactor into eight different compartments with a total working volume of 7.5 *l*. The upflow chamber in each compartment is twice the width of the downfow chamber. Each baffle is angled at about 45^{o} to distribute the flow towards the centre of the upcomer. The ABR was set up in a controlled temperature room. The critical difference between the CMAR and the ABR is that the CMAR is essentially a mechanically stirred reactor whereas the ABR is an upflow hybrid reactor.

A batch of raw sewage from Umbilo sewage works (Durban, South Africa) was used in the reactors for experiments to compare the start-up of the CMARs and the ABR. The two CMARs were operated first whilst the ABR was started up later since it has a shorter HRT. Although the CMARs had been in operation for a long time they did not achieve steady state because the acid solution used to measure their gas production through displacement entered the reactors resulting in a decrease in pH. The pH decreased to values of about 6.3 which is out of the optimum range of 6.5 to 7.8 for methanogens (Haandel and Lettinga, 1994). This accidental influx of acid occurred up to day 32. The Test reactor had calcium hydroxide added to it to enable it to recover from acid contamination. The Control reactor was left to recover without any chemical additions. The HRT was reduced to 37 d from 20 d so as to reduce the amount of volatile acids in the reactors. Eventually the CMARs were fed with the sucrose feed to enable comparison with the ABR and also to increase stability of the reactor contents. They finally achieved steady state at an organic loading rate (OLR) of 0.2 kg/m^3.d. The ABR was first sparged with nitrogen and then the reactor was inoculated with ca. 7.5 *l* of the raw sewage with the outlet sealed to prevent air contamination. A litre of sucrose feed was then added and the reactor left to for 3 days to stabilise and allow the biomass to settle. Feeding began at a HRT of 60 h. This gave a volumetric organic load of 1.6 kg/m^3.d. After the reactor achieved steady state at this volumetric organic load the HRT was reduced to 35.7 h (2.7 kg/m^3.d). There was an increase in gas production as a result of the increased organic load. The HRT was then reduced to 20 h (4.8 kg/m^3.d) and was maintained at this loading rate until the reactor reached steady state. The experiments maintained an initially long retention time (60 h), which was reduced, in a stepwise fashion during which time substrate concentration was kept constant. This provided better reactor stability and superior performance than a reactor with a constant and low retention time coupled with a stepwise increase in substrate concentration.

The operational parameters monitored were the pH, total solids, volatile solids, alkalinity and gas production. At a later stage gas composition and COD measurements were also taken. During the start up period for the Test reactor a total of ca. 14.99 g (calcium hydroxide) were added to correct the pH. The pH eventually stabilised at about 7.15. The Control reactor had a steady increase in pH to the same value. This demonstrated the ability of anaerobic reactors to recover from upset without chemical additives. The ABR had a pH higher than 7 at all times. This was mainly due to a sample collection error. The Ripley Ratio (RR) for the CMARs was initially below 0.3, which is the maximum value for an efficiently operating water treatment system. However, due to the acid contamination they increased to values of about 0.5. This was eventually corrected to values below 0.3 due to the reduced loading rates. The TS of the CMARs showed a downward trend from a value of about 3.2 gTS/*l* to 1 gTS/*l*. This decrease in total solids was due to the change in feed fromraw sewage to the synthetic sucrose feed, which had a lower TS content. There was a gradual increase in the TS of the ABR as the poor settling biomass was removed. It eventually leveled of at about 4 gTS/*l*. The VS of the CMARs were reduced from about 2 gVS/*l* to about 0.5 gVS/*l*. The CMARs had a decrease in gas production due to acid contamination. This was followed by erratic gas production, as the conditions in the reactors had not stabilised. The gas production finally stabilised to about 2500 m*l*/day. The ABR operated for some time without any gas production. When gas production began there was a gradual increase in gas production to average at about 2000 m*l*/day at the initial loading rate. Each increase in the loading rate brought about a sudden anomalous increase in gas production due to the increased mixing.

The research by many scientists has shown that Monod's equation or some variant of it can describe the breakdown of many organic compounds by the bacteria in the different anaerobic processes. In sewage treatment, however, the micro-organism are in a suspended solid mass. In this mass inorganic and inert biological organic matter are intermingled with the different types of anaerobic bacteria. This

makes it feasibly difficult to determine the bacterial concentration experimentally. This also makes it difficult to measure the concentration of the different groups of active bacteria in an anaerobic biomass. The biodegradable matter cannot be distinguished from the non-biodegradable mass making it difficult to quantity its exact mass. Furthermore, the concentration of substrate at the surface of the bacteria differs from that in the bulk of the liquid phase due to absorption. As a result the concentration of the bulk liquid phase of the reactor or the effluent is not indicative of availability of substrate to the micro-organism in the treatment system. Thus the empirical approach of evaluating the observed experimental results is the only alternative for design and optimisation of anaerobic digestion systems. In the design of an anaerobic reactor, the maximum organic load it can withstand is related to the biomass retention capacity of the reactor. The biomass retention capacity is a factor dependent on the retention time of the sewage in the reactor.

The organic loading tests were undertaken with a stepwise increase in the influent substrate concentration. The feeding commenced at an OLR of 4.8 kg/m^3.d for the ABR. The OLR was doubled when the reactor reached steady state. The flow rate (HRT) into both reactors and other parameters were kept constant. The substrate concentration was increased from 4 gCOD/*l* (4.8 kg/m^3.d) to 64 gCOD/*l* (76.8 kg/m^3.d) for the ABR. For the CMAR it was increased from 4 gCOD/*l* (0.25 kg/m^3.d) to 32 gCOD/*l* (2 kg/m^3.d). The method used was to increase the organic loading rate until the reactors failed. Since the two reactors had different operating HRTs, the tests began when both had the same COD removal rate of about 60 % COD reduction. The same parameters as in the start-up period were monitored for both reactors. Prior to reactor failure the pH for the ABR fluctuated but was always above 7.3 while the pH in the CMAR was constant at 7.5. The CMAR was able to produce a maximum of 4000 m*l*/day of biogas at an organic loading rate of 0.5 kg/m^3.d. The ABR produced ca. 15000 m*l*/day of biogas at an organic loading rate of 38.5 kg/m^3.d. Both reactors showed an increase in gas production with an increase in the loading rate. Thus the acclimated biomass in the reactors was shown to have increased activity with an increase in organic loading rate. The biogas produced at steady state contained 10 to 15 % nitrogen, 50 to 62 % methane and 25 to 30 % carbon dioxide. The CMAR had a COD removal efficiency ca. 70 %, which did not fluctuate when OLR was increased. The ABR reached a maximum COD removal of 80 %. An increase in the OLR led to an initial decrease in the COD removal until the biomass recovered and the high COD (80 %) removal rates resumed. The ABR reached a maximum OLR of 76.8 kg/m^3.d whilst the CMAR reached a maximum OLR of 2.0 kg/m^3.d. The investigations showed that the ABR could be operated at higher organic loads than the CMAR and give the same organic removal rate. This verified the importance of increasing the SRT/HRT ratio in anaerobic reactors. The CMAR, however, proved to be stable to changes in the influent feed strength, as there was no immediate noticeable changes in the gas production.

Chapter 3: Wastewater Treatment Systems

Chapter 4: Laboratory Equipment and Analytical Methods

Chapter 7: Conclusions and Recommendations

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ABR	anaerobic baffled reactor
Acetyl CoA	acetyl coenzyme A
ALK	alkalinity (mg/*l* as $CaCO_3$)
ATP	adenosine triphosphate
BRV	biochemical reaction vessel
CMAR	completely mixed anaerobic reactor
CRT	cell retention time (s)
CSTR	completely mixed tank reactor
COD	chemical oxygen demand (mgO_2/l)
DF	downflow (part of ABR compartment)
DW	dry weight (g)
E	enzyme
EMP	embden meyerhof pathway
ES	enzyme-substrate complex
FAD	flavin adenine dinucleotide
$FADH_2$	flavin adenine dinucleotide (reduced)
F/M	food to micro-organism ratio
g	gaseous
GDP	guanosine diphosphate
h	hour
K	Kelvin
k_x	rate constant
k_m	Michelis constant
k_d	specific endogenous decay rate
HABR	hybrid anaerobic baffled reactor
HRT	hydraulic retention time
NAD	nicotinamide adenine dinucleotide
min	minute
MLSS	mixed liquor suspended solids
MLVSS	mixed liquor volatile suspended solids
RBC	rotating biological contactor
RR	ripley ratio
S	substrate
S_{min}	minimum substrate (mg/*l*)
SCFA	short chain fatty acids
SCOD	soluble chemical oxygen demand (mgO_2/*l*)
SEM	scanning electron microscopy
SRT	solids retention time (h/min/d)
t	time (h/min/d)

T	temperature in degrees Celsius
TS	total solids (gTS)
TSS	total suspended solids (gTSS)
μ	specific growth rate (d^{-1})
μ_m	maximum specific growth rate (d^{-1})
UASB	upflow anaerobic sludge blanket reactor
UF	upflow (part of ABR compartment)
VA	volatile acid
VFA	volatile fatty acid (g)
VS	volatile solids (gVS)
VSS	volatile suspended solids (gVSS)
WRMSTS	Water Resources Management Strategy Technical Secretariat
x	concentration of micro-organisms ($mg l^{-1}$)
Y	yield coefficient (g/e^- eq)
ZINWA	Zimbabwe National Water Authority

anabolism	The biosynthesis of new cellular material
acid-forming bacteria	The group of bacteria in a digester that produce volatile acids
aerobic	Bacteria use O_2 as the terminal electron acceptor
anaerobic	Bacteria use terminal electron acceptors other than O_2 or NO_3
active mass	Active biomass
adenosine triphosphate	Energy rich molecule
biodegradation	Breakdown of compounds by biologically mediated reactions
biogas	A mixture of gases, predominately methane and carbon dioxide produced by anaerobic fermentation
buffer capacity	A measure of the capacity of wastewater for offering resistance to changes in pH.
biomass	Bacterial cells
Carbon/Nitrogen Ratio	The ratio of organic carbon to that of total nitrogen.
catabolism	breakdown of complex molecules for metabolic processes
Continuos-feed Digester	A digester which is regularly charged with small amounts of fresh slurry at short intervals; the freshly charged slurry automatically displaces an equal volume of effluent and the process continues without interruption.
degradation	The breakdown of substances by chemical, physical and/ or biological action.
dewatering	The process of removing water from effluent slurry of a digester by evaporation or filtration.
effluent	The sludge or spent slurry from a continuos fed digester
enzyme	A complex organic substance (protein) produced by living cells and having the property of accelerating transformations such as digestion processes.
fermentation	Mechanism of energy generation in which ATP synthesis occurs during catabolic reactions and organic molecules act as both electron donors and electron acceptors.
guanosine triphosphate	Energy rich molecule
hydraulic retention time	The average time that a liquid stays in a reactor before it is discharged. It is equal to the active volume of the reactor divided by the flow rate of the liquid entering it. It is usually expressed in days but maybe as short as hours.
hydrolysis	The process by which complex organic compounds are broken down to simpler organic compounds. This usually involves the work of a single enzyme or a multitude of enzymes.
inert	Unreactive
influent	The feed into a digester.
mesophilic	Within a moderate temperature range
methane	A colourless, odourless, flammable gas and the main constituent of natural gas, coal gas and biogas.
Plug flow	Movement without mixing in an axial direction in a digester.
soluble	Able to dissolve in a certain liquid (water).
solids retention time	The average time that a solid stays in a reactor before it is discharged. (h/min/d)

Chapter 1: Introduction

Southern Africa is by all standards a drought prone area. The rainfall pattern is erratic. Due to man's activities large tracts of land are turning into deserts. Without water life would not survive, as water is the solvent for all chemical reactions taking place in nature. It takes part in beneficial reactions as well as destructive reactions. However, despite its destructive capabilities water is essential in human society. Humans use water for drinking, cooking, washing and a host of other applications. In industry water is used as a solvent, a coolant and for transport purposes. In the mines it is used to leach minerals and dilute acids after use. Most of these activities result in water that is high in undesirable elements. As the rains in Southern Africa are erratic, dams have been built to maintain a perennial source of water for man.

The activities of man pollute the already scarce water resources making it unfit for human usage. Thus it is essential to:

i. have better treatment facilities to purify water from rivers.
ii. minimise pollution in rivers by having better wastewater treatment facilities

Another factor is the large population increase in the urban areas as people move to these areas in search of jobs. This creates a demand on water resources, which are already scarce. There is then a need to protect the water resources by preventing pollution through wastewater treatment. Unfortunately in Southern Africa the water treating activities are usually left to the individual municipalities. Most of these municipalities are usually poor and can not afford to update their plants to keep in line with modern trends in water treatment.

1.1 Water in Zimbabwe

Zimbabwe is a landlocked country in Southern Africa. Zambia borders it to the north, Botswana to the west, Mozambique to the east and South Africa to the south. The Limpopo River flows between Zimbabwe and South Africa partially demarcating the southern border between these two regional trading partners. On the northern border we have the Zambezi River which is an important tourist, economic and social feature in Zimbabwe. On the Zambezi is the Kariba dam, which is used for hydroelectric power generation and as a water resource. The great Dyke is a range of mountains that stretch across the centre of the country creating the highveld. On the highveld we find the sources of most of the major rivers found in Zimbabwe. These are the Save, Mazowe, Manyame, Sanyati, Gwayi, Mzingwane and Runde rivers.

The capital city of Zimbabwe is Harare, which is located on the highveld. Harare is situated in a region of high rainfall and it has the largest urban population. The city draws its water from Lake Mcllwaine and Lake Chivero. Lake Chivero is the major source of potable water for the city. This lake became eutrophic as a result of the discharge of nitrogen and phosphorus-rich sewage effluent, causing excessive growth of algae. This algae then died and decomposed, depleting the dissolved oxygen levels, killing fish and making the water difficult and expensive to treat. The next major city in Zimbabwe is the city of Bulawayo. Bulawayo is situated in the drier southern part of the country. Historically King Lobengula chose Bulawayo as a city as it offered the best site in the Matabeleland region for establishment of his capital. Lobengula's Ndebele tribe at the time did not comprise of a large population as he was fleeing from the Zulu king Tshaka. Thus the availability of water was not a major issue on the suitability of the site at the time. However, with the increase in population of the city in recent times coupled with the frequent droughts in that part of the country, the city has

developed problems of finding adequate potable water. This has led to the setting up of a committee to look into harnessing water from the Zambezi River. The basic idea is to pump water from the Zambezi River to the city. This technologically simple project has been hampered by political and financial difficulties. The city of Mutare is situated in the eastern district of the country and draws its water from the Odzi Dam. Although the city is in a region with abundant rain it has experienced difficulties with water during years of drought because there are insufficient dams to store enough water to meet its demands. The city of Masvingo is situated in the southern part of Zimbabwe and is serviced by the Kyle Dam. Masvingo like Bulawayo has problems of supplying adequate potable water to its growing population. The last major city in Zimbabwe is the city of Gweru, which is situated, in the central part of the country. Gweru draws its water from the Manyame Dam. Like all other cities in Zimbabwe, Gweru has problems with a growing urban population and lack of funds for expansion or creation of new water reservoirs.

Besides population growth the other major threat to the water resources in Zimbabwe is the effect of sewage and industrial effluents. With the progress of development in most parts of the country, the quality of water in most rivers has deteriorated due to the greater pollution loads. Initially, the problem was one of biologically treated waters that still contained biological nutrients and caused eutrophication. With the increased population in the urban centres this has now developed into a situation where the current water treatment facilities can no longer cope with the increased loads and this has resulted in the discharging of partially treated effluents into the rivers. The greatest hindrance to the development of adequate wastewater treatment facilities has been the lack of finance by local authorities rather than a lack of planning. Zimbabwe has a population of 11 000 000 with an annual rate of natural increase of 1.02 %. The population density is 29 people/km^2 (Ministry of Education, 1989).

1.1.1 Water regulations

Until the 1960s, water pollution in Zimbabwe (formerly known as Southern Rhodesia) was not perceived to be a national problem and only local pollution occurred. As development progressed the quality of water in the rivers started to deteriorate as a result of the discharge of sewage and industrial effluent. The original Water Act of 1927 could not deal with pollution, since it was not even defined in the Act. The periodic blooms of blue-green algae in Lake Chiveroin 1960 made it necessary to review this aspect of the Act. As a result of the perceived long term pollution threat to Zimbabwe's water resources as a whole and the short-term threat to Harare's water supplies, a number of committees were set up to tackle the problem of water pollution.

The Ministry of Water Development in 1964 set up the Water Pollution Committee. In its final report in 1966 the committee recommended that Government should adopt the following basic principles:

i. Pollution should not only be defined clearly and precisely, but also comprehensively.
ii. The relevant legislation should be under one act - the Water Act.
iii. The legislation should provide for pollution control by effluent standards which relate to the effluent discharge and not to the receiving water.
iv. The Ministry of Water Development should be the responsible Ministry and a Technical Advisory Board should be established to advise the Minister.

Most of these principles were adopted by the Government and were subsequently incorporated into the Water Act No. 41/1976, which was based on the following principles:

i. Water rights were granted in perpetuity.
ii. Water rights were issued on a priority system where the people holding the oldest water rights had control of the water.
iii. Water was supervised and managed by the River Boards.

With the advent of independence in 1980 to Zimbabwe many experts and ordinary people called for changes in the 1976 Water Act (WRMS Technical Secretariat, 1999). As a result the Minister of Rural Resources and Water Development set up a committee which included people from various government ministries, the private sector and others. After the committee had come up with its recommendations, the changes to the Water Act were approved by the Cabinet Committee on Legislation in 1994 leading to the new Water Act of 1999. This Act is governed by the following principles:

i. The State owns all surface and groundwater, except for primary purposes mainly domestic such as drinking, cooking and washing), any use of water must be approved by the state.
ii. People with interests in the use of water should be involved in making decisions about the use and management of Zimbabwe's water resources.
iii. Water is to be managed by catchment areas because rivers do not match provincial or district boundaries. Catchment and sub-catchment Councils will be set up for all river systems and aquifers. Sub-catchment areas will be based on sub-hydrological zones. The committees will include rural representatives from the communal, small-scale commercial farms, large commercial farms, mines, urban representatives from industry, manufacturing representatives and municipalities.
iv. Use of water resources must be carried out in a way that protects and sustains the environment. The environment is seen as a legitimate user of water competing with other users such as industrial, agricultural and domestic users. Some amount of water should be left to flow back into the environment and there should be provision for this in the catchment plan.
v. People who use water and people who pollute water must pay. Fines up to Z$ 100 000 or twice the value of the advantage gained or prejudice caused will be levied. Imprisonment of up to two years can be imposed. People who cause pollution of water may have to pay all the costs of removing the pollution. When a user is ordered to stop an offence he or she must comply immediately, even if the matter is to be heard in court. Water prices must be socially acceptable.
vi. Managing water as an economic good is the best way of achieving efficient and fair use, and of encouraging conservation and protection of water resources. The Zimbabwe National Water Authority (ZINWA) and Water Resources Management Strategy (WRMS) will operate as a commercial enterprise. However, the Government has to ensure that the poor and disadvantaged will still have fair access to water.

It is apparent that the water resources of Zimbabwe will in the future come under more stress as the population grows. The fact that the Water Act of 1999 stipulates heavy fines for pollution offenders means that methods should be found for local treatment of wastes for major polluting industries. In line with international trends it is imperative that the waste effluent should be reduced at source. This involves introducing and implementing waste minimisation and cleaner production practices in these major polluting industries. Low strength waste effluents do not pose a major threat to the water resources as they can be effectively degraded by aerobic treatment processes which has a sho-t residence time. However, high strength waste effluents from agricultural products manufacturers (4000 mgCOD/*l*) slaughter house wastes(480-780 mgCOD/*l*) and brewery waste (990 000 mg COD/*l*) do create a problem as they can only be effectively decomposed by anaerobic treatment processes (McCarty, 1964; Barber and Stuckey, 1999). These wastes streams are defined as high strength wastes because they contain a high level of fats and carbohydrates. Their decomposition by the acidogenic bacteria gives rise to a high concentration of fatty acids which may render the methanogens inactive in a poorly designed reactor. This is because their acidity lowers the pH level until it is below the optimum for methanogenice degradation. At present in Zimbabwe there are no high rate treatment processes that can effectively remove the high strength effluents from these industries. These industries

have been some of the major contributors to the problem of eutrophication in Lake Chivero. Theoretically, the anaerobic baffled reactor has all the necessary attributes to have a major impact on the treatment of these high strength effluents in conjunction with well-implemented waste minimisation and cleaner production practices. The assessment of the suitability of the ABR, in this study, for the treatment of high strength effluents could create a basis for its implementation on a larger scale.

1.2 Objectives

- From the objectives of the New Water Act of 1999, the Zimbabwean government seeks to protect the environment. The presence of technology that reclaims polluted water is one of the ways the environment can be effectively protected. In this study the Anaerobic Baffled Reactor (ABR) was studied as an alternative wastewater treatment technology to the Completely Mixed Anaerobic Reactor (CMAR). The CMAR was chosen as it is the most common reactor in use in Zimbabwe and it is one of the most well researched reactors. In comparing the ABR to the CMAR one is able to see wether the ABR can be a suitable replacement for the CMAR. Such comparability can only be started on a laboratory level as in this work.

- The Water Act of 1999 also emphasises the need for a cost-effective means of making water available to the majority of the people. The study also compared the CMAR with the ABR in terms of their COD removal efficiency, start-up periods and stability during start-up. If the ABR proves to have a better efficiency on all these elements on a laboratory level then chances are that it will be a more cost effective replacement for the CMAR. This is because it will be able to treat larger and higher strengths of water for the same energy input as the CMAR.

- In the Water Act of 1999 (Zimbabwe), users and polluters of water are expected to pay heavy fines. This makes it imperative that the anaerobic wastewater treatment systems in place should be able to withstand higher organic loads. Thus, organic loading tests were carried out on the Completely Mixed Anaerobic Reactor (CMAR) and the Anaerobic Baffled Reactor (ABR) and a comparison of their organic loading capacity was determined.

- The comparison of the two reactors was based upon the parameters of chemical oxygen demand, pH, alkalinity, total solids, total suspended solids, volatile solids, volatile suspended solids, gas composition and gas production rate. This study aims to critically evaluate the reactors according to these parameters so as to be able to graphically observe their stability behaviour when different conditions are exerted on the CMAR and ABR.

- The study aims to seed the ABR and CMAR with municipal waste. The two reactors will then be started up until steady stae is achieved. The achievement of steady state will be seen by the stability of the parameters mentioned above over three or more HRTs. Once steady state is achieved the feed to the reactors will be increased stepwise from 4 gCOD/*l* until steady state is achieved for each reactor. This will be done until the reactors fail and from this it will be possible to see which reactor has a higher organic loading rate. Considering the fact that the ABR has a higher SRT/HRT ratio it is likely that it will have a higher organic loading rate. However, it must be noted that this ability to separate the SRT from the HRT might lead to build up of solids in the ABR and clog the system.

1.3 Approach

The general approach in this study was to review existing water treatment systems, and to distinguish between aerobic and anaerobic so as to show the advantages inherent in anaerobic systems. The

analytical methods were modified to fit the conditions of the study and a critical evaluation of the CMAR and ABR was undertaken to assess the reactor with the better performance under the conditions in the study.

Chapter 2*, Overview of the Biochemical Process of Biological Digestion*, is a review of the literature and associated theory. It discusses the various biochemical pathways involved in biodegradation of organic compounds by bacteria. It also highlights the different micro-organisms involved in biological degradation and their characteristic features.

Chapter 3, *Wastewater Treatment Systems*, is a literature review of the processes used in wastewater treatment systems. It discusses the various aerobic and anaerobic reactors and tries to show the advantages and disadvantages of each system. The review also highlights the advantages of anaerobic digestion to developing countries especially the fact that biogas is produced. The biogas can be used to heat the anerobic system or harnessed to provide heat for cooking, washing or any other work that requires heat. A comprehensive literature review is made of the Anaerobic baffled reactor and its advantages over other systems are stated.

Chapter 4, *Laboratory Equipment and Analytical Methods*, gives a detailed description of the laboratory equipment used in the study. It also focuses on the various analytical methods undertaken and explains their importance in the evaluation of the two reactor systems.

Chapter 5, *The Start-up of the Reactors*, investigates the start-up of the reactors. It follows the stages of each reactor on a day by day basis so as to show the variations of the parameters under study. The main parameters monitored were the alkalinity, pH , gas production and gas composition as it is this parameters that give an early warning of impending failure of the system. The chapter focuses mainly on interpretation of the graphs of the various parameters under study. It tries to explain the changes of the various parameters in relation to each other by obvserving their graphical behaviour.

Chapter 6, *Organic Loading Tests*, shows the results of the two reactors during varying organic loading tests. The reactors were loaded from 4 gCOD/*l* to 64 gCOD/*l*. As with the start-up, the results mainly involve an analysis of the various graphs of the parameters studied.

Chapter 7, *Discussion and Recommendations*, is a discussion of the two reactors as pertains to the study. The significance of the study is summarised and suggestions for future work are made.

Chapter 2: Overview of Biochemical Process of Digestion

The most abundant and important group of micro-organisms in biological wastewater treatment are the bacteria. Fungi, protozoa and a range of other invertebrate organisms are found in biological treatment systems but they play a comparatively minor role. The dominant bacteria are the heterotrophs that degrade and mineralise organic compounds present in the wastewater to carbon dioxide and water i.e. the importantance of the bacteria is in the utilisation of the organic matter present in wastewater. Bacteria are small in size, have an impressive ability to reproduce, are able to mutate and to live almost in any environment. The small size of the bacteria and their resultant surface area to volume ratio makes them efficient nutrient and catabolic exchangers with the liquid media in which they are either suspended or are in contact. Most of the bacteria found in wastewater systems are prokaryotes that belong to the kingdom Mauna. The prokaryotic cells possess a cell wall surrounded by the cell membrane. Most are unicellular but often form colonies of independent cells. The cell membrane governs the passage of molecules and ions into and out of the cell.

2.1 Bacteria in biological treatment plants

Bacteria form the major proportion of the solids in the biological treatment systems. The bacterial community found in biological treatment systems have a variety of genera present that are aerobic, heterotrophic, Gram-negative, rod-shaped with polar flagella. The bacteria flora of aerobic treatment systems are basically the same. The major genera are *Zoogloea, Pseudomonas, Chromobacter, Achromobacter, Alcaligenes and Flavobacterium. Escherichia Coli* and other faecal indicators are present but are not indigenous members of the microbial community (Gray, 1989). The two most important aerobic heterotrophic bacteria in biological treatment systems are *Zoogloea* and *Sphaerotilus natans*. *Sphaerotilus natans* is a Gram-negative non-sporing bacterium made up of individual rod-shaped cells with rounded ends enclosed in a sheath of varying thickness (Phaup 1968).

The bacteria present in activated sludge plants are more specialised than those usually associated with fixed film reactors. The bacteria in activated sludge are either free-swimming or flocculating bacteria. The free-swimming species grow faster than the flocculating species. The activated sludge process selects the flocculating bacteria in preference to the free-swimming bacteria. The latter play a major role in substrate utilisation. Fixed film reactors have a greater range of micro-organisms and thus support a less specialised flora of bacteria comprising a greater diversity.

Bacteria are also the major group of micro-organisms involved in anaerobic digestion. Other groups of micro-organisms include fermentative ciliate and flagellate protozoa and some anaerobic fungi. During hydrolysis, the major substrates in the sludge are degraded to basic monomers. Proteins are hydrolysed to smaller units such as polypeptides, oligopeptides or amino acids by extracellular enzymes called proteases. A small proportion of bacteria produce proteases. The majority of bacteria are able to utilise these smaller peptides which pass through the cell wall and are broken down intracellularly. The production of protease is far in excess of that required and the over-production could be in the order of 50 times more than is required (Hattingh and Kotze, 1967). The most active proteolytic bacteria are the spore-forming *Clostridium* sp. Proteolytic bacteria in anaerobic sludge can be spore formers, cocci, non-sporing rods and bifid-like bacteria. Lipolytic (breakdown lipids) bacteria are highly effective in anaerobic digesters but they are difficult to isolate (Crowther and Harkness, 1975). The most common cellulolytic (degrade cellulose) bacteria is *Bacteroides ruminicola* which is a Gram-negative coccobacci. The heterogeneous group of facultative bacteria that carry out hydrolysis are also involved in the acid formation. In the acid formation stage the hydrolysed substrate is converted to organic acids and alcohols, with new cells being produced. The major acid-forming organisms are *Bacillus* sp., *Micrococcus* sp. and *Pseudomonas* sp. Propionic acid and longer chain fatty acids are degraded by an intermediate microbial group called the obligate hydrogen-producing acetogenic bacteria.

The third and final stage is methane production. The end-products of acid fermentation are converted to methane and carbon dioxide with smaller proportion of other gases. Obligate anaerobic bacteria carry out this conversion. Most methanogenic bacteria belong to the genera *Methanobacterium* (rod-shaped), *Methanococcus* (coccoid), *Methanosarcina* (sarcina-like), *Methanospirillum* (spirillar). Methanogens can be classified into three groups according to their energy source i.e. hydrogenotrophs (*Methanobrevibacter*), acetoclastic methanogens (*Methanothrix*) and hydrogen/acetate utilisers (*Methanosarcina*). They differ from other bacteria in their type of metabolism and also in a number of characteristic features in the composition of their cell constituents. They lack a typical peptidoglycan skeleton e.g. *methanococcus* has only a protein envelope while *methanosarcina barkeri* has a cell wall which consists of a polysaccharide composed of uronic acids, neutral sugars and amino sugars. The methanogenic bacteria are not subject to growth inhibition by penicillin. Methanogens contain a number of unique coenzymes. They contain coenzyme 420 which is involved in electron transfer , co-enzyme M which is used in methyl transfer reaction and factor B which is required for the enzyme formation of methane from methyl co-enzyme M. There is a close relationship between gas production and the numbers of methanogenic bacteria present in a digester. Methanogenic bacteria are usually present in numbers between 10^6 - 10^8 mg l^{-1} (Zeikus, 1980). The methane bacteria can ferment only a small range of simple organic compounds viz:, formate, acetate, methanol, carbon dioxide and hydrogen. Most of the methane is produced from propionic acid and acetic acid with a larger amount of methane produced from acetic acid. Formic acid fermentation and methane fermentation associated with ∃-oxidation of long-chain fatty acids probably accounts for the major proportion of the methane not derived from acetic and propionic acids.

Methanothrix and *Methanosarcina* are two types of methanogenic bacteria that utilise acetate for their metabolism. The maximum specific growth rates of these acetate-consuming organisms are $\mu_m = 0.1$ d^{-1} and 0.3 d^{-1}, respectively. *Methanothrix* has a K_s of 200 mg l^{-1} and *Methanosarcina* has a K_s of 300 mg l^{-1} acetate (Lettinga and van Haandel, 1994) (figure 2-1).

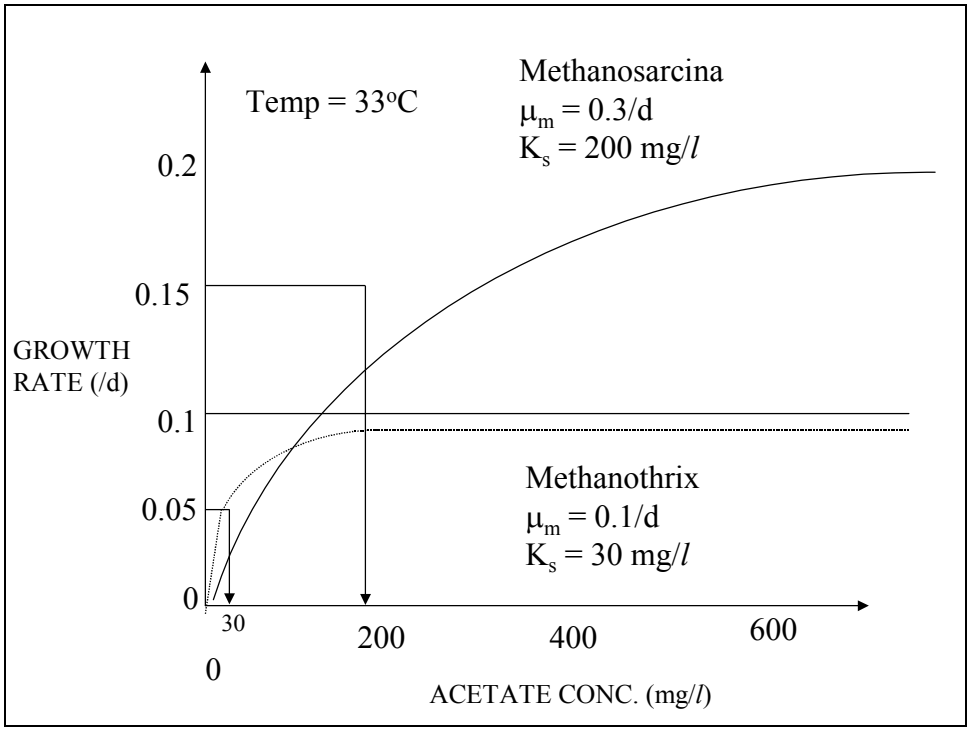

Figure 2-1: Specific growth rates against acetate concentration for *Methanothrix* and *Methanosarcina* (After: Lettinga and van Haandel, 1994).

At a low acetate concentration (< 55 mgl^{-1}), the specific growth rate of *Methanothrix* becomes higher than that of *Methanosarcina*. As a result the methanogenic organism mass will be composed of the former bacteria. At concentrations of acetate exceeding 55 mgl^{-1}, the *Methanosarcina* out-compete *Methanothrix* and are the prevailing acetate-consuming organism (Lettinga and van Haandel, 1994).

2.2 Bacterial growth

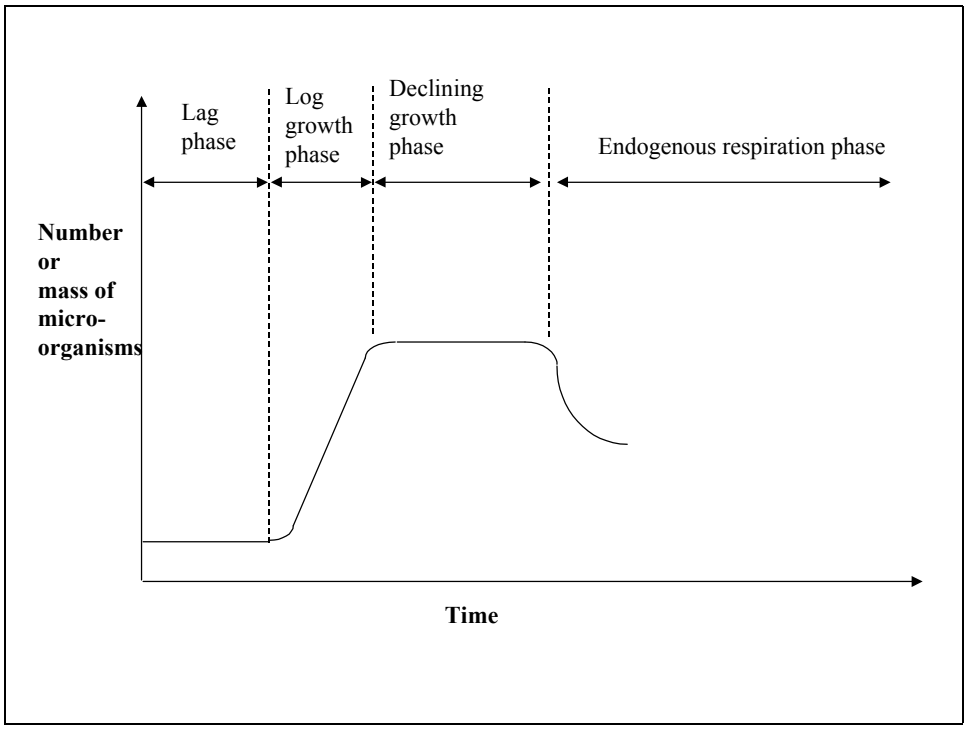

Figure 2-2: Bacterial growth curve (After: Gray, 1989).

The lag phase represents the acclimatisation of the organisms to the substrate with bacterial cells having a long generation time and zero growth (figure 2-2). During the lag phase there is no significant increase in cell numbers as the bacteria are preparing for the synthesis of DNA and various inducible enzymes required for cell division. The length of the lag phase depends on the size and degree of adaptation of the bacterial cells. In the lag phase each cell increases in mass, but there is no increase in the number of cells. When the substrate is broken down and taken into the cell, the size and mass of the bacteria increases as the amount of enzymes and nucleic acids increases. A bacteria inoculum for a wastewater treatment system normally comprises of cells in the stationary growth phase. Cells only begin to divide when a sufficient concentration of the appropriate enzymes has built up (Gray, 1989).

In the log growth phase, the cells begin to divide and there is a discernible increase in the growth rate leading to the exponential phase (figure 2-2). The substrate conversion rate is at its maximum. Biochemically, the steady state condition of growth is indicated by a near constant ratio of DNA/cell, RNA/cell and protein/cell as well as constant cell density and minimum cell size (Gray, 1989). The ability of the microbial population to degrade the substrate determines the growth rate at this phase.

When the substrate becomes limiting the cells enter the declining growth phase. Here the specific growth rate declines as the substrate concentration is depleted and this causes the generation time to increase. At the declining growth phase the mass of the microbial protoplasm is greater than the mass of viable cell as most of the viable micro-organisms have stopped reproducing because of the limiting substrate.

When the death rate of viable micro-organisms equals the rate of production, the cells are now in the stationary phase. This is where there is maximum microbial density as this signifies the largest number of cells the specific substrate can produce under the given conditions. In the stationary phase the substrates and nutrients are exhausted and there is a build up in toxic metabolites. The micro-organisms are still capable of reproduction but lack the necessary substrate or environmental conditions (Crowther and Harkness, 1975).

The final phase is the endogenous death phase. The substrate is now completely exhausted and the build up of toxic metabolites has become unfavourable for cell survival. There is a increase in the death rate of the viable cells. This leads to a reduction in the microbial density. As the substrate is now exhausted, the cells

utilise their own protoplasm as a source of energy thereby decreasing the mass of the total protoplasm.

The microbial growth curve is not a characteristic of bacterial cells. It is more a response of the cells to their environment and energy requirements. From the microbial growth curve it is seen that cells react differently according to the availability of growth substrate and nutrients (figure 2-2). It will also be seen that the same cells will give different products with different substrates. The microbial growth curve clearly demonstrates that for a microbial community to survive there must be a constant input of substrate and the environmental conditions must be kept favourable. In biological treatment systems the microbial growth curve is manipulated by having continuous flow systems that are maintained at a particular growth phase. This is done by controlling the food to micro-organism ratio (F/M). The growth of micro-organisms depends on their ability to obtain energy from the system. Energy is required for the production of new protoplasm and motility. Micro-organisms obtain their energy from the metabolism of inorganic and organic compounds.

2.3 Enzymes and co-enzymes

Chemotrophs in wastewater derive free energy from the oxidation of the organic matter present in the wastewater. Electrons are transferred from the organic matter to the breakdown products by pyridine nucleotides or flavins. The organic matter in wastewater is utilised by micro-organisms in a series of enzymatic reactions. Biochemical reactions occur with great rapidity through the mediation of natural catalysts (enzymes). Enzymes have a high degree of specificity and a bacterial cell must produce different enzymes for each substrate. The high degree of specificity and the great efficiency of enzymes, direct transformations of organic compounds through defined reaction sequences. Enzymes are proteins combined with either an inorganic or low molecular weight organic molecule. Although enzymes are composed mainly of proteins they are characterised by the chemical reactions that they catalyse (Crowther and Harkness, 1975).

Many enzymes require certain organic substances as co-enzymes in order to function. Co-enzymes are usually complex organic molecules or metal ions usually referred to as nucleotides. A nucleotide consists of a nitrogenous base, a sugar and one or more phosphate groups. These co-enzymes generally act as acceptors or donors of a functional group of atoms, electrons or specific atoms that are removed from or contributed to the substrate. The co-enzyme component of given enzymes determines what chemical reaction will take place.

2.3.1 Adenosine triphosphate (ATP)

In biological systems the co-enzymes responsible for the transport of free energy is adenosine triphosphate (ATP). ATP is a nucleotide consisting of an adenine, a ribose and a triphosphate unit. The active form of ATP is usually a complex of ATP with Mg^{+2} and Mn^{+2}. ATP is a high energy phosphate compound and its phosphoanyhydride bonds are high-energy bonds. They are termed to be high-energy bonds as they release a lot of energy when they are hydrolysed. Conventionally in thermodynamics the oxidation of organic and inorganic compounds releases heat energy yet micro-organisms are not heat engines. As a result micro-organism are forced to prevent the loss of the chemical energy in the form of heat. This is done with coupled chemical reactions in which energy is given up from one compound to another compound with only a minor heat loss. In this way, a thermodynamically unfavourable reaction can be converted into a favourable one by coupling it to the hydrolysis of a sufficient number of ATP molecules. As the chemical reactions release energy inorganic phosphate is added to ADP to form ATP. In this way energy is stored in the ATP rather than lost as heat. When the micro-organism require the energy the ATP is reduced back to ADP with a transfer of energy to the chemical reaction needing it. This is achieved without the use of an electron acceptor making it a fermentation reaction.

2.3.2 Nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD)

The other co-enzyme useful in the metabolism of organic material is NADH. Nicotinamide adenine dinucleotide (NAD^+) is a major acceptor in the oxidation of fuel molecules. The reactive part of NAD^+ is its nicotinamide ring. In the oxidation of a substrate, the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons, which are equivalent to a hydride ion. The reduced form of this carrier is called $NADH_2$. The other co-enzyme is flavin adenine dinucleotide ($FADH_2$). $FADH_2$ does not play a major role in the

metabolism of organic matter as it is found in the latter stages of the metabolism. The NADH and $FADH_2$ formed in glycolysis, fatty acid oxidation and the citric acid cycle are energy rich molecules because they have a pair of electrons with a high transfer potential. When these electrons are donated to an external electron acceptor, a large amount of free energy is released. This energy can be harnessed to generate ATP in heterotrophs.

2.4 Heterotrophic metabolism

The two fundamental ways of metabolism for heterotrophs are fermentation and respiration. These two ways differ in that respiration requires an external electron acceptor. All the carbon sources utilised by heterotrophic micro-organisms are converted to a small number of intermediates such as pyruvate and acetyl-co-enzyme A (acetyl-CoA). The anabolic pathways of cell synthesis are the same in all micro-organisms whether heterotrophs or autotrophs. The majority of bacteria can metabolise carbohydrates, which means that using glucose, it is possible to compare all the major steps in heterotrophic metabolism. In the conversion of carbohydrates to glucose and finally to pyruvate, the substrate is broken down in a series of enzyme-catalysed reactions with small amounts of energy released at each oxidation stage and recovered by substrate-level phosphorylation (Crowther and Harkness, 1975). This degradation sequence is called glycolysis or the Embden Meyerhof Pathway (EMP). Micro-organisms utilise energy from the system through the use of a phosphate enzyme system. Glucose is broken down to pyruvate through three stages. In the first stage there is an endergonic reaction using energy from ATP. In this first stage glucose is converted to fructose 1,6-bisphosphate. In the second stage the six-carbon sugar is cleaved to form two interconvertible three-carbon molecules (glyceraldehyde 3-phosphate). The last stage is energy-yielding, with substrate-level phosphorylation occurring when glyceraldehyde 3-phosphate is converted to 1,3-bisphosphoglycerate. In the next steps the high phosphoryl transfer potential of 1,3-bisphosphoglycerate is used to generate ATP. Two moles of ATP are generated with the release of water resulting in the formation of pyruvate. The net reaction in the conversion of glucose to pyruvate is:

$$GLUCOSE + 2Pi + 2ADP + 2NAD^+ \rightarrow 2PYRUVATE + 2ATP + 2NADH + 2H^+ + 2H_2O \qquad [2\text{-}1]$$

If there is no external electron acceptor pyruvate may undergo a further transformation in the fermentation pathway. There are a number of reactions, which regenerate NAD and NADH with the production of a wide range of fermentation products. The reaction that occurs depends on environmental factors and the presence of specific enzymes. The possible products are ethanol, lactate or acetic acid. The end-products of fermentation are still rich in energy even though the fermentation pathway is complete.

Only a small fraction of energy is released in the fermentation reactions. More energy can be extracted aerobically by means of the citric acid cycle and the electron transport chain. This takes place when an external electron acceptor is available. The citric acid cycle is the final common pathway for oxidation of fuel molecules. The entry point to this oxidative pathway is acetyl CoA. Acetyl CoA is formed by the oxidative decarboxylation of pyruvate.

$$PYRUVATE + NAD^+ + CoA \rightarrow ACETYL - CoA + CO_2 + NADH + H^+ \qquad [2\text{-}2]$$

In the citric acid cycle a two-carbon acetyl-unit condenses with a four-carbon compound (oxaloacetate) to yield a six-carbon tricarboxylic acid (citrate). The first oxidation-reduction reaction is the oxidative decarboxylation of isocitrate to α-ketoglutarate. This results in the release of NADH and carbon dioxide forming a five-carbon compound. The α-ketoglutarate is oxidatively decarboxylated to form succinyl-CoA a four-carbon compound. The cleavage of the energy rich succinyl thioester bond of CoA is coupled to the phosphorylation of guanosine diphosphate (GDP). The reactions of four-carbon compounds constitute the final stage of the citric acid cycle. Succinate is converted to oxaloacetate through an oxidation, a hydration and a second oxidation. Oxaloacetate is regenerated for another round of the cycle. Energy is trapped in the form of $FADH_2$ and NADH. FAD is used as the hydrogen acceptor because the free-energy change when succinate is oxidised to fumarate is insufficient to reduce NAD^+. The net reaction of the citric acid cycle is:

$$ACETYL - CoA + 3NAD^{+} + FAD^{+} + GDP + Pi + 2H_2O \rightarrow 2CO_2 + 3NADH + FADH_2 + GTP + 2H^{+} + CoA \quad [2\text{-}3]$$

The citric acid cycle is the intermediate pathway between anaerobic and aerobic metabolism. In the cycle a hydrogen ion and electrons are released which enter the electron transport system where they react with oxygen or an inorganic electron acceptor. The nature of the external electron acceptor determines whether there is aerobic or anaerobic digestion taking place in wastewater bacteria. In other words, respiration maybe *aerobic* or *anaerobic*. The presence of oxygen means that the aerobic bacteria will digest the wastewater. In the absence of oxygen, anaerobic bacteria will digest the wastewater. Oxygen is the sole electron acceptor in aerobic respiration while carbon dioxide, sulphate or nitrate are the electron acceptors in anaerobic respiration.

2.5 Anaerobic digestion

The rate of engineering and scientific research development in the anaerobic process has been slow with developments in one field having to await developments in another (Mosey, 1982). Anaerobic digestion is a microbial fermentation by which organic matter is converted to carbon dioxide and methane. It is a phenomenon, which occurs naturally in river sediments, marshes and the rumen of herbivorous animals. For anaerobic digestion to occur methanogens must be present and there must be little or no oxygen present. And in all these environments these conditions are met and methanogens are present in considerable numbers. The process of anaerobic digestion consists of three discrete stages: hydrolysis, acid formation and methane formation. These three stages normally occur simultaneously in an anaerobic reactor but for convenience they are discussed separately

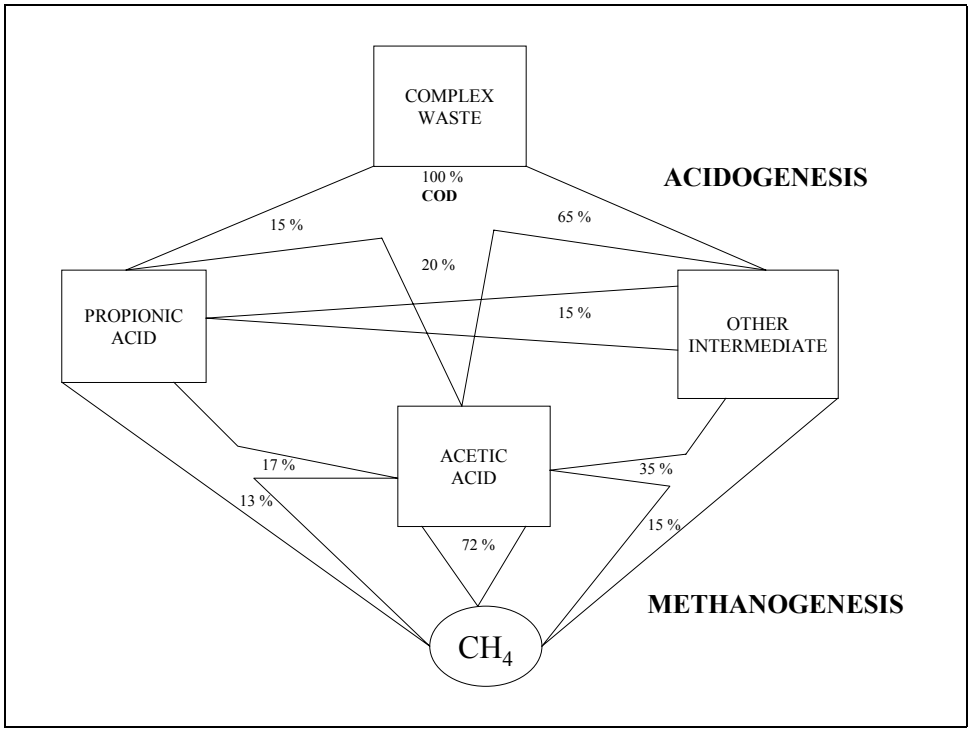

Figure 2-3: Pathways in methane fermentation of complex wastes (After: McCarty, 1964).

There is the hydrolysis and partial dehydrogenation of a wide range of complex organic compounds by both anaerobic and facultative bacteria. This stage is non-methanogenic as no methane is produced. The compounds formed in this stage are volatile fatty acids, carbon dioxide and gaseous hydrogen. In the second stage, the volatile fatty acids, carbon dioxide and gaseous hydrogen are converted by a specialised group of bacteria to form methane and more carbon dioxide.

Anaerobic digestion is a process that different researchers approach in different ways. McCarty and

Lawrence (1969) describe three stages namely:

i. hydrolysis of complex material,
ii.
iii. acid production,
iv. methane fermentation.

Mosey (1981) considered it to consist of four stages i.e.

i. solubilisation - hydrolysis of complex material
ii. acidogenesis - conversion of organic compounds from long chain to short chain compounds
iii. acetogenesis - conversion of propionate and butyric acids to acetate.
iv. methanogenesis

Gray (1989) and Speece (1983), simply consider two stages :

i. a non-methanogenic stage
ii. a methanogenic stage

These may seem like different ways of analysing the anaerobic fermentation in reality the basic principal is the conversion of complex organic material to organic acids which are in turn converted to carbon dioxide and methane.

$$COMPLEX\ ORGANIC\ MATERIAL \rightarrow ORGANIC\ ACIDS \rightarrow CARBON\ DIOXIDE + METHANE \quad [2\text{-}4]$$

In order to facilitate the discussion that gives a detailed description of the process of anaerobic fermentation it is preferable to use the concept of the non- methanogenic and the methanogenic stages coupled with the four-stage approach.

2.6 Non-methanogenic stage

Usually wastewaters contain lipids, proteins and carbohydrates. Xenobiotics and inorganic heavy metals are also found in some wastewaters. Anaerobic digestion was initially applied to complex feedstock, such as municipal wastewater sludge. These contained a wide range of nutrients and alkalinity sources. Other feedstock included food-processing wastewater, such as effluents from meat-packing plants and sugar beet plants. This wastewater contains readily biodegradable organics and the carriage water has a normal component of inorganic metal ions that are usually found in surface or groundwater. De-ionised wastewater arising from evaporative condensates such as pulp and paper mill black liquor evaporation condensate, coal conversion condensate and de-ionised process wastewaters have also been used as feedstocks (Speece, 1983). These feedstock contain complex organic material comprising carbohydrates, amino acids or long chain fatty acids. The first stage in anaerobic digestion is the hydrolysis of these complex organic materials into simple organic compounds. This hydrolysis involves the solubilisation of the waste particulates (complex organic material) and fermentation into volatile acids (simple organic compounds). The complex organic materials are usually insoluble in water. Hydrolysis not only breaks them into simpler organic molecules but makes them more soluble in the wastewater. This makes it easier for the acidogens to utilise them. Extracellular enzymes carry out the process of hydrolysis. The degradation of lipids starts with hydrolysis to fatty acids and glycerol. The degradation of long chain fatty acids follows the path of β-oxidation where the production of each molecule of acetic acid results in the transfer of four hydrogen atoms to intracellular hydrogen-carrier molecules namely, FAD and NAD^+. These carrier molecules are subsequently returned to their oxidised state by liberation of extracellular hydrogen gas. Proteins are hydrolysed by extracellular enzymes into peptides and then into their component amino acids which are absorbed into the bacterial wall. They are then degraded intracellularly to ammonium salts and a variety of short chain carbon compounds. The pathways for carbohydrate degradation are the Embden Meyerhoff pathway (EMP) and the Hexose Monophosphate Shunt (HMS). The EMP is the most important of the two (Mosey, 1974).

2.6.1 Acidogenesis

The soluble simple organic compounds are generated in the first stage while the acidogenic bacteria (acidogens) utilise those already present in the wastewater. The acidogens transform them into short chain fatty acids (SCFA). The principal SCFAs are acetic acid, butyric acid and propionic acid. Other products of the transformation are carbon dioxide and gaseous hydrogen.

The biochemical pathways and end products for acidogenesis depend on:

i. type of feed substrate
ii. hydrogen partial pressure (pH_2)

To fully understand the degradation of simple, soluble organic molecules by acidogens, consider the substrate, glucose. Initially, the glucose is converted to pyruvic acid and then to acetic acid. During this degradation process the partial pressure of hydrogen is a very important parameter as it determines the end product of the process. This is because there is a need to regenerate the NAD^+ for the EMP to remain operative. In order to regenerate NAD^+ there has to be dehydrogenation of the $NADH_2$. This reaction is thermodynamically unfavourable and only becomes thermodynamically favourable when $pH_2 < 10^{-4}$ atm. (Speece, 1983).

At $pH_2 < 10^{-4}$ atm, $NADH_2$ can be dehydrogenated to NAD^+ and H_2. This means the EMP can proceed and glucose is degraded to pyruvic acid and so:

$$2CH_2COCOOH + 2NAD^+ + 2ADP + 2Pi \rightarrow 2CH_3COOH + 2ATP + 2NADH_2 \quad [2\text{-}5]$$

$$2NADH_2 \rightarrow 2NAD^+ + H_2 \quad [2\text{-}6]$$

Hence under low pH_2

$$GLUCOSE \rightarrow ACETATE + CO_2 + H_2 \quad [2\text{-}7]$$

$$C_6H_{12}O_6 + 2H_2O + ADP + 4Pi \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 + 4ATP \quad [2\text{-}8]$$

In anaerobic digestion, the methanogenic and non-methanogenic bacteria normally grow in close association to form a tightly knit symbiotic community of micro-organism which co-operate together to form a self regulating fermentation which automatically controls its pH values, redox potential and oxygen tension. The acetoclastic methane bacteria (those that produce methane from acetic acid by cleavage) co-operate with the acid-forming bacteria (acidogens) to control the concentration of acetic acid and hence the pH value of fermentation (Mosey, 1982). The growth rate of acetoclastic methane bacteria is relatively slow (minimum doubling times of 2 to 3 days at 35^{o}C) and that of acid forming bacteria is significantly fast (minimum doubling times of 2 to 3 hrs at 35^{o}C) (Mosey, 1982) This form of control is relatively crude and may lead to acid overload under conditions of shock loads. This is because the slow growing methanogens will be unable to remove the acetic acid that is produced by the faster growing acid formers. Fortunately, the obligate hydrogen utilising methane bacteria offer a more subtle form of control. Take for example the reaction (Figure 2-3):

$$PYRUVIC\ ACID + HYDROGEN \rightarrow PROPIONIC\ ACID \quad [2\text{-}9]$$

This reaction is speeded up by the presence of hydrogen, as it is one of the percursors of the end product. Now if the concentration of hydrogen increases the reactions like (2-10) slow down as they result in the introduction of more hydrogen in the digester gas.:

$$GLUCOSE + WATER \rightarrow ACETIC\ ACID + HYDROGEN \quad [2\text{-}10]$$

As a result by controlling these reactions with monitoring the traces of hydrogen in the digester gas, the methanogenic bacteria control the internal metabolism of the acid forming bacteria. When the hydrogen

concentration increases the reaction (2-11).

$$2CH_3COOH + 2H_2 \rightarrow CH_3CH_2CH_2COOH + 2H_2O \quad [2\text{-}11]$$

becomes more favourable. This means that the acid load on the system is reduced as one mole of butyric acid is produced instead of two moles of acetic acid (2-12).

$$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + CO_2 + H_2 \quad [2\text{-}12]$$

This also provides more time for the slow growing acetogens to metabolise the large amounts of acetic acid now present in the digester. This is known as 'acetic acid overload' and the operator does not need to reduce the loading rate to adjust the pH value as the reactor will recover on its own. When the surge loads persist for a long time, the hydrogen partial pressure (pH_2) becomes very high. If $pH_2 > 10^{-4}$ atm then $NADH_2$ cannot be dehydrogenated directly. The organism finds a sink for the electron and H^+ by reducing pyruvate to propionic acid. This triggers a large-scale production of propionic acid. Initially there is the reaction (2-13).

$$\begin{aligned} &GLUCOSE + NAD^+ + 2ADP + 2Pi \rightarrow 2PYRUVATE + 2ATP + 2NADH_2 \\ &C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2Pi \rightarrow 2CH_3COCOOH + 2ATP + 2NADH_2 \end{aligned} \quad [2\text{-}13]$$

Then when $pH_2 > 10^{-4}$ atm., pyruvate is reduced to propionic acid with dehydrogenation of $NADH_2$:

$$\begin{aligned} &PYRUVATE + 2NADH_2 + ADP + Pi \rightarrow PROPIONATE + 2NAD^+ + ATP + WATER \\ &CH_3COCOOH + 2NADH_2 + ADP + Pi \rightarrow CH_3CH_2COOH + 2NAD^+ + ATP + H_2O \end{aligned} \quad [2\text{-}14]$$

So instead of capturing the energy in ATP, use it to dehydrogenate $NADH_2$. The overall reaction becomes:

$$GLUCOSE \rightarrow PROPIONATE + ACETATE + CO_2 + H_2 \quad [2\text{-}15]$$

$$C_6H_{12}O_6 + 3ADP + 3Pi \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2 + 3ATP \quad [2\text{-}16]$$

Then ther is reversal of hydrogen production. The metabolism and growth of the acetogenic bacteria, which would, otherwise reverse this process by converting the propionic back into acetate is simultaneously switched off by the accumulated hydrogen in the system. This situation is known as *propionic acid overload* and the plant operator must act to overcome the overload. This means that if $pH_2 > 10^{-4}$ atm, propionic acid and other SCFAs will accumulate and increase the acidity of the system thereby dropping the pH value. There is the possibility of washing out the small population of acetogenic bacteria before the obligate H_2-utilising bacteria have time to clear the accumulated hydrogen.

2.7 Methanogenic stage

Methane bacteria are fastidious anaerobes having strict requirements for redox potentials and absence of dissolved oxygen. However, in nature, methane bacteria are rarely found on their own. They usually form a tightly knit symbiotic community of micro-organisms which operate together to form a self regulating fermentation which automatically controls its own pH values, redox potentials and oxygen tension. Methanogenesis takes place in two major routes:

a) Hydrogen (H_2) - oxidation

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad [2\text{-}17]$$

b) Acetate (CH_3COOH) - cleavage

$$CH_3COOH + H_2O \rightarrow CH_4 + CO_2 \quad [2\text{-}18]$$

Tetrahydrofolate and vitamin B_{12} coenzymes are involved in the pathways of methane formation.

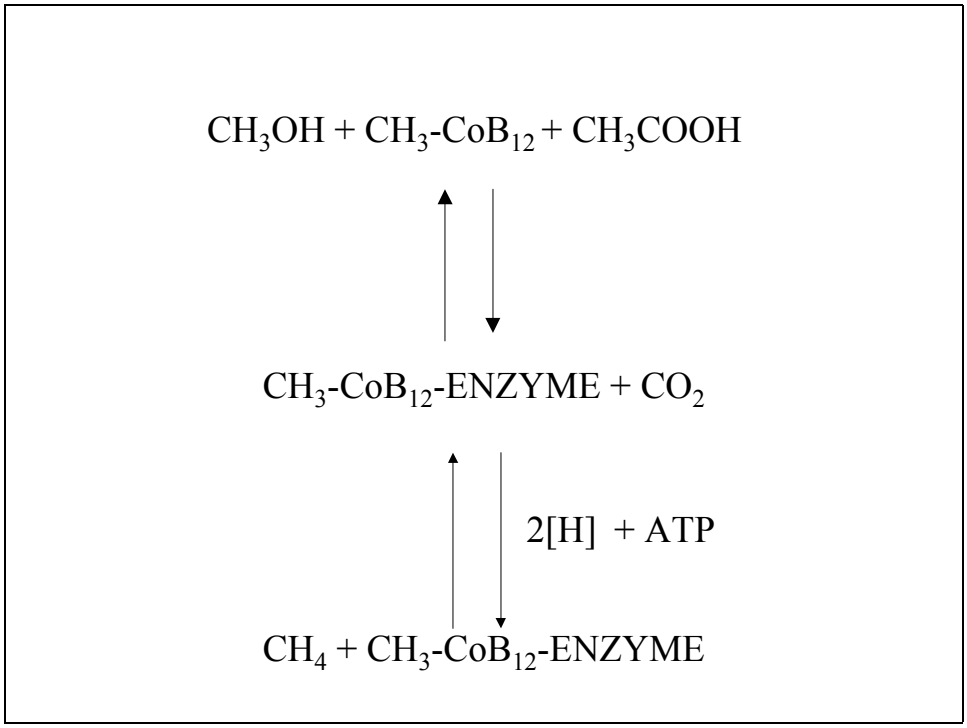

Figure 2-4: Formation of methane by acetate cleavage through vitamin B_{12} (After: Mosey, 1974).

Methanosarcina barkeri uses acetate as the sole carbon and energy source instead of H_2/CO_2. Here methane is formed mainly from the methyl group of acetate. No external electron donors are required. About 70 % of methane comes from acetate and a large proportion comes from carbon dioxide and gaseous hydrogen in sewage sludge digesters. (McCarty, 1964). Isotopic labelling has shown that the methyl group of acetic acid is transferred to methane intact, while the protons come from the solvent.

$$C^{14}H_3HCOOH \rightarrow C^{14}H_4 + CO_2 \quad [2\text{-}19]$$

$$CH_3C^{14}OOH \rightarrow CH_4 + C^{14}O_2 \quad [2\text{-}20]$$

$$CH_3COOH \xrightarrow{D_2O} CH_3D + CO_2 \quad [2\text{-}21]$$

$$CD_3COOH \xrightarrow{H_2O} CD_3H + CO_2 \quad [2\text{-}22]$$

The best substrate for the growth of all methanogens is hydrogen and carbon dioxide (H_2/CO_2). In the reaction [2-16] between carbon dioxide and hydrogen, carbon dioxide is always present in excess and very little gaseous hydrogen is in the digester gas. The energy available from the coupled reactions of carbon dioxide and hydrogen is sufficient to support reasonable growth. The hydrogen dependent reduction of carbon dioxide to methane requires an anaerobic electron transport pathway. The electron carrier F_{420} does not play a direct role in methanogenesis. It is a deazaflavin with a long side chain ending in two glutamate residues. It accepts two electrons (Effelson and Wolfe, 1981). Its main role is as an electron acceptor for

formate, pyruvate and 2-oxoglutarate dehydrogenases and NADPH. The carbon dioxide reduction process occurs with the C_1 compounds (methanol, formaldehyde and formate) bound to a carrier. One carrier is Coenzyme M that has a 2-mercaptoethane sulphonic acid and the functional part of the molecule is the –SH group. Coenzyme M occurs in all methanogenic bacteria (Keltjens and Vogels, 1981). An early-labelled reduction product of $^{14}CO_2$ that can be detected in whole cell extracts of methanogenic bacteria is methyl-coenzyme M (CH_3-S-CoM). This compound is the substrate for the final step in methane production catalysed by methyl coenzyme M reductase. Addition of CH_3-S-CoM to an extract of methanogens under an atmosphere of H_2/CO_2 results in the stimulation of methane formation. This indicates that the methyl coenzyme M reductase reaction is coupled to activation and reduction of carbon dioxide. This results in the following cyclic system:

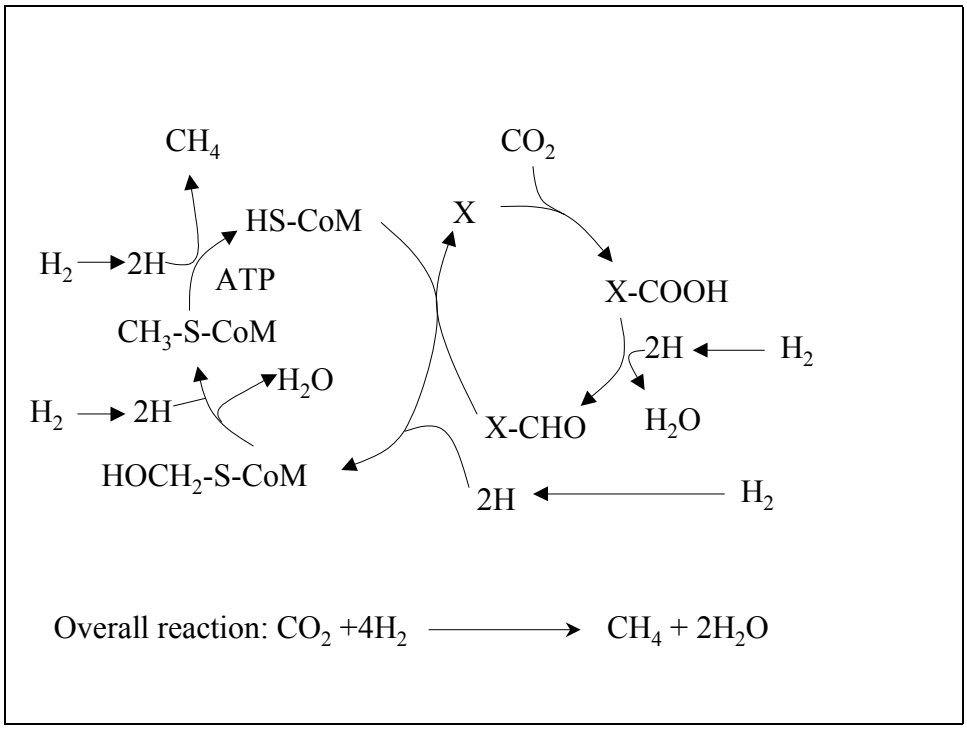

Figure 2-5: Reduction pathway for carbon dioxide with the formation of coenzyme M (After: Romesser, 1978).

The final step in methane reduction is

$$CH_3 - S - CoM + H_2 \xrightarrow[A,B,C]{ATP, Mg^{2+}} CH_4 + HS - CoM \qquad [2\text{-}23]$$

Three components A, B, and C (2-23) are involved in addition to ATP, Mg^{2+} ions and the substrates. Component A is a membrane bound, oxygen sensitive hydrogenase, component B is an oxygen labile low molecular weight coenzyme and component C is the methyl reductase itself. The ATP phosphorylates and adenylylates one of the enzymes of the system rather than act as a substrate (Kell et al., 1981). It has been shown that in *Methanobacterium thermoautotrophicum* grown on H_2/CO_2, the hydrogen atoms in the methane formed arise exclusively from water and not from hydrogen. This means during methanogenesis hydrogen serves only as a source of electrons.

Most methanogens can metabolise carbon monoxide to methane during growth on H_2/CO. This process proceeds via oxidation of carbon monoxide to carbon dioxide and finally reduction of carbon dioxide to methane. The oxidation of carbon monoxide involves a carbon monoxide dehydrogenase. The enzyme uses Coenzyme F_{420} as an electron acceptor.

Chapter 3: Wastewater Treatment Systems

Biological processes have long been used for wastewater treatment. Biological processes can be classified into aerobic and anaerobic biological treatment systems based on the type of microbial metabolism. Several versions of these basic processes are in current use with differences in such factors as influent flow rate, type of reactor, contacting patterns between the biological and liquid phases, amount and type of recycle and degree of mixing. These processes are continuous-flow, enrichment cultures of micro-organisms creating complex biological systems that are not fully understood. In an individual process there are considerable temporal fluctuations in the species of micro-organisms predominating since sizeable variations occur in the process inputs (Andrews, 1971).

3.1 Enzyme reactions

To have an appreciation of the different biological treatment processes, it is essential to have an understanding of the fundamental kinetic relationships of the biomass concentration, the substrate concentration (influent wastewater) and the enzymes involved. It is appreciated that in this study there is little use of kinetic analysis yet an understanding of the kinetic issues involved in the growth and ultimate death of the bacteria is essential to create an understanding of why the reactors fail. The following reactions will show how the bacterial reactions are affected by substrate concentration. In the organic loading tests the substrate concentration is increased and the resultant increase in the mass of the organisms is understood by referring to the monod equations. The kinetics of substrate removal show why the CMAR and the ABR have different SRTs. The catalytic activity of the enzymes of the prominent reaction within a reactor determines the overall rate of biological reaction. The rate of an enzyme catalysed reaction in which a substrate, S, is converted into products, P, is found to depend on the concentration of the enzyme, E, even though the enzyme undergoes no net change. Enzyme-catalysed reactions involve the reversible combination of the enzyme and substrate in the form of an enzyme substrate complex, ES, with the irreversible decomposition of the complex to a product, P, and the free enzyme, E. The proposed mechanism (Gray, 1989) is:

$$E + S \underset{k_2}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_3}{\Rightarrow} E + P \qquad [3\text{-}1]$$

k_1,k_2,k_3 - *rate of the reactions*

$$ES = \frac{k_1[E][S]}{k_2 + k_3} \qquad [3\text{-}2]$$

If [E] and [S] are the concentration of the free enzyme and the free substrate, and if $[E]_o$ is the total concentration of the enzyme and substrate:

$$[E] + [ES] = [E]_o \qquad [3\text{-}3]$$

As only a little enzyme is added, the free substrate concentration is almost the same as the total substrate concentration. This then means that:

$$[ES] = \frac{k_1([E]_o - [ES])[S]}{k_2 + k_3} \qquad [3\text{-}4]$$

This rearranges to:

$$[ES] = \frac{k_1[E]_o[S]}{k_1 + k_2 + k_3[S]} \qquad [3\text{-}5]$$

Under steady state conditions the various rate constants k_1; k_2; k_3 can be expressed as the Michealis constant, K_m(3-6):

$$\frac{k_2 + k_3}{k_1} = k_m \qquad [3\text{-}6]$$

k_m - saturation/Michealis constant

3.1.1 Equations of bacterial growth

The different phases of the bacterial growth curve (see figure 2-1) can be represented quantitatively. The growth rate of the micro-organisms is proportional to the rate of substrate utilisation:

$$\frac{\partial x}{\partial t} = \mu x \qquad [3\text{-}7]$$

x - concentration of micro-organisms (mgl^{-1})
μ - specific growth rate (d^{-1})

Equation 3-7 assumes that all the micro-organisms present in the reactor are viable. This is not true for wastewater treatment systems. In a batch reactor there will be a change in the environmental conditions as the substrate is depleted by the micro-organisms. This will cause a change from the exponential phase into the declining growth phase. Most expressions that have been formulated to describe the kinetics of micro-organism metabolism are based on the Monod model (Harper,1991). This model assumes that the growth rate $\delta x/\delta t$ is a function of organism concentration and some limiting substrate. The concentration of the growth limiting substrate and the specific growth rate of biomass is related by:

$$\mu = \frac{\mu_m S}{k_s + S} \qquad [3\text{-}8]$$

μ_m - maximum specific growth rate at saturation concentration of growth limiting substrate (d^{-1})

It can be seen from equation 3-8 that the specific growth rate can be any value between zero and μ_m provided the substrate is kept constant. The substrate concentration controls the overall rate of metabolism in continuous flow treatment processes used in biological wastewater treatment. In the Monod relationship the microbial growth rate increases as the availability of substrate increases until the maximum specific growth rate is achieved at which point a factor other than substrate becomes growth-limiting. The specific growth rate equation of microbial growth under exponential conditions can be replaced by the Monod function (McCarty and Lawrence, 1969):

$$\frac{\delta x}{\delta t} = \frac{\mu_m S x}{k_s + S} \qquad [3\text{-}9]$$

At low substrate concentrations, the Monod ratio approaches S/k_s and the growth rate is directly proportional to substrate concentration. This is characteristic of a first order process.

$$\frac{\delta x}{\delta t} = \mu_m x \qquad [3\text{-}10]$$

At high substrate concentrations, the Monod ratio approaches unity where the growth rate is independent of substrate concentration. This is typical of a zero order process.

In most biological wastewater treatment systems the retention time of the micro-organisms is such that the endogenous phase occurs (Lettinga and van Haandel, 1994). Some unit processes are designed to operate in this phase. Incorporating the endogenous decay into the equation (3-7) for microbial growth:

$$\frac{\delta x}{\delta t} = (\mu - k_d)x \qquad [3\text{-}11]$$

k_d - specific endogenous decay rate

When the retention time is short, k_d is an order of magnitude less than μ and so is insignificant. When the system is operated in the endogenous phase, k_d is used in the calculation of the net amount of organisms produced and the oxygen utilisation rate. This is because the rate of accumulation of dead cells becomes large enough to compete with the rate of growth of new cells. The oxygen utilisation rate is a measure of the activity and quantity of viable cells. The net production of micro-organisms in the treatment system is equal to the difference between bacterial growth and decay. In order to maintain a viable biomass at a constant load, a minimum substrate concentration is necessary. The lowest net growth rate is zero. At this point an equal amount of viable micro-organisms is formed as the ones that are dying. Therefore, the net growth rate is zero (Gray, 1989) (3-12):

$$\frac{\delta x}{\delta t} = (\mu - k_d)x = 0 \qquad [3\text{-}12]$$

substituting the Monod relationship equation for μ gives:

$$\frac{\delta x}{\delta t} = \frac{(\mu_m S_{\min} - k_d)x}{k_s + S_{\min}} \qquad [3\text{-}13]$$

S_{min} – minimum substrate concentration (mg/l)

and so

$$S_{\min} = \frac{k_s k_d}{(\mu_{\min} S_{\min} - k_d)} \qquad [3\text{-}14]$$

In water treatment processes there are a series of sequential processes taking place to convert the complex organic material to more energetically stable products. And so the minimum substrate concentration *(S_{min})* is the sum of the minimum concentrations for the various processes.

The mass of organisms produced is related to the mass of substrate utilised by the expression:

$$\frac{\delta x}{\delta t} = -Y\frac{[\delta S]}{[St]} \qquad [3\text{-}15]$$

x - concentration of micro-organisms (mg/l)
S - concentration of substrate (mg/l)
Y - yield coefficient
t – time (s)

The yield coefficient is expressed as mass of organism produced per mass of substrate consumed. The yield coefficient is a function of the type of substrate, species of micro-organisms and environmental conditions. The yield coefficient is usually assumed to be constant for a given biological treatment

process treating a specific wastewater. The expression of yield can be combined with the constant growth rate equation to give the substrate utilisation:

$$\frac{\delta S}{\delta t} = \frac{-\mu x}{Y} \quad [3\text{-}16]$$

At high temperatures, long residence times and high cell concentrations the substrate utilisation for cell maintenance is important and equation 3-16 is modified to:

$$\frac{\delta S}{\delta t} = \frac{-\mu x - k_m x}{Y} \quad [3\text{-}17]$$

k_m - *specific maintenance coefficient*

$$\mu = \frac{\delta x}{\delta t} = (Y(-\frac{\delta S}{\delta t}) - k_d)x \quad [3\text{-}18]$$

The reciprocal of the specific growth rate is the solids retention time (SRT) (McCarty and Lawrence, 1969):

$$\frac{1}{\mu} = SRT \quad [3\text{-}19]$$

To maintain the slow growing bacteria and allow them to multiply the SRT must be maintained above the minimum value defined by μ_m :

$$SRT > SRT_{\min} = \frac{1}{\mu} \quad [3\text{-}20]$$

The kinetics of substrate removal will determine the actual SRT required for a given treatment efficiency. To maximise the removal capacity, the SRT is maintained at the highest possible value (McCarty, 1964). In order to increase the SRT and maximise reactor biomass concentration suspended growth processes are designed such that biomass is separated from the treated effluent and returned to the reaction zone. This disconnects the control of the SRT from the operating hydraulic retention time (HRT) of the process. High SRTs reduce reactor volume. Operation of the treatment process at high SRT affords a safety factor to protect against system failure and to promote biological acclimation to inhibitory material (Speece, 1983).

3.2 Aerobic wastewater treatment

The primary objective of biological wastewater treatment processes is the conversion of biodegradable organics into a microbial biomass which can be separated by appropriate solids/liquids separation processes (Casey, 1997). Organic wastewater, which contains a low concentration of organic matter, can be treated efficiently and economically by aerobic treatment. Part of the organic matter is converted to carbon dioxide through microbial respiration and part is converted to microbial biomass residue. The more concentrated wastewater and organic suspension can also be effectively stabilised anaerobically. The organic waste matter is converted to carbon dioxide and methane as well as to an anaerobic biomass residue (McCarty, 1964).

3.2.1 Monitoring and Control

The two important control objectives of all anaerobic treatment systems are:

i. the achievement of a consistently high degree of waste stabilisation.

ii. the highest conversion of organic waste to methane (Hickey and Wu, 1991).

The basic aim of any waste treatment system is the stabilisation of organic waste by the removal of energy and energy containing substances from the system. The treatment system must enable anaerobic digestion to take place effectively reducing the energy content of the organic waste. The treatment system attains this removal in energy through energy losses from the system to the atmosphere. These losses arise in accordance with the second law of thermodynamics, which states that for a spontaneous reaction to occur at constant temperature and pressure there must be a heat loss to a thermal sink (Weilande and Rozzi, 1991). In biological systems, such as those found in anaerobic treatment systems, these energy losses occur in both anabolic and catabolic pathways. Energy, which is bound in organic waste molecules, is retained when those molecules are incorporated in biological protoplasm. This creates a solution containing dissolved organic solid wastes. The dissolved organic solid wastes are removed from solution (system) by physical separation of the solid and liquid phases. This liquid-solid separation results in the removal of the energy rich organic solid wastes from the solution concurrently removing their energy from the system. The removal of energy from the system also removes unwanted odours from the organic waste (Hickey and Wu, 1991). Treated organic waste that is ready for dewatering and drying usually has a 'tarry' smell.

The ultimate product of the stabilisation process is methane. As a result the two objectives of removing energy from the waste and producing methane are related by the fact that for any one of them to take place efficiently the other must be occurring as well. To accomplish these objectives, a balance must be maintained between the microbial populations in the system, i.e., there must be a balance between the acidogens, acetogens and methanogens. There is no definite answer to the question of which are the most appropriate feed back variables for the control of a water treatment system (Weiland and Rozzi, 1991). This is due to the complex mass of microbial biomass found in an anaerobic reactor. Each type of bacterial species has its own optimum environmental conditions for growth. It is also important to note that not all the processes involved in anaerobic digestion are fully understood and the inflexible design of anaerobic treatment processes can limit the capacity to implement control actions (Speece, 1983).

In order to provide reliable information for process control, a monitoring system must combine available on-line data, intermittent off-line measuring and an understanding of the mechanisms controlling process behaviour. Monitoring of conventional anaerobic treatment systems involves a combination of intermittent manual sampling followed by off-line analysis and quantitative observations (Hickey and Wu, 1991). The process variables used in anaerobic process control are defined as:

i. stability indicators

ii. control variables

Stability indicators are the parameters that should be monitored to assure an early warning of oncoming unstable conditions. A good stability indicator displays a large variation in the shortest possible time after the factor, which upsets the process, has been applied. Stability variables should preferably refer to the gas phase. This is because the gas compounds have a higher kinetic energy as compared to compounds dissolved in the liquid phase (Hickey and Wu, 1991). The control variables should be monitored to keep or restore stable conditions. The environment to be controlled is the mixed liquor which contains the anaerobic micro-organisms and so control variables are related to the liquid phase. The response of a control variable to a change in the liquid phase environment depends on reactor hydraulic behaviour and the waste to be treated. Plug flow systems give a delayed response while completely mixed systems will give a much faster but dampened response (Weiland and Rozzi, 1991).

3.2.2 Percolating filters

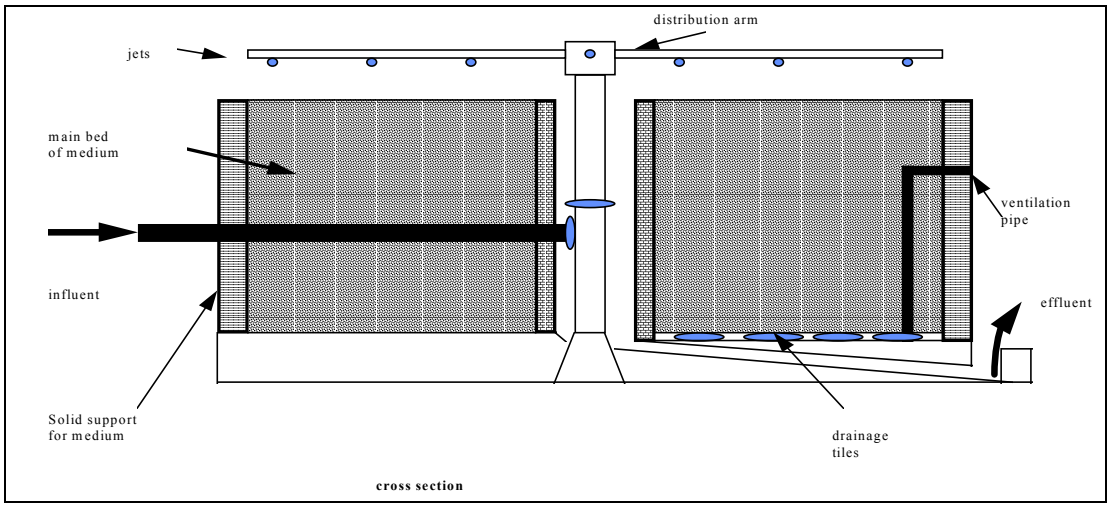

Figure 3-1: Schematic representation of a percolating filter (After: Gray, 1989).

The percolating filter consists of a bed of graded hard material about 2 m deep. The depth of filters ranges from 0.9-15 m. With high-rate filters using modular plastic medium, the medium is stocked into a tall tower housed in lightweight prefabricate material as high as 12-15 m. The majority of the COD removal occurs within the top 750 mm. The major limiting factor in the selection of depth is the loss of hydraulic head. The depth of the filter does not affect performance, as does the total available surface area of the media. Therefore regardless of depth, filters of the same volume will have similar performance (Greeley, 1948).

The filter medium should have satisfactory grading, durability, roughness of texture, satisfactory shape characteristics and be of low cost (Gray, 1989). The medium has intersites that allow air and applied wastewater to access all parts of the bed. The main purpose of the medium is to provide adequate voidage. Smaller media of about 50 mm with rough surfaces provides a better compromise between surface area and voidage. The most frequently used media are granite, clinker and blast furnace slag. The other type of media in use is plastic media. It has a high voidage, wide interstitial spaces and is about 10 % of the weight of mineral medium when in operation. It allows tall filters to be built using prefabricated and lightweight structures. High organic loads can be applied, as blockages do not occur due to the high voidages. This means that air can reach all areas of the filter. There are two main types of plastic media, modular and random (Gray, 1989).

Percolating filters are built on a concrete base covered with drainage tiles to provide a raised floor on which the medium rests. It is important to ensure that the under-drainage area is deep enough to allow the passage of air from ventilation pipes lining the base of the filter bed. The filter has a ventilation system to ensure access of the air to the bed The ventilation pipes are connected to the atmosphere to ensure that there is a free flow of air throughout the whole filter bed. Some aeration is caused by natural convection currents due to the temperature difference between the air temperature and the internal temperature of the bed. Filter beds are built partially or fully in the ground as they have to support a huge weight from the medium, attached film and wastewater (Gray, 1989).

The distributor regulates the flow rate and frequency of the application of the influent wastewater over the surface of the filter. The distribution system can be static or moving. Moving distributors offer the advantage of uniformity of dosing and completeness of wetting of the filter surface. There are two types of moving distributors, the rotary and the reciprocating distributors. The outlet holes of the rotary distributors are spaced along the arm with the holes becoming more closely spaced further away from the centre. Simple holes, instead of spray nozzles, with splash plates to ensure maximum distribution of the water are used to avoid blockages

The non-motile micro-organisms (bacteria and fungi) form a film on the medium. The motile organisms (micro- and macroscopic) are found in the intersites and feed on this film. They prevent heavy film growths blocking the intersites. The microbial film takes 3-4 weeks to become established in warm temperatures and up to two months in colder temperatures (Gray, 1989). The necessary micro-organisms for the treatment of the wastewater are already present in the sewage. This means that no seeding is essential. A film quickly forms over the surfaces of the medium that receive a constant supply of nutrients. The organic matter in the wastewater is degraded aerobically by the heterotrophic micro-organisms that dominate the film. The grazing fauna makes the spongy film more porous as they feed on it. In low-rate filters the clear effluents are a result of the wastewater being strained as it flows through the film matrix. This also increases the micro-organism to wastewater contact time (Schaetzle, 1953).

The first stage of purification of the wastewater is the adsorption of nutrients onto the film. The fine particles are flocculated by the extracellular polymers secreted by the micro-organisms and adsorbed on to the surface of the film. Here extracellular enzymes secreted by heterotrophic bacteria and fungi break down the organic material. The micro-organism that comprise part of the film take in the soluble nutrients together with those nutrients broken down by the extracellular enzymes and synthesise them. Carbon dioxide and other end products of aerobic respiration diffuse in the other direction (Gray, 1989). The thickness of the film determines the extent of oxygen diffusion. The film grows due to an increase in microbial biomass and accumulation of particulate waste by flocculation and physical entrainment. This occurs when the rate of accumulation of particulate waste exceeds the rate of solubilisation and intake by micro-organisms. The thickness of a film depends on whether the influent wastewater is soluble or not. However the film eventually exceeds its critical thickness and becomes anoxic. Eventually the film becomes so thick that the underlying layers become anaerobic but as less nutrients become available to the lower layers they go into an endogenous phase of growth. This means that the lower micro-organism lose their ability to hold on to the medium and portions of the filter become detached and are washed away in the wastewater flow. This process is known as sloughing (Casey, 1997).

3.2.3 Rotating biological contactors

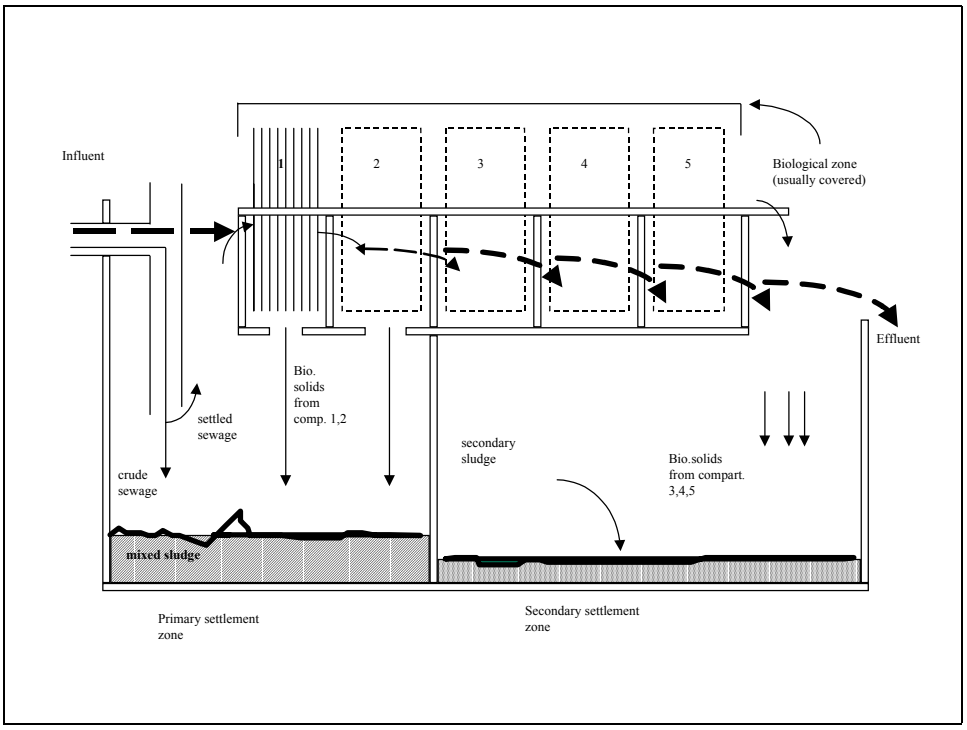

Figure 3-2: Schematic representation of a rotating biological contactor (After: Borchadt, 1970).

Rotating biological contactors (RBC) became commercially available in 1965. The basic design consists of a series of flat or corrugated discs 2-3 m in diameter mounted on a horizontal shaft. The discs rotate at right angles to the flow of settled matter. The waste passing between the discs flows parallel to the adjacent faces of the discs, which support the microbial film. The disc are usually made of plastic, corrugated polythene, PVC or expanded polystyrene. Each disc is 10-20 mm thick and spaced about 20 mm apart. The mounted discs are placed in a contoured tank that fits fairly closely to the rotating medium. This allows about 40 % of their area to be immersed as they are slowly but continuously rotated. This also helps to force a thin film of fluid to pass over the disc face, forestall circuiting and to cause enough local velocities to carry all sloughed solids out of the tank and into the final clarifier. Lattice constructions and wire mesh containers can also be used as support media (Tseng and Connor, 1993).

The flow of wastewater through the tank and the action of the rotating medium produces a high hydraulic shear on the microbial film that ensures efficient mass transfer from the liquid into the microbial film on the disc. Thus the discs rotate slowly imparting a lifting action to the wastewater through the drag forces generated. This in turn causes the wastewater adjacent to each disc face to flow in a circular pattern over the submerged portion of the disc. Contact between the wastewater and the discs is not a single pass between adjacent surfaces but a rapid circulation of waste many times over several quadrants of the disc before the wastewater leaves the tank for the next stage of discs (Tseng and Connor, 1994). This helps to increase the contact time between the micro-organism and the medium, thereby increasing the retention time of the wastewater. This then increases the removal efficiency of the RBC. To minimise short-circuiting, discs are arranged in groups separated by baffles.

To simulate a plug flow reactor, a minimum of four compartments separated by baffles is used. However, to simulate plug flow in larger installations several complete RBC units in series are utilised. The spacing of the discs ensures that the lightweight plastic discs are evenly balanced throughout the drive shaft and so use very little energy. The media should be covered to protect the exposed film on the disc from the weather. Covering insulates the system, reduces heat loss and increases the rate of oxidation (Borchadt, 1970).

As the shaft rotates the discs, wastewater enters the spaces between the discs when immersed and is then replaced by air when out of the liquid. Purification occurs with the film alternatively adsorbing organic nutrients from the wastewater and then obtaining oxygen from the atmosphere for oxidation. The water then passes through a settlement tank to remove any solids before discharge. The first disc develops a heavy growth of heterotrophic bacteria and fungi. Protozoan are found on successive discs. Film is sloughed off continuously and will remain in the liquid phase adding to the overall treatment process (Tait and Friedman, 1980).

The RBC is an intensification of the percolating filtration process with the density of the biomass on the discs reaching 200 g (DW)m^2. This is equivalent to sludge loading of 40-60 kg MLVSS m^{-3}. The advantages of the RBCs over the percolating filter are:

i. complete wetting of the media
ii. no clogging of media due to excessive film accumulation.
iii. regular exposure to air ensures unlimited oxygen
iv. the area of land used is only 10 % of that required by the equivalent treatment capacity supplied by low rate filtration
v. lower sludge production
vi. excellent process control
vii. ease of operation
viii. high COD removal and substantial nitrification.
ix. good settleability of solids
x. lower power consumption
xi. no distribution problems
xii. no recirculation required

The RBCs have some disadvantages like;

i. inability to handle surges of flow.

ii. sensitivity to overloading.
iii. power failure causes loss of efficiency.
iv. power failure causes uneven film growth on disc as half is left immersed in wastewater.
v. uneven growth results in imbalance of discs and severe motor wear.
vi. requiring frequent motor drive and bearing maintenance.

RBCs are expensive to run but are used because they do not cause odour problems, have a low fly nuisance and are quiet. This makes them ideal for treatment of waste near domestic houses. The RBC is a secondary treatment device with relatively short detention period. It has a very low food to micro-organism (F/M) ratio and yields very good COD removal efficiencies (Borchadt, 1970).

3.2.4 Activated sludge process

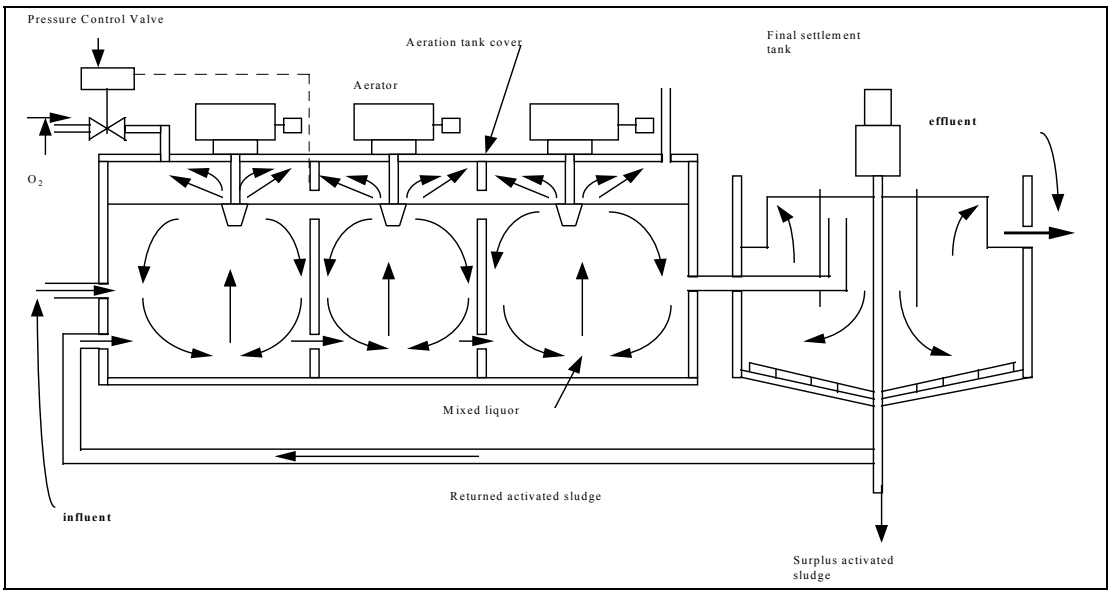

Figure 3-3: Schematic representation of an activated sludge system (After : Gray, 1989).

The activated sludge process is the most widely used biological wastewater treatment process. It is used to treat both industrial and domestic wastewater. It was first developed into a full-scale plant in 1913 by Ardern and Lockett at the Davyhulme Treatment Works in Manchester (Gray, 1989). The activated sludge process takes advantage of the dispersed bacterial flocs and free-living micro-organisms that are suspended within the body of water.

The principal unit in all activated sludge is the biochemical reaction vessel (BRV). The waste organic matter is mixed with the active sludge producing the mixed liquor in the BRV. This mixed liquor is aerated for several hours. During this time the micro-organisms in the sludge utilise the organic matter in the waste for energy and cell synthesis. The process relies on a dense microbial population being mixed with the wastewater under aerobic conditions. In the BRV, the substrate and oxygen concentration allows high rates of microbial growth, respiration and biosynthesis of new micro-organisms. Initial treatment is primarily physical in nature with the particulate and colloidal material being rapidly absorbed on to the microbial floc. The most important function in the activated sludge process is the flocculent nature of the microbial biomass. Each floc is a cluster of several million heterotrophic bacteria bound together with some inert organic and inorganic material. New flocs contain actively growing and dividing heterotrophic bacteria with a high metabolic rate. Naturally older flocs, have a lower proportion of active cells. They consist mainly of dead bacterial cells surrounded by a viable bacterial layer. Despite having a reduced metabolism older flocs have a greater settleability as compared to younger floc. The slower growing autotrophs (e.g. nitrifying bacteria) become established as the flocs age. This is why the concept of sludge age is important in terms of overall efficiency (Parker et al., 1971). The floc is grazed on by the higher trophic micro-organisms in the BRV and this reduces the overall biomass. A well-flocculated sludge is in a state of dynamic equilibrium between the flocs aggregating into larger flocs and being broken into smaller flocs. The

flocs are broken into smaller pieces by the shear stress imposed on them by the aeration system. The turbidity of the final effluent is reduced by ciliate protozoan, which feed on the dispersed bacteria. As a result of the strong binding forces of the calcium, magnesium and other multivalent cations the flocs exhibit great resistance to shear forces (Parker et al., 1972). There is a rapid adsorption of suspended and colloidal matter on to the flocs when the sludge and wastewater are mixed. This results in a sharp fall in the residual COD of the wastewater. The adsorption capacity of the flocs depends on the availability of suitable cell surfaces. When all the adsorption sites are occupied and the adsorption capacity of the floc is reduced, breakdown and assimilation of the agglomerated material proceeds more slowly. This means that the flocs are close to their maximum adsorption capacity and they have removed most of the organic matter from the wastewater (Coackley, 1985). If the HRT is too short there will be insufficient time for the adsorbed material to be stabilised. This means that if the flocs have not removed enough organic material from the wastewater to reduce their adsorption capacity there is a progressive reduction in COD removal. Low rate processes produce good flocculation while high rate processes result in poor flocculation. The well-formed flocs settle out well in the sedimentation tank with the smaller flocs, dispersed micro-organisms and solids being carried out of the tank with the final effluent. In this way the system is self-regulating with the best flocs being recirculated. The flocs have to efficiently adsorb and absorb the organic fraction of the wastewater and rapidly and effectively separate from the treated effluent within the settlement tank. The activated sludge biomass is then transferred to the secondary settling tank where it is separated from the mixed liquor. The separated sludge is recycled back to the aeration tank. A proportion of the settled sludge is intermittently removed from the system to maintain a predetermined concentration of active biomass (Gray, 1990). The concentration of suspended solids in the aeration tank is usually referred to as the mixed liquor suspended solids (MLSS) concentration. The MLSS is a measure of the organic and inorganic fraction of the activated sludge. It is not an accurate measure of the organic fraction or biochemical sludge activity. Changing the sludge wastage rate controls the MLSS. The higher the MLSS the greater is the biomass available to utilise the nutrients of the wastewater and the greater the efficiency of the process. The availability of oxygen in the aeration tank and the capacity of the sedimentation unit to separate and recycle activated sludge limit the upper value of the MLSS (Gray, 1990).

The major advantage of the activated sludge process over other processes is its ability to allow operation over a wide range of loadings to suit specific treatment objectives. This is accomplished by using different combinations of the main operating parameters. The most important parameter in the manipulation of the activated sludge process is the F/M ratio. High-rate processes have a high sludge loading, a short SRT, a high sludge activity and a short hydraulic retention time. The COD removal per unit volume of reactor is very high but there is a relatively high concentration of organic matter remaining in the final effluent. On the other hand, low-rate processes have a low sludge loading, a long SRT, a low sludge activity and a long hydraulic retention time. The sludge is in the endogenous respiration phase so food is limited. The rate of microbial decay is high compared with the rate of microbial growth and there is very little excess sludge produced. This all results in a low residual concentration of organic matter in the final effluent.

3.3 Anaerobic wastewater treatment

Anaerobic digestion is used for the treatment of high strength wastes such as sludges and manures that have a high concentration of suspended solids. In cases where the major portion of the wastewater is insoluble organic material, lengthy digestion periods are required to allow for the relatively slow biological process of hydrolysis and solubilisation. For these insoluble particulates to be completely digested, total digester times of at least 10-20 days are required (Malina and Pohland, 1992). The main hindrance to the more widespread use of anaerobic digestion in waste treatment has been a lack of the fundamental understanding of the process itself. The understanding of the process is essential in order to explain and control the occasional upsets that occur. A better understanding of the process is required in order to extend it successfully to a wider variety of industrial and domestic wastes. The microbial biomass responsible for anaerobic digestion can be 'packaged' in a variety of process configurations. Each configuration has different implications for the ratio of SRT/HRT. Maximal SRT is desirable for process stability and minimal sludge production. Minimal HRT minimises reactor volume and reduces capital costs (Speece, 1983). The ability to choose the most appropriate configuration is critical to successful operation.

3.3.1 Advantages of anaerobic wastewater treatment

i. Low production of biological sludge as compared to aerobic systems. In aerobic systems a lot of energy is given out on oxidation of the wastewater to carbon dioxide. As a result there is a lot of energy available for anabolic processes. The absolute quantity in kg of organic matter is low and the dewatering capacity is very high.
ii. High treatment efficiency.
iii. No oxygen requirements.
iv. Methane is produced which is a useful source of energy. The methane is an energy rich end-product because on formation of methane there is very little energy produced. The sludge is generally well stabilised.
v. Low nutrient requirements.
vi. Low capital costs because technically plain and relatively inexpensive reactors are used which can be operated with little consumptive high-grade energy.
vii. Low operating costs.
viii. Anaerobic organisms can be preserved for long periods of time (exceeding one year) without any serious deterioration of their activity (Lettinga, 1995). Other important characteristics of anaerobic sludge generally remain unaffected like the settleability of the sludge.
ix. Very high loading rates can be applied in modern anaerobic wastewater treatment systems. As a result the space requirements of the system are relatively small.

The ultimate decision to develop anaerobic treatment systems maybe made when anaerobic treatment is economically advantageous in comparison to the available alternatives. It is also essential that the waste treatment system chosen meet the required specifications of the treated effluent. It can be combined with post-treatment methods by which useful products like ammonia or sulphur can be recovered.

3.4 Low rate systems

In the low rate anaerobic systems the removal of biodegradable organic matter was based on the settling of suspended organic matter. Only a small fraction of the influent organic matter is settleable and so the maximum removal efficiency in these systems does not exceed 30-50 % (settleable fraction) of the biodegradable matter of a wastewater. If the ratio of total COD/soluble COD is greater than 1.0 then complete removal of COD can only be achieved by removal of both soluble and particulate organics during treatment (Malina and Pohland, 1992). The poor performance of these primary treatment systems was due to the lack of sufficient contact between the anaerobic micro-organism and the non settleable part of the organic matter in the influent wastewater. This means that the main part of the dissolved or hydrolysed organic matter cannot be metabolised and leaves the treatment system untouched by the micro-organisms (Speece, 1983).

3.4.1` Septic tank

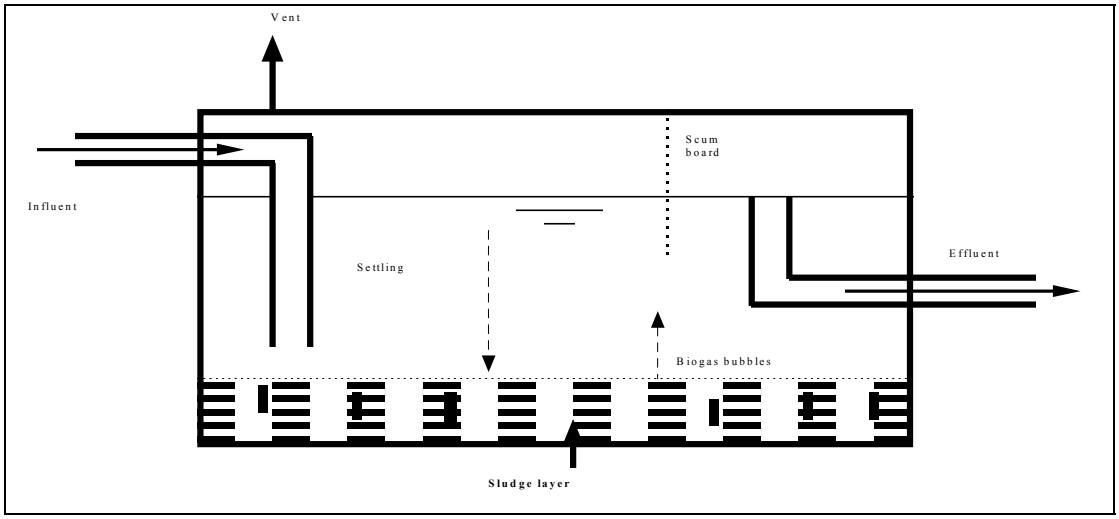

Figure 3-4: Schematic representation of a septic tank (After: Lettinga and van Haandel, 1994).

In the septic tank the wastewater flows through the upper part of the tank while the anaerobic sludge rests at the bottom of the tank. The system depends on the settleability of the solids present in wastewater. The settleable solids will sediment and are degraded by the anaerobic sludge at the bottom of the tank. The water above this anaerobic sludge helps to keep air out of the sludge as in the bottom layer of a lake. The efficiency of retention of settleable solids maybe hampered due to the agitation of decomposing solids by biogas bubbles. The liquid retention time of the septic tank is one to two days, which is sufficient for removal of settleable solids (Lettinga and van Haandel, 1994).

3.4.2 Imhoff tank

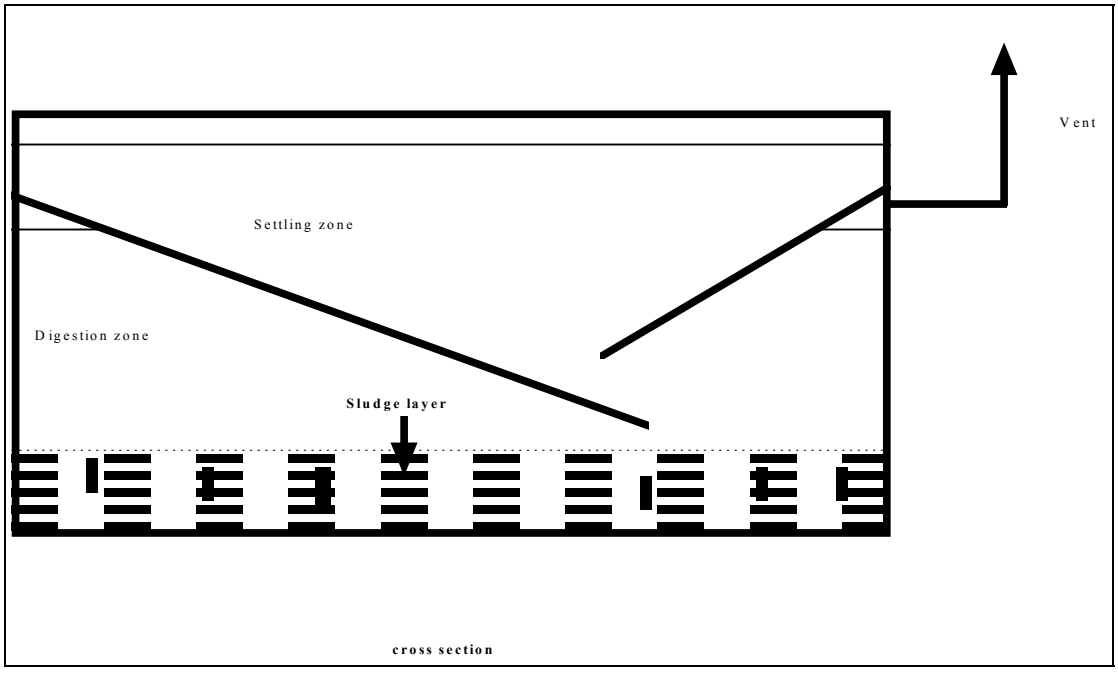

Figure 3-5: Schematic representation of an Imhoff tank (After: Lettinga and van Haandel, 1994).

The Imhoff tank has the same basic operating principles as the septic tank as the primary aim is removal of settleable solids from the bulk liquid fraction. It also has a liquid retention time of one to

two days. The main difference is that the Imhoff tank has improved efficiency of retention of settleable solids as it is divided into a settling zone and a digestion chamber through the use of baffles. This means that the evolved gas from the anaerobic sludge layer does not enter the settling zone and agitate the settling solids. In other Imhoff tanks the accumulated solids are conveyed to a heated digester. This increases the rate of anaerobic digestion. The Imhoff tank and the septic tank are in effect primary treatment systems with biological treatment of settled solids (Lettinga and van Haandel, 1994).

3.5 High rate systems

The efficient removal of organic matter requires the presence of a high concentration of active bacterial mass. In high rate systems, the suspended material is separated from the bulk liquid fraction of the wastewater through the use of several physical processes. These physical processes ensure that a portion of the influent suspended solids will be trapped and retained in the high-rate treatment processes. The particulates that are not retained will pass through the reactor and contribute to the residual COD of the treated effluent. These inert particulates will accumulate in the treatment system causing serious dilution of the active anaerobic biomass. In order to treat raw wastewater with high levels of suspended solids either the wastewater should be pre-treated before application or the organic loading rate should be reduced. Two mechanisms of sludge retention are applied:

i. System based on immoblisation of the sludge. This is usually achieved by attachment on a solid carrier matter. The solid carrier matter acts to trap only the solid matter of the wastewater. This solid fraction adheres to the organic biomass already trapped on the solid carrier matter e.g. anaerobic filter.

ii. Systems based on liquid-solid separation with the return of the separated solids to the reactor. This is achieved by taking advantage of the setlleability properties of the solid fraction of the wastewater. The settler can be external e.g. contact process, or internal e.g. UASB.

The anaerobic digestion system should have a high organic matter removal efficiency at the shortest possible hydraulic retention time. This means the volume of the system should be as small as possible. In all high rate systems there is a separation of solids from the bulk liquid phase of the wastewater. This helps to increase the SRT of the solids. The bulk liquid phase invariably flows through the system with little impedance. This means that the SRT/HRT ratio is now greater than one. The value of SRT/HRT depends on how well the system is able to retain solids.

3.5.1 Anaerobic ponds

An Anaerobic pond is also a flow through system with anaerobic accumulated sludge at the bottom. An Anaerobic pond is usually about 2 to 5 m deep and uncovered. Mixing of the liquid phase may occur due to agitation caused by rising biogas bubbles and also due to the wind and the sun. Anaerobic ponds are extensively used as sewage treatment in developing countries because of the low costs of setting them up. There are usually used as a pre-treatment step in a series of stabilisation ponds. The retention time of sewage in anaerobic ponds is about 2 to 5 days. As a result the removal efficiency of organic matter is higher than in the primary treatment systems such as the septic and Imhoff tanks (Lettinga and van Haandel, 1994). Organic loading rates are usually less than 1 or 2 kg COD/m^3.d. The large reactor volumes provide a good degree of equalisation for toxics and organic shock loads (Landline et al., 1991). The process may suffer from mixing inefficiencies and non ideal contact between incoming wastewater and the anaerobic biomass.

3.5.2 Conventional Completely mixed anaerobic digester

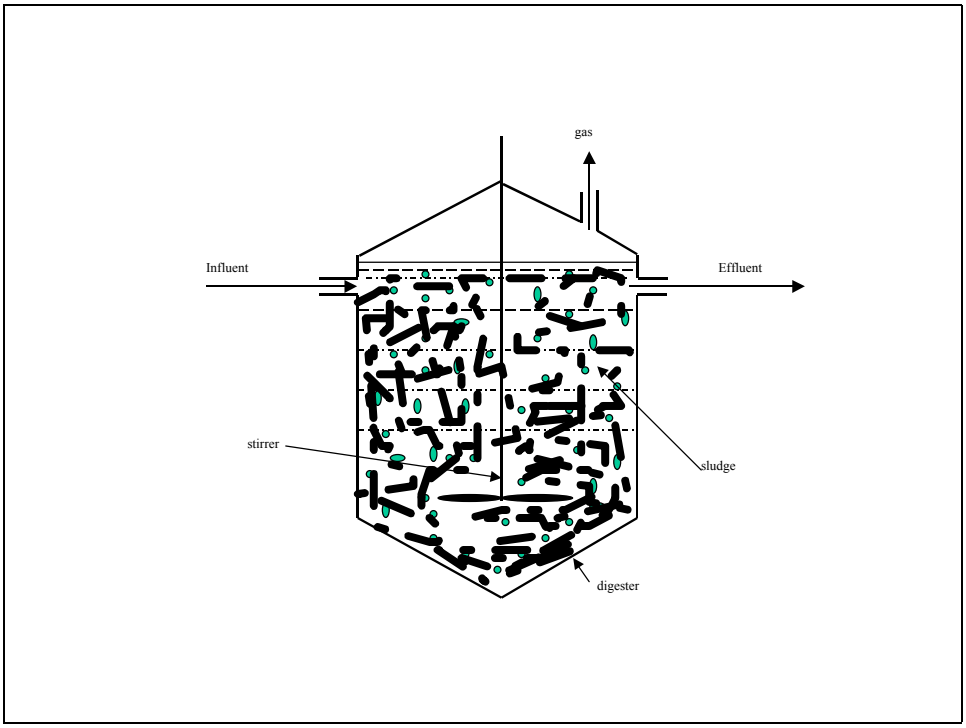

Figure 3-6: Schematic representation of a Completely Mixed Anaerobic Reactor.

The most widely used anaerobic reactor is the Completely mixed anaerobic reactor. The design of the CMAR is very simple and once in operation it is relatively simple to operate. The conventional completely mixed anaerobic digester consists of a heated digestion tank containing waste and bacteria responsible for anaerobic treatment. The raw waste material is usually introduced periodically. Although it is preferable to introduce the raw waste material continuously, this is usually not possible because of the long retention time required for the completely mixed anaerobic digester. In anaerobic digestion the limiting retention time is reached when the bacteria are being removed from the system faster than they can reproduce themselves. The anaerobes reproduce themselves after about three to five days at 35 ^{o}C (McCarty, 1964). For practical control and reliable treatment a retention time of about ten to thirty days is normally used. The full-scale CMAR can be mechanically or gas mixed. The mechanically mixed digester is equipped with an impeller mixer in a draft tube. The gas-mixed digester has recirculated sludge introduced together with compressed sludge gas into a draft tube (Pagilla *et al.*, 1997). There are many variations to the shape of the digesters. The main precaution to take when using CMARs is to avoid overloading them, as they are susceptible to hydraulic and organic overloads. The frequent upsets associated with these types of anaerobic digesters, previously, where mainly due to a lack of understanding of the fundamentals of the anaerobic process (McCarty, 1964).

3.5.3 Anaerobic Contact Process

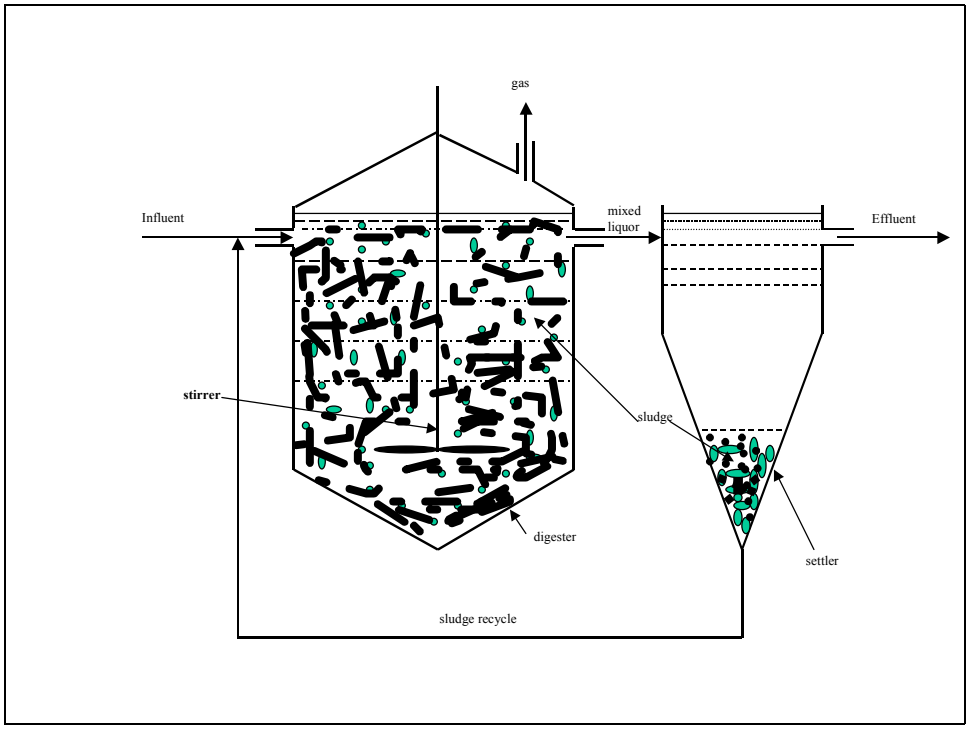

Figure 3-7: Schematic representation of a Contact process.

The anaerobic contact process can be used to overcome some of the disadvantages of the conventional digester process by separating and recycling effluent suspended solids back to the mixed anaerobic reactor. This enables the system biomass to be controlled independently of the wastewater flow, the system SRT can also be controlled separately from the HRT. It is then possible to maintain the required SRT for biomass growth while increasing the applied loading rate and reducing the HRT for capital cost (Pavlostathis and Giraldo-Gomez, 1991). The biomass separation system will retain both the active micro-organism and the undigested influent suspended solids. As a result there is more extensive biodegradation of wastewater particulates. Besides having most of the advantages of the conventional mixed digester, the anaerobic contact process has the extra benefits of increased SRTs and smaller required reactor volumes. These advantages are completely dependent upon the production of an anaerobic biomass with satisfactory properties for adequate solid-liquid separation as the whole system relies on the recycled effluent suspended solids. A settling tank with a liquid upflow velocity of less than 1 mh^{-1} is a common device used for solids separation (Pavlostathis and Giraldo-Gomez, 1991). Membrane filtration of the reactor effluent has also been used as a more possible method of bio-solids control. Anaerobic contact systems that use gravity settling for solids separation are dependent on the separation properties of the anaerobic floc. However, solids settleability can often be problematic as active anaerobic flocs usually have biogas associated with them. The biogas increase the buoyancy of the floc forcing it to float to the surface of the settler. A number of approaches have been developed to enhance sludge settleability such as gas stripping, stirred or vacuum de-gasification, inclined plate or lamella settlers, and the addition of coagulants and flocculants to promote floc formation. An alternative design approach is a short-term reduction in temperature of the settler influent to temporarily reduce the rate of biogas production in anaerobic flocs. In a system that is sensitive to sludge settleability characteristics, it is imperative to minimise transient inputs of toxic and organic shock loads that can impair floc settleability.

The anaerobic contact process can be applied over a wide range of wastewater concentrations. The lower practical limit of wastewater concentrations is probably in the range 1000 to 2000 mg COD/*l* (Malina and Pohland, 1992). There is no well-established upper concentration limit.

3.5.4 Anaerobic filter

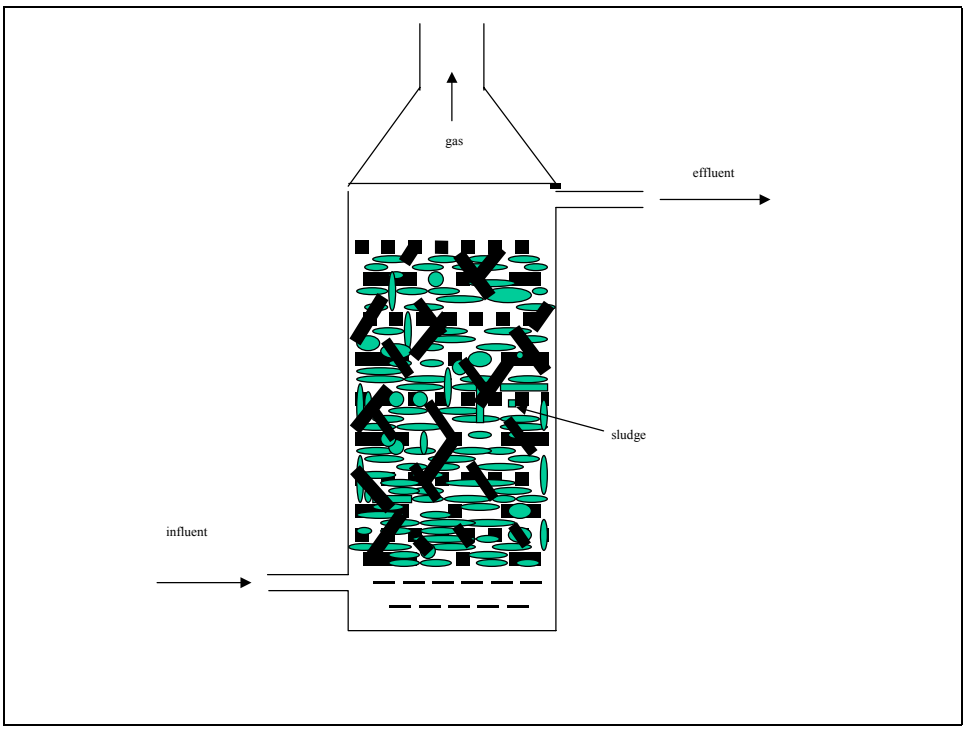

Figure 3-8: Schematic representation of an anaerobic filter.

Young and McCarthy introduced the anaerobic filter in 1969 (Young, 1991). The anaerobic filter is mainly used for industrial wastewaters. Biofilm reactors utilise a fixed film approach for the development of the high biomass concentration required for the treatment of wastewater. An inert media is used to entrap and accumulate micro-organism in the reactor. The anaerobic filter is usually operated in the upflow mode but it can be operated in the downflow mode (Kennedy and Berg, 1982). Full-scale anaerobic filter configurations have included rectangular and cylindrical tanks. The tanks had diameters ranging from 6 to 26 m, heights from 3 to 13 m and volumes of about 100 to 10 000 m^3 (Young and Yang, 1989). The shape and diameter of both the reactor and the media can have important effects on the stability of the attached film. The filters can be operated anaerobically or aerobically using aeration units or anoxically with a separate carbon input (Young and Yang, 1989).

Anaerobic filters are operated using a wide variety of media. These may be mineral or random packed plastic filter media. Mineral media used is porous stones, gravel and pottery fragments. These tend to have a large bulk density and relatively low surface areas. Plastic media have a higher specific surface area and a lower density. Commercial media available are pall rings (loose-fill) and modular block formed from corrugated plastic sheets. The channels in the modular-block media maybe tubular so that no lateral flows occur throughout the height of a block. It may also be counter-stacked so that a cross-flow effect occurs at the contact points within the media matrix. The specific surface area of media used in full-scale anaerobic filters averages about 100 m^2/m^3. Laboratory and full-scale data show lower efficiencies for loose-fill and tubular media than for cross-flow media. Other considerations like economic and operating factors should be the ultimate determining factors as to which media to use (Young, 1991).

The entire reactor can be filled with media or it maybe restricted to the upper part of the reactor only. Young (1991) found that the media heights ranged from full-depth to placement only in the upper 50 to 70 % of the reactor height. The packing configuration of the upflow anaerobic filter means you can have a fully packaged reactor, a modular reactor or a hybrid reactor. The packaged reactor is usually filled with randomly packed mineral or loose-fill plastic media. The modular reactor contains

plastic media packaged in a specific pattern. In the hybrid reactor an unpacked zone is provided beneath the media to permit accumulation of granulated sludge. The performance of the hybrid reactor depends on the contact of the wastewater with the suspended growth in the sludge layer and the attached biofilm in the media matrix. The media is an integral part of the anaerobic filter reactor and the media/height ratio is a critical factor on the performance of the reactor. Young (1991) recommended that at least the upper two-thirds of the reactor be filled with the media in a reactor.

The physical attachment of bacteria to the medium prevents washout of bacteria at high volumetric loading rates. This leads to high values of reactor biomass concentration and SRT. The biological biomass is retained as solids entrapped within the interstital voids of the media or suspended beneath the media as a granulated sludge mass. Removal efficiency is not interrelated to specific surface area of media because a significant portion of the active biomass is present as unattached dispersed growths in the interstices of the medium. The suspended solids flocculate as they move up through the filter forming larger particles that finally settle back down the filter column. The movement of solids and gas bubbles being released introduces an element of mixing into this plug-flow system. The high SRT/HRT ratio of the anaerobic filters gives them greater stability and excellent resistance to inhibitory compounds. The media is an important parameter in the anaerobic filter as it is responsible for:

i. gas-solid separation
ii. providing uniform flow through the reactor
iii. improving contact between the waste water constituents and biomass contained within the reactor
iv. permitting the accumulation of the large amounts of biomass needed to produce long solids retention times.

3.5.5 Fluidised bed

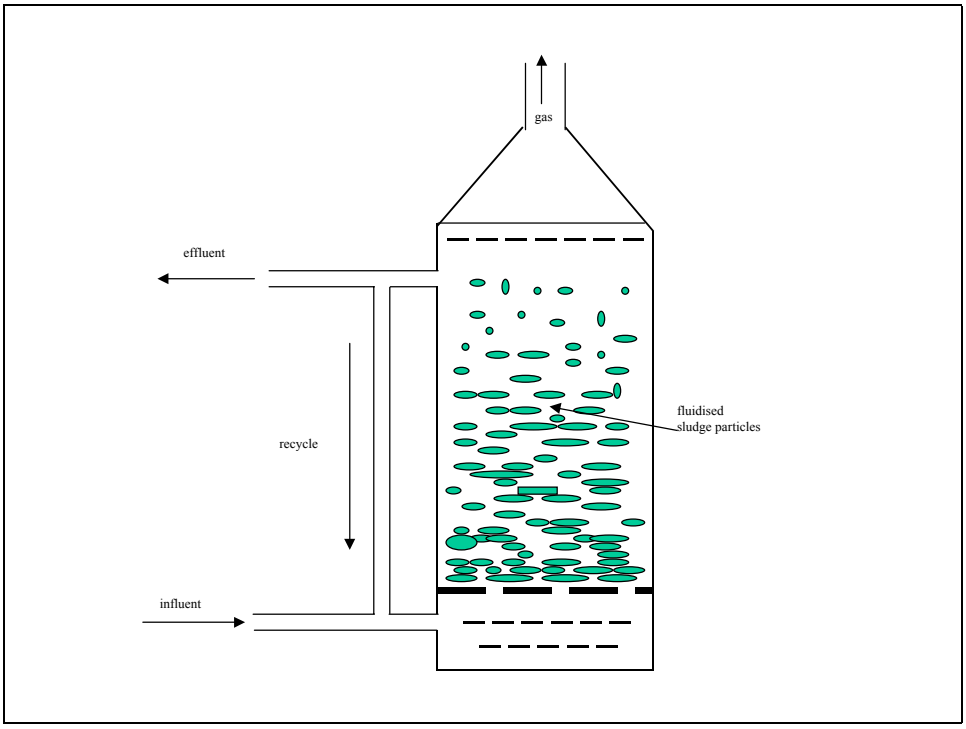

Figure 3-9: Schematic representation of a fluidised bed.

In the fluidised bed system, the carrier consists of granular medium, which is kept fluidised as a result of the frictional resistance of the waste flow. When an increasing flow of liquid passes through the bed of particles. Initially, the bed expands and the particles get suspended and are free to move relative to other particles. The bed is now fluidised. When the flow threshold for fluidisation is surpassed, two different types of behaviour occur according to Iza (1991):

i. the distance between the particles increases as the bed expands (particulate).
ii. the excess flow passes through the bed forming bubbles (aggregative).

Through fluidisation, the bed behaves as a fluid with new physical properties and follows hydrostatic and hydrodynamic fluid laws. By increasing the flow of liquid through a settled bed of particles, the fixed bed starts to expand. If the flow rate is further increased the particles start to move suspended on the upflow liquid separated from other particles. A further increase in the flow rate causes the particles to become more separated from each other and their hydrodynamic behaviour resembles the behaviour of particles settling. The limit of this phenomenon is called fluid transport, where particles are carried out of the bed by liquid flow (Iza, 1991). Fluidised bed can also be a bed of particles lighter than the liquid flow. The bed is then fluidised downward by a fluid of lower density. To achieve this lower density, biogas is injected at the bottom of the column producing a fluid mixture whose overall density is able to drag down the floating particles.

Sand was first used as the granular medium in fluidised bed reactors (FBR) but its high density meant higher upflow liquid velocities were required. This also increased pumping costs. Lower density media such as anthracite and plastic are now used. More recently, lighter products like ion exchange resin beads, granular activated carbon, natural or baked clays, pumice and reticulated polyurethane have been used (Iza, 1991). The size distribution of the particles used on a fluidised bed reactor is usually very narrow. The idea is to get a homogenous particulate fluidisation. Due to the biological nature of the process, biofilm growth affects the size, overall density, shape and roughness of the particles, as well as its chemical and adsorptive characteristics. Fluidisation can be two phase or three-phase fluidisation. A two-phase fluidisation is involves a solid-liquid interaction while a three-phase fluidisation involves a solid-liquid-gas interaction. With three-phase fluidisation, there is a noticeable contraction of the fluid bed on introduction of the gas phase. Many theories have been postulated to explain the contraction (Rigby and Capes, 1970; Darton and Harrison, 1975).

The main problem associated with FBR is the control of the biomass growth. The high velocities used in the FBR mean that non-attached biomass leaves the reactor with the effluent. If there is a significant loss of biomass the effluent quality is reduced. Thicker biofilms are not attached to the support carrier as firmly as the thin biofilms. This creates problems as the thicker biofilms break up or promote formation of granules that are not attached to the support carrier. As the biofilms grow at different rates they create bioparticles of varying diameters. This creates segregation in the bed or mixing in extreme cases. As the bed segregates the lighter bio-particles are mostly found on the top part of the reactor with the heavier bio-particles at the bottom. These differences cause a non-homogeneity on the fluidisation of the bed which can cause particle wash-out, bed compaction and stagnant areas. To over come this problem the thicker particles are usually wasted and scrubbed and then recycled to the bed. In practical applications differences were experienced in controlling the partial size and density of the flows.

Inlet wastewater concentrations range between less than 100 mg COD/*l* to 2 000 mg COD/*l* without adverse effects. The fluidised bed reactors have been operated with different substrates (sewage to vinasses) under widely dispersed loading rates of a 5 to 10 kg COD/m^3.d to 150-180 kg COD/m^3.d (Iza, 1991). The advantages of the fluidised reactor are:

i. high concentration of biomass, attached to a dense carrier, which cannot be washed out of the reactor.
ii. a very large surface area for biomass attachment.
iii. initial dilution of influent with effluent, which provides alkalinity, reduces substrate concentration and helps reduce the shock effects of toxic compounds.
iv. a high mass transfer properties.
v. no plugging, channelling or gas hold-up.
vi. an ability to control and optimise biofilm thickness.
vii. the biomass carrier can be tailored to a specific application to enhance performance.

The restart-up of the systems is very easy and so can be used on a seasonal wastewater. However the bed does have certain disadvantages due to its dependency on a fluidised carrier system. The energy requirements of keeping the bed fluidised means that lighter particles are used, which make hydrodynamic control of the bed more difficult and reduce the mass transfer characteristics of the system. The very high loading rate in these systems is often related to very low retention times. This

means the systems must be closely monitored. The scale-up of the system is very difficult and many compromises between technical and economic aspects must be made.

3.5.6 Upflow anaerobic sludge bed reactor (UASB)

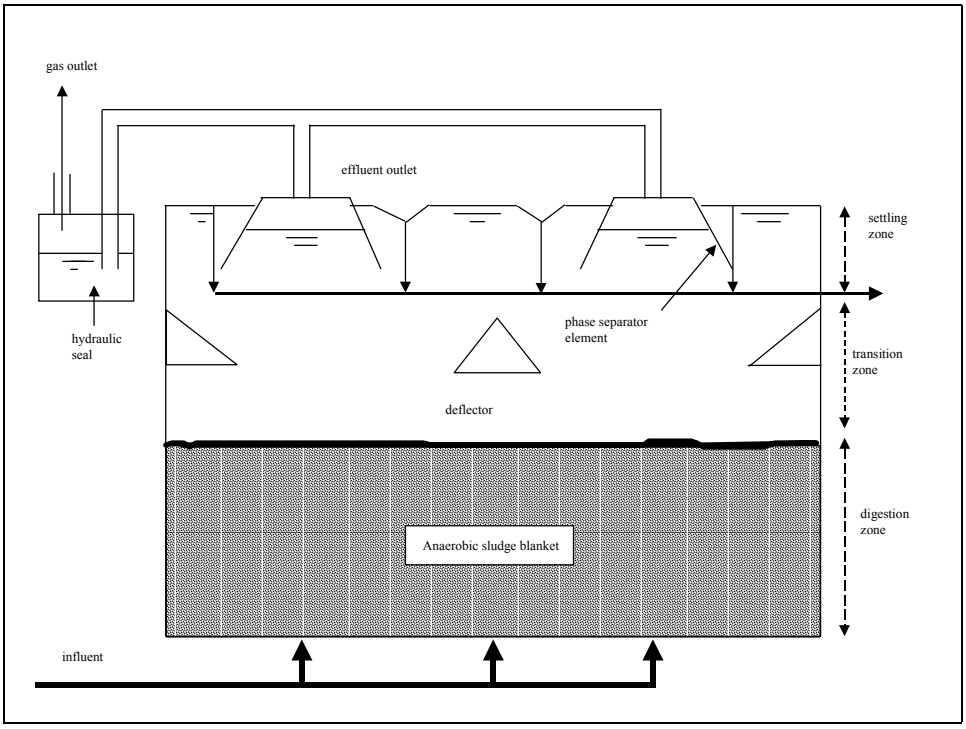

Figure 3-10: Schematic representation of an upflow anaerobic sludge bed reactor (After: Lettinga and van Haandel, 1994).

Lettinga and co-workers in the Netherlands developed the upflow anaerobic sludge bed reactor (UASB) in the 1970s. This reactor had no internal packing and yet still incorporated the immobilised cell feature of the anaerobic filters (Speece, 1983). It is the most widely used high rate anaerobic system for anaerobic sewage treatment. The most characteristic device of the UASB reactor is the phase separator. It divides the reactor into a settling zone (upper part) and a digestion zone (lower part). The wastewater is introduced uniformly through the bottom of the reactor. It then passes the sludge bed and enters the settling zone via the aperture between the phase separators. The inclined walls of the phase separator increase the area of the liquid flow in the settling zone as the wastewater approaches the water surface. This decreases the upflow velocity of the liquid as it flows towards the discharge point. This reduced upflow liquid velocity means that sludge drawn into the settling zone can flocculate or settle out. With time the mass of accumulated sludge on the phase separator will exceed the frictional force that keeps it on the inclined surface and it slides back into the digestion zone and reparticipates in the digestion of the incoming wastewater and its organic matter. The presence of the settler on top of the digestion zone enables the system to maintain a large sludge mass in the UASB. The effluent discharged is relatively free of suspended solids.

The biogas bubbles rise up to the liquid-gas interface under the phase separator. Sludge flocs with adhering gas bubbles may rise up to the interface in the gas collector, but will settle when the gas bubbles are released to the gas phase at the interface. Baffles that are placed under the apertures of the gas collector units operate as gas deflectors and prevent the biogas bubbles from entering the settling zone. This stops them from creating turbulence in the settling zone that would hinder the settling process (Lettinga et al, 1980).

An important feature of the UASB process is that a granular type of sludge develops. These granules are usually 1-5 mm in diameter (Haandel and Lettinga, 1994). The granules have a high density and

exceptional mechanical strength. The granules also combine a high settling velocity with a high specific methanogenic activity. The formation of granules is related to the operational conditions prevailing in a UASB. A granular type of sludge develops on mainly soluble types of wastewater. With raw sewage a flocculent sludge develops and the reactors achieve high removal efficiencies. Lettinga and van Haandel (1994) noted that, although, granulation is not a prerequisite for successful anaerobic treatment the use of granular sludge may offer some specific benefits. In a reactor seeded with granular sludge the flocculent sludge from the raw sewage influent settles on top of the better settling granular sludge and is removed from the UASB separately. The UASB has gained precedence over the conventional CMAR. Like all other modern high rate reactors the UASB is able to separate SRT/HRT (Grobicki and Stuckey, 1991) through the use of the sludge blanket that develops as a result of granulation (Lettinga, 1995).

For treatment of wastewater at temperatures lower than 18-20 ^{o}C the UASB system is modified to enable attainment of reasonable removal efficiencies. The expanded granular sludge bed (EGSB) reactor is operated in expanded mode at high upward velocities of about 6-12 mh^{-1}. The EGSB is fairly efficient in the removal of soluble organic matter even at low temperatures. This is attributed to the intensive contact between the incoming organic matter and the sludge granules. The EGSB is useful at lower temperatures and lower strength wastes when the gas production rate and mixing induced by it is relatively low. This is because the higher kinetic energy of the influent waste water and the extended height of the expanded granular bed contribute to a better performance as compared to the usual UASB reactor (Van der Last, 1991). The other variant of the UASB is the two-stage UASB reactor. In this reactor configuration the first stage involves entrapping the organic matter and partially converting it into soluble compounds. Particulate influent matter can be absorbed onto the flocs. The particulate matter is then hydrolysed and mixed with the liquid phase for discharge from the reactor. The first hydrolytic reactor will contain a flocculent sludge and is operated at a relatively low upflow velocity. Little if any methanogenesis will develop in the hydrolytic reactor because the pH is depressed by acid fermentation. Only a part of the entrapped matter will be hydrolysed and so the excess sludge will have to be discharged from the reactor at a relatively high frequency. This means that the sludge age will be too low for the slow growing methanogens. The effluent from the hydrolytic reactor will be mainly dissolved compounds, so that it can be conveniently treated in an EGSB reactor (Lettinga and van Haandel, 1994).

3.5.7 Anaerobic baffled reactor (ABR)

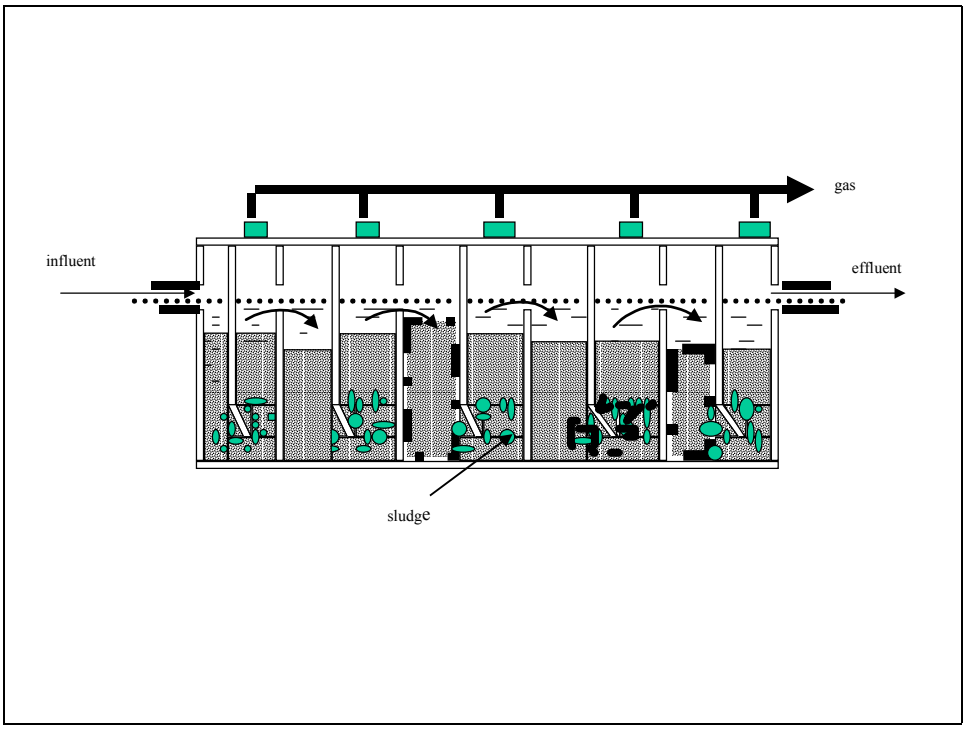

Figure 3-11: Schematic representation of an anaerobic baffled reactor.

In recent years the treatment of large volumes of wastewater has been facilitated by the development of high-rate anaerobic reactors that separates the HRT from the SRT (Iza et al., 1991). This means that the slowly growing methanogens are able to remain in the system independent of the wastewater flow, thus making it possible to have higher volumetric flow rates. The main objectives of efficient reactor design must be high retention time of bacterial cells within the reactor, together with good mixing to ensure a high rate of contact between the cells and substrate (Grobicki and Stuckey, 1991). The first type of high rate digesters were the anaerobic filters but these had long term plugging problems as the active biomass had to cling to the filters to increase the retention time (SRT) of the bacteria in the reactor.

McCarthy (1982) developed a modified version of the UASB, namely, the Anaerobic Baffled Reactor (ABR). McCarty and co-workers at Stanford University, USA noticed that most of the biomass present within an anaerobic rotating biological contactor (RBC) was actually suspended and when they removed the rotating discs they developed the ABR (Barber and Stuckey, 1999). This process has a series of vertical baffles to make the wastewater flow under and over them as it moves from reactor inlet to reactor outlet. The basic reactor design is a rectangular box, with internal vertical baffles alternately hanging and standing. These baffles divide the reactor into a number of compartments (Grobicki and Stuckey, 1991). On its upward passage the waste flows through an anaerobic sludge blanket and does so for each compartment in that specific ABR. The physical geometry of the baffles extends the path length of the wastewater to be treated and so increases the bacterial contact. The angle baffle is beneficial as the flow is diverted towards the centre of the upflow chamber (Bachmann et al., 1985: Grobicki et al., 1990). More importantly it is a physical mechanism that exists for increasing the superficial velocities, hence improving the micro-mixing within a natural laminar flow system (Holt et al. 1994). Hence the waste is in intimate contact with active biomass, but due to the design of the reactor most of the biomass is retained in the reactor. The bacteria within the reactor tend to rise and settle as a result of gas production but are moved horizontally relatively slowly by the wastewater flow (Boopathy and Tilche, 1991). The baffles also enhance biomass cell retention and mixing.

Holt et al. (1994) described the ABR as intermediate between plug flow and completely mixed with a greater tendency toward plug flow. The wastewater comes into contact with a large active biological mass as it goes through the reactor and the effluent is relatively free of biological solids due to the action of the baffles (Bachmann et al., 1985) The most important advantage of the ABR is its ability to separate acidogenesis and methanogenesis longitudinally down the reactor (Barber and Stuckey, 1999). This allows the reactor to behave as a two-phase system and increases the acidogenic and methanogenic activity by a factor of about four.

Bae *et al.* (1997) compared the UASB with the ABR and found the ABR to be superior in terms of soluble chemical oxygen demand (SCOD) removal, methane production and maximum organic loading rate. They attributed its superior performance to its greater ability to separate the different bacterial populations of anaerobic digestion. The configuration of the ABR coupled with the fact that it operates like a number of tanks in series means that various profiles of microbial communities may develop within each reactor compartment. The type and amount of substrate as well as external parameters like temperature and pH will determine the microbial ecology found in a particular chamber. The fast growing bacteria capable of growth at high substrate levels and reduced pH will dominate the front compartments of the reactor. This partial separation of acidogens and methanogens longitudinally down the ABR allows it to behave as a two-phase reactor without the associated control problems and high costs (Barber and Stuckey, 1999). Many workers have provided evidence of this phase separation using various techniques like Scanning Electron Microscopy (SEM) and ATP analysis. Holt et al. (1997) used SEM to analyse the bacterial morphology of the biomass in the different chambers of a 5-chamber ABR and two 5-chamber hybrid ABRs (HABRs). One HABR had filter media in the downflow section while the other had the filter media in the upflow section of its chambers. The substrate they used was a synthetic phenolic wastewater and samples were taken from the first and fourth chamber of each reactor. They found that the HABRs and the ABR had a visible difference in the density and domination of species in its chambers. The scans showed that the chambers of all the reactors had irregular granules with gas vents covered by single rod-shaped bacteria. The micrographs illustrated that there was no one dominant phenol degrading species of bacteria existing within each chamber. Although the micrographs did manage to show evidence of the different bacteria being dominant in the different chambers, classification of individual species was difficult. Boopathy and Tilche (1991) also applied SEM techniques to a 3-chamber ABR with upflow of wastewater in the first

chamber. The feed substrate used was high strength molasses (molasses alcohol stillage and raw molasses). This meant the first chamber received a higher acetate loading than the rest of the reactor. The predominant bacteria observed by SEM where similar in structure to *Methanosarcina* and *Methanothrix*. Since *Methanosarcina* has a lower affinity for acetate but a higher growth rate at a higher concentration of acetate, the first chamber was dominated by *Methanosarcina* and the remaining chambers by long filamenteous *Methanothrix* species. Other morphotypes similar to a variety of heterotrophs, methanogens and desulfovibro were also observed in the biomass and granules. Polprasert (1992) contradicted the findings of Boopathy and Tilche (1991) and reported that *Methanothrix* were found to be the dominant species in the first chamber. The reason for this discrepancy was that the low strength slaughter house wastewater used by Polprasert gave a low acetic acid concentration (20 mg/*l*) in the first chamber unlike the high strength molasses wastewater used as a substrate by Boopathy and Tilche (3000-5500 mg/*l*).

Although granulation is not necessary in the ABR for optimum performance, various reports have noted the appearance of granules in the ABR. Boopathy and Tilche (1991) applied a low initial loading rate (0.97 kg COD/kg VSS.d) and liquid upflow velocity 0.46 m/h to encourage the development of a suitable mass of flocculent and granular organisms. After about 30 days, stable granules of 0.5 mm appeared in all the chambers. SEM showed that the granules were comprised primarily of acetoclastic methanogens. Xing and Boopathy (1991) also noticed granulation after a month of reactor operation. The reactors used in both studies had the same geometry, size and substrate. Holt (1997) observed the presence of large granules of 1-4 mm in the early chambers of the HABR with a downward-low filter (DF) and ABR only. The size of the granules decreased in subsequent chambers. This decrease is due to the fact that the size of the granular sludge is related to the imposed sludge loading. At lower loads a decrease of the strength of the granular sludge will occur (Lettinga, 1995). From these three studies of bacterial morphology using SEM it is apparent that the SEM method is not very accurate in determining the specific bacteria in the biomass under study. All the researchers acknowledged that the bacterial biomass was too complex for a complete and thorough identification of individual species. This means that a smaller species could have been dominant in all the chambers but due to its size it went unidentified.

Xing *et al.* (1991) used adenosine triphosphate (ATP) analysis to determine the relative position of the most active bacteria in the ABR. Samples were taken from the top, middle and bottom of all three chambers from a 150 *l* working volume reactor treating molasses at a loading rate of 20 kg COD/m^3.d. ATP was found to be highest at the bottom of the first chamber, then the third and finally the second chamber. The results show that these chambers had 92 %, 88 % and 85 % activity respectively. The amount of ATP is a measure of the quantity of energy available at that stage in the reactor as bacteria use ATP as a source of energy. Polprasert *et al*.(1992) found an opposite trend to this one. The difference in substrate concentration means that more dilute wastewater (as in the work of Polprasert et al., 1992) have lower acetate levels in the front compartments. As a result *Methanosaeta* is likely to dominate at low strength wastewater. *Methanosaeta* grows at a slower rate than *Methanosarcina* and is more sensitive to environmental conditions such as reduced pH (figure 2-2).

Xing and Boopathy (1991) noticed that the volatile suspended solids concentration was greater in the first chamber as compared to subsequent chambers. They also observed that the biomass concentration increased with time in each chamber as the substrate was loaded into the reactor. The same trend was observed for the granular sludge weight. Holt et al (1997) had a lower biomass concentration (9.62 gVSS/*l*) as compared to the HABRs downflow and upflow (DF & UF) with (24.33 and 15.45 gVSS/*l*) respectively (Boopathy and Tilche, 1991). In this study the first chamber of all three reactors had a lower pH value and slightly higher temperature due to the higher microbial activity in this chamber. The comparison of the effluent COD removal for each chamber showed a gradual increase in COD removal efficiency for each chamber. Bachmann (1991) also observed a similar trend were the highest biological solids concentration was found in the first chamber. The experimental data showed a gradual decrease in COD with successive compartments. (Polprasert, 1992; Holt, 1991: Boopathy; 1992) also found that most of the COD was removed in the first compartment. The hydrodynamics of the reactor aids the development of granulation in the higher loaded initial chambers of the ABR. This then enhances COD removal. Boopathy and Tilche (1991) discovered that in an HABR (UF) the hydrodynamics and sludge blanket encouraged the retention of solid mass resulting in an effluent virtually free of solids.

Grobicki and Stuckey (1991) calculated the solids retention time [given as cell retention time (CRT)], biomass yield (Y_{obs}) and wash out of biomass under varying loading and HRT conditions. The CRT varied from 7-734 days with a HRT of 5-80 h. The authors attributed the large deviations in their results to the varying degrees of granulation in the reactor. They found a strong correlation between CRT and HRT with a linear regression $r^2 = 0.89$. However, they suggested that caution must be exercised when using the calculated figures due to the assumption of perfectly mixed behaviour in the ABR. They calculated from theory and mass balances that the observed yields were very low (about 0.03 kg VSS/kg COD). This implies constant biomass concentration profiles over time. This contradicts the findings of Boopathy and Tilche (1991), Xing *et al.* (1991) as these researchers found increasing biomass concentrations profiles with time across their reactors (Boopathy and Tilche, 1991) found that by increasing the organic loading rate from 6 to 17.5 kg COD/m^3.d the VSS effluent concentration increased. However a linear increase up to 28 kg COD had an effluent concentration of 27 gVSS/*l*.

The hydrodynamics and degree of mixing that occur within the ABR strongly influences the extent of contact between substrate and bacteria. This controls mass transfer and potential reactor performance. In 1992, Grobicki and Stuckey conducted a series of residence time distribution studies by tracking the fate of an inert tracer (Li^+) in the effluent of a number of ABRs with 4-8 chambers. Some of the ABRs had biomasss while the rest did not have any biomass in them. They were all operated at various HRTs. The data obtained was incorporated into the 'Dispersion' and 'Tanks In Series' models (Levenspiel, 1972). They found low dead space in comparison with other anaerobic reactor designs e.g. 50-93 % in an anaerobic filter (Young and Young, 1988) and > 80 % in a CSTR (Stuckey, 1983). On addition of biomass the dead space increased but no correlation was found between hydraulic dead space and HRT. Hydraulic dead space was found to be a function of flowrate and number of baffles in the reactor. Biological dead space, on the other hand, was found to be a function of biomass concentration, gas production and flow rates. The contradictory effects of hydraulic dead space and biological dead space prevented a correlation being derived between HRT and overall dead space. At high HRT biological dead space was found to be the major contributor to overall dead space with its effects diminishing at lower HRT.

Grobicki and Stuckey (1995) performed similar RTD studies to an ABR with effluent recycle to study the effects of recycle on the hydrodynamic performance of the ABR. They found that the flow pattern of the ABR was intermediate between plug flow and perfect mixing tending towards plug flow at recycle ratio 0 and then tending towards perfect mixing at recycle ratio = 2.0. A positive correlation was observed between the inverse of the dimensionless peclet number (D/uL) and recycle ratio. The percentage of dead space (18.8 %) was similar to that in Grobicki's (1991) earlier work even though the biomass was seven times higher in concentration. Grobicki concluded that the biomass present in an ABR has no effect on the amount of dead space and that recycling the effluent of the reactor improves mixing within the reactor. Recycling, however, was found to increase the amount of dead space. Holt (1997) carried out similar studies on a HABR (DF & UF) with baffle angles of 45^o and 60^o. He found from C-Curve analyses that the HABR (UF) provides a greater retention of tracer within the intersitial spaces. The media improved the reproducibility of the results. A greater correlation between the Tanks In Series (N) model and the experimental results was found for the (45^o , 25 mm) arrangement. This showed that the ABR could be modelled against the Tanks in Series model as it had a small deviation from plug flow. In the work of Grobicki (1991; 1997), the hydraulic dead space was found to be lower than that found by Holt (1997). Holt concluded that the ABR is sensitive to changes in the physical arrangement of the divide baffle. He also found that the location of the media within the chamber has a significant effect on the hydrodynamics of the system.

The correlation of the predictions of the tanks in series model by Grobicki (1991) and Holt (1997) shows that the ABR can be modelled as a series of CSTRs with little dead volume. Grobicki and Stuckey (1992) found that with biomass in the reactor and at higher HRTs there was significant channelling in the reactor. This together with short-circuiting will affect the accuracy of any model (Holt, 1991). In order to take channelling and short-circuiting into account it is essential to calculate the number N of ideally mixed reactors in series using tracer studies. The results of these experiments will then be input as the number of real compartments into a reactor in series model.

Bachmann *et al.* (1983) found similar treatment behaviour under identical conditions in an ABR, AF and RBC. This led to the hypothesis that the ABR can be adequately modelled as a fixed film reactor.

Bachmann *et al*. (1985) used an influent substrate of 8 gCOD/*l* to model the ABR on the fixed film model. The model was used to predict a decrease in treatment efficiency with:

i. decreasing influent substrate concentration at constant loading rates.
ii. an increase in organic loading at constant influent substrate concentration.
iii. an increase in recycle ratio at constant HRT.

Reactor efficiency improved with reducing substrate concentration at constant HRT. Xing and Tilche (1992) modelled a HABR with a working volume of 150 l, with a molasses wastewater influent substrate with a strength of 20 kgCOD/m^3.d. The ATP testing employed in the model concluded that the active biomass was found in the base of the reactor. The model used the biomass weight and not its concentration. The model assumed that all substrate consumption occurred within a granular sludge bed which was perfectly mixed due to gas evolution. The model accurately predicted that:

i. at constant organic loading the treatment efficiency increased with increasing influent substrate concentration.
ii. as HRT was reduced the performance deteriorated with increasing loading with a constant sludge weight.
iii. an improvement in COD removal efficiency was observed with increasing sludge weight until a certain concentration was achieved beyond which reactor performance becomes independent of biomass concentration.
iv. an increase in recycle ratio coincided with a subsequent decrease in COD removal.

From the above discussion it is apparent that the low rate systems like the sceptic tank and the imhoff tank were based on the natural settling of suspended organic matter resulting in poor contact between the anaerobic microorganisms and the non settleable part of the organic matter. This meant that these systems did not adequately treat the soluble portion of the organic waste. The high rate wastewater treatment systems are an improvement on the low rate systems. However, the anaerobic ponds also relies on natural mixing with increased solid retention time making it no different from the low rate systems. The development of the CMAR offered a system with better mixing at an increased cost due to the artificial mixing employed. Its SRT/HRT ratio of one, made the system susceptible to hydraulic and organic overload. The contact process is an attempt ot increase the SRT/HRT ratio but the success of the system is dependent on the production of an anerobic biomass with adequate solid-liquid separation properties which is difficult to achieve and reproduce. Other high rate systems like the anaerobic filter also try to address this problem through the use of a carrier system to immobilise the sludge and improve the SRT/HRT ratio. However, these systems suffer from clogging after sometime in use and then the filter needs to be regenrated again. The fluidised bed tries to over come these clogging problems but the energy requirements coupled with the control and monitoring needs of these reactors renders them less cost effective.The UASB has an internal settler brought about by the granulation of the wastewater. The UASB has managed to overcome most of these problems. Its main difference to the ABR is that the ABR is a series of UASBs. The advantages of the ABR over well established systems include:

i. better resilience to hydraulic and organic shock loading.
ii. longer biomass retention times.
iii. lower sludge yields.
iv. ability to partially separate between the various phases of anaerobic catabolism.

The ABR combines the advantages of high stability and reliability with a high void volume. The risk of clogging and sludge bed expansion with resulting high microbial losses is reduced and there is no need for special gas collection or biological solids separation (Bachmann *et al*., 1985). These effectively means the ABR has most of the advantages of the other reactors combined the only major draw back to the ABR appears to be the high SRT/HRT ratio which may cause problems when the wastewater has a high solid fraction.

3.6 Sludge Treatment

The effluent discharged from biological treatment systems consists of the liquid phase and the solid phase. The liquid phase is mainly water that contains dissolved organic and inorganic matter. The solid phase consists mostly of dead micro-organisms, recalcitrant compounds and inorganic minerals. It is the necessary to have in place some form of liquid-solid separation device to reduce the sludge volume. Several processes are commonly used for liquid-solid separation of biological sludges. The simplest thing to do is to use the discharged effluent for agricultural use without any separation. The best method is to dry the effluent sludge naturally. This produces a true solid with a very low water content. Artificial drying is carried out by centrifuging, vacuum filtration and filter pressing. All these artificial methods produce thick cakes that are inferior to the end product of natural drying. The choice of the liquid-solid separation system depends on:

i. desired final solids fraction.
ii. available financial and technological resources.
iii. available land area

The natural drying method is most appropriate in hot climates as the prolonged period of sun exposure removes a considerable amount of the pathogens present in the effluent sludge. The investment and operational costs are significantly lower than those of mechanised options.

Chapter 4: Laboratory equipment and analytical methods

Anaerobic digestion has provided a technological solution for the stabilisation of wastewater in human society. In the CMAR, the new biomass grown is completely mixed and becomes evenly distributed throughout the contents of the reactor. In this study conventional flow-through reactors with no solids recycle were used. Therefore the solids retention time (SRT) to hydraulic retention time (HRT) ratio was one (Speece, 1983). In the ABR the biomass is retained by the use of baffles coupled with the effluent mixing in the reactor. This increases the SRT/ HRT to be greater than one.

4.1 The Laboratory Completely Mixed Anaerobic Reactor (CMAR)

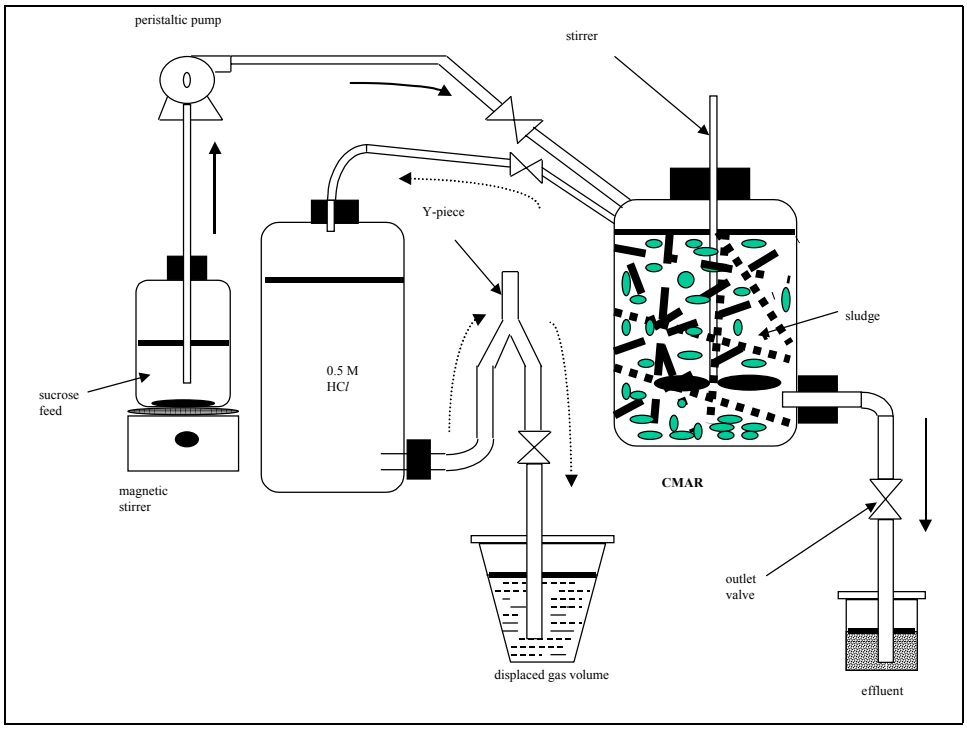

Figure 4-1: Schematic representation of the laboratory-scale completely mixed anaerobic reactor.

The completely mixed reactor consisted of a 25 *l* aspirator bottle that was maintained in a water bath at 37 ^{o}C. A magnetic stirrer continuously stirred the feed to improve homogeneity of the feed concentration. It was then fed to the reactor using a peristaltic pump controlled by a timer. Small volumes of sludge were pumped three times a day to give a residence time of approximately 20 days. A valve sealed the sludge inlet, except during feeding. The aspirator bottle was stirred by an overhead stirrer (140 rpm) to completely mix the contents of the reactor. The digested sludge was withdrawn by gravity from the base of the digester through an outlet pipe. A valve to prevent loss of the reactor contents through gravity sealed the outlet pipe. The gas was collected daily through a glass bottle containing acidified water. The acidified water was displaced for gas measurement. The anaerobic reactor was completely sealed except for a gas outlet pipe that was connected to the bottle. A Y-piece was used to maintain the contents of the reactor at a pressure higher than atmospheric pressure to prevent air leakage into the reactor. Two completely mixed anaerobic reactors were set up. The organic loading tests were done using a sucrose feed in the Test reactor while the Control reactor had constant feed.

4.2 Laboratory Anaerobic Baffled Reactor (ABR)

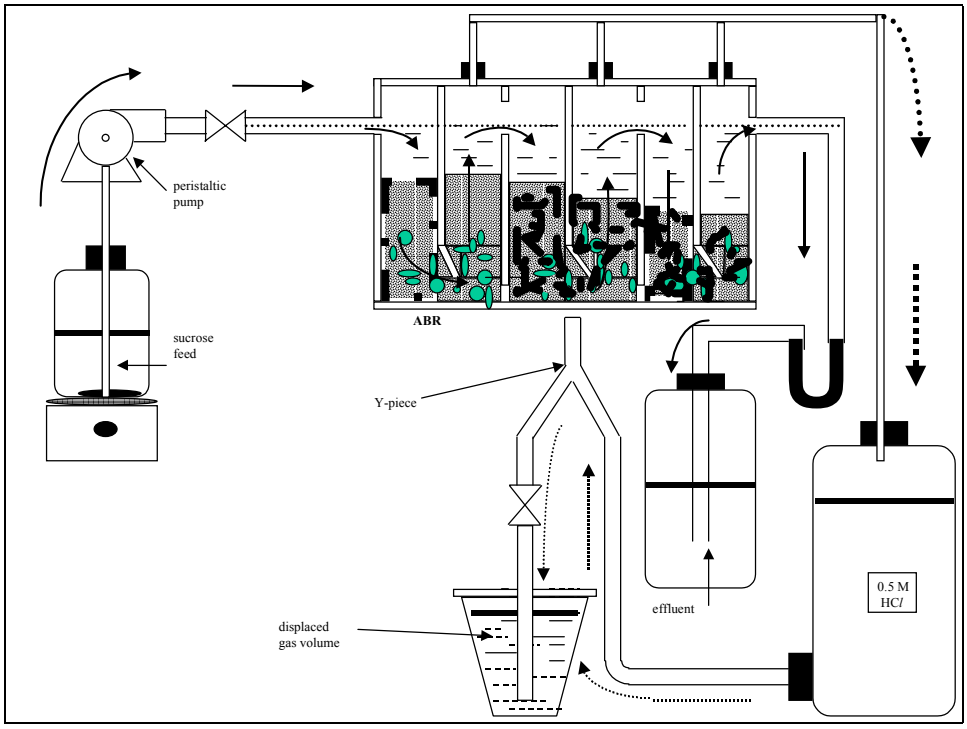

Figure 4-2: Schematic representation of the laboratory-scale 3-compartment anaerobic baffled reactor.

The laboratory anaerobic baffled reactor (ABR) consisted of rectangular perspex box that was maintained in temperature control room at 37 ^{o}C. The baffles divided the reactor into 8 discrete compartments with a total working volume of 7.5 litres. The upcomer in each compartment was twice the width of the downcomer. The feed was continuously stirred by a magnetic stirrer to improve homogeneity of the feed concentration and was fed to the reactor using a peristaltic pump controlled by a timer. The timer was set so that the reactor was fed approximately 0.075 *l* every 3 minutes to give a residence time of approximately 20 h. The sludge inlet had a valve that was closed during cleaning of the inlet tubes. The digested sludge was withdrawn continuously from the digester through a U-tube. As with the laboratory–scale CMAR the gas was collected daily through a glass bottle containing acidified water (0.5M HCl). The ABR was completely sealed except for a gas outlet pipe that was connected to the glass bottle with acidified water. A separate outlet collected the gas from the reactor for each compartment. A Y-piece was used to maintain the contents of the reactor at a pressure higher than atmospheric pressure to prevent the introduction of air into the reactor.

4.3 Feed and Nutrient Medium

The two CMAR were fed with raw sewage from Umbilo Sewage works (Durban, South Africa) from day 1 to day 238. This feed had a total solids (TS) content of about 23 gTS/*l* and a volatile solids (VS) content of about 19 gVS/*l*. Although this feed seemed to function well in bringing up the reactors from upset it was changed for the sucrose feed to enable comparison with the ABR (Table 4-1). The raw sewage feed would have been unsuitable for the comparison studies as it fluctuated in composition and strength. The feed to the two CMARs and the ABR was changed to a synthetic carbohydrate-protein feed with the following composition:

Table 4-1: Constituents of sucrose feed (5*l*)

Chemical	Mass (g)

Sucrose	13.34
Peptone (Oxoid)	4.00
Meat extract (Lab-Lemco)	1.34
Di-potassium orthophosphate	0.40
Sodium hydrogen carbonate	16.25

Table 4-2: Constituents of Mineral solution (2*l*)

Chemical	Mass (g)
Iron chloride-4-hydrate	15.70
Cobalt chloride-6-hydrate	2.38
Manganese chloride-4-hydrate	0.75
Sodium molybdenate-2-hydrate	0.75
Nickel chloride-6-hydrate	0.90

The feed solution used was a soluble, complex organic mixture which has been well characterised for work with anaerobic organic mixed cultures (Boopathy and Tilche, 1991). The feed was made up at ten times the concentration and autoclaved for 20 min. at 121^{O}C.The sucrose feed (500 m*l*) was added to 100 m*l* of the mineral solution and then was diluted to 5*l*. The sucrose in the feed is for the catabolic requirements of the bacteria while the meat extract and peptone are for the anabolic requirements of the bacteria. Sucrose hydrolyses to glucose and fructose (Rawn, 1989). These soluble organic molecules are taken up by the acidogens and degraded rapidly to acetate (Noike *et al*., 1985). This necessitates the buffering di-potassium orthophosphate and the addition of sodium hydrogen carbonate. The trace elements Fe, Co, Ni, Mg and Na, are for the various conditions of active methanogenesis. These elements are implicated in the enzyme systems of acetogenic and methanogenic bacteria.

4.4 Analytical Methods

The CMAR and the ABR were monitored for the following stability indicators

i. Gas production
ii. Gas composition

and the following control variables:

i. pH
ii. Total solids/Total suspended solids and Volatile solids/Volatile suspended solids
iii. Volatile acids/alkalinity ratio (Ripley's Ratio)
iv. Chemical oxygen demand reduction

The advantage about using all these parameters is that they supply complementary information about the system. In this study these parameters were monitored at least five times a week. Charting of the trends of the parameters was also carried out. In a high rate process the most important processes are the gas production and gas composition as these two parameters change quickly enough to keep up with the rapidly changing contents of the reactor. As this study involved a high rate ABR and a low rate CMAR the composition of the gas and the gas production changes were the main parameters monitored for control of the systems.

4.4.1 Temperature

Temperature governs the rate of reaction in wastewater treatment systems. Changes in temperature give rise to significant alterations in the microbial community structure. Higher temperatures up to 35-40 ^{o}C

give rise to higher rates of COD removal. After this range bacterial cells become reduced in size and number, thus growing in a dispersed phase resulting in turbid effluents. Although anaerobic digestion proceeds over a wide range of temperatures, the mesophilic process at least is sensitive to sudden temperature changes. The usual limit is about 2 ^{o}C/d. The maximum for thermophilic anaerobic digestion is 55 ^{o}C. For sewage treatment only mesophilic digestion is relevant due to the fact that the sewage temperature will have to be raised from room temperature to the thermophilic range. This involves using more energy making it economical unfeasible. Temperature also affects the extent of digestion for settled sewage solids. The decrease in the fraction of organic matter degraded can be attributed to a low rate of hydrolysis. A major economic consideration is whether or not the digestion of any particular wastewater produces enough methane to satisfy the heat requirements of the process at the desired temperature (Mosey, 1981).

The reactors in the study were operated at 37 ^{o}C. This temperature falls within the range for mesophilic anaerobic digestion. The ABR was kept in a temperature-controlled room. The CMARs were kept immersed in water baths that were thermostatically maintained at 37 ^{o}C. There were no temperature variations observed when the water was exchanged as all the water added was first brought to 37 ^{o}C. The two methods of temperature control maintained the temperature efficiently at 37 ^{o}C.

4.4.2 pH

The pH is not ideal as either a control or a stability indicator because it is not considered to be very sensitive due to its log derivation (Weiland and Rozzi, 1991). The value and stability of the pH of an anaerobic reactor is important because methanogenesis only proceeds at a high rate when the pH is maintained in the neutral range (pH = 7.0). Different authors have different values for the optimum pH for methanogenesis. Lettinga and van Haandel (1994) suggest the range for methanogenesis is between 6.3 and 7.8, Eastman and Ferguson (1981) place it in the range 6.8 - 7.5, Mosey (1974) states that it occurs between 6.0 and 8.0 while Malina and Pohland (1992) assert that *Methanosarcina barkeri* and *Methanosarcina vacuolata* degrade acetate at a pH of 5 when grown with hydrogen and methanol as the catabolic substrates. In general most researchers agree that the optimum pH is near neutrality (Mosey, 1974: Iza *et al*., 1991). Acidogenic populations are significantly less sensitive to low and high pH. Hence, acid fermentation will predominate over methanogenic fermentation. This results in 'souring' of reactors as the pH decreases and less gas is produced. Notably less methane is monitored in the gas analysis. For a given increase of volatile acid production, the pH variation in the mixed liquor will depend on the bicarbonate buffer. It will decrease as the bicarbonate buffer increases. It is wrong to only monitor the pH of the feed, as it is the pH within the reactor that is important. If the system is sufficiently stirred, the pH of the feed stream becomes irrelevant as it is sufficiently diluted by the reactor contents.

The pH of the reactors was monitored using a pH electrode (Toledo 400 series). The pH meter was calibrated initially using buffer solutions. The electrode was first placed in the pH 7.0 buffer solution. The temperature of the solution was taken and the temperature control on the pH meter was adjusted to that of the buffer solution. The pH was calibrated to a pH of 7.0. The electrode was then cleansed using distilled water. The electrode was placed into the pH 4.0 buffer solution and calibrated as before. After cleansing with distilled water the electrode is ready for pH measurements. The effluent from the reactors was placed into a 50 m*l* beaker and the pH of the solutions recorded. The pH of this effluent was immediately measured so as to avoid loss of carbon dioxide. The loss of carbon dioxide results in higher pH readings as the remaining effluent is less acidic.

With the ABR, the last compartment had a syringe needle inserted through the top part of the compartment. When not in use the needle was covered by parafilm so as to avoid introduction of air into the reactor. A syringe was fitted into the needle and used to withdraw the effluent from the last compartment. This enabled fresh effluent to be collected from the reactor. When not in use, the needle was left above the water level of the ABR so that it would not interfere with the flow dynamics of the reactor.

4.4.3 Alkalinity

The alkalinity of a solution is its ability to resist a change in pH. The alkalinity of a solution is considered to be a mixture of CO_2 and a strong base. The solution is titrated with a strong acid to neutralise this strong base thereby determining the alkalinity of the solution. After this titration, the pH of the solution is obviously equal to that of a pure CO_2 solution. This is called the CO_2 equivalence point. The equivalence point of a solution is determined by titrating the solution to a value of about 4.2-4.5 as it is known the pH value is within this range (Lettinga and van Haandel, 1994). The following expression can be used for the alkalinity in a solution with only carbonate species:

$$Alk = 2\left[CO_3^{-2}\right] + \left[HCO_3^{-}\right] + \left[OH^{-}\right] - \left[H^{+}\right] \qquad [4\text{-}1]$$

Alk - alkalinity of the solution of carbon species and a strong base.

In an anaerobic reactor the pH is around 7 and the ionic species of water dissociation and the carbonate concentration are much smaller than the bicarbonate concentration. This means that the alkalinity due to the carbonate system can be taken to be the concentration of bicarbonate - *bicarbonate alkalinity*:

$$Alk = \left[HCO_3^{-}\right] \qquad (6.5 \le pH \le 7.5 \qquad [4\text{-}2]$$

The carbonate system is the main determinant of the buffering capacity of most wastewaters. The carbonate system is not only important for ionic equilibrium in the water phase, but also has implications for the liquid-gas and the liquid-solid equilibrium with respect to calcium carbonate precipitation or dissolution. pH is also an important parameter in the post-treatment of anaerobically digested sewage. At high pH, phosphate tends to precipitate, nitrogen is removed by stripping or precipitation of the poorly soluble mineral, struvite (Lettinga and van Haandel, 1994).

Various methods are available to determine the volatile acids and alkalinity concentrations of the digesting sludge. Ripley et al (1986) developed a very simple titration procedure (see Appendix A.4). The sludge was first centrifuged and 50 m*l* supernatant was transferred to a 250 m*l* volumetric flask. The calibrated pH meter was then placed into the volumetric flask. The pH meter was set to the temperature of the supernatant and this was then titrated with 0,5 M hydrochloric acid. The supernatant was titrated until the pH reached 5,75 and the titre recorded as P. The titration was continued until the pH reached 4.3 and the additional titre recorded as I. The Ripley Ratio was then calculated as follows;

$$Ripley\ ratio = \frac{VA}{ALK} = \frac{I}{P} \qquad [4\text{-}3]$$

The 'Ripley ' VA/ALK ratio is a useful digestion monitoring tool which increases rapidly with process upset, then decreases with recovery (Ross et al., 1992). This is because as the volatile acids increase and the alkalinity falls when a problem is developing, the ratio will change faster than individual values. The Ripley ratio gives early warning of an impending problem.

4.4.4 Solids Content

Solids may affect water or effluent quality. The wastewater to be treated consists of a solid phase and a liquid phase. The wastewater treatment system besides stabilising the influent wastewater energetically creates an effluent that has an easily separable solid-liquid interface. The solid phase of the influent wastewater has three distinguishable categories of solids:

i. dissolved solids
ii. colloidal solids
iii. particulate solids

Dissolved solids consist of all the soluble inorganic and organic salts. The colloidal solids and particulate solids are insoluble and form the suspended solids. As a rough guide it can be expected that one third of the solids in the influent wastewater are particulate and settle out, one third are particulate and do not settle out, and the last third are dissolved in water (Lettinga and van Haandel, 1994).

A distinction can be made between dissolved and suspended solids by filtering the wastewater. In this way the dissolved solids will be found in the filtrate while the particulate and suspend solids are left on the filter paper. The type of filter holder, pore size, porosity, area, thickness of filter and the physical nature, particle size and amount of material deposited on the filter are the principal factors affecting separation of suspended from dissolved solids. In wastewater analysis the solids left on the filter paper, after heating it at 105^{o}C, are termed total suspended solids (TSS). They quantify the amount of solids in the wastewater less the dissolved solids. If the filter paper is heated at 550^{o}C, the solids that evaporate are the volatile suspended solids (VSS). The remaining solids represent the fixed total, dissolved and suspended solids. These determinations of fixed and volatile solids do not distinguish between inorganic and organic matter because the loss on ignition is not confined to organic matter. It includes loss due to decomposition or volatilisation of some mineral salts. The determination is useful in control of wastewater treatment plant operation because it offers an approximation of the amount of organic matter present in the solid fraction of wastewater.

Errors in the volatile solids measurement may arise due to loss of volatile matter while drying at 550^{o}C. When the concentration of the volatile suspended solids is low as compared to the concentration of fixed solids, there maybe considerable errors in the results obtained. Temperature is very important in order to have more accurate results as weight losses due to volatilisation of organic matter, mechanically occluded water, water of crystallisation and gases from heat-induced chemical decomposition, as well as weight gains due to oxidation, depend on temperature and time of heating. If the residues have high oil and grease contents then it is difficult to dry them to constant weight as oil and grease have a high boiling point. Residues dried at 103 -105 ^{o}C may contain water of crystallisation and some mechanically occluded water. Bicarbonates may be converted to carbonates with concomitant release of carbon dioxide. Loss of organic matter at this temperature is marginal and attainment of constant weight is slow as the occluded water is removed slowly from the solid mass.

The biomass concentration was determined by taking a 50 m*l* sample of the feed and effluent from each reactor and drying it at 105 ^{O}C giving the dry mass of the sample. From the dry mass the total solid concentration of the sample was calculated. The sample was then heated at 600 ^{O}C for 15 min. in an muffle furnace to give the fixed solids mass. The difference between the dry mass and the fixed solids mass gave the volatile solids (VS) mass from which the amount of volatile solids (VS) in the sample was calculated (see Appendix A.1).

4.4.5 Chemical oxygen demand

The COD in the reactor can be categorised into the following fractions: Unbiodegradable soluble and particulate, readily and slowly biodegradable, and finally, heterotroph active biomass. Readily biodegradable COD consists of relatively small molecules that are readily transported into microbial cells whereas slowly biodegradable COD comprises larger and more complex molecules that require extracellular breakdown (hydrolysis) to smaller units before uptake and utilisation by the cells.

The COD test is based on the oxidation of organic material. In the test, the organic material oxidised and its concentration is evaluated from the decrease in the oxidant concentration during the test. In the COD test a sample of wastewater is mixed with a solution of dichromate and concentrated sulphuric acid. Silver sulphate is added as a catalyst and the temperature increased to the boiling point of the mixture (150^{o}C) to accelerate the redox reaction. The catalyst aids in the oxidation of fatty acids. Chloride ions will react with the dichromate and so are removed by adding some mercuric sulphate to the refluxing mixture (Sundstrom, 1931). After a period of two hours the oxidation of almost all the organic compounds present in the sewage is complete. Thus the concentration of the organic matter originally present in the sample can be calculated

from the decrease in dichromate concentration. This decrease can be determined titrimetrically or spectrophotometrically. The completeness of the oxidation may be verified experimentally by using a solution of a known concentration of an organic compound. For theoretical COD values, the oxidation reaction between the general molecule $C_xH_yO_z$ maybe written as:

$$C_xH_yO_z + \left(\frac{4x-y-2z}{4}\right)H_2O \rightarrow \left(\frac{4x-y-2z}{8}\right)CO_2 + \left(\frac{4x+y-2z}{8}\right)CH_4 \quad [4\text{-}4]$$

The atomic weight of H(1 g/mol), C (12 g/mol) and O (16 g/mol) means that 1 mol of organic material exerts a demand of 8(4x + y - 2z)g O_2. As a result the theoretical oxygen demand of organic material can be expressed as:

$$COD = \frac{8(4x+y-2z)}{12x+y+16z} mgCOD \setminus mgC_xH_yO_z \quad [4\text{-}5]$$

It follows from the above discussion that if oxygen is used for oxidation of organic material, then the mass of oxygen used is numerically equal to COD. Hence the mass of oxidised organic material in a wastewater system can be measured by determining the consumption of oxygen for that particular oxidation. The equation 4-6 for the oxidation by dichromate shows that for each mole of dichromate, three atoms of oxygen atoms are released.

$$Cr_2O_7^{2-} + 8H^+ \rightarrow 2Cr^{3+} + 4H_2O + 3O \quad [4\text{-}6]$$

The oxidation of organic matter, by dichromate, follows the following reaction pathway (Sawyer et al., 1994):

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d+c)H^+ \rightarrow nCO_2 + \frac{a+8d-3c}{2}H_2O + cNH_4^+ + 2dCr^{3+}$$

where d=2n/3+a/6-b/3-c/2. [4-7]

From this equation, theoretical COD (COD=*3/2d*) of a compound can be calculated based on the equivalent amount of oxygen required to oxidise it. The organic material (COD) present in the effluent wastewater will have one of the following forms:

i. sludge - COD
ii. methane - COD
iii. mineralised - COD
iv. COD remaining in the effluent

At steady state the daily mass of influent COD is equal to the daily mass of COD leaving the system as methane in the excess sludge produced, in the effluent and daily amount of COD oxidised.

$$MS_i = MS_e + MS_x + MS_d + MS_o \quad [4\text{-}8]$$

MS_i - daily mass of influent COD
MS_e - daily mass of effluent COD
MS_x - daily mass of COD in discharged sludge
MS_d - daily mass of digested sludge
MS_o - daily mass of oxidised sludge

Normally, COD measurements for a reactor are calculated for the influent wastewater, the effluent wastewater and the gas production. In equation 4-8 MS_e and MS_x are contained in the effluent wastewater

(COD_{OUT}) while the daily mass of oxidised sludge (MS_o) is incorporated into the biomass. For anaerobic bacteria, the growth rate is very slow that this amount is negligible. The daily mass of digested (MS_d) is released as methane $COD_{CH4.}$ The COD mass balance then consists of:

$$COD_{IN} \rightarrow COD_{OUT} + COD_{CH4} \qquad [4\text{-}9]$$

The method used to test for chemical oxygen demand (COD) was the standard dichromate open reflux, as set out in the Standard Methods. The sample used was 50 m*l* of a solution which had been appropriately diluted to give a COD value below 500 mg/*l*. Potassium hydrogen phthalate was used as a standard in the COD test. The theoretical COD of this solution was 500 mg/*l*. Mercuric sulphate (1g), sulphuric acid (5 ml) and standard potassium dichromate solution (25 ml) were added to the sample in the reflux flask. The flask was then attached to a condenser and the mixture refluxed for 2 hours. A blank consisting of distilled water was refluxed at the same time for the same length of time. After coooling the samples and blank were titrated with ammonium sulphate using ferroin indicator solution. After refluxing the sample should be green. For calculations see Appendix A.2.

4.4.6 Gas production and composition

The gas composition was determined using a Gow-mac GC 150 series. The carrier gas was helium at 30 m*l*/min. The oven and detector temperatures were left at room temperature. The peaks obtained were for nitrogen, methane and carbon dioxide. The separated components were detected using a thermal conductivity detector (TCD). Waste stabilisation in anaerobic digestion is directly related to methane production. Buswell and Mueller (1952) gave the following formula to predict the quantity of methane from knowledge of the waste chemical composition:

$$C_n H_a O_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 \qquad [4\text{-}10]$$

This formula shows that the ultimate oxygen demand of the waste being degraded is equal to the ultimate oxygen demand of the methane gas produced.

Chapter 5: Start-up of reactors

Reactor start-up is a very important economic process step, because during this period the productivity of the wastewater supplier must be adapted to the capacity of the wastewater treatment plant. The start -up of an anaerobic reactor is very important as it impacts on the maximum removal rates the reactor will achieve. The failure to start-up a reactor efficiently may lead to souring of the reactor and it has to be restarted. Due to the slow growth of anaerobic bacteria the start-up period may take up to seven weeks or more. A complete start-up of an anaerobic reactor system needs a period of 3 to 5 months (Lettinga, 1995). The reduction of the duration of start-up and the improvement of process control are important factors in order to increase the competitiveness of the anaerobic system. A comparison of the start-up of the CMAR and the ABR is very difficult because of the differences in the configurations of the reactors.

5.1 General start-up of anaerobic reactors

The duration of the start-up of an anaerobic reactor depends on numerous biological, chemical and physical parameters.

Table 5-1: Important parameters for reactor start-up.

Innoculum	Reactor	Wastewater	Environment	Operation
volume activity adaptation	configuration geometry size	composition strength	temperature pH-value nutrient contents trace elements	loading retention time mixing

All the parameters in Table 5-1 are in strong interaction during start-up. The start-up of the anaerobic reactor depends on all these factors but it is necessary that the bacterial biomass volume is as high as possible. The aim is to have a biomass with a high concentration and activity. The seed biomass should contain a mixture of different methanogenic genera For an effective start-up it is preferable to have a mixture of several sources of active biomass. Even if a seed biomass is available that has adapted to the specific wastewater properties but which comes from another reactor system, the addition of some municipal anaerobic biomass maybe beneficial. It is always advisable to use municipal anaerobic biomass as seed biomass due to the increased spectrum of different methanogenic genera naturally found in it. It is also essential that the bacterial biomass must not be in the endogenous phase as this prolongs the start-up time (Chapter 2, Section 2.2). If the innoculum consists mainly of bacteria in the exponential growth phase, and depending on their adaptation to the intended feed, the start-up period can be shortened considerably. However, in practice it is difficult to determine the phase of microbial growth of an bacterial biomass. Fortunately, anaerobic bacteria can withstand long periods without feeding and this does not significantly reduce their activity. This is because anaerobic bacteria are capable of functioning and growing under conditions of extremely low free energy (Hickey et al., 1991). This means that if the biomass is taken from an operating digester the length of the start-up is greatly reduced. It is also essential that the micro-organism in the biomass adapt and acclimatise to the feed. This will enable them to degrade the organic compounds in the target wastewater. For xenobiotic wastewater it is better to produce seed material in a separate reactor using a synthetic wastewater. Synthetic wastewater is easily metabolised by the anaerobic bacteria to provide a ready source of energy during the lengthy digestion period required for the degradation of the xenobiotic compounds.

The microbial factors that influence reactor start-up.

i. dominant bacterial groups
ii. growth rate of methanogenic species
iii. biomass yield coefficient
iv. morphology of the methanogenic species
v. half velocity constant K_s of the micro-organisms
vi. adaptation of the micro-organisms to wastewater properties

vii. ability to excrete polysaccharides

The properties of the microbial seed biomass are fundamental to the rapidity of start-up of the anaerobic reactor. The growth rate and biomass yield of the anaerobic bacteria is important because the acetogenic/methanogenic phase is rate-limiting. In the initial phase of start-up the high rate of wash out must be balanced by the formation of new micro-organisms. The washing out of the biomass is due to the poor integration of the bacterial biomass. The anaerobic process is highly dependent on the interaction of the acetogenic and methanogenic bacterial colonies. These two forms of bacteria interact in a way that is mutually beneficial to them (Chapter 2, section 2.6.1). However, as the methanogens are more susceptible to environmental changes, the environmental conditions are optimised to enable their survival. For achieving fast growth of the starter culture the reactor temperature for mesophilic anaerobic digestion should be between 33 to 37 ^{o}C, the pH about 7.2 to 7.6 and the nutrient content must be balanced. A COD:N:P ratio in the range 100: (10-1): (5-1) seems to be beneficial (Kennedy, 1985). In the case of unbalanced wastewater, addition of nutrients is necessary during the start-up period (Hickey and Wu
, 1991). Trace elements like iron, cobalt, nickel and molybdenum considerably affect the duration of the start-up as they are essential for methanogenic growth. The shape of the bacterial cells, their half velocity constant and their ability to excrete polysaccharides are significant factors. The polysaccharides compose a major part of bacterial composition and so are important in the increase of bacterial mass. However their role depends on reactor configuration as the reactors differ in their separation of SRT and HRT, which influences the level of COD removal achieved by the reactor.

5.2 Laboratory procedure for start-up

The start-up procedure was designed so as to be able to compare the start-up of two reactors that have different configurations, geometry and size. The two reactors, namely, the CMAR and the ABR have different mixing patterns. The CMAR is closer to being an ideal backmix reactor while the ABR is closer in its flow patterns to the ideal plug flow reactor. For an effective comparison of these two reactors from the different poles of the mixing patterns of flow reactors, it is imperative that all the other parameters are similar in the reactors. If the reactors are fed the same influent wastewater, have the same innoculum, function in similar environments and have the same operating conditions, it is possible to compare their differences in reactor coordinates i.e. volume and shape. Unfortunately, the reactor coordinates determine the operating conditions such as retention time, loading and mixing. The only parameter that is not affected by the reactor coordinates but is a measure of the operation of the reactors, is the COD removal efficiency of the reactor.

During start-up of the CMAR and the ABR, it was not possible to compare them on the basis of their removal efficiencies. The two reactors by virtue of their configurations have different retention times. Basically the CMAR has no physical mechanism of separating the SRT from the HRT unlike the ABR. This effectively means that the ABR will have a lower HRT than the CMAR in order to achieve the same removal efficiencies. The strategy was to start-up each reactor separately until it achieved steady state. Therefore the two reactors were compared according to how quickly they achieved steady state, their operational stability and ease of operation during start-up.

5.2.1 Start-up of completely mixed anaerobic reactors (CMAR)

Initially the CMARs were started-up using digested sludge and was fed with raw sewage from Umbilo Sewage Works (Durban, South Africa). The temperature was kept at approximately 37 ^{o}C. The reactors were fed at 21 d HRT. The reactors were very unstable and did not achieve steady state as the acid solution (0.5 M HCl) used to measure the gas production through displacement entered the reactors resulting in a decrease in pH. This was because as the effluent was let out of the reactor, the pressure of the reactor decreased below atmospheric pressure, allowing the acid solution to flow into the reactor. The addition of acid to the reactors occurred up to day 32 after start-up. It was not possible to determine how much acid had entered the reactors as the contamination occurred unnoticed. The Test reactor had calcium hydroxide added to it to enable recovery from the low pH conditions caused by the influx of acid. Meanwhile the Control was left to recover without any chemical additions so as

to evaluate the significance of chemical addition during start-up of the CMARs. The hydraulic loading rate to both reactors was increased to 37 d so as to reduce the amount of volatile acids in the reactor. This is because the volatile acids add to the high acidity of the reactor and reduce the activity of the methanogens. As discussed in Chapter 3, section 3.4.2, the CMAR has a retention time of ten to thirty days. Due to the nature of the experiments to be carried out on these laboratory reactors and their size it was decided to operate them at 20 d HRT. The HRT was reduced sequentially from 37 d to 30 d and finally to 20 d. This avoided upsetting the feeding pattern of the bacteria.

5.2.1.1 Results for the Completely Mixed Anaerobic Control reactor

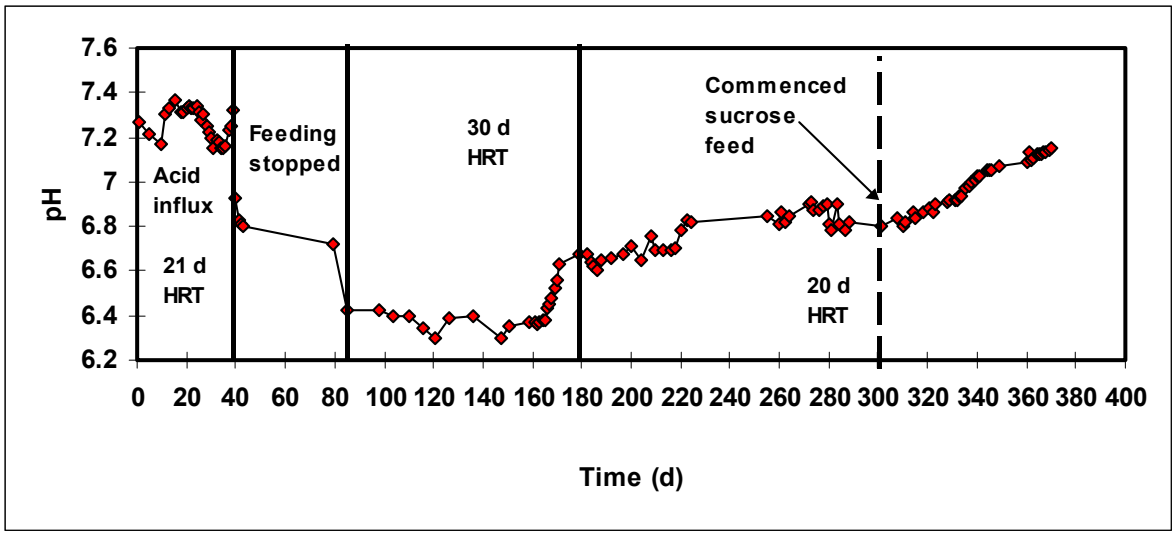

Figure 5-1: Plot of variation of pH of the effluent with time of the (CMAR) Control reactor.

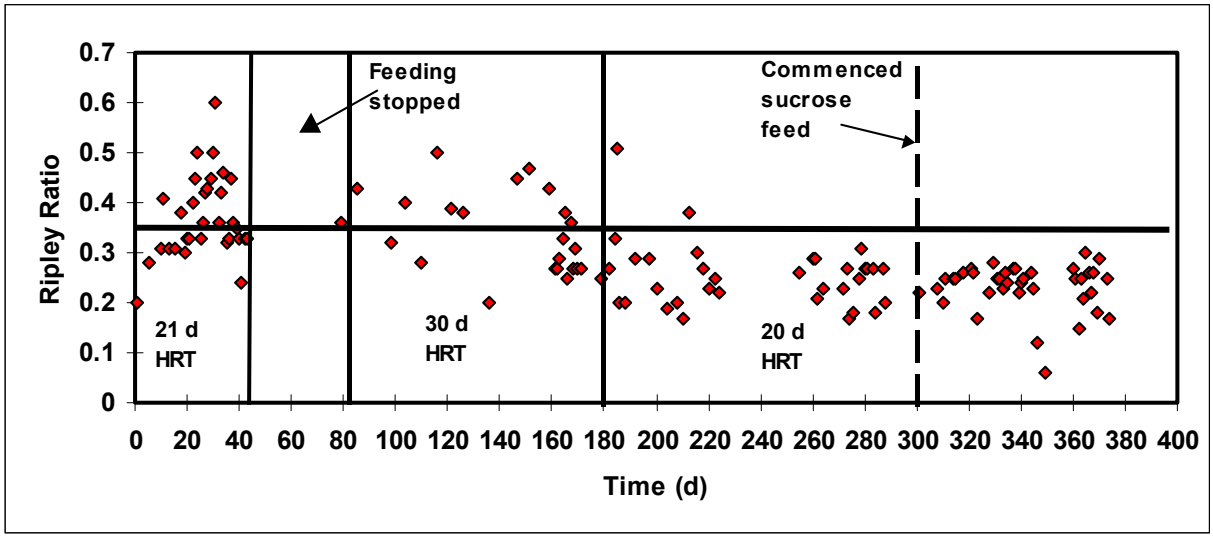

Figure 5-2: Plot of variation of Ripley Ratio with time for the (CMAR) Control reactor.

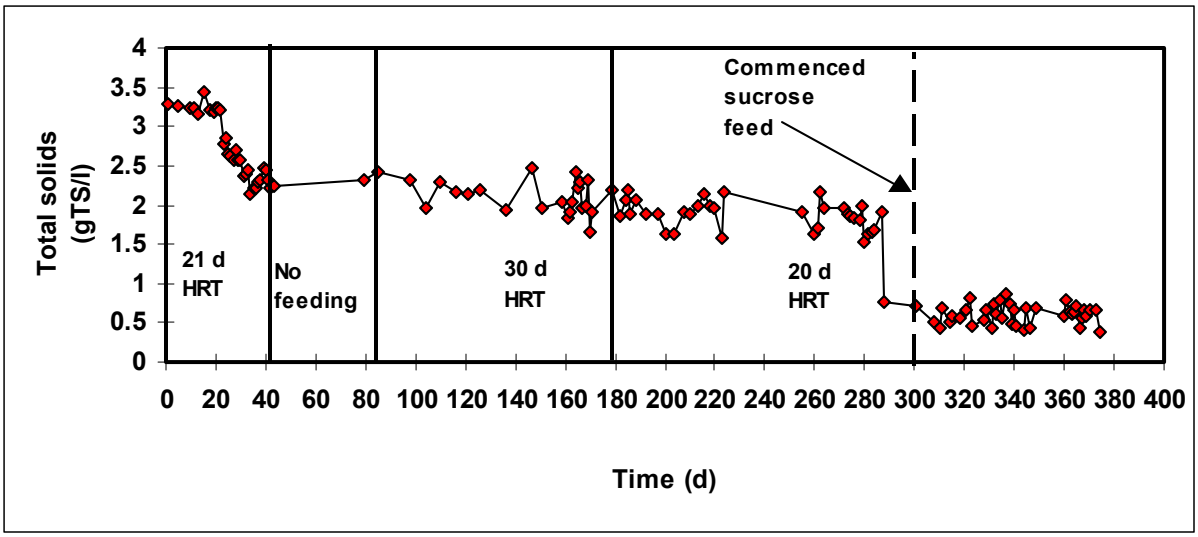

Figure 5-5: Changes in the total solids content in the effluent of the Control (CMAR).

The gradual increase in the total solids (TS) can also be attributed to the reduced hydraulic loading rate. The reactor was run at 30 d HRT. This was done to reduce the amount of volatile acids in the reactor as the acidogens now had less organic solids to digest. Also, the reduced HRT means that less solids are added and therefore, less solids will come out of the reactor since the SRT: HRT ratio is one for a CMAR.

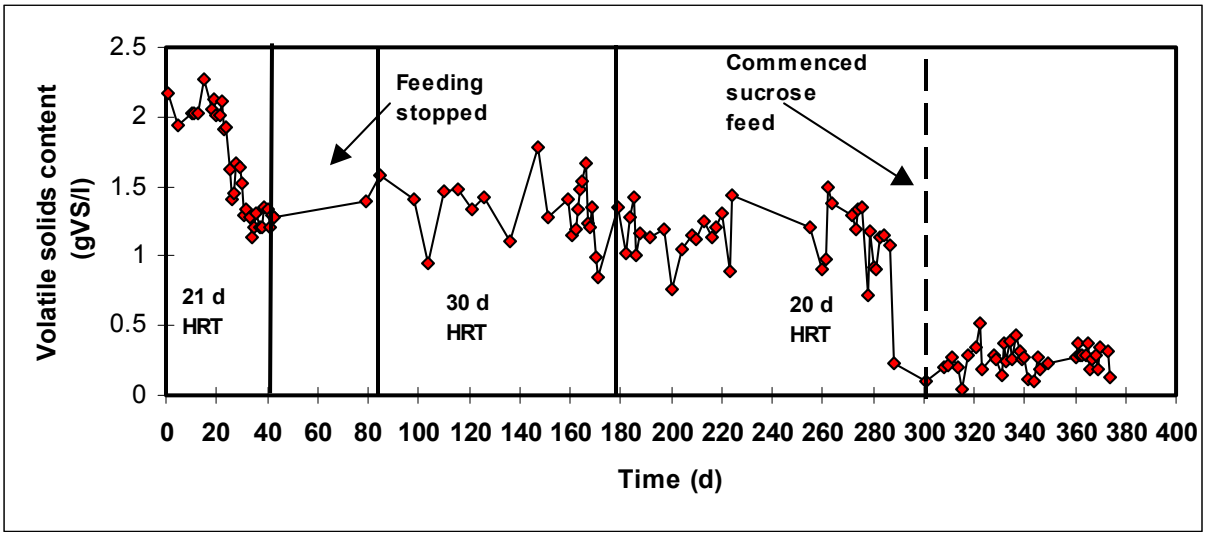

Figure 5-6: Changes in the volatile solids content in the effluent of the Control (CMAR).

Initially there was a decrease in the volatile solids content of the Control reactor from 2.2 gVS/*l* to 1.2 gVS/*l*. The feeding to the reactor was then stopped to allow the reactor to recover from the acid contamination. After feeding began at 30 d HRT there was an initial decrease in the volatile solids (VS) content. The volatile solids content then stabilised at a value of about 1.1 gVS/*l*. This stable value continued until the feed was changed from the raw sewage to the synthetic sucrose feed. When the new feed was added there was a decrease in volatile solids content but the VS stabilised at a value of 0.3 gV/*l*.

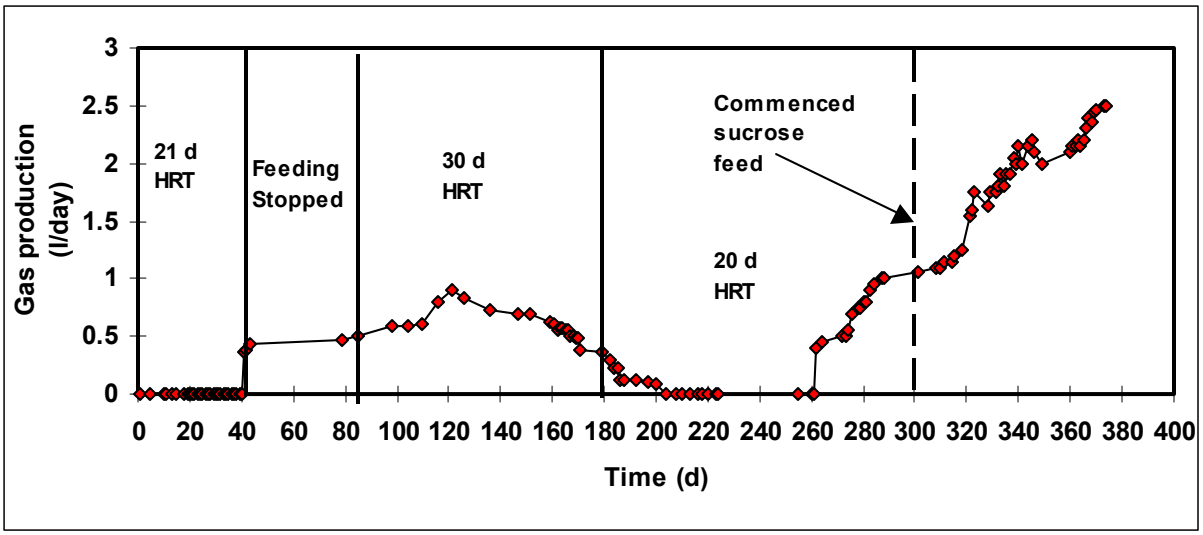

Figure 5-7: The gas production profile for the Control reactor.

Initially there was no gas production in the Control reactor. Gas production began on day 40 but the Control reactor produced only 0.5 *l*/day. Its gas production rate peaked at 0.9 *l*/day on day 120. This shows that although the CMARs were started at the same time and with the same seed sludge, the active biomass concentration of the Test reactor was higher than in the Control reactor. The Control reactor had a lapse in gas production from day 200 to day 260. The introduction of the sucrose feed on day 300 resulted in increased microbial activity in the reactor. This could be a result of the added trace metal nutrients. This reasoning must be treated with caution as the sucrose feed degrades rapidly to glucose and glucose is a soluble organic compound that is rapidly fermented to acetate and finally to carbon dioxide, hydrogen and methane. According to Eastman and Ferguson (1981) soluble substrates like glucose and fructose have a rapid fermentation step with minimum cell residence times of a few hours for acid-forming bacteria. For the Control reactor there was a high nitrogen content in the digester gas. The reasons for this were outlined previously. In general the gas production profile and the gas composition data (Figure 5-4 & 5-7) show that there was a constant change in the internal reactor conditions and microbial population dynamics.

5.2.2 Start-up of anaerobic baffled reactor

The Perspex rectangular reactor was inoculated with digested sludge from Umbilo Sewage Works (Durban, South Africa). Exactly 7.5 *l* of sludge was added to the reactor with the outlet sealed to prevent air contamination. One litre of the sucrose solution was added to the ABR. It was left to stand for 3 days to allow the biomass to settle. Thereafter, the sucrose solution was fed at a concentration of 4 gCOD/*l* and a hydraulic retention time of 60 h. Only the gas composition of each compartment was sampled and analysed. The pH rose above 7.5. The hydraulic retention time was then increased to 35.7 h. There was an increase in gas production as a result of the change. When the system stabilised at this hydraulic retention time, the HRT reduced to 20 h. This HRT was maintained until it achieved steady state again

Results for the Anaerobic Baffled Reactor (ABR)

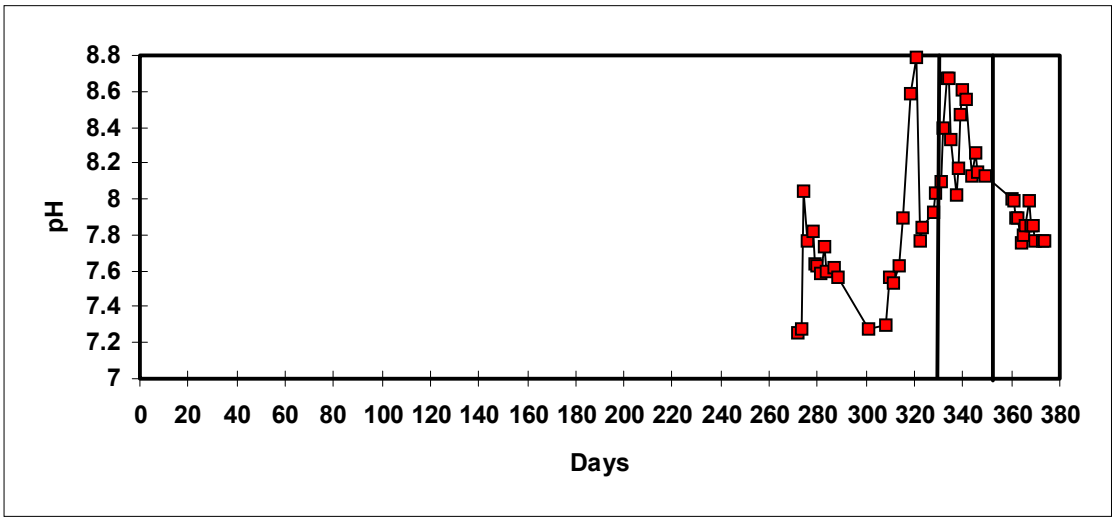

Figure 5-15: Plot of the variation of the effluent pH of the ABR during start-up.

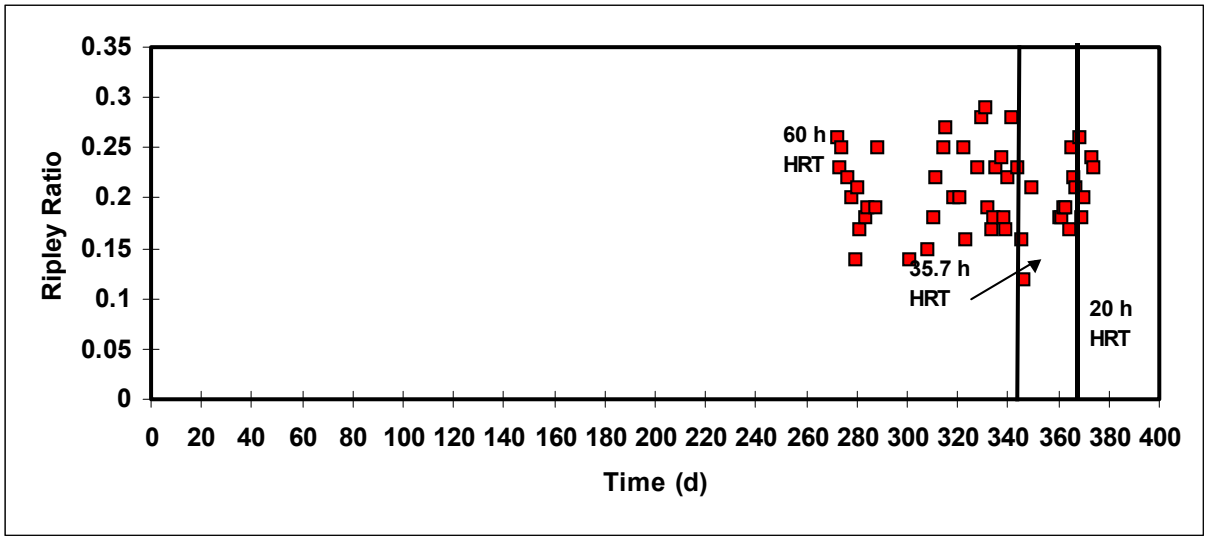

Figure 5-16: Plot of the variation of the Ripley Ratio from the effluent samples of the ABR during start-up.

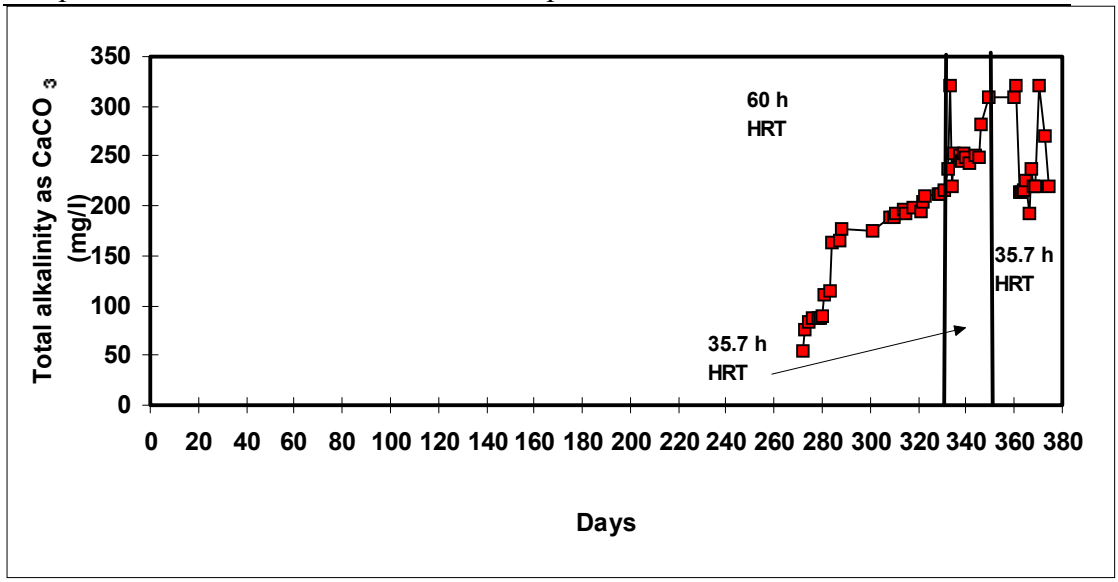

Figure 5-17: Plot of the variation of the total alkalinity of the ABR during start-up.

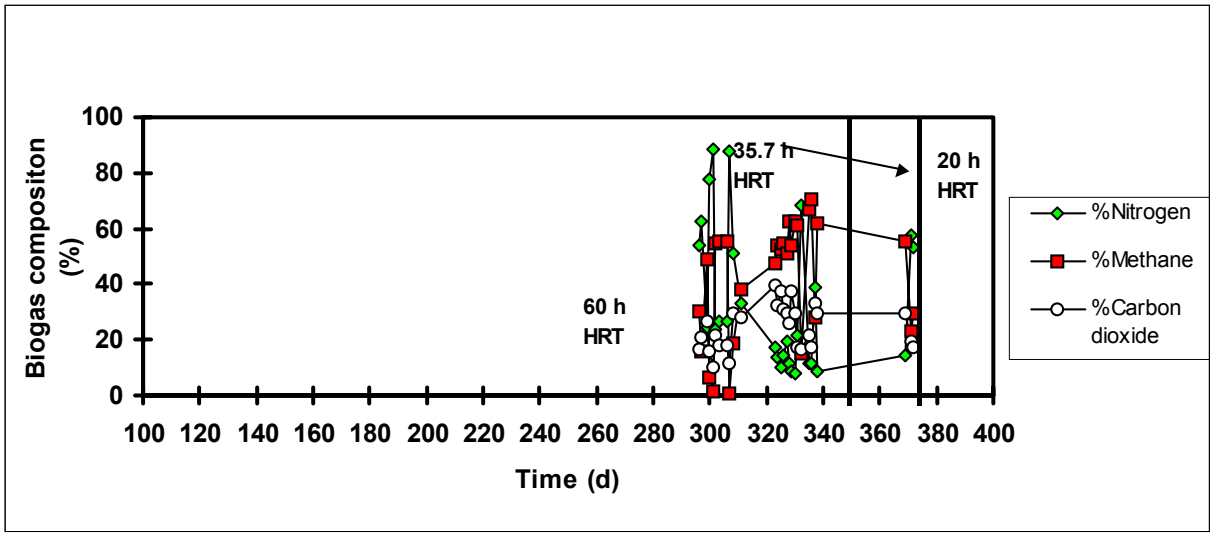

Figure 5-18: Gas composition variations of the ABR during start-up.

It can be seen (Figure 5-15) that the pH of the effluent of the ABR was always above 7.0. During some instances the pH rose to values above 8.0. The reasons for this are attributed to the fact that it was not possible to take the pH of the inner contents of the reactor. As there was no means to collect samples from within the reactor, effluent was collected from the outlet of the reactor. This meant there was a long period between collection of the sample and measurement of the pH of the sample. As a result the carbon dioxide in the effluent diffused into the atmosphere increasing the pH of the effluent from the ABR. The high pH values are also due to the highly buffered sucrose feed which is the same as the one used in the CMAR (Grobicki and Stuckey, 1991). The alkalinity of the ABR was initially at a value of 50 mg/*l*. The alkalinity of the effluent from the reactor increased sharply because the sucrose feed was heavily buffered and contained a high concentration of sodium bicarbonate. This means that due to the flow hydrodynamics of the ABR, which separates SRT from HRT, there was a gradual increase in the bicarbonate concentration in the reactor. When the loading rate was changed from 60 h HRT to 35.7 h HRT, there was a sharp increase in the effluent alkalinity. This is attributed to the increased mixing, as the flow rate through the baffled reactor was increased (Grobicki and Stuckey, 1991). The alkalinity was stable at around 250 mg/*l* for 30 days before there was an increase in alkalinity to 300 mg/*l*. This maybe due to the continued increase in the accumulation of the bicarbonate in the baffled reactor. On

changing to a loading rate of 20 h HRT there was a slight increase in alkalinity to 325 mg/*l*. The alkalinity then decreased to a minimum of 150 mg/*l*. This decrease in alkalinity is due to the fact that,

initially, the increase in the loading rate increased the mixing of each individual compartment. This coupled with the increased flow of the reactor means that more bicarbonate ions are lost from each compartment. With time the bicarbonate ions are now less in the reactor resulting in a lower effluent alkalinity. This reduced alkalinity is short lived as more bicarbonate ions are fed into the reactor and this coupled with the high retention capability of the reactor result in the re-accumulation of the bicarbonate ions.

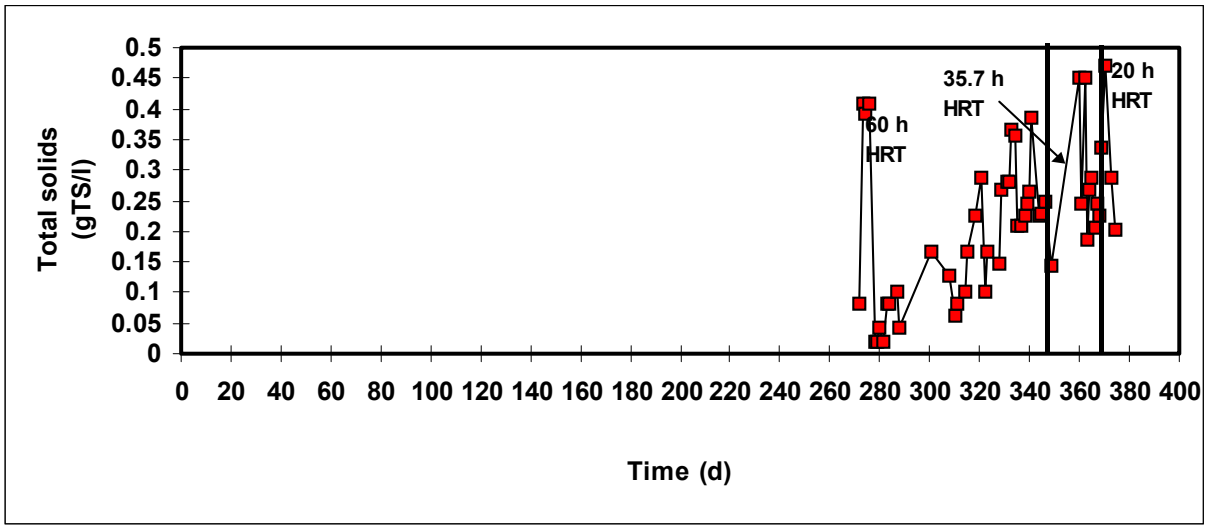

Figure 5-19 Plot of the variations in the volatile solids content with time of the effluent of the ABR.

The total solids from the ABR effluent were always below 0.5 gTS/*l*. This is to be expected as the ABR has a high solids retention time (SRT). The baffles together with the high local mixing in the individual chambers result in a high retention of the solids. Grobicki (1992) described the effluent from the ABR as being 'relatively free of solids' while Holt et al. (1997) found very low solids wash out with hybrid ABRs. Initially, there was a downward trend as the bacterial biomass is washed out. Once all the weakly attached biomass had been washed out there was a gradual increase in the total solids (TS) coming out of the reactor. It should also be remembered that at this stage it is unlikely that the biomass had granulated thoroughly to form stable beds. This increase occurs when the reactor is at steady state. This cycle of events occurred each time the HRT was reduced. It seems that the increased flow rate washes out particles with a settling velocity below the upflow velocity they experience. In addition, increased shear stresses cause more granules to fragment, creating more small particles to be washed out.

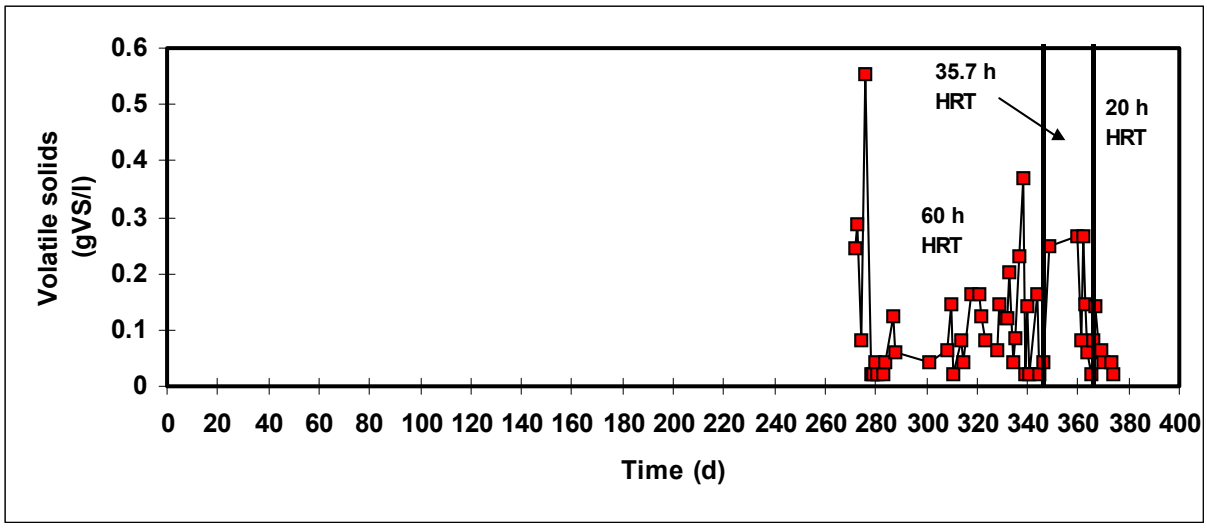

Figure 5-20: Plot of the variations in the volatile solids content with time of the effluent of the ABR.

The effluent from the ABR was found to have a volatile solids content of less than 0.3 gVS/*l*. As the sucrose feed is largely a soluble feed it is expected to pass through the ABR with little solids accumulation. This means that the effluent VS content will not represent a true picture of the biomass variation within the reactor. Another factor which makes VS measurements from the ABR with sucrose feed useless for biomass representation is that the quantitative composition of the sucrose feed varied daily as it had to be made up everyday.

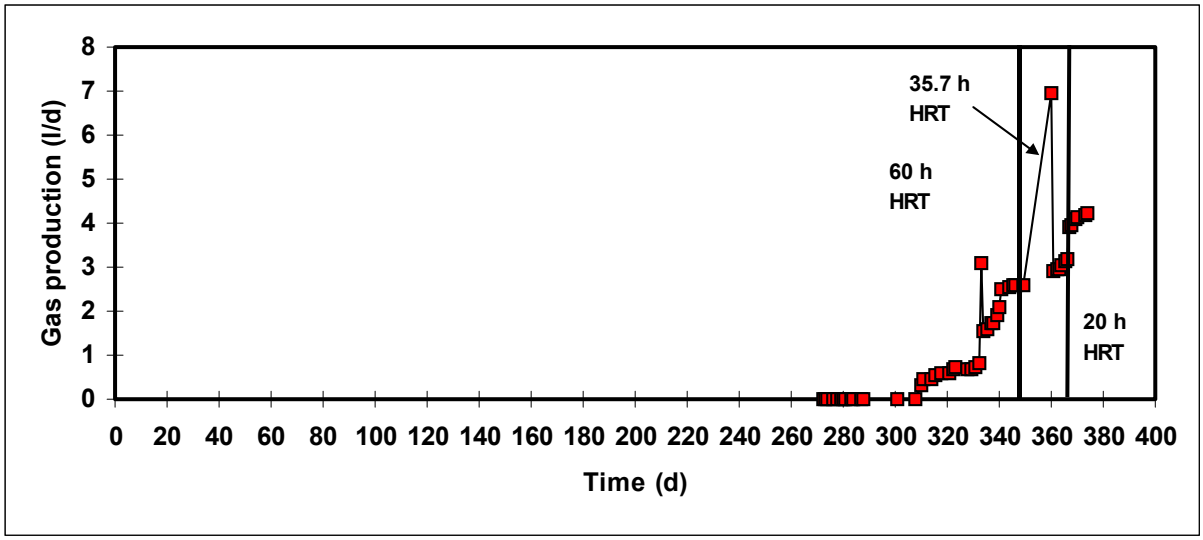

Figure 5-21: Gas production profile of the ABR.

As expected of any anaerobic reactor there was no gas production in the beginning. Since the bacteria had to acclimate to the complex feed. Grobicki and Stuckey (1991) also noticed this slow acclimatisation of mixed anaerobic cultures to this sucrose feed while undertaking steady state and shock loading analyses of the ABR. The reactor was started of at 60 h HRT. There was a general increase in the gas production to about 1.0 *l*/day. When the HRT was reduced to 35.7 h there was a sudden increase in the daily gas production to 3.0 *l*/day. Similarly, when the HRT was reduced to 20 h there was a sudden increase in the daily gas production to 6.95 *l*/day from a steady state value of about 2.5 *l*/day. These sudden increases in gas production are due to the increased mixing at the higher flow rate. Grobicki and Stuckey (1992) noticed that the movement of granules in the ABR resulted in gas bubbles travelling to the gas-liquid interface carrying a biomass granule and finally disengaging from it allowing it to drop back into the reactor. This means that as the biogas is attached to the biomass granules in the compartments that their increased movements results in shear forces that disengage the granules from the biomass without having to get to the gas-liquid interface. Many researchers have confirmed that the individual compartments are well mixed because of this movement of the gas bubbles (Holt et al, 1997; Grobicki and Stuckey 1991).

5.3 Conclusions

The following conclusions can be drawn from these results:

The accidental introduction of 0.5 M HCl acid into the reactors did upset the microbial balance necessary for anaerobic digestion. This was noticeable in that the pH value fell to values below the optimum of 7.0, the Ripley Ratio increased showing that the reactor was heading for upset and alkalinity decreased. The experiment to observe the necessity of lime addition to neutralise acidic conditions in the reactor was carried out with both reactors at the same HRTs, temperature and with raw sewage feed. The Control reactor was able to recover from this upset without the addition of chemicals. From its stability indicators it was found that the reduction of the feed rate into the reactor resulted in a gradual return to optimum conditions within the Control reactor. The addition of lime to the Test reactor did result in an increase in pH but when compared to the results for the Control reactor it was seen that the results had a similar trend. The addition of lime introduces –OH ions into the reactor and these are expected to react with the carbon dioxide from the acidogens to give bicarbonate ions. This will then increase the alkalinity of the reactor. Since at pH values around 7.0 only the concentration of bicarbonate ions is significant for alkalinity purposes. In the results for the alkalinity of the Test reactor it was found that the alkalinity did not increase with the addition of lime. This factor is also evident from the VA/ALK ratio results where the addition of lime did not result in any change in the ratio. The reason for this stem from the fact that at pH values near 7.0 carbon dioxide is most likely to be precipitated as calcium carbonate rather than as calcium bicarbonate.

It was found that:

i. It is not worthwhile using lime to maintain a pH above 6.5.
ii. The addition of lime did not result in an increase in alkalinity.

The strategy followed in the ABR, which involved the use of an initially long HRT which was subsequently reduced whilst maintaining a constant feed strength (4 gCOD/*l*) resulted in a short and stable start-up. The same strategy was used in the CMARs as there were changed from an HRT of 30 d to 20 d at steady state. The longer retention times encouraged the growth of hydrogen scavenging bacteria, which remained at quasi-steady state after the start-up period was over. These kept the hydrogen levels low ($pH_2<10^{-4}$) and so enabled the conversion of pyruvic acid to acetate. Since in Chapter 2, it was stated that the hydrogen levels determine the end product of acidogenesis. This subsequently determined whether there was a build up of short chain fatty acids and souring of the reactor or not. The long retention times meant a higher SRT which from the discussion in Chapter 3, means that the reactors were able to maintain the slow growing bacteria and allow them to multiply. This also promoted the biological acclimation to the sucrose feed. The graphical evidence from the results of the pH, RR, alkalinity and gas production chows that the strategy allowed the reactors to slowly move towards steady state. The ABR (160 days) had a shorter start-up period than the CMARs (240 days). It was not possible to conclusively say that the ABR had a shorter start-up time as the CMARs contained micro-organisms that had been inhibited by toxic chloride ions from the hydrochloric acid. It must be noted that the ABR has a HRT of 20 h compared to that of the CMAR (20 d) which means that it probably had a higher SRT than the CMAR resulting in it have better stability indicator graphs. Thus, from their start-up from these differing conditions the ABR was seen to have a more stable start-up operation. The significance of the graphical evidence is that it shows that for a steady and stable start-up of an anaerobic reactor,

i. the reactor must start off at a longer retention time to allow the bacteria to acclimate to the feed. This also aids bacterial integration and reduction of biomass wash-out.
ii. separation of the HRT from the SRT leads to higher SRTs which result in more efficient start-ups.

In the ABR, the lowering of the HRT and the resultant channelling caused more washout of biomass as compared to the CMARs that did not show any increase in their TS as the HRT was lowered. The introduction of the more soluble sucrose feed (2.5 gTS/*l*) resulted in a lower TS effluent value. According to Ross *et al*. (1992) the TS content of the feed to a CMAR should be above 4 gTS/*l*. However, with the sucrose feed the CMAR still managed to produce the same volume of methane as

the raw sewage sludge at the same HRT. By adding the necessary nutrients for catabolic, anabolic metabolism to the feed, it was possible to operate the CMARs with the soluble feed. Furthermore, it was noticed that the introduction of the synthetic feed increased the activity of the anaerobic bacteria in the CMARs (Figures 5-7 & 5-12). The presence of the di-potassium orthophosphate buffer and the sodium hydrogen carbonate in the feed solution resulted in an increase in pH and alkalinity making the sucrose feed even better at starting-up the upset reactors. The soluble feed was better for the ABR because the baffles and high mixing in the ABR result in a high SRT which makes feeding substrates with a high TS content difficult. The sucrose feed was found to be a better feed for the ABR but did not help with the performance of the CMAR as it resulted in a lower biomass concentration within the reactor. The solids content results showed that the ABR is a reactor that separates the SRT from HRT, as the solid content of the ABR was relatively free of solids. The study also showed that the effluent results of the ABR could not be used to predict the internal biomass composition of the ABR. This is because the ABR is divided into compartments and also its separation of HRT and SRT means that the biomass is left inside the reactor. This means the effluent quality of the two reactors was compared rather than the variation of the bacterial biomass during start-up. This showed that the ABR effluent had less solid matter as it had a TS content of less than 0.5 gTS/*l* as compared to that of the CMAR which averaged 0.8 gTS/*l*. The ABR effluent was seen to have less biomass as it had a lower VS content than that for the CMAR.

Chapter 6 Organic loading tests

The ABR and the CMAR are reactors that are dissimilar in two respects. Firstly the ABR has a series of vertical baffles that enable it to increase the SRT/HRT ratio. This increases its process stability and economics (Speece, 1983). A double pedal stirrer is used to mix the contents of the CMAR and it has no solids recycle making the SRT/HRT ratio one. This fundamental difference means that the ABR has a lesser HRT and therefore a smaller volume (7,5 *l*) than the CMAR (20 *l*). This also means that the CMAR is limited by the doubling times of the anaerobic bacteria, which is 3-5 days at mesophilic temperatures. For practical control and reliable treatment, the minimum allowable SRT or HRT is 10-30 days for the CMAR (McCarty, 1964). In this study, a HRT of 20 days was used for the CMARs. The ABR, on the other hand, can be operated efficiently at a HRT of 20 h. The ABR has a higher SRT than its HRT since the solid fraction of the influent wastewater is entrapped by the baffles and moves with the biomass at a slower rate than the liquid fraction. From this discussion it is obvious that it is not possible to operate the two reactors at the same HRT. Operating the ABR at HRT 20 days would remove its major advantage over the CMAR. As a result the two reactors where initially allowed to attain the same COD removal efficiency before any tests were commenced.

Table 6-1: The literature values of COD removal values attained by CMARs.

Type of waste	COD removal (%)	Reference
Penicillin broth	75	Heukelekian, 1949
raw sewage sludge	57	Li et al, 1996

Table 6-2: The literature values of COD removal values attained by ABRs.

Type of waste	COD removal (%)	Reference
Carbohydrate-protein	95	Grobicki and Stuckey, 1991
Glucose	72-99	Bae et al., 1997
Phenolic	83-94	Holt et al., 1997
Slaughterhouse wastewater	75-90	Polprasert et al., 1992
Molasses wastewater	70	Boopathy and Tilche, 1992

From the results in Tables 6-1 and 6-2 it is clear that the CMAR does not have the same removal rate as the ABR at steady state. In this study, the CMAR was first brought to a COD removal of about 62 % at steady state and then the ABR was allowed to attain the same COD removal efficiency.

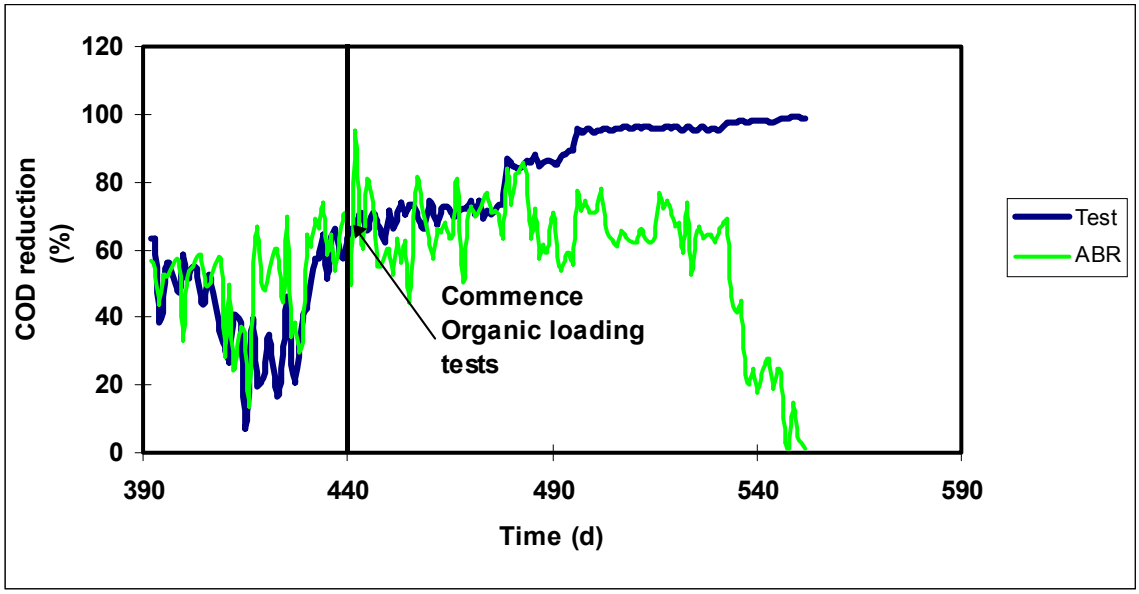

Figure 6-1: Comparison of the COD reduction for the Test (CMAR) and ABR during the tests with synthetic sucrose substrate.

From Figure 6-1 it can be seen that the two reactors had the same COD reduction on day 440. This is when the organic loading tests were begun. It is also noticeable that there were times before day 440 when the two reactors had the same COD reduction but it must be born in mind that at these instances the two reactors had not attained steady state. Besides, the ABR still had a COD reduction of less than 60 % which is below the predicted steady state COD reduction of the CMAR of about 57-75 % (Table 6-1).

6.1 Results for the Completely Mixed Anaerobic Control reactor

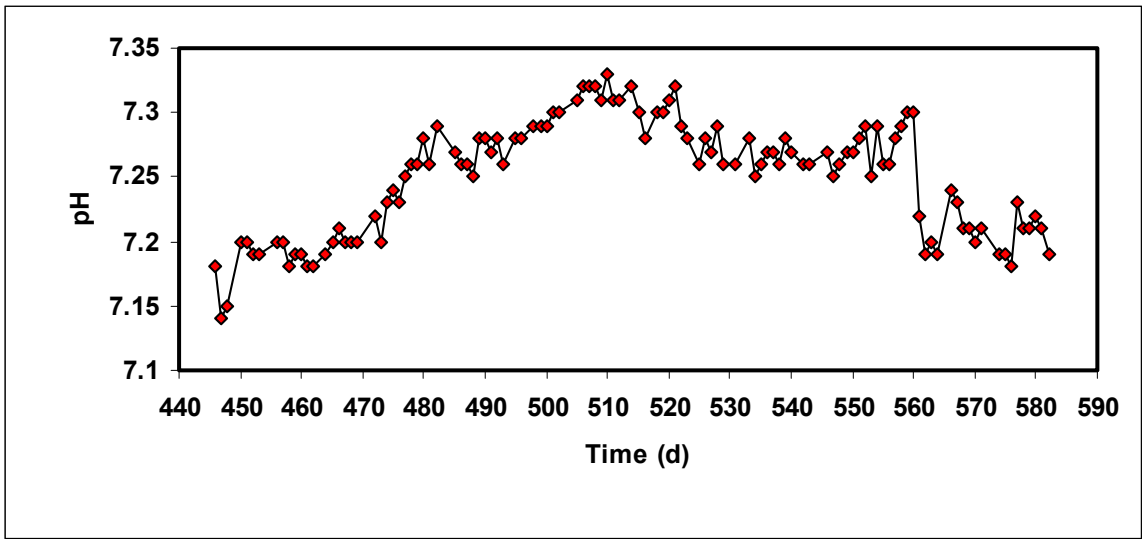

Figure 6-2: Plot of the variation in the effluent pH with time of the Control (CMAR).

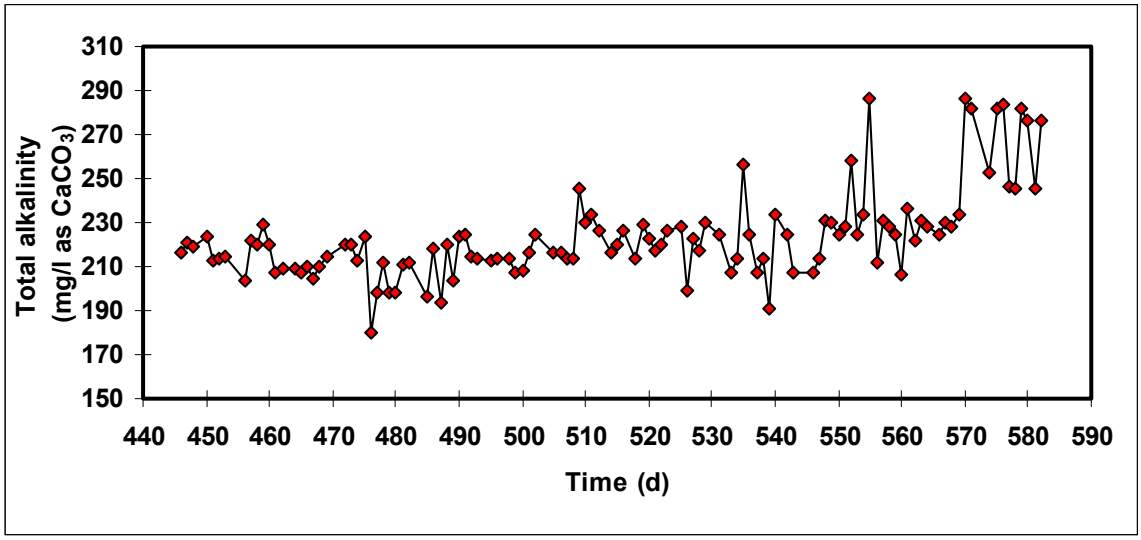

Figure 6-3: Plot of the variation in the total alkalinity of the effluent with time of the Control (CMAR).

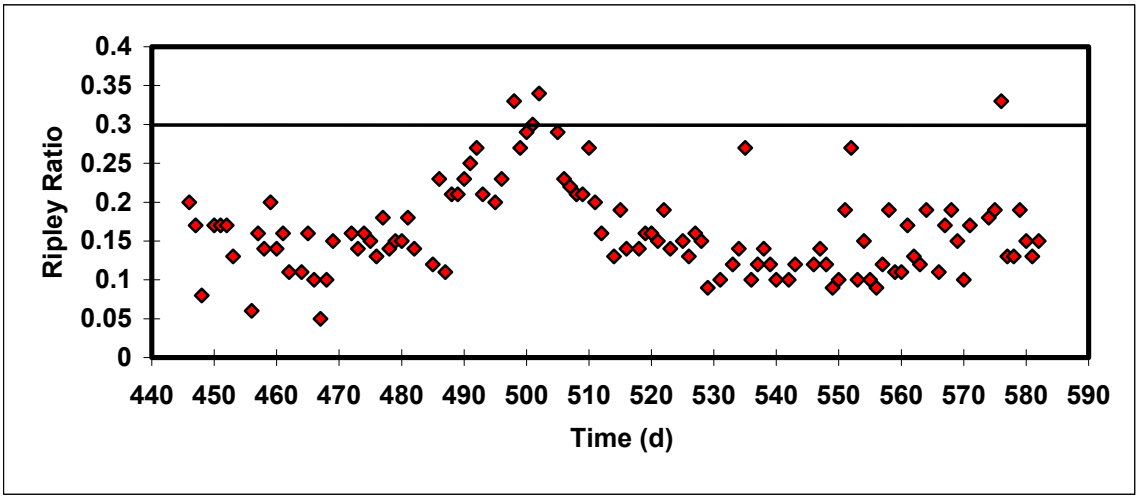

Figure 6-4: Plot of the variation if Ripley Ratio with time of the Control (CMAR).

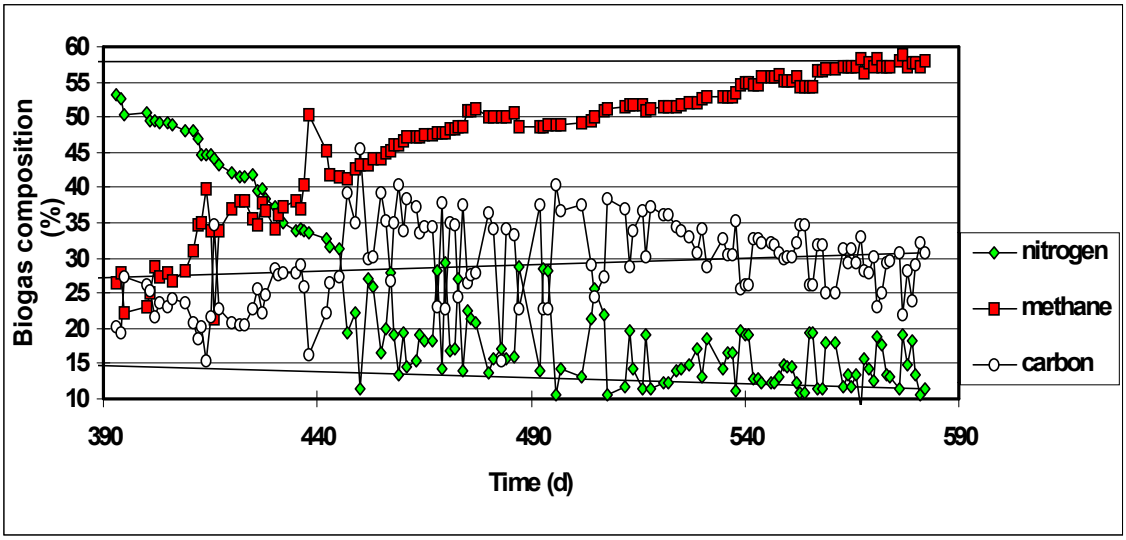

Figure 6-5: Plot of the variation of the gas composition profile for the Control (CMAR).

The pH of the control reactor was always above 7 due to the high buffering in the feed. Grobicki and Stuckey (1991) observed the same trend using a similar feed. This is well within the region for effective anaerobic digestion (Eastman and Ferguson, 1981; McCarty and Mosey, 1991). From figure 6-2, there is a increase in pH during the first days. After this the pH stabilised at a value 7.25 and 7.35. Eventually the pH dropped to a value around 7.2. This shows that the contents of the reactor had a fairly stable environment as the reactor was operating at steady state. Caution must be exercised when using pH as a stability indicator because of it log derivation c.f. Chapter 4, section 4.3.1. However, figure 6-3 shows that the alkalinity of the reactor was stable confirming the steady state assumption. The Ripley's Ratio (RR) of the Control reactor was generally below 0.3, which is indicative of a well functioning anaerobic reactor. This means that despite the generally agreed view that the CMAR should have a feed with a total solids content > 5 gTS/*l* the reactor will still be able to function on a feed with the necessary constituents for growth. After day 480 the Volatile acid (VA)/alkalinity ratio increased sharply to 0.30. This resulted from the fact that there was an increase in the acidogenic population, which are the main producers of carbon dioxide and volatile acids. From the gas composition graph (figure 6-5), it is seen that the carbon dioxide content had risen to about 45% on day 450, initially. Although the RR finally stabilised below 0.2 the effect of the increase in carbon dioxide is noticeable in the drop in pH on Day 560 on figure 6-2 . Generally, the RR indicated that the Control reactor was fairly stable. Initially the Control reactor had a high nitrogen content and very low methane and carbon dioxide contents. This had been noticed previously on figure when the reactor was started-up. From the decrease in the nitrogen content in figure 6-5 it becomes apparent that the high nitrogen

content was probably due to the internal biological population of the reactor. This makes it less likely to be due to air contamination as the nitrogen content decreased to values of about 10 % which are in agreement with that found by other researchers (Lettinga *et al*., 1983; Polprasert *et al*., 1992). As the reactor achieved steady state the methane content rose to an average value of ca. 52 % CH_4. The carbon dioxide content rose from a value of 25 % at unsteady state to an average value of about 30 % CO_2. The nitrogen content at steady state was found to be 12 % N_2.

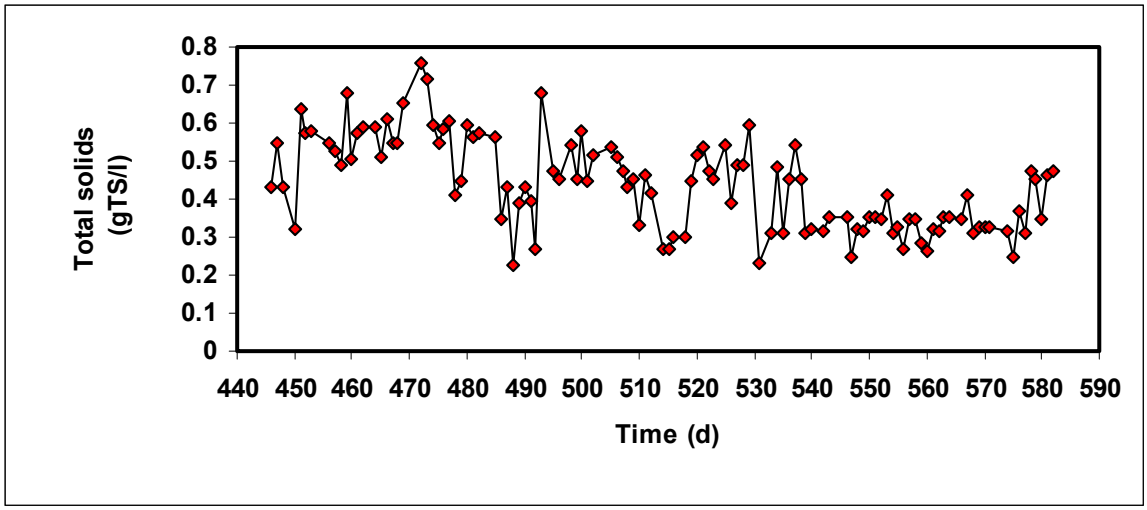

Figure 6-6: Plot of the variation of the TS content for the effluent of the Control (CMAR).

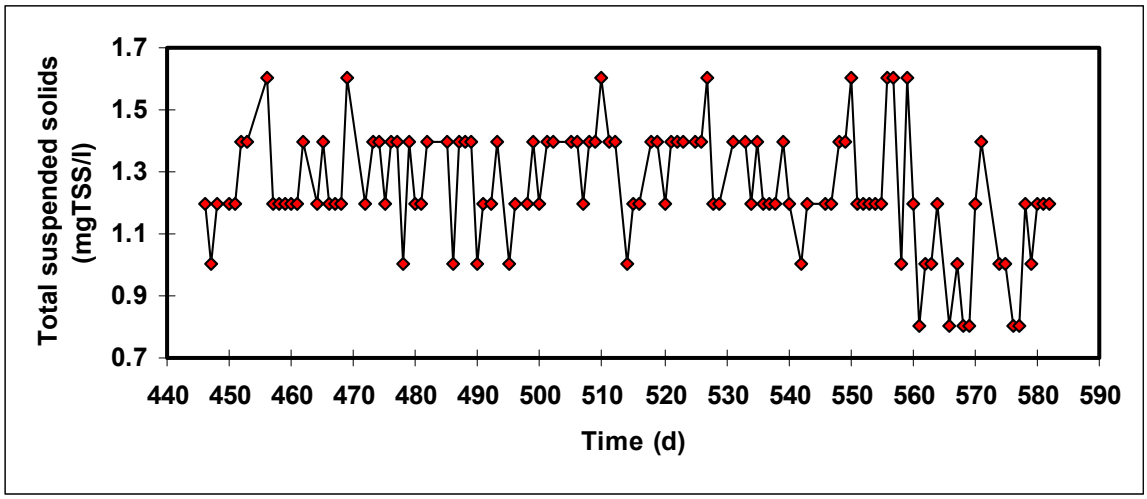

Figure 6-7: Plot of the variation of the TSS content for the effluent of the Control (CMAR).

The biomass concentration of the Control reactor decreased from a TS content of ca 0.6 gTS/*l* to 0.35 gTS/*l*. *T*his is due to the fact that, although, the bacteria were at steady state they continue to acclimatise to the feed and so digest it more efficiently with time. This value of TS is rather too low for a CMAR as the value is usually >30 gTS/*l* (Li et al., 1997). The reason for this is that the sucrose feed used had a low TS of about 2.55 gTS/*l* which diluted the reactor constituents. It is advisable to have a feed with a total solid content > 5 gT/*l*, the sucrose feed had all the nutrients necessary for anaerobic bacterial growth making it suitable for use even though it had a lower TS content.. The profile of the TS plot shows that the contents of the reactor were fairly stable. Figure 6-4 shows that the total suspended solids (TSS) was very low compared to the TS. The average value for the TSS was 1.25 mgTSS/*l*. *T*his suggests that the effluent of the CMAR had become diluted by the sucrose feed, which had a low total solids content.

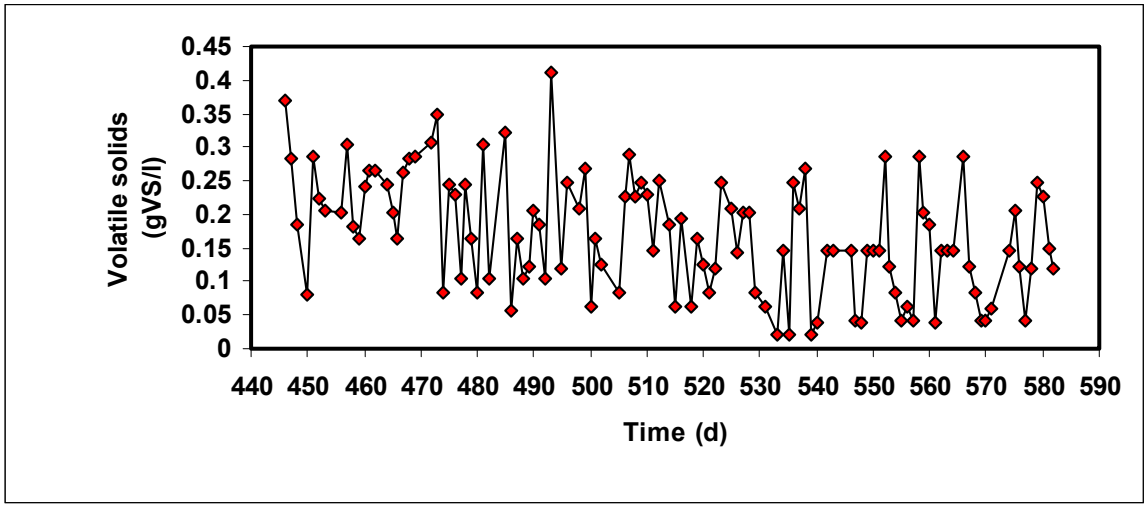

Figure 6-8: Plot of the variation of the volatile solids content for the effluent of the Control (CMAR).

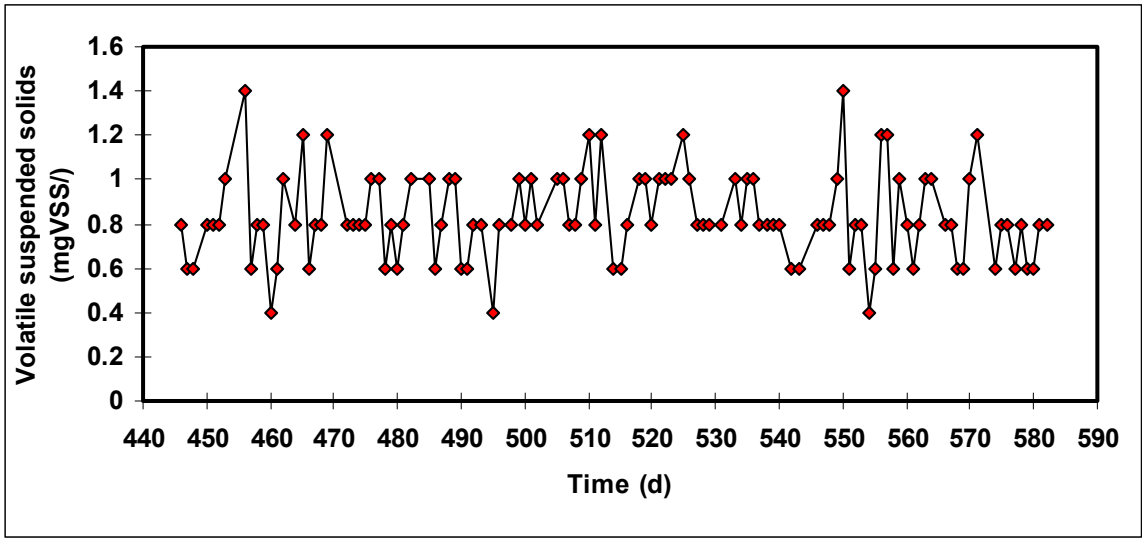

Figure 6-9: Plot of the variation of the volatile suspended solids content for the effluent of the Control (CMAR).

The VS graph profile shows that the amount of volatile solids was stable. The value of the VS was between 0.08-0.4 gVS/*l* with an average of 0.17 gVS/*l*. The VSS had an average value of 0.83 gVSS/*l*. *T*he difference between the two values shows that the reactor had a very low biomass concentration. The reactor was assumed to be at steady state as the biomass concentration is seen to be constant over 15 HRTs. Given the slow adaptation of mixed anaerobic cultures to change coupled with the influent sucrose feed concentration, this is only a quasi-steady state (Grobicki and Stuckey, 1991).

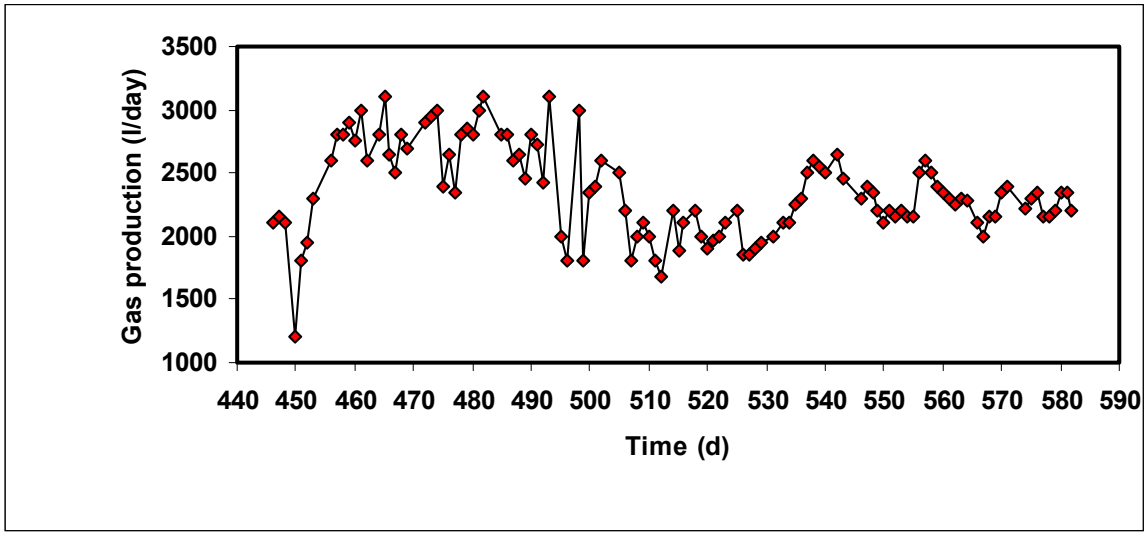

Figure 6-10: Plot of the variation with time of the gas production for the Control reactor (CMAR).

Initially the gas production was as low as 1.0 *l*/day. The amount of gas produced rose to steeply to average at a value of about 3.0 *l*/day. There was a slight decrease in the average gas production to about 2.25 *l*/day. The average gas production during this period was 2.35 *l*/day. From the gas production it could be seen that the Control reactor was at steady state. It was also noticeable that even when the reactor was at steady state the internal microbial environment was not stagnant but is continually changing as the gas production rate varies from day to day. This coupled with the size of a 20 *l* reactor vessel accounts for the varying average gas production at quasi-steady state.

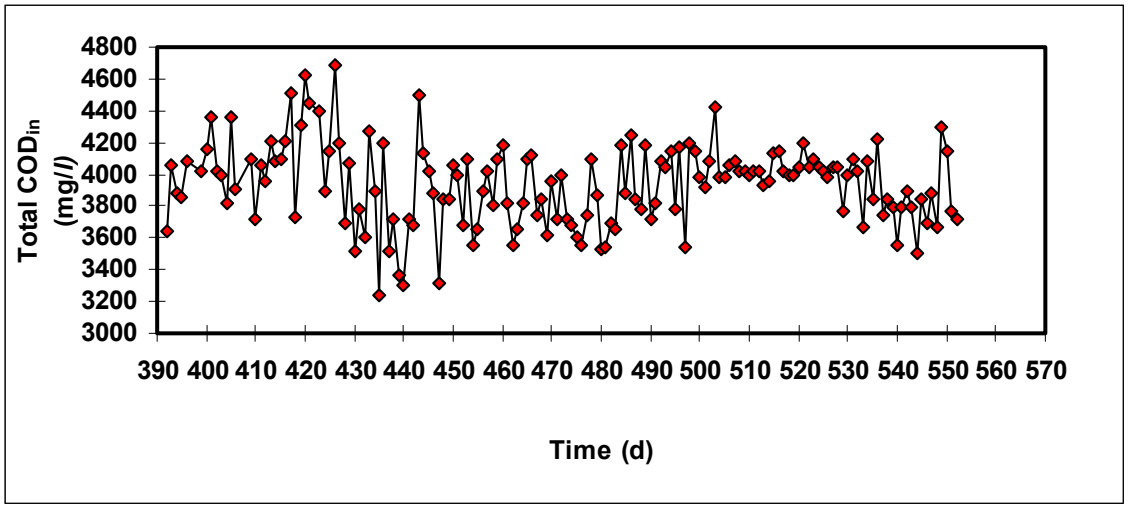

Figure 6-11: Plot of the variation with time in the total COD of the feed of the Control reactor (CMAR).

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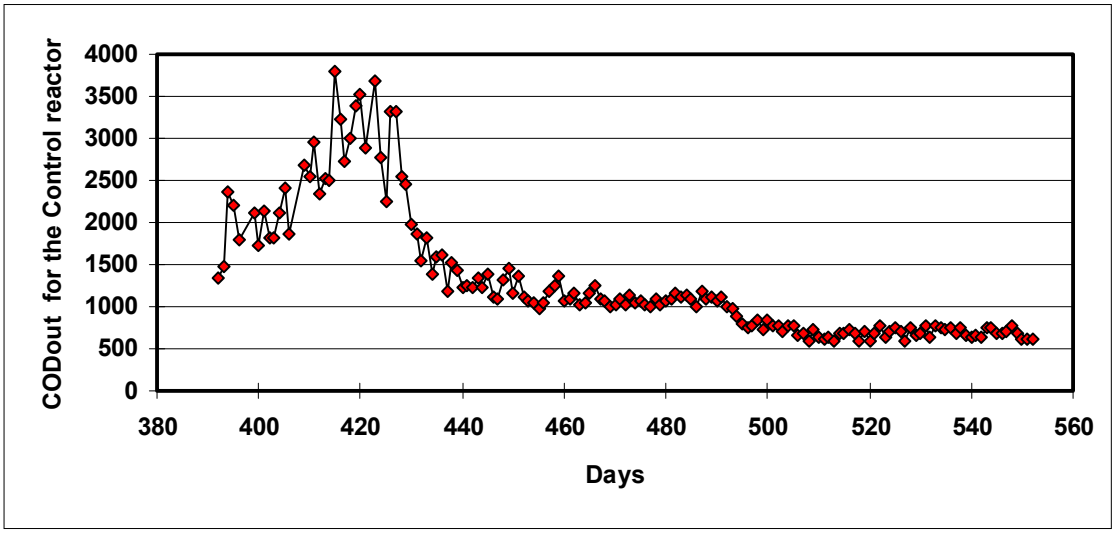

Figure 6-12: Plot of the variation with time in the total COD of the effluent of the Control reactor (CMAR).

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Figure 6-11 shows that the influent to the Control CMAR did not have a constant value. The average influent COD concentration (COD_{in}) for the for the Control reactor was 3935 ± 255 mg/*l* . This explains why the various parameters of the Control reactor where never stable even though it was at steady state. From figure 6-12, the reactor initially had an effluent COD of about 3500 mg/*l*. As it approached steady state the effluent COD concentration decreased to a value of about 550 mg/*l*. The fairly stable value of the effluent COD concentation (COD_{out}) best indicates that the biomass in the CMAR was fairly constant.. This is because the COD measurements provide a better characterisation of the organic component of a wastewater as they involve the oxidation of the organic component of the wastewater only.

6.2 Results of the Completely Mixed Anaerobic Test reactor.

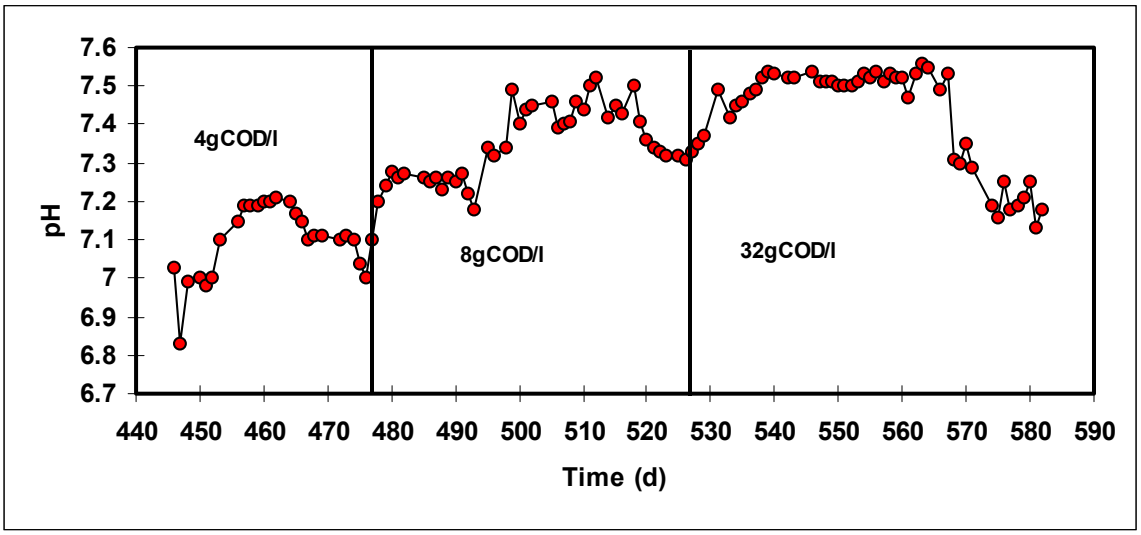

Figure 6-13: Plot of the variation in the pH of the effluent of the Test (CMAR).

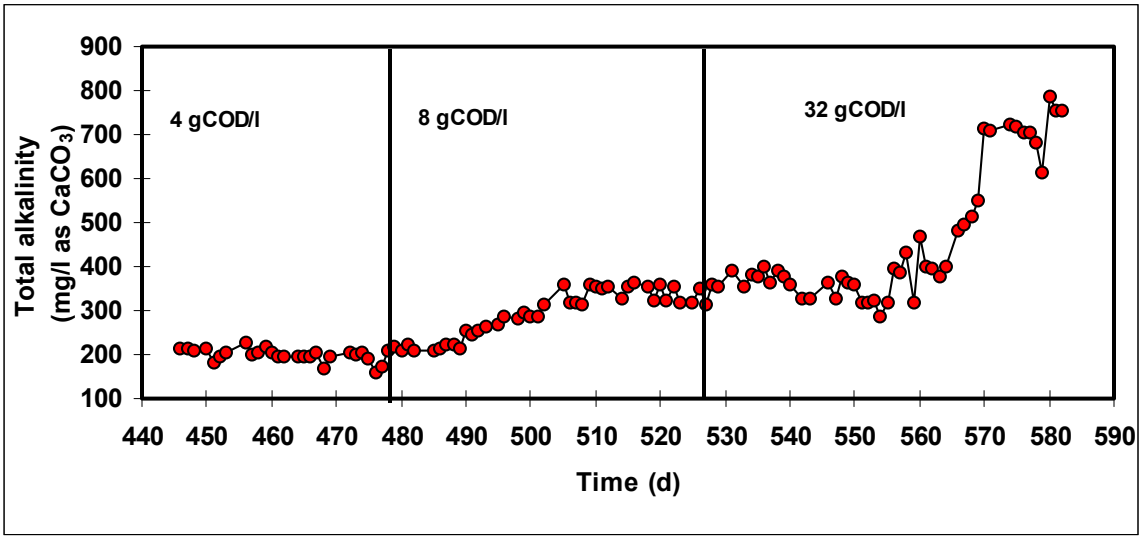

Figure 6-14: Plot of the variation in the total alkalinity of the effluent of the Test (CMAR).

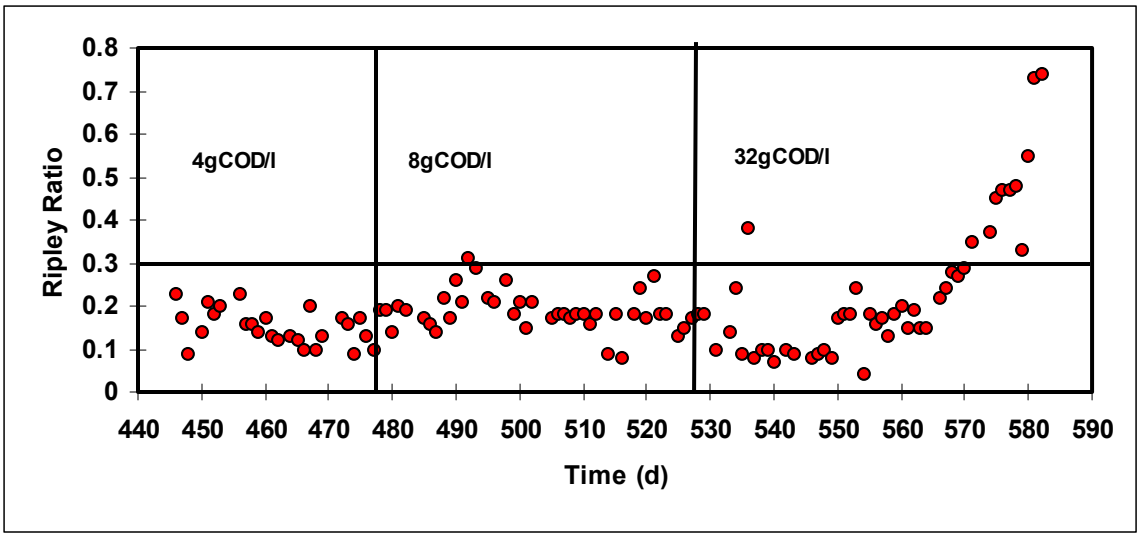

Figure 6-15: Plot of the variation in the Ripley Ratio with time of the Test (CMAR).

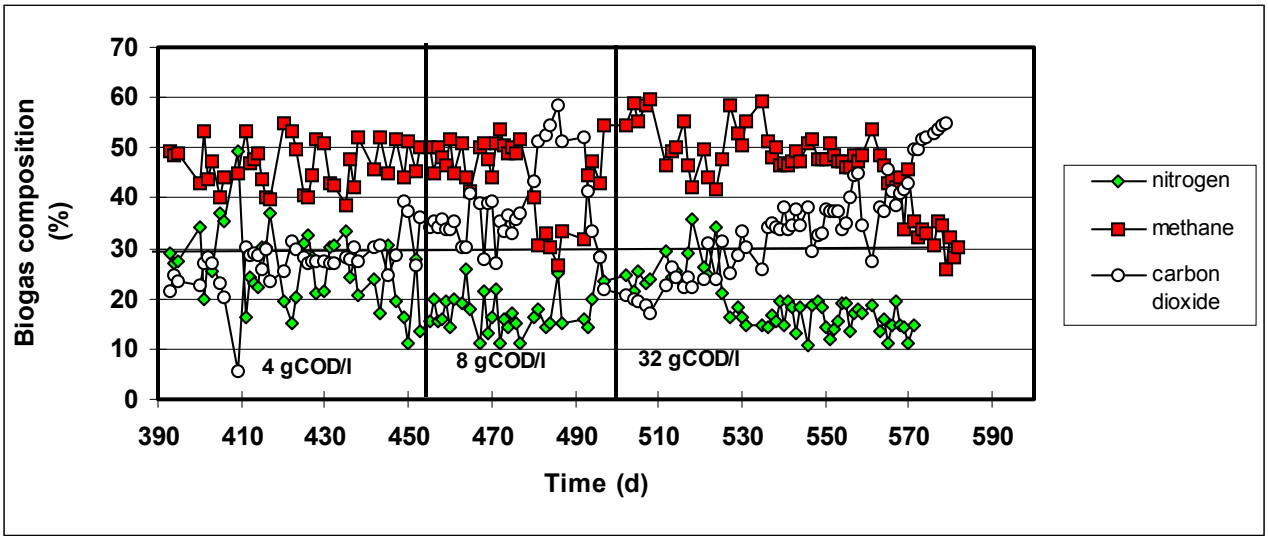

Figure 6-16: Plot of the variation of the gas composition with time of the Test (CMAR).

At a feed concentration of 4 gCOD/*l* the pH increased from about 7.0 to stabilise at about pH 7.2. The reactor achieved steady state with a Ripley Ratio below 0.3. Increasing the feed strength concentration to 8 gCOD/*l* led to an initial reduction in pH to 7.0. This was followed by an increase to 7.3 and then eventually stabilised at 7.5. From Figure 6-13 it can be seen that the decrease in pH occurred at a time when there was an increase in the carbon dioxide content of the reactor. This reduction in pH is due to the fact that the increased organic load (8 gCOD/*l*) resulted in an increase in the proportion of acidogens in the reactor. From figure 6-14 it is noticeable that there is an increase in the alkalinity of the reactor resulting in the organic overload in the reactor. There is an increase in pH to 7.5. This upward trend in pH is due to the increase in alkalinity in the reactor as seen in figure 6-14. With the feed concentration at 8 gCOD/*l*, the RR rose until it was at 0.31. This increase is due to the increased volatile acids but as the alkalinity of the reactor increased the RR also decreased to about 0.2. The decrease in RR continued to values around 0.1 showing that had the reactor continued operating at a feed concentration of 8 gCOD/*l* it would have improved its operating efficiency. When the feed concentration was changed to 32 gCOD/*l* the pH dropped to 7.3. There was again an increase in carbon dioxide from biogas composition (figure 6-15) and the pH rose to 7.5 again. This increase is partly due to the increased alkalinity as seen from figure 6-14 and also

due to the fact that the bacteria have acclimated to the sucrose feed and so are able to recover from an acid overload more quickly. From the gas composition, it seen that there is an increase in carbon dioxide in the reactor and a decrease in methane content. This is a sign of the increase in acidogens and acetogens, which produce carbon dioxide as they form acetate. From the RR figure 6-15, it is seen that there is an increase in volatile acids confirming this increase in acidogenic bacteria. Mosey (1982) and Wentzel (1987) found that the pH eventually fell to values below 6.5 where there is no more production of acetate. They assume that the methanogens, now only produce methane from carbon reduction and there is only a small population of methanogens in the reactor. So eventually the reactor stops producing methane and it fails. When the feed concentration was increased to 32 gCOD/*l* the RR rose to 0.7 showing that the reactor was heading towards upset as the ratio shows the amount of volatile acids exceeded the alkalinity level that is able to buffer them sufficiently. From the gas composition graph (figure 6-5) the level of carbon dioxide is now > 50 % while the methane content of the digester gas has dropped to < 30 %. Bae et al. (1997) also found a similar trend in the methane content while carrying out organic loading tests on a UASB.

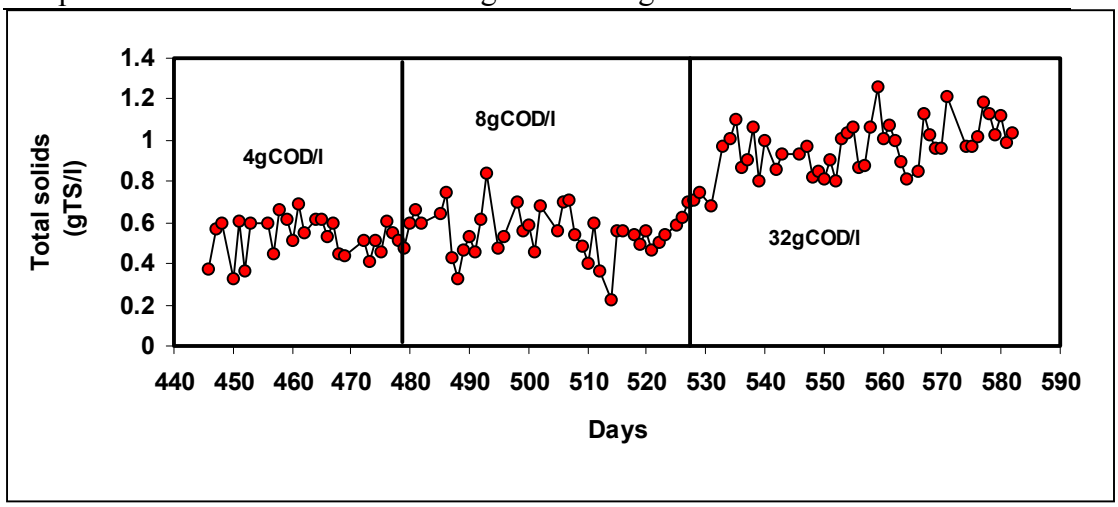

Figure 6-17: Plot of the variation in the total solids with time of the effluent of the Test (CMAR).

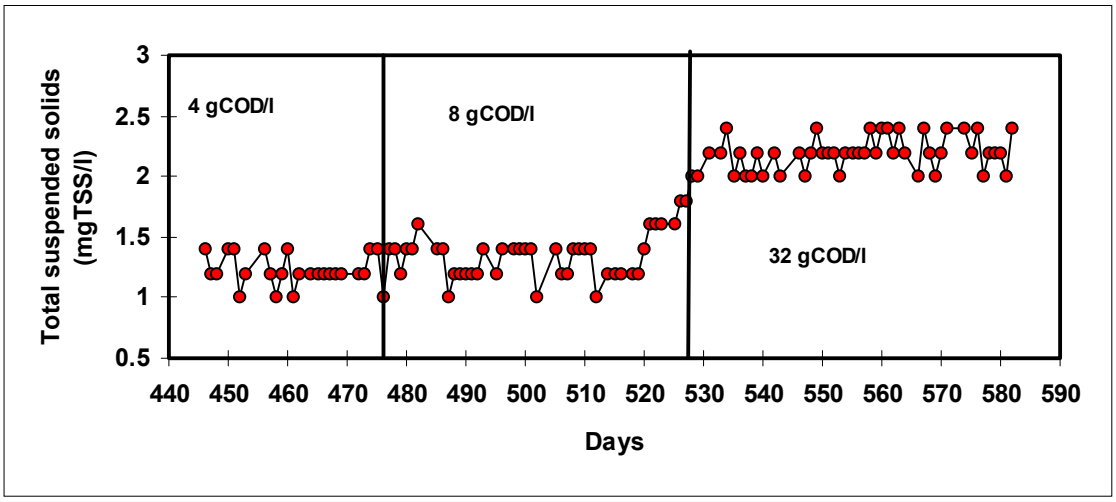

Figure 6-18: Plot of the variation in the TSS with time for the effluent of the Test (CMAR).

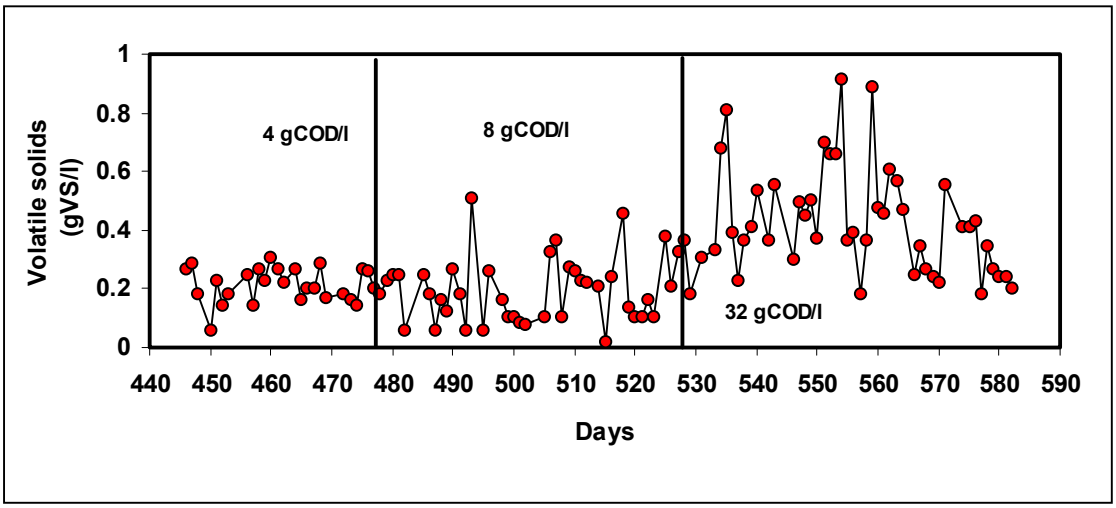

Figure 6-19: Plot of the variation in the volatile solids with time of the effluent of the Test (CMAR).

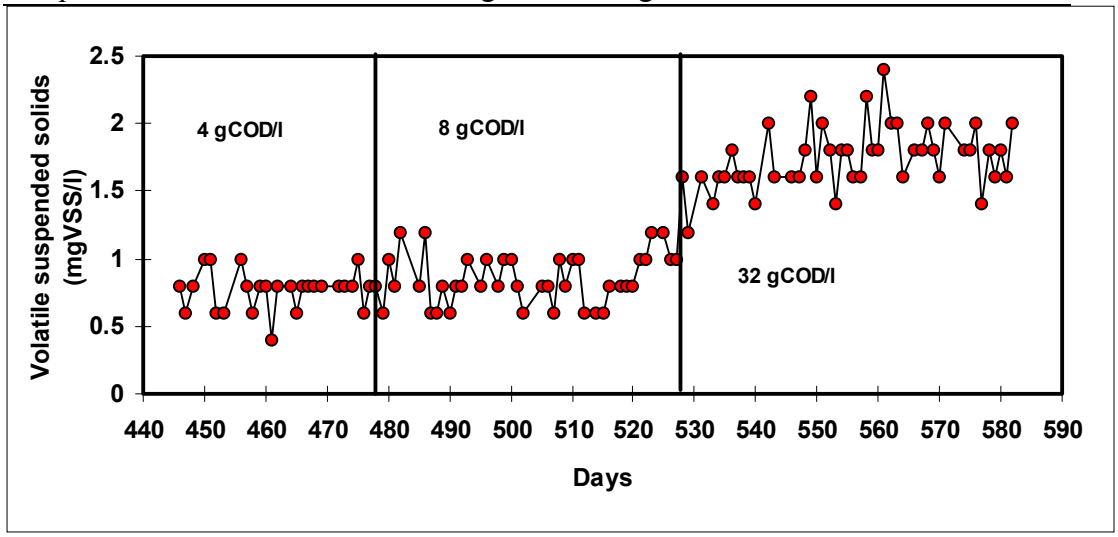

Figure 6-20: Plot of the variation in the VSS with time of the effluent of the Test (CMAR).

The total solids (TS) of the reactor remained stable when the feed concentration was increased from 4 gCOD/*l* to 8 gCOD/*l*. As anaerobic digestion is a very slow process, there is a delayed response to the effect of the increased feed concentration. Initially the TS averaged 0.54 gTS/*l* after an increase in the feed concentration the average TS content was 0.98g TS/*l*. The same effect is seen with the TSS, which had an average of 1.27 mgTSS/l. This rose to 2.2 mgTSS/*l* after the sucrose feed concentration was raised to 8 gCOD/*l*. The volatile solids (VS) also showed a similar trend to the TS. The average VS content of the reactor was about 0.2 gVS/*l* at a feed concentration of 4 gCOD/*l*. On increasing the feed concentration to 8 gCOD/*l* the VS content doubled to 0.44 gVS/*l*. The VSS content for the reactor initially had an average of 0.8 gVSS/*l* and this doubled to an average of 1.76 gVSS/*l*. This suggests that as the amount of substrate for growth was increased. There was an increase in the bacterial biomass since there were more nutrients available for growth. Changing the feed to 32 gCOD/*l* did not bring about a change in the TS content as the reactor was now heading towards upset. The bacteria were no longer reproducing at the same rate since the balance between the non-methanogenic bacteria and the methanogenic bacteria was upset due to the overload (Mosey, 1981).

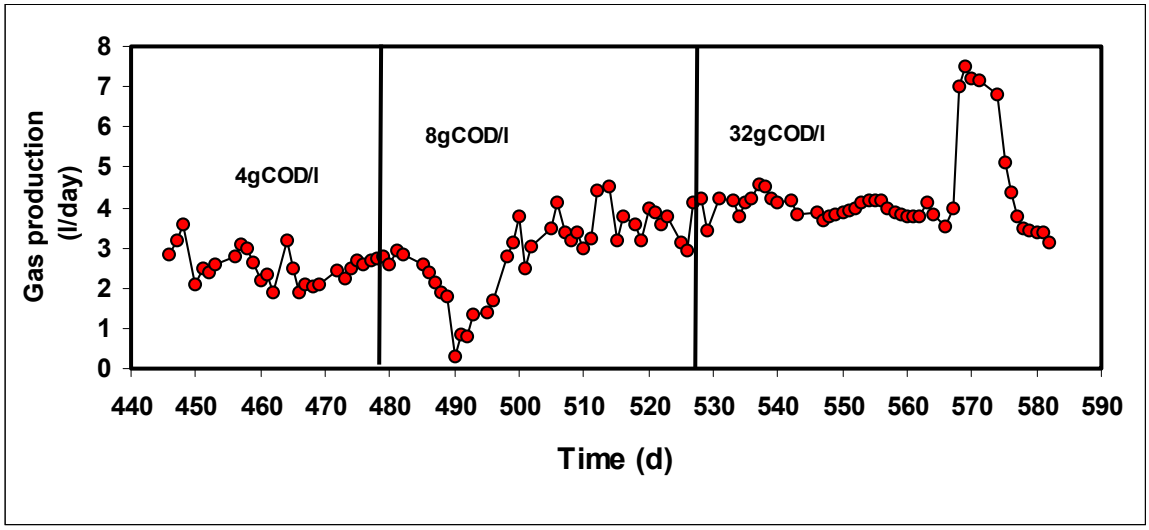

Figure 6-21: Plot of the variation in the gas production of the Test (CMAR) with time.

The gas production at a feed concentration of 4 gCOD/*l* averaged 2.5 *l*/day. When the feed concentration was increased to 8 gCOD/*l* there was decrease in gas production to below 1.0 *l*/day. This is due to the increased organic load resulting in an increase in volatile acids as seen from figure 6-15 of the Ripley Ratio. A similar trend was not observed when the organic load was quadrupled because the bacteria had acclimated to the sucrose feed and there was a better balance between the non-methanogenic and methanogenic species. The gas production did eventually increase to average around 4.0 *l*/day. When the feed concentration was increased to 32 gCOD/*l* there was no decrease in gas production although there was an increase in the RR because carbon dioxide production increased as can be seen from figure 6-16. There was an increase in gas production on day 567 to 7.5 *l*/day. This is a result of the increased feed concentration and carbon dioxide production. Most of the gas produced after this was carbon dioxide indicating that the reactor was heading towards process failure (figure 6-16).

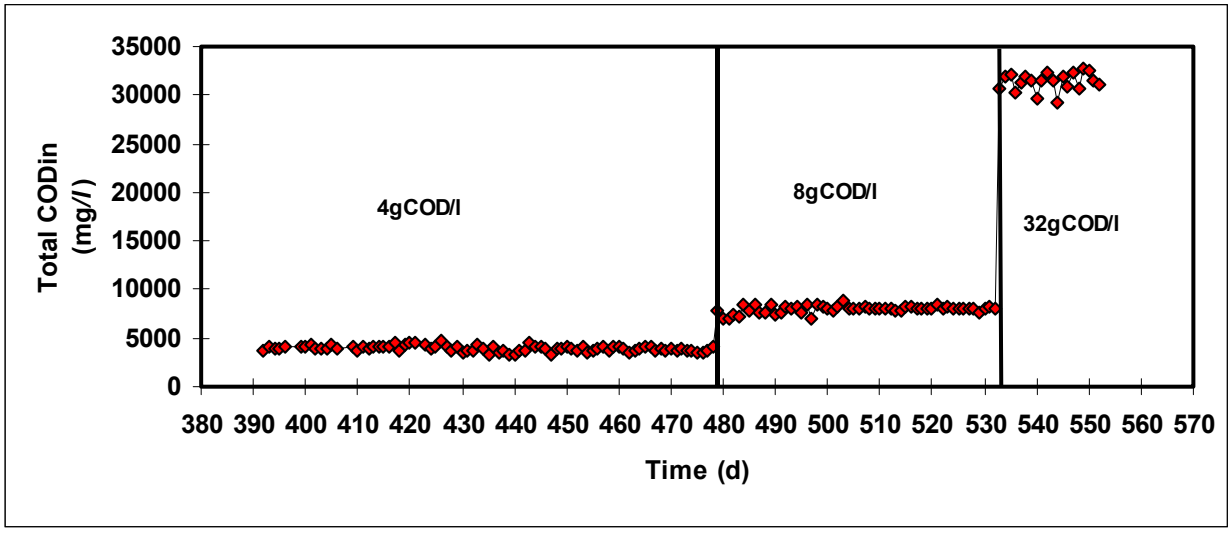

Figure 6-22: Plot of the variation in the total COD of the feed of the Test (CMAR).

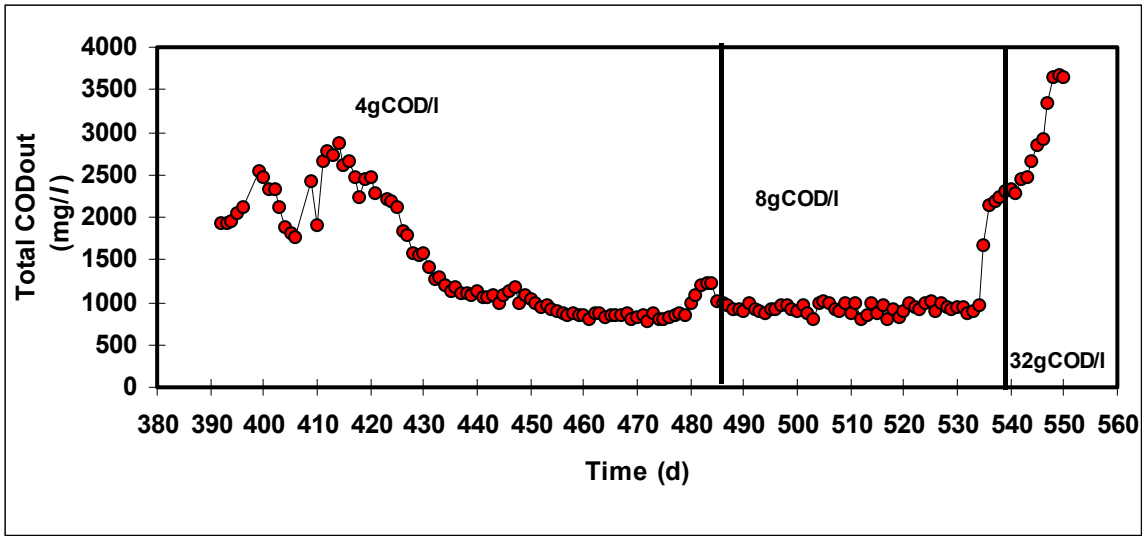

Figure 6-23: Plot of the variation in the total COD of the effluent of the Test (CMAR) with time.

At a feed concentration of 4 gCOD/*l* the reactor achieved a steady state effluent concentration of about 900 mg/*l*. This is a COD reduction of about 70%. When the feed strength was increased to 8 gCOD/*l* the effluent COD concentration remained at about 900 mg/*l* and as a result the COD reduction rose to about 90%. This is because with the CMAR the SRT/HRT ratio is one. This means that the effluent and the reactor contents will eventually have the same COD concentration. Considering that the reactor has a HRT of 20 d, this means that the effect of the change in influent concentration will be noticeable after about three retention times at steady state. Furthermore, bacteria carry out the process of anaerobic digestion with very low doubling times (Mosey, 1982; McCarty, 1964). When the feed concentration was changed to 32g COD/*l* there was an increase in the effluent COD concentration to 3500 mg/*l*. From figure 6-20 it is seen that this increase is due to the growth of micro-organisms in the reactor. There is a drop in the COD reduction percentage of the reactor showing that the reactor was heading towards upset.

6.3 Results for the Anaerobic Baffled reactor (ABR)

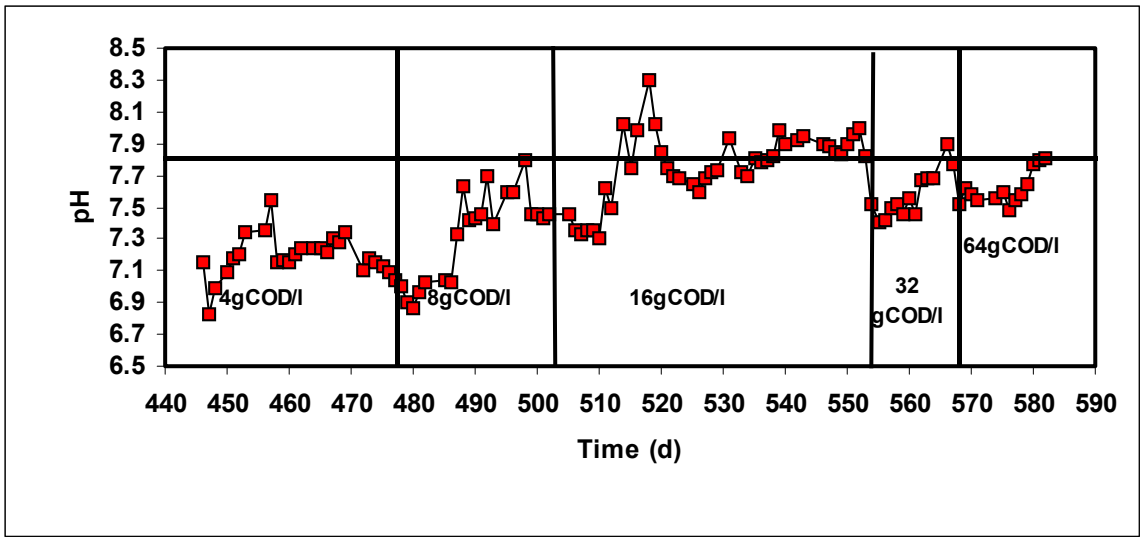

Figure 6-24: Plot of the variation in the pH with time of the effluent of the ABR.

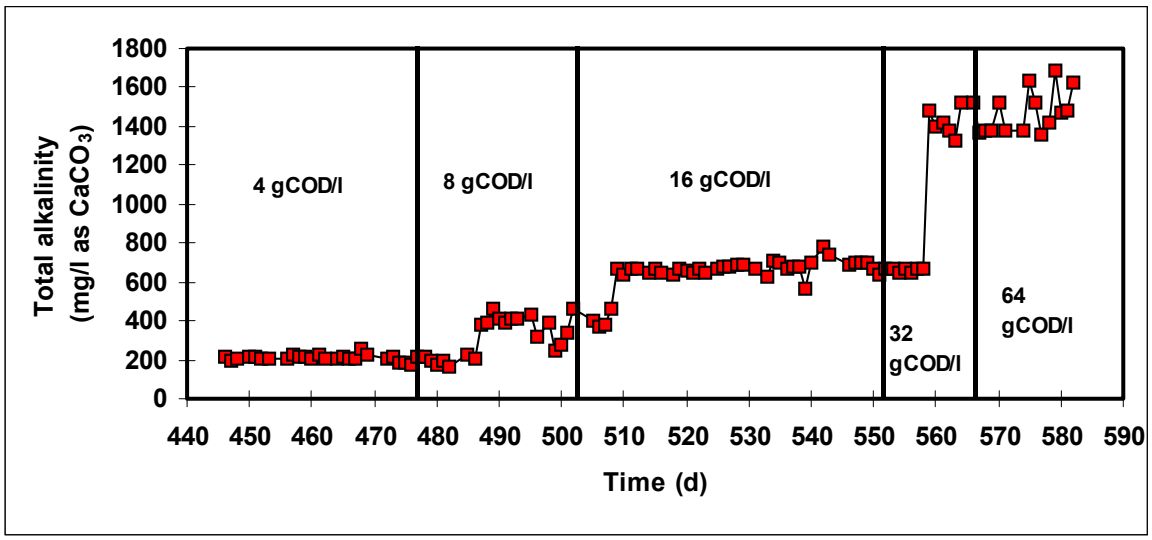

Figure 6-25: Plot of the variation in the total alkalinity with time of the effluent of the ABR.

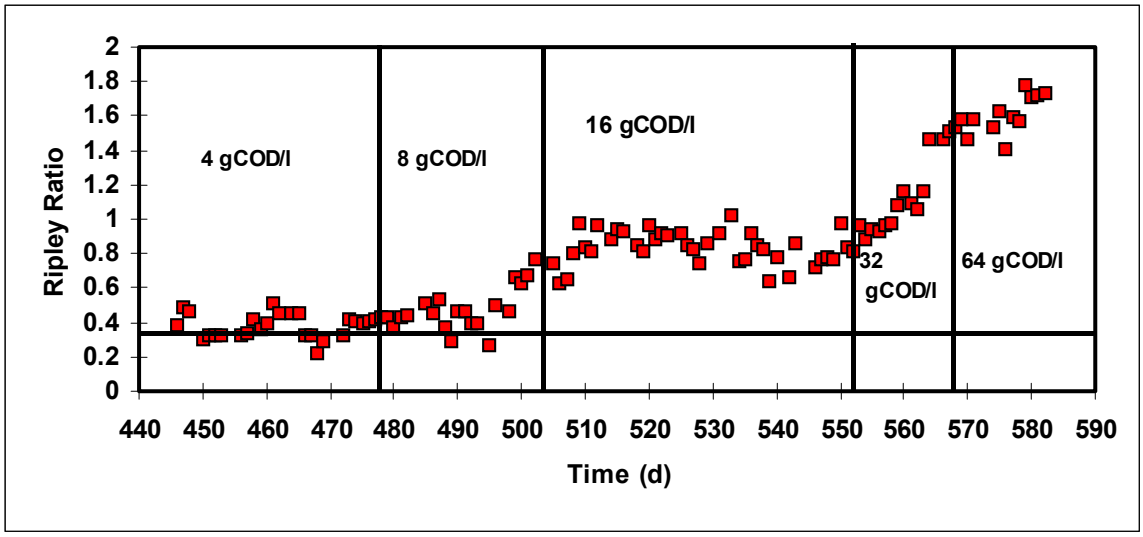

Figure 6-26: Plot of the variation in the Ripley Ratio of the ABR.

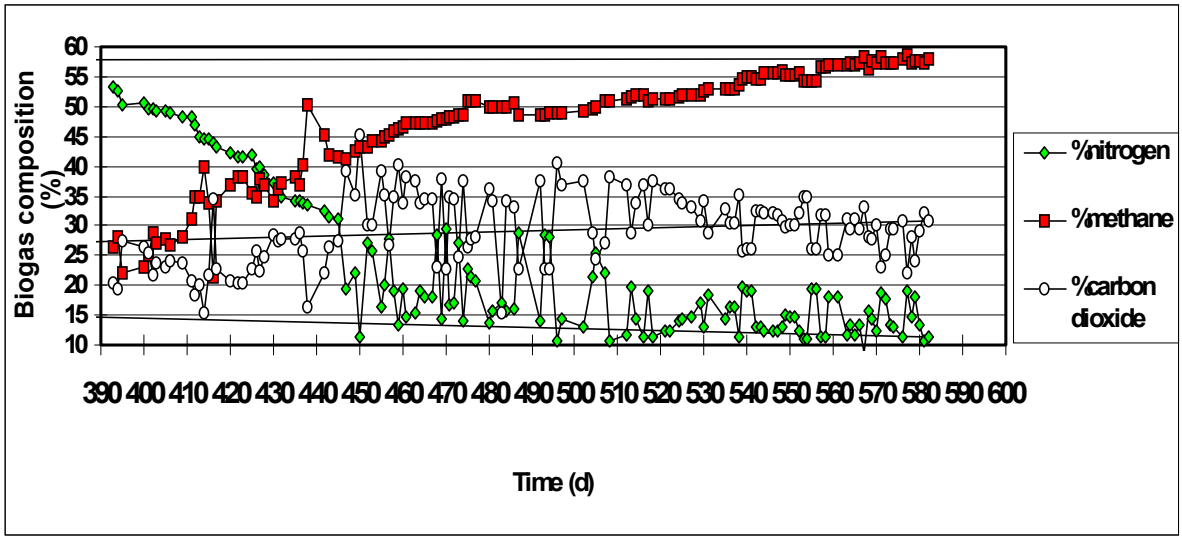

Figure 6-27: Plot of the variation in the gas composition of the ABR.

The pH was always above 7.5 and even went to a value as high as 8.3. As mentioned earlier the high pH values are because of the highly buffered sucrose feed. It is generally agreed that the ABR is a reactor that partially separates between the various phases of anaerobic catabolism (Barber and Stuckey, 1999) and further evidence of this is seen in the pH profile of Holt *et al*.(1997) where the pH increased across the reactor. This suggests that in the final chamber there is mainly methanogenesis taking place. Methanogenesis results in the removal of acetic acid and other acidic end-products of acidogenesis (c.f. chapter 2, section 2.5). This means that in the final chamber there are less H^+ ions in solution and so the solution has a higher pH. It is noticeable that after each change in the organic loading rate (OLR) there is a decrease in pH. This followed by an increase in carbon dioxide content as seen from figure 6-27. This decrease in pH results from the fact that acidogens have a higher doubling time than methanogens (Speece, 1983). This means that there is an initial increase in the short chain fatty acids, which are the products of acidogenesis. Eventually, the pH increases because the bacteria have a symbiotic relationship that is self-regulating (Mosey, 1982; McCarty, 1964; Speece 1983). The methane content decreases as the carbon dioxide content increases. This shows that the increase in pH decreases the activity of the methanogenic bacteria while increasing the activity of the acidogenic bacteria. Since the acidogens operate at a lower optimum pH than the methanogens.

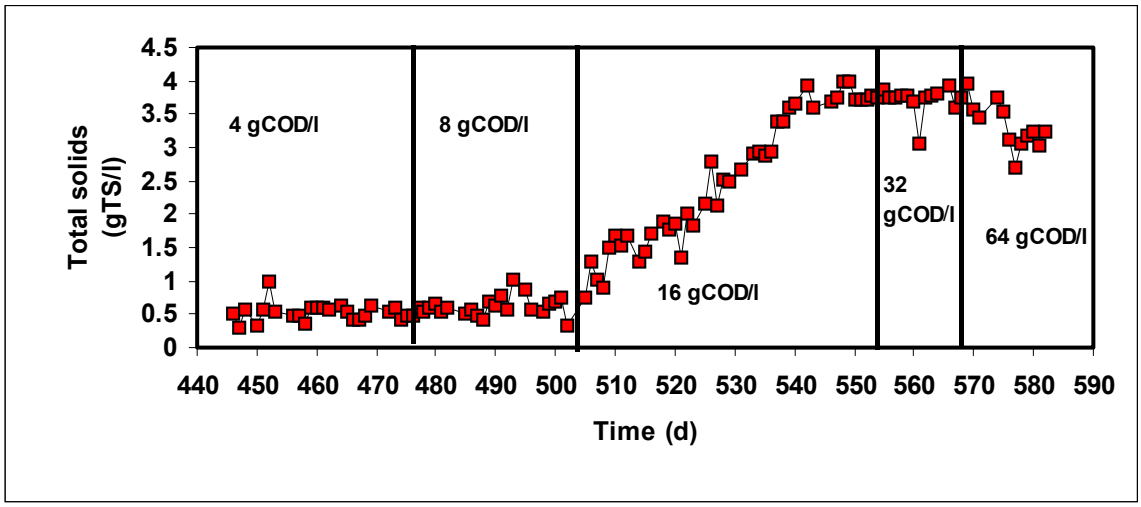

Figure 6-28: Plot of the variation in the total solids content with time of the effluent of the ABR.

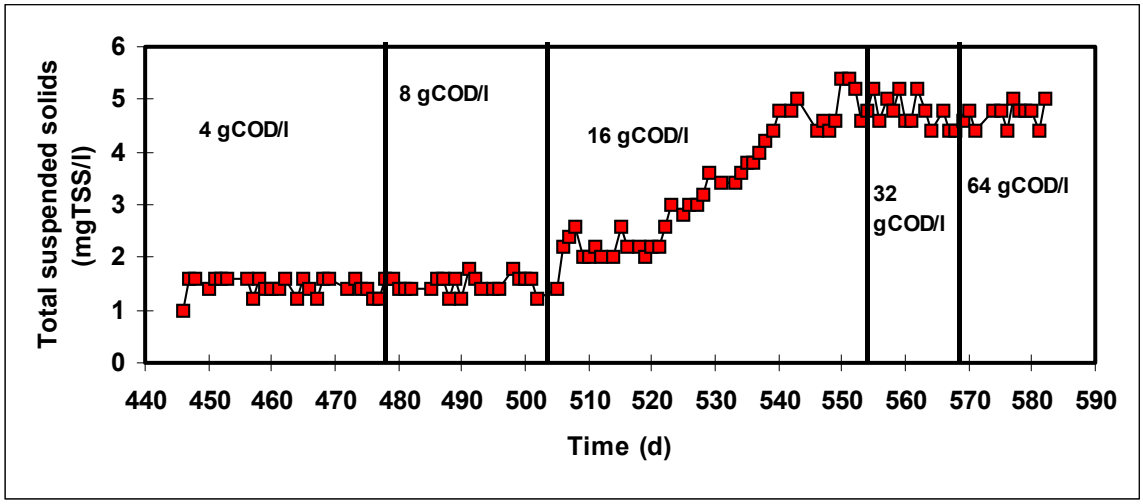

Figure 6-29: Plot of the variation in the TSS content with time of the effluent of the ABR. .

In general, the TS and TSS graphs reflect that there was an increase in the amount of solids in the reactor with time as the OLR was increased. After an increase in the feed strength concentration from 4 gCOD/*l* to 8 gCOD/*l* there was no increase in the TS content of the ABR. The average TS content was 0.5 gTS/*l* while the TSS had a value of about 1.5 mgTSS/*l*. This is due to the slow doubling times of anaerobic bacteria as bacterial biomass growth contributes the major portion of the total solids in the reactor. The amount of total solids in the reactor is also increased as a result of cell decay or endogenous metabolism (Eastman and Ferguson, 1981). When the feed strength was increased to 16 gCOD/*l* there was an exponential increase in TS to 3.5 gTS/*l* and 5 mgTSS/*l.* This increase shows that there was an exponential growth in the number of micro-organism in the reactor since the refractory material in the bulk liquid is expected to pass unaltered through the reactor (Iza *et al.,* 1991). This also represents unsteady conditions in the reactor at this time as the rapidly increasing biomass meant that the conditions in the reactor changed continually. This is also shown by the unstable gas composition, pH, gas production and RR results.

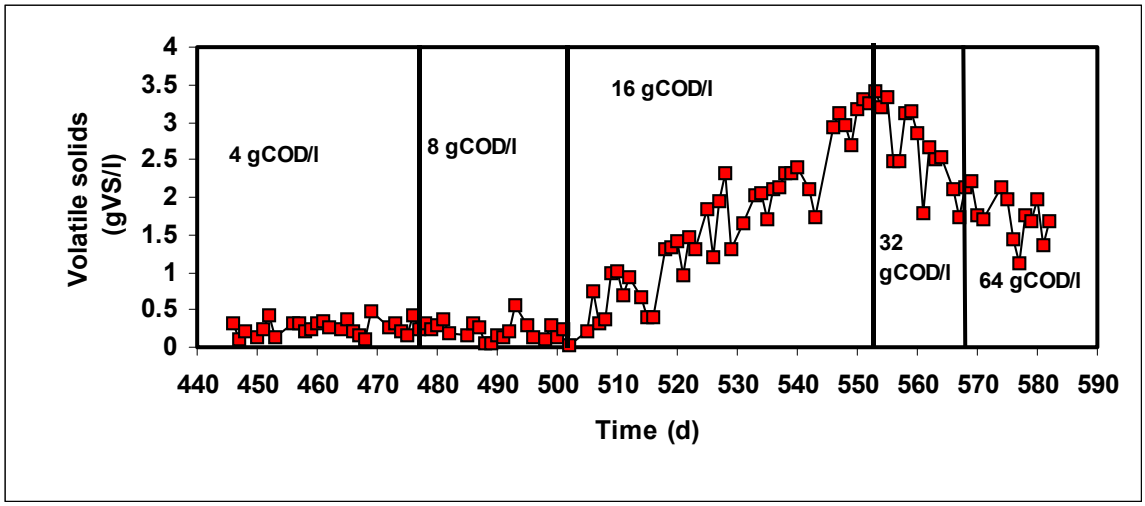

Figure 6-30: Plot of the variation in the volatile solids content of the effluent of the ABR with time.

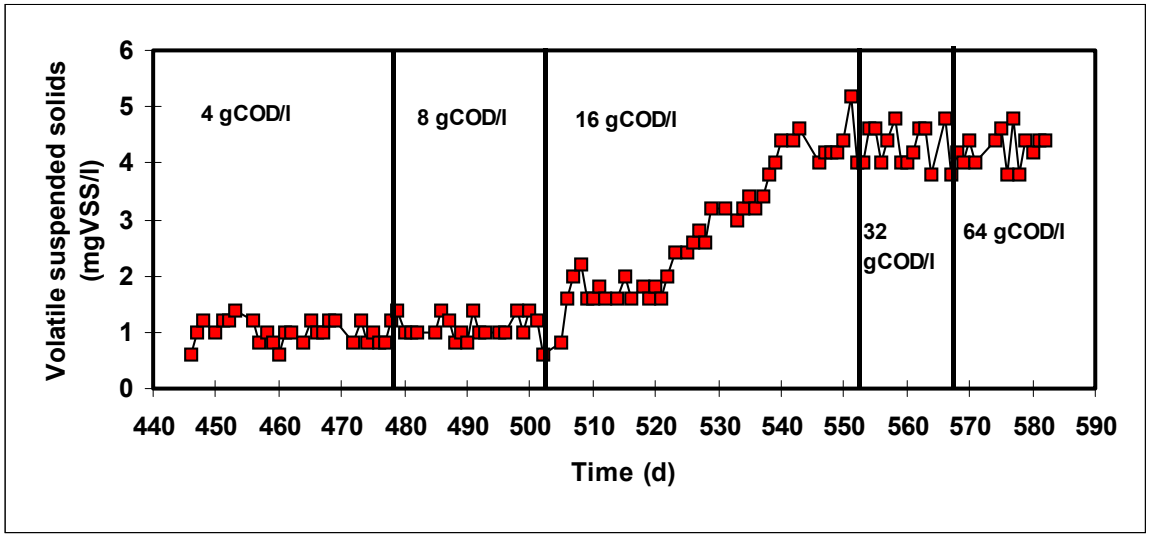

Figure 6-31 : Plot of the variation in the volatile suspended solids content of the effluent of the ABR with time.

Figures 6-30 and 6-31 also show an exponential increase in the VS and VSS of the ABR. This confirms that the exponential increase in the TS and TSS was due to the exponential growth of the bacterial biomass. The VS initially had an average value of 0.46 gVS/*l* and this increased to about 3.5 gVS/*l*. When the feed concentration was changed to 32 gCOD/*l* there was a decrease in VS which continued when the feed concentration was changed to 64 gCOD/*l*. The VSS has an initial value of about 1.0 mg/*l* and this increased at 16 gCOD/*l* to average at 4.12 gVSS/*l*. The VSS did not decrease like the VS and this is attributed to the fact that the VSS concentration is a better characterisation of the microbial biomass than the VS (Iza et al., 1991). Furthermore, the exponential increase in bacterial mass (Figure 6-31) means there are less volatile mineral salts available as the increased number of bacteria has used them up. This results in a lower VS value after the exponential increase. The fact that the VSS had a constant value after the exponential increase phase suggests that the microbial biomass had a constant mass and that the rate of bacterial growth was roughly equal to the rate of bacterial cell decay. Figure 6-31 shows that the bacteria in the reactor followed Monod kinetics since VSS represents the bacterial growth which reached a saturation level at a certain substrate level (c.f. Chapter 3, section 3.1.2). Usually a reactor is operated at one of the phases of bacterial growth (Gray, 1989) but with the ABR it was operated at all these phases as the test involved varying organic load. This is noticeable from the figure 6-30 which shows the lag growth phase, exponential growth phase and the declining growth phase.

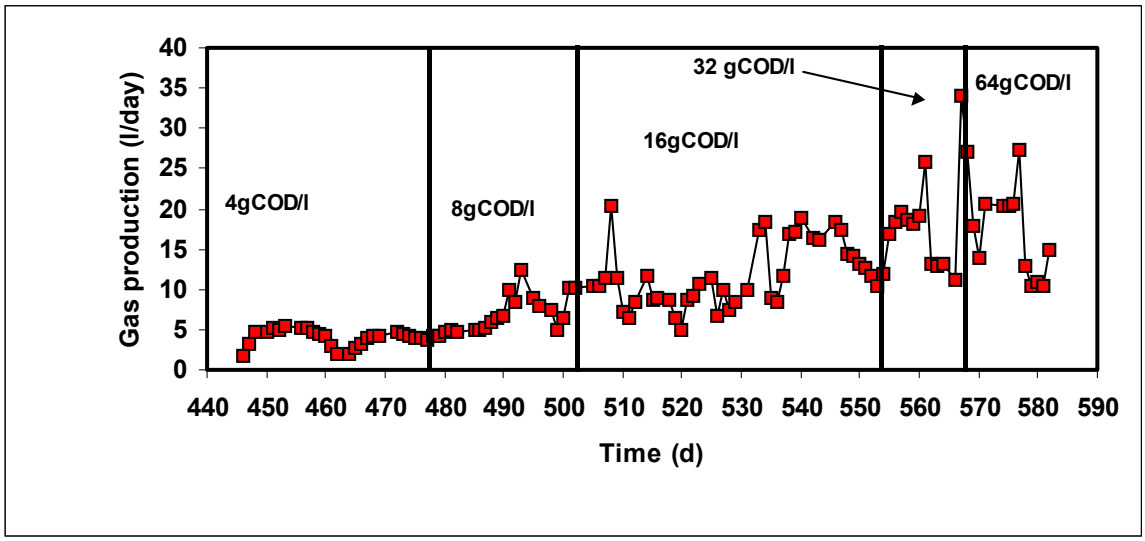

Figure 6-32: Plot of the variation in the gas production of the ABR with time.

With each change in organic loading rate (OLR) there was a increase in the carbon dioxide content of the biogas. This was due to an increase in volatile fatty acids. At the feed concentration of 4 gCOD/*l* the average methane content at steady state was about 50% with a carbon dioxide content of about 36%. At 32 gCOD/*l* the average methane and carbon dioxide content was about 40%. When the feed concentration was increased to 64 gCOD/*l* the carbon dioxide content increased to above 60%. The methane content fell to 20%, which showed that the reactor was heading towards failure. The percentage of nitrogen was below 20 %. The presence of nitrogen is not surprising as the feed substrate contains nitrogen for cell growth and various authors have noticed similar concentrations of nitrogen using the same feed (Eastman and Ferguson, 1981; Polprasert *et al*., 1992; Grobicki and Stuckey, 1991; Lettinga *et al*., 1983). As expected, the gas production profile reflects an increase in gas production with each increase in the OLR. At a feed concentration of 4 gCOD/*l*, the average gas production was 5.4 *l*/day at steady state. When the feed concentration was increased to 8 gCOD/*l,* the steady state gas production averaged 10.0 *l*/day. However, at 16 g COD/*l* the gas production was irregular but it eventually stabilised at about 17.0 *l*/day. There was no exponential increase in the gas production with the exponential bacterial growth (figure 6-31). Not all the bacteria present in the biomass are active making bacterial activity evaluation by gas production inaccurate. There was an increase in VA/ALK ratio during this period of instability. This shows that there was an increase in volatile fatty acids (VFAs) for this period. The increased carbon dioxide composition (Figure 6-26) suggests that most of the acidogens are increasing faster than the methanogens. As expected the acidogens react faster to change and multiply faster than the methanogens. The VA/ALK of the ABR is always above 0.3. Since the ABR is a staged reactor which separates the HRT from the SRT of the waste influent (Barber and Stuckey, 1999; Boopathy and Tilche, 1991; Holt *et al*., 1997; Bae *et al*., 1991). The soluble VFAs produced in the first compartments will flow out with the bulk liquid component of the wastewater leaving behind the solid mineral salts that increase the alkalinity of the solution. This results in a higher VA/Alkalinity ratio than if the reactor were a batch reactor with a HRT/SRT ratio of one. This also means that the RR of 0.3 is not a very good parameter to determine the stability of the ABR.

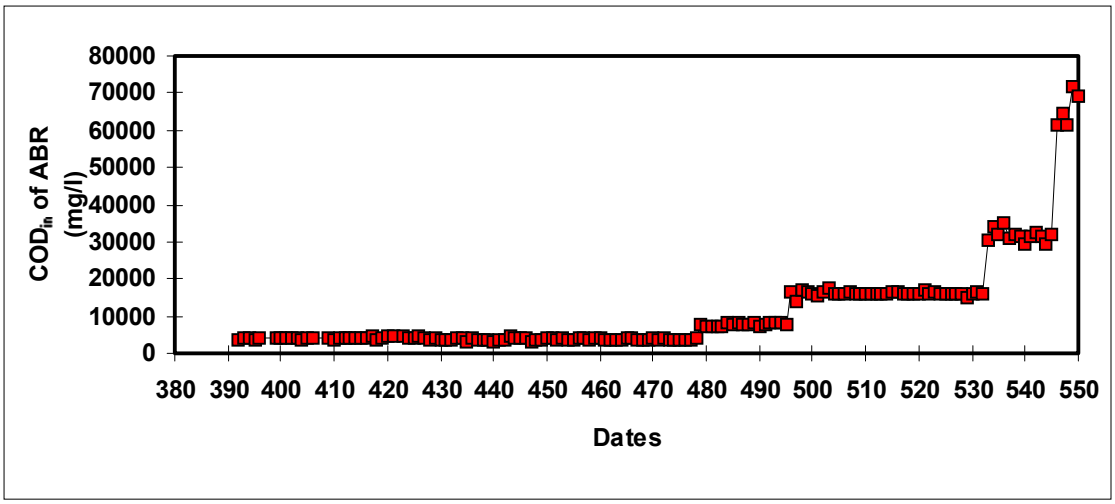

Figure 6-33 : Plot of the variation in the COD of the feed of the ABR.

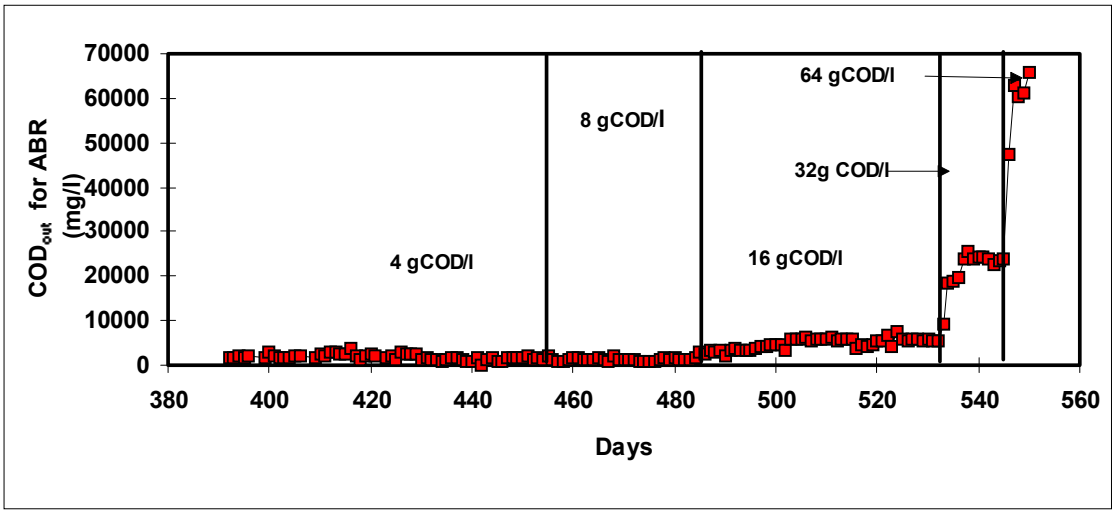

Figure 6-34 : Plot of the variation in the COD of the effluent of the ABR.

At a feed concentration of 4 gCOD/*l,* the effluent COD concentration was about 1000 mg/*l* at steady state. This gave a COD reduction percentage of about 75%. The constant COD concentration confirms that the ABR had achieved a quasi-steady. Increasing the feed concentration to 8 gCOD/*l* increased the effluent concentration to about 2000 mg/*l* and the COD removal remained at about 75%. Each increase in the feed concentration led to an increase in the effluent concentration. The COD reduction decreased as the organic load was varied. It is also interesting to note that there was no exponential increase evident in figure 6-34 unlike in figure 6-31. This is because the COD measurements measure the carbon content of the effluent in terms of the amount of oxidant or oxygen needed to oxidise the organic matter in the effluent. The COD value will include the value of the organic and inorganic matter that can be oxidised by the potassium dichromate-acid mixture. This means the higher the value of the inorganic oxidisable matter in the sucrose feed the higher the COD value of the effluent. This is noticeable in figure 6-34, which now shows that COD_{out} value increased with the increase in the sucrose feed concentration.

6.4 Results of the COD reduction for all the reactors

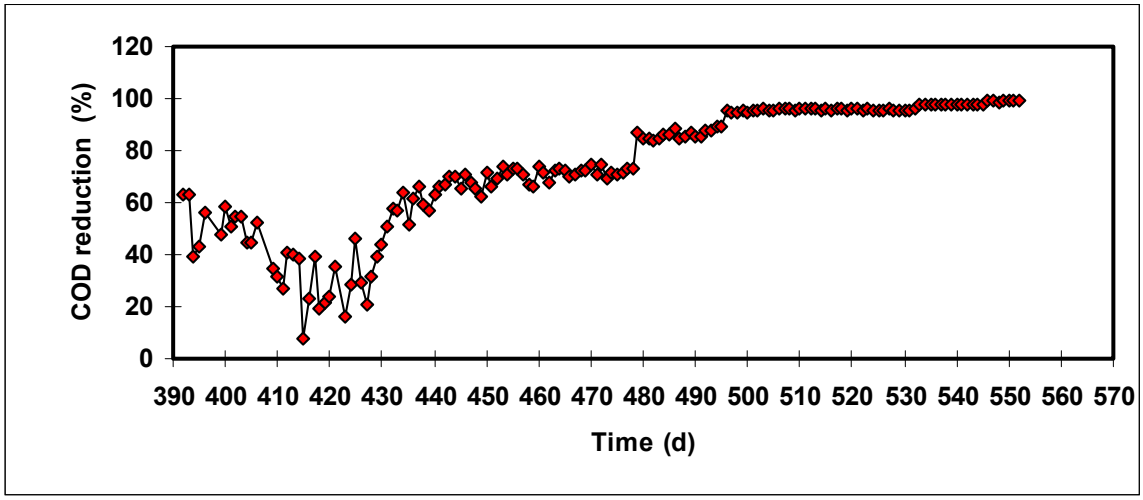

Figure 6-35: Plot of the COD reduction for the Control (CMAR) with time.

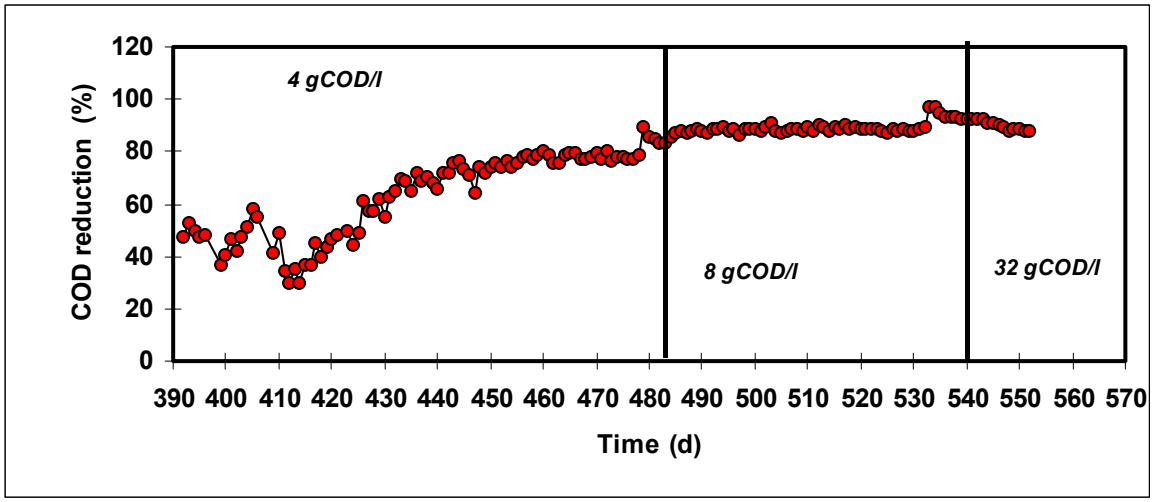

Figure 6-36: Plot of the COD reduction for the Test (CMAR) with time.

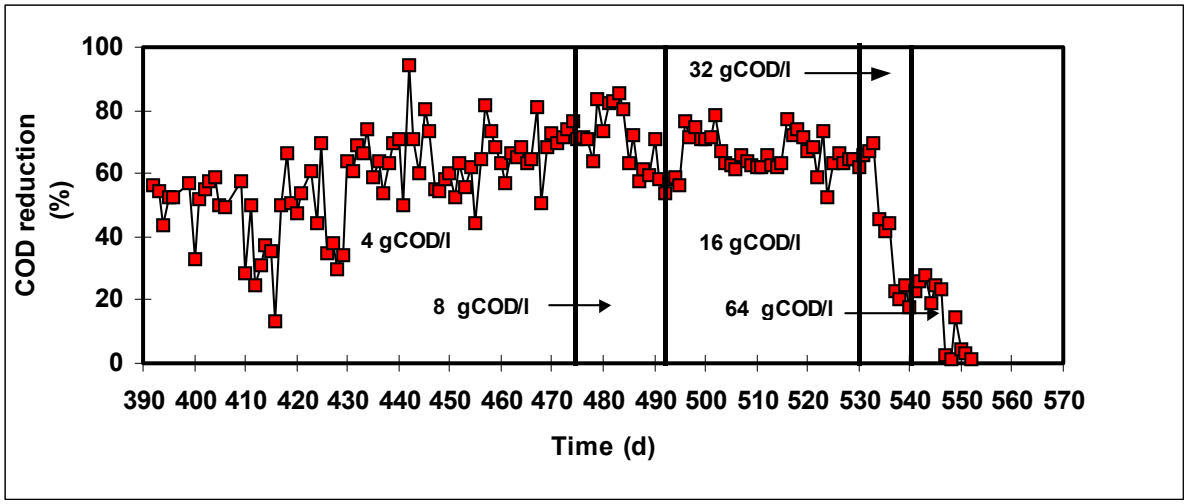

Figure 6-37: Plot of the COD reduction for the ABR with time.

The Control reactor had a low COD reduction percentage up to day 430. This shows that the reactor had not achieved steady state contrary to the TS reduction results. This clearly demonstrates that the COD measurement is more sensitive to changes within the reactor. COD determines the amount of oxidisable organic matter in the biomass and waste whereas the TSS includes refractory material which is not oxidisable. After day 430 the COD reduction initially increased and then stabilised at 67.9 %. This was followed by a second stable period where the COD reduction was 96.8 %. The reason for the change in the COD reduction is that as the bacteria acclimatise to the sucrose feed, their metabolism rate increases and they are able to utilise more of the feed. The Test reactor showed a similar pattern in the COD reduction at a feed concentration of 4 gCOD/*l,* although it had a slightly higher average COD reduction of 74.7 %. The ABR did not exhibit a similar pattern, as its initial COD reduction was fairly high at about 56.5 %. Its average COD reduction at steady state for the feed concentration at 4 gCOD/*l* was 66.02 %. From figure 6-37 it is noticeable that the COD reduction for the ABR was variable. Furthermore, as Grobicki and Stuckey (1991) discovered the sucrose (carbohydrate-protein) feed is quite complex and it takes time for the biomass to acclimatise to it. Now, the ABR had been in operation for a shorter period than the CMARs and the symbiotic relationship of the various trophic groups of bacteria had not fully developed (McCarty, 1964; Mosey, 1982) making the acclimatisation of its biomass to the feed even more difficult. The Control reactor had an average COD reduction of 95.8 % at a feed concentration of 8 gCOD/*l* while the ABR had average COD reduction of 68.4 %. The ABR was progressively fed the sucrose feed at concentrations of 16, 32 and 64 g COD/*l* giving COD reductions of 64.4, 23.1 and 4.58 % respectively. The COD removal percentage deteriorated with an increase in the feed concentration because as outlined in Chapter 2, section 2.3, the bacteria utilise the free energy from the breaking down of the molecular compounds in the feed. When one mole of a molecular compound is broken down it releases a certain amount of energy. Given that the bacteria reproduce at a slow rate, this means that if there is a large number of the molecular compounds available only a given number are utilised by the bacteria for their energy requirements and the rest are left in the effluent. This finding is in agreement with the results of Xing et al. (1991), Polprasert et al.(1992), Bachmann et al.(1985) and Bae et al.(1997) who all found a decrease in COD removal with increasing organic load.

Table 6-3: Comparison of the COD removal efficiencies for the ABR of this study with those found in the literature with all reactors operating at 20 h HRT.

	COD removal efficiency (%)				
OLR (kg COD.m^{3}d)	This study	Xing *et al*. (1991)	Polprasert *et al*.(1992)	Bachmann *et al*. (1985)	Bae *et al.* (1997)
4.8	66.02	71	75	88	95
9.6	68.4	88		81	88
19.2	64.4	67		75	72
38.4	23.1			60	
76.8	4.58				

From table 6-3 it is noticeable that the COD removals in this study where lower than those found in the literature. The reasons for this maybe that the seed sludge used in this feed did not have a high enough initial biomass concentration as the number of bacteria present in the reactor affect the COD removal rate.

6.5 Results of the COD mass balances for all reactors

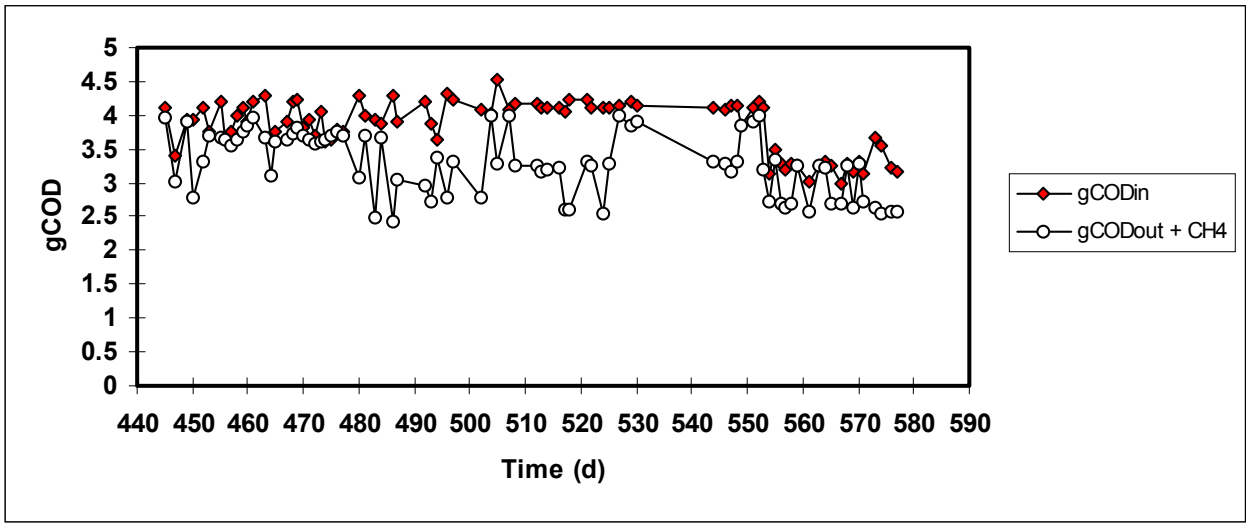

Figure 6-38: plot of the mass balances for the Control (CMAR).

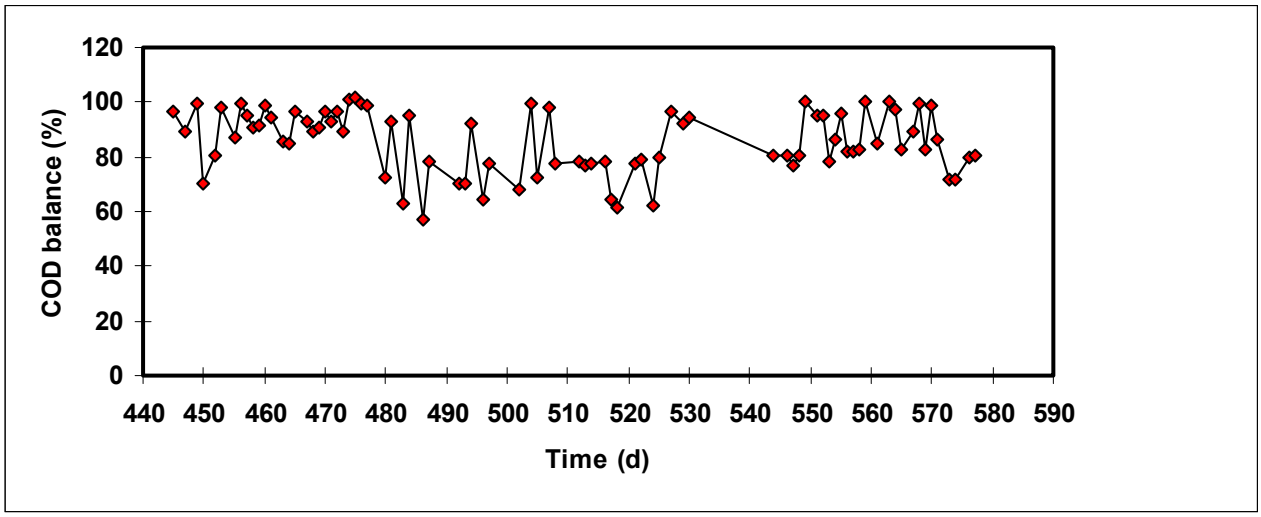

Figure 6-39: Plot of the mass balance percentage of the Control (CMAR).

From Figure 6-38 it can be seen that the Control CMAR was at steady state. A COD mass balance revealed that about 80.2 % of the COD taken in was accounted for. This is observed from the average of figure 6-39. This indicates that the efficiency of the reactor was 80.2 %. The rest that was not accounted for is the COD fraction that is incorporated into the biomass as this is assumed to be negligible in the COD mass balance equation.

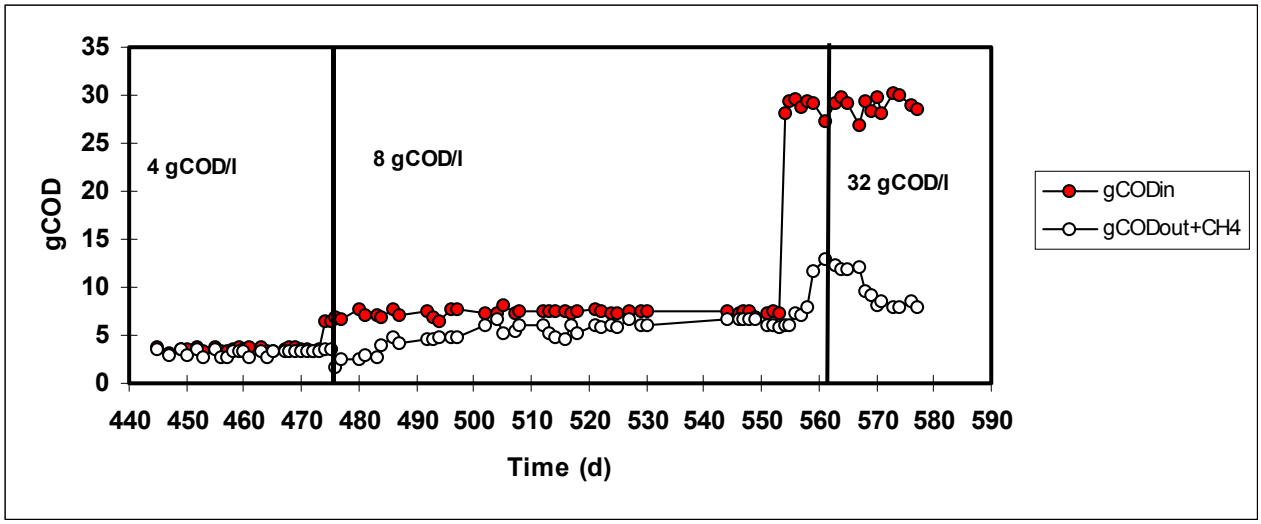

Figure 6-40: Plot of the mass balances for the Test (CMAR).

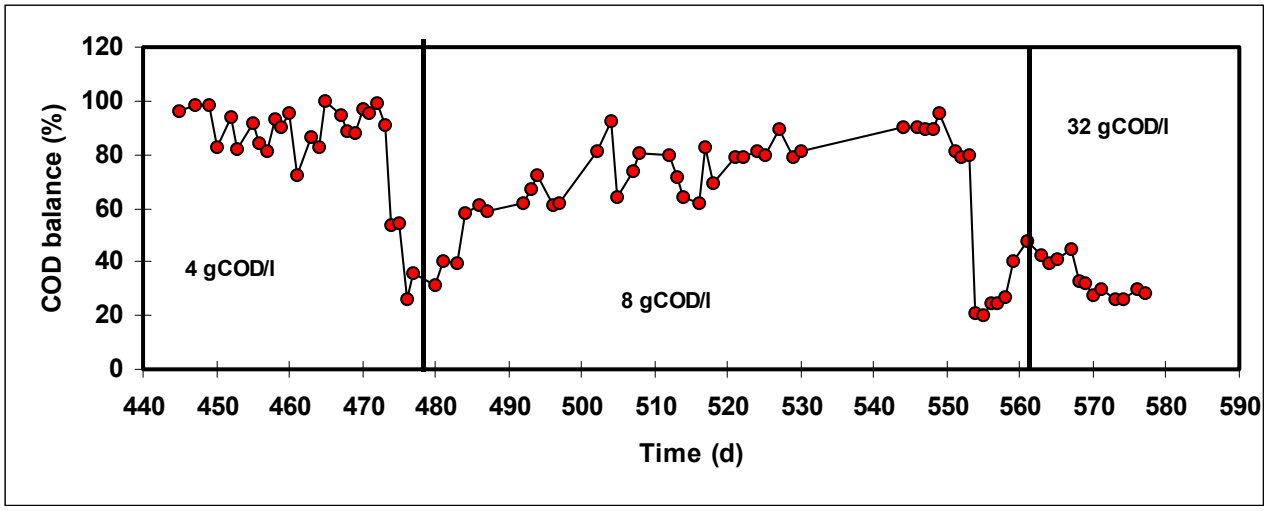

Figure 6-39: Plot of the mass balance percentage of the Control (CMAR).

Figure 6-40 shows that initially at 4 gCOD/*l* the Test reactor was at steady state, with an average of 88.6 % of the COD that was added to the reactors was accounted for. The COD mass balance decreased when the feed concentration was changed to 8 gCOD/*l*. The COD mass balances show that some COD that is taken into the reactor is unaccounted for. It is assumed that the COD that makes up the difference has not been converted to methane or any other oxidisable form. The COD balance improves with time as the bacteria acclimatise to the new feed concentration and as the reactor approaches steady state. An average of about 80.8 % of the COD into the reactor is accounted for. At the feed concentration of 32 gCOD/*l* only about 35.6% of the COD into the reactor is accounted for. This shows that the reactor has become overloaded and is heading for upset as the amount of COD accounted for decreases with time.

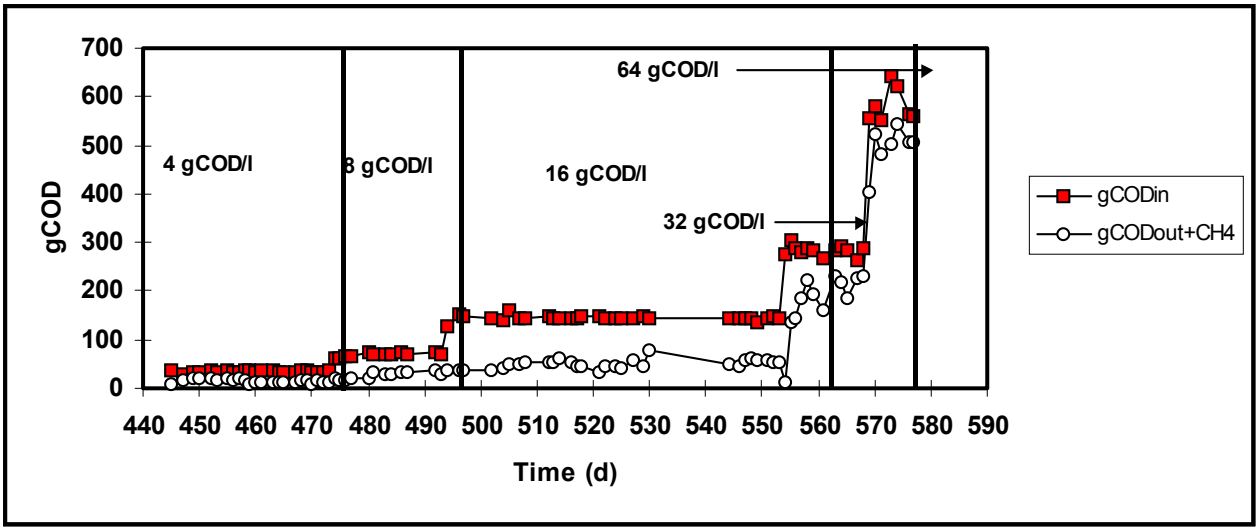

Figure 6-42: Plot of the mass balances for the ABR during tests.

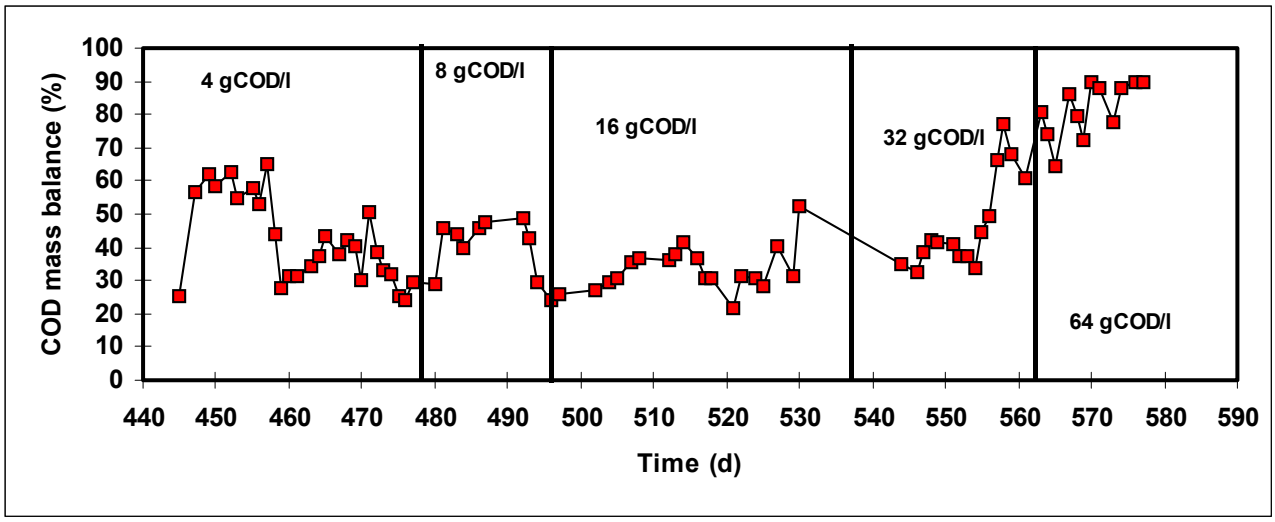

Figure 6-43: Plot of the mass balance percentage of the ABR.

The ABR did not have good COD mass balances as the average COD accounted for was at 60.5 % at 4 gCOD/*l*. This decreased to an average of 34.6% for the concentrations of 8, 16 and 32 gCOD/*l*. The reason for this is that the ABR is a baffled reactor that strains the effluent and separates the liquid fraction from the solid fraction of the influent. This means that the COD into the reactor that is taken up by the slow moving bacteria is unaccounted for in the effluent as the bacterial biomass moves slowly across the reactor. This means that there is a slow build up of solids in the ABR leading to higher TS content values (figure 6-17). This is unlike in the CMAR where the SRT/HRT ratio is one and all the COD taken up by the bacterial biomass is present in the effluent as well as the reactor contents providing a better balance. At 64 gCOD/*l* there appeared to be an improvement in the COD mass balances as figure 6-43 depicts an average of about 90 %. This is misleading because on looking at figure 6-42 it is seen that the value of the COD_{in} equals that of the COD_{out} with very little methane gas as seen from the gas composition graph (figure 6-27). This shows that the influent was going through the reactor with little interaction with the biomass. This suggests that as the reactor becomes overloaded the bacteria have enough free energy from the excess organic compounds present. The addition of more moles of organic molecules will result in these extra molecules passing through the system untouched, as their free energy is not required. The difference between the SRT/HRT ratio of the CMAR and ABR accounts for the difference in the profile of their COD mass balance graphs (figure 6-41 and figure 6-43) at overload. The plot for the CMAR (figure 6-41) decreases as more sucrose feed is applied as the supplied COD is converted to an unoxidisable form within the reactor. The ABR shows an increase in the COD mass balance percentage (figure 6-43) as the reactor is overloaded. This is because its high HRT means that the slow growing bacteria do not have sufficient time to metabolise the excess organic compounds in the sucrose feed and so the COD value remains the same.

Chapter 7 Conclusions and Recommendations

The extent to which this work has been able to fulfil the scientific objectives outlined in chapter 1 section 1.4 is analysed in this chapter. Then the main conclusions that have been drawn from this study are presented, providing the basis for examining the importance and subsequent implications of the work with respect to comparison of the CMAR and the ABR. Finally, based on the findings of this work, areas in which future work might proceed are suggested.

7.1 Conclusions

In the New Water Act of 1999, the Zimbabwean government seeks to protect the environment. The presence of technology that reclaims the polluted water is one of the ways the environment can be effectively protect. This requires a water treatment technology that releases a good quality effluent. The anaerobic reactor of choice will have to be able to withstand greater organic shock loads to avoid releasing high strength loads into the environment. In the study, the CMAR was able to withstand an organic load of 0.82 kgCOD/m^3.d. The ABR on the other had was able to handle an OLR of 38.4 kgCOD/m^3.d (see Appendix B.2 for calculations). This means that the ABR was able to take a load 47 times that of the CMAR. The main reason for this great difference is that the ABR separates the HRT from the SRT by the use of its baffles. The baffles help to retain the solid fraction of the effluent and reactor contents within the reactor. This leads to a higher SRT and exceeds the minimum specific growth rate allowing higher concentrations of suspended bacterial biomass. The ABR is a reactor, which separates the process of acidogenesis from that of methanogenesis along its length. In anaerobic digestion three main trophic groups of bacteria function concomitantly to degrade organic matter (Ferry and Wolfe, 1977; Zender and Guijer, 1981). The structure of the ABR results in the physical separation of the various key trophic groups resulting in a higher resistance to organic loading. This means that in the ABR each trophic group is able to undergo its reactions with little interference from the end products of the other trophic groups. From the discussion in Chapter 2, the short chain fatty acids from the acidogenesis reduced the pH of the reactor and so inhibited the methanogenic bacteria, which function better at higher pH. The CMAR usually operates on an influent with a TS concentration > 4 gTS/*l* (Ross et al., 1992). In the study the sucrose feed had a TS concentration of about 2.5 gTS/*l*. Since the HRT/SRT ratio for the CMAR is one this means that the reactor contents became more dilute with time and so decreased the bacterial biomass in the reactor. With a reduced bacterial biomass concentration the CMAR did not function under optimal operating conditions. However, the study was based on comparison using the sucrose feed which has all the nutrients for bacterial growth. Normally a CMAR can take up an OLR of 1-10 kgCOD/m^3.d (Malina and Pohland, 1992). In the study the OLR fell below this range because of the dilute feed concentration. The increased bacterial biomass concentration means that the ABR took a higher organic load than the CMAR. The conclusions from this study were that:

i. The dilute feed can be used to run an anaerobic reactor provided it has all the essential nutrients for bacterial growth.
ii. The ABR can withstand a greater shock load than the CMAR.

An important advantage of anaerobic digestion is that it is a source of energy, namely biogas. In this study the ABR had a higher gas production rate than the CMAR The CMAR had an average gas production of 0.2 *l*/d per litre volume as compared to the ABR which had 2.27 *l*/d per litre volume (see Appendix B.3). This is expected as the ABR had a higher organic loading rate than the CMAR. This also points to the fact that the ABR had a higher biomass concentration and as a result showed a greater bacterial activity. This is direct evidence of the ability of the ABR to retain biomass and increase its SRT thereby increasing its efficiency. Both reactors showed a deterioration of biogas quality as organic load was increased. They both had a methane content of 26 % at overload. This is very poor quality biogas since ideally the methane content should be about 70 %. In this study the reactors only attained a maximum methane content of about 53 %. The reactors also showed a high nitrogen content averaging 9 % of the total biogas. This is unusual for anaerobic digestion but biogas quality also depends on the feed and the nature of the seed sludge. The two possibilities are that the seed sludge already contained denitrifying bacteria or there was stripping of nitrogen in the reactors. Generally the biogas quality in both reactors was of a poor quality.

The quality of the effluent of an anaerobic reactor is also monitored by looking at it solids content. This also takes into consideration the biomass concentration as depicted by the volatile solids and volatile suspended solids. In the study the CMAR has a TS and TSS of 0.5 gTS/*l* and 2.5 mgTS/*l* respectively at feed concentration 32 gCOD/*l*. The ABR had a TS and TSS 3.5 gTS/*l* and 4.5 mgTS/*l* at 64 gCOD/*l*. The values of the VSS graphs of the ABR and CMAR both showed the phases of microbial growth (Chapter 2, section 2.2) in their profiles. At lower loading rates the bacteria where in the lag phase as there was no significant increase in the growth rate. When the CMAR was loaded at 0.82 kgCOD/m^3.d there was an exponential increase in VSS. The ABR experienced a similar growth at 19.2 kgCOD/m^3.d. Taking into account that bacterial growth in wastewater treatment systems follows Monod kinetics, at such high substrate concentrations for both reactors the growth rate becomes independent of substrate concentration. This means that it becomes a zero order process. In such a system, which has been operating for a long time, the endogenous phase undoubtedly occurs. The fact that the VSS graph stabilised at a certain value after exponential growth means that the rate of accumulation of dead cells equalled the rate of growth of new cells. Thus for both reactors an increase in substrate after this does not result in an increase in VSS but the degradation of the substrate by acidogens results in an accumulation of volatile acids. This means that more cells become non-viable. As a result the rate of accumulation of dead cells is greater than the rate of growth of new cells and this results in a decrease in VSS. From this evidence it can be seen that;

i. The bacterial biomass in an anaerobic reactor will increase with an increase in organic load.
ii. The solids content of a reactor gives a good characterisation of the changes of the biomass in the reactor.

A good parameter for monitoring the quality of the effluent of an anaerobic reactor is COD. However this is better characterised by considering the COD removal efficiency of a reactor. The CMAR had a COD reduction of about 81 % while the ABR had a COD removal of about 66.3 %. This is lower than that reported in the literature but this is due to the fact that it was never allowed to operate at a certain loading rate long enough for the bacteria to adjust to the new loading rate. On the other hand the CMAR had a higher COD removal rate than literature due to the feed with a low solids concentrate. This is so since the effluent COD had less organic matter. The COD balances for the Control (CMAR), which had a constant feed concentration show, an efficiency of about 80.2 %. With the Test (CMAR) doubling the feed concentration reduced the efficiency to about 60.5 % and when the feed concentration was at 32 gCOD/*l* it fell to 20 %. A similar trend was noticed with the ABR. Eventually, when the feed concentration was at 64 gCOD/*l* the COD mass balances revealed that most of the COD into the reactor came out unoxidised. This was reflected by the high COD_{out} values. The ABR tended to have a poorer COD mass balance than the CMARs but it should be appreciated that the ABR has a higher biomass retention capacity and so the COD retained by it is higher. From the results of the COD reduction and COD mass balances it can be concluded that both anaerobic reactor can be used to produce an effluent of appreciable quality provided the influent is applied at the correct HRT.

Finally, the results of all the investigations in the ABR and CMAR have shown that:

i. The ABR had a shorter start-up period as compared to the CMAR.
ii. The ABR proved to be superior in terms of maximum allowable organic load when compared to the CMAR.

From these conclusion it is evident that on a laboratory scale the ABR equals and sometimes is better than the CMAR. This means that it can function as a laboratory substitute for the CMAR. On a larger scale more comparative investigations need to be carried out on the economics of their operation. It is also important to investigate more on the ability of the ABR to deal with less soluble feed substrates.

7.2 Recommendations for future work

Since the HRTs of 20 d for the CMAR and 20 h for the ABR were taken based on results from other researchers, it would be interesting to bring both reactors down to their shortest possible HRT. This would enable a comparison of both systems operating close to their stress levels. Alternatively, the

ABR would have its HRT increased to the minimum HRT of the CMAR thus comparing both reactors at the same HRT.

This study did not investigate the amount of volatile acids in the different chambers. Such a study would allow the researcher to see how the composition of the volatile acids changed as the reactors were overloaded. This will involve re-designing the reactors to allow the removal of samples from within the reactors as the reactions that determine the efficiency of the process are taking place within the reactors. For the ABR the ability to monitor the contents of each chamber separately will enable the pH profile of the ABR to be elucidated. It is recommended that more work be done to discover what is the maximum TS value that can be used to operate both reactors. With the ABR the aim would be to see at what value it becomes clogged up while for the CMAR it would be interesting to note the minimum TS value it could operate.

A more practical approach would be to have a pilot plants of the CMAR and ABR operated on domestic sewage or any effluent of choice. This would give better evidence on whether the ABR can effectively replace the CMAR as the anaerobic reactor of choice. In the pilot plant, in addition to the parameters investigated in this study, a further comparison of economic factors will also have to be undertaken.

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A Analytical methods

Appendix A outlines the calculations for the analytical methods used in the study. The calculations for the solids content, COD measurements and alkalinity are also shown. These are the calculations that were used to draw up the spreadsheets used to formulate the graphs with the results.

A.1 Solids Content

Total Solids Dried at 103-105°C

$$mg total solids / l = \frac{(A - B) \times 1000 l}{sample volume, ml}$$

where:

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

Total Suspended Solids Dried at 103-105°C

$$mg\ total\ suspended\ solids / l = \frac{(A - B) \times 1000}{sample\ volume, ml}$$

where:

A = weight of dried residue + filter, mg, and

B = weight of filter, mg.

Volatile Solids Ignited at 550°C

$$mg\ volatile\ solids / l = \frac{(A - B) \times 1000}{sample\ volume, ml}$$

where:

A = weight of residue + dish before ignition, mg, and

B = weight of residue + dish after ignition, mg.

Volatile Suspended Solids Ignited at 550°C

$$mg\ volatile\ su\,\mathrm{sup}\,ended\ solids / l = \frac{(A - B) \times 1000}{sample\ volume, ml}$$

where:

A = weight of residue + filter before ignition, mg, and

B = weight of residue + filter after ignition, mg.

A.2 COD measurements

Standardisation of FAS titrant

$$10 \times 0.25 / ml\ FAS = 2.5 / ml\ FAS$$

COD in mg/*l*

$$\frac{(a-b) \times N \times 8000}{ml\ undiluted\ sample}$$

where:

a = titration of blank
b = titration of sample
N = normality of FAS solution

A.3 Alkalinity

0.05 M HCl acid was used for the titration.

1 mole of HCl =	36,5 g
0.05 moles of HCl =	1.825 g

Now:

$$0.05M = 1.825 g / l \times \frac{1000\, mg}{g} = 1825\, mg / l$$

The HCl acid is used to neutralise the organic acids thus:

$$HCl + XOH \rightarrow XCl + H_2O$$

Cl^- has a charge of 1 eq/mole which means the equivalent weight of HCl is:

$$\frac{36{,}5\, mg / mole}{1\, meq / mole} = 36{,}5\, mg / meq$$

Then for HCl:

$$N = \frac{1{,}825\ mg / l}{36{,}5\ mg / meq} = 50\ meq / l \times \frac{1000\ meq}{eq} = 0{,}05$$

Total alkalinity = the meq of acid required/litre of sample to reach pH 4.3

$$= 7{,}8\,ml \times 50\,meq / l \times \frac{1000\,meq}{eq} \times \frac{1}{(50\,ml\,sample\,volume)}$$

$$= 7{,}8\,meq / l$$

$$\therefore \quad 7{,}8\,meq / l \times 50\,mg\,CaCO_3 / meq = 390\,mg / l\ as\ CaCO_3$$

B COD Mass Balances

This Appendix shows the calculations for the COD mass balances. It shows how the COD is calculated for the influent, effluent and biogas fractions. It concludes these calculations by showing how the COD out of the reactor accounts for the COD into the reactor. It also outlines the calculations of the organic loading rate (OLR) and the gas production rate for the ASBR as an example.

B.1 COD Balances

COD_{IN}

Influent COD = 3980 mg/*l*
measured flow rate = 1,025 *l*/day

$$\therefore 3980\frac{mg\,COD}{l}\times\frac{g\,COD}{1000\,mg\,COD}\times 1{,}025\frac{l}{day}=4{,}08\,g\,COD/day$$

COD_{OUT}

Effluent COD = 780 mg/*l*
Effluent removed = 1.025 *l*/day

$$\therefore 780\frac{mg\,COD}{l}\times\frac{g\,COD}{1000\,mg\,COD}\times 1{,}025\frac{l}{day}=0{,}8\,g\,COD/day$$

COD_{CH4}

Fixed constants were set as follows:

Temperature : 37°C = 310 K
ρH_2O : 47mmHg = 0.06184211 atm
R (gas constant) : 82,0587 m*l*.atm/K

Measured pressure = 1003 mb = 1.016 atm
Corrected pressure = 1.016-0.06184211 atm = 0.9744 atm

Measured Gas production = 2,2 *l*/day
Proportion of methane in gas = 58.5 %

$$\therefore 0.585\times 2{,}2\,l/day=1{,}29\,l/day$$

$$PV=nRT$$

$$n=\frac{PV}{RT}=\frac{0{,}9744\ atm\times 1{,}29\ l/day}{82{,}057\ ml.atm/K\times 310K}=0{,}05\ moles/day$$

1 mole of methane = 16g
0,05 moles of methane = 0,8 g

The theoretical COD of a compound can be calculated:

$$Cr_2O_7^{2-} + 8H^+ \rightarrow 2Cr^{3+} + 4H_2O + 3O$$

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2} H_2O + cNH_4^+ + 2dCr^{3+}$$

d = 2n/3+a/6-b/3-c/2

For methane,

CH_4 n = 1
a = 4
b = 0
c = 0

$$d = \frac{2n}{3} + \frac{a}{6} - \frac{b}{3} - \frac{c}{2} = 1{,}33$$

These values were substituted into Eq. to give:

$$CH_4 + \frac{4}{3} Cr_2O_7^{2-} + \frac{24}{3} H^+ \rightarrow CO_2 + 11H_2O + \frac{8}{3} Cr^{3+}$$

The oxygen equivalent was then calculated.

I mole CH_4 = 3/2 *d* moles O_2 = 2 moles O2

1 mole CH_4 = 16 g

1 mole O_2 = 32 g

Therefore, complete oxidation of 16 g CH_4 requires 64 g O_2

So, 1 g CH_4 requires 4 g O_2

Theoretical COD of 1 g CH_4 is 4 g

$$\therefore 0{,}8\, gCH_4 / day \times 4\, gCOD / gCH_4 = 3{,}2\, gCOD / day$$

COD_{IN} = **4,08 gCOD/day**

COD_{OUT} + COD_{CH4}= 0,8 gCOD/day + 3,2 gCOD/day = **4,0 gCOD/day**

C.1 Calibration of the gas chromatograph

The accuracy of a gas chromatography in practice is limited by the accuracy of the peak area evaluation and by the accuracy of the calibration method. In this study the calibration curves were prepared by injecting volumes of high purity methane or nitrogen with known volumes of carbon dioxide (Fedgas) using a syringe with a valve. Carbon dioxide was used as the reference gas. Mixtures of nitrogen and carbon dioxide, and methane and carbon dioxide were injected into the GC at ambient temperature (25^{o}C). The following are the results of the calibration curves.

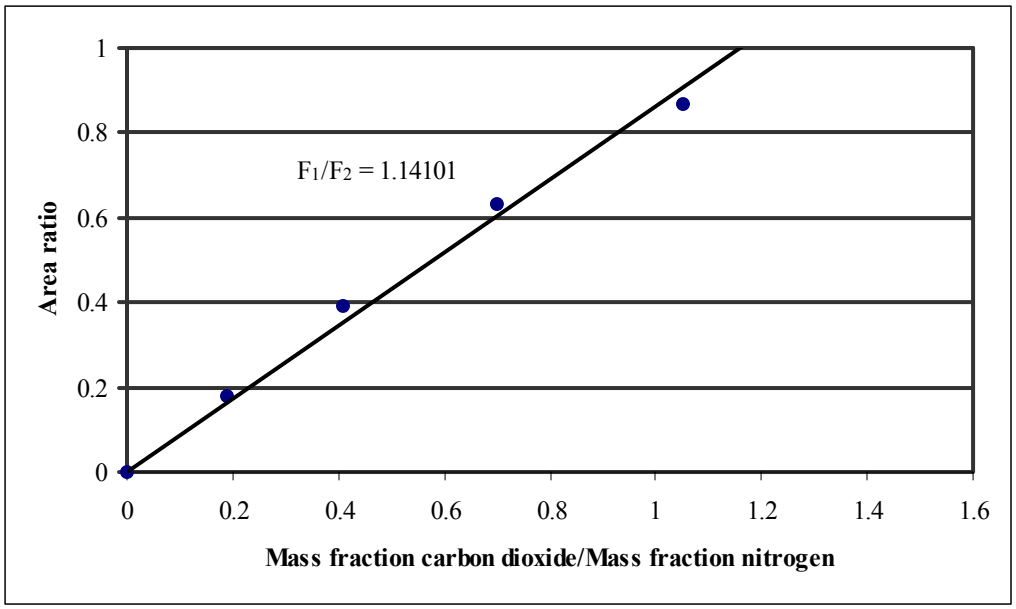

Figure C-1: Gas chromatograph calibration curve for nitrogen and carbon dioxide mixture at oven temperatures of 25°C.

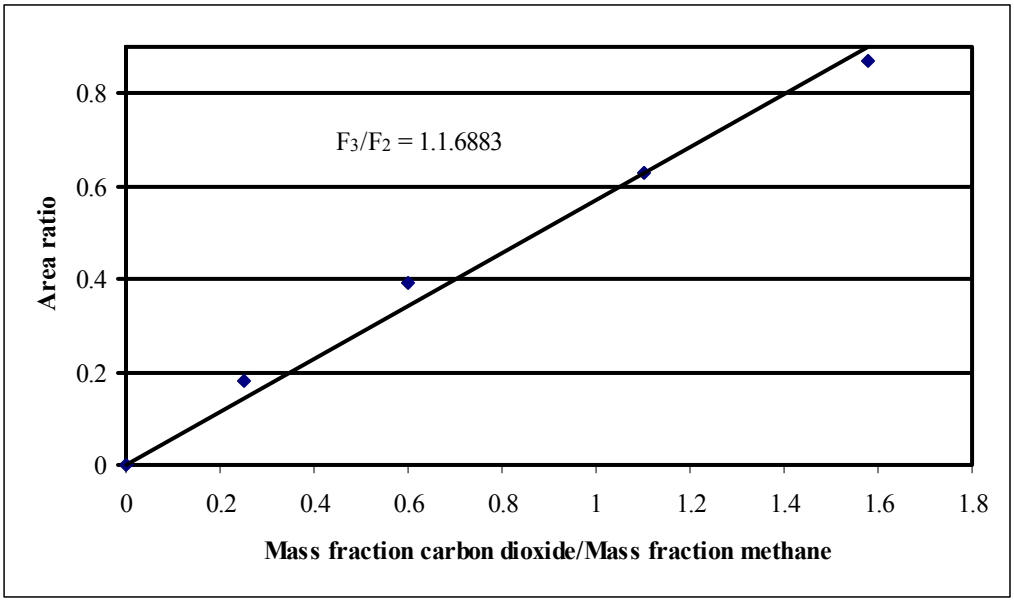

Figure C-2: Gas chromatograph calibration curve for methane and carbon dioxide mixture at oven temperatures of 25°C.

C.2 Gas Chromatography Method

A Gowmac 150 Series gas chromatograph equipped with a thermal conductivity detector (TCD) was used to analyse the biogas for nitrogen, methane and carbon dioxide. A stainless steel column (Hayesep D, 4 m by 3,2 mm, 80/100 mesh) was used for the separation with the following conditions:

Conditions for the Gowmac 150 Series

Column oven : 25°C

Detector : 25°C

Filaments : 25°C

Injection port : 25°C

The carrier gas was helium (HP) at a flow rate of 30 m*l*/min. The nitrogen, methane and carbon dioxide had residence times of 1.4, 2.8 and 6.6 min, respectively. The serum bottles were equilibrated to atmospheric pressure and then samples of digester gas were withdrawn from each bottle by inserting the needle of a gas tight syringe (100 μ*l*) through the rubber septum and withdrawing 100 μ*l* of headspace gas. The peak area was recorded with a Varian integrator with the attenuation set at 8 and the chart speed at 0.5 cm/min.

C.3 Gas Composition Analyses

1. The biogas composition was determined from the gas chromatograms.

2. The volume of biogas was corrected to STP.

3. Biogas samples were analysed, by gas chromatography, for nitrogen, methane and carbon dioxide.

4. The peak area of each component was recorded.

5. Carbon dioxide was used as the reference gas in the GC calibration. Area ratios were calculated with carbon dioxide as the common denominator, i.e. N_2/CO_2 and CH_4/CO_2.

6. The area ratio was multiplied by the inverse of the fraction ratio (F_1/F_2) for each calibration curve:

$$\frac{area\ N_2}{area\ CO_2} \times \frac{1}{1.14101}$$

$$\frac{area\ CH_4}{area\ CO_2} \times \frac{1}{1.16883}$$

7. The resultant **M values** were used to calculate the mole fraction for each biogas component. The proportions of each component were calculated on a mass basis since mass is conserved.

8. Fixed constants were set a s follows;

Temperature	:	310 K
pH_2O	:	47 mmHg = 0.06184
R (gas constant)	:	82.057 m*l*.atm/K
Headspace	:	25 m*l*

Molecular masses

Water vapour	:	18 g/mol
Nitrogen	:	28 g/mol
Methane	:	16 g/mol
Carbon dioxide	:	44 g/mol

9. The total gas production was calculated by summing the head space volume and the volume produced by metabolism.

10. The atmospheric pressure (atm) at the time of gas wastage was recorded.

11. The partial pressure of each gas component was calculated:

(Atmospheric pressure - pH_2O) × gas mole fraction

12. The number of moles of each component, in the biogas sample, was calculated from the gas equation:

$$PV = nRT$$

$$n = \frac{PV}{RT}$$

P was the calculated partial pressure of each constituent and *V* was the total gas volume.

13. The number of moles produced, by metabolism was calculated by correction for the headspace.

14. The mass of each gas component was calculated (mg):

mass = moles × molecular mass

15. The fraction of each component in the biogas was calculated as a percentage (m/m):

$$\frac{gas\ mass}{total\ mass} \times 100$$

C.4 Reproducibility Experiments

In order to asses the accuracy of the gas chromatograph, increasing volumes of pure methane were injected into the GC. Replicate injections were undertaken so as to assess the reproducibility of the GC. The recorded peak areas were plotted against the gas volumes.

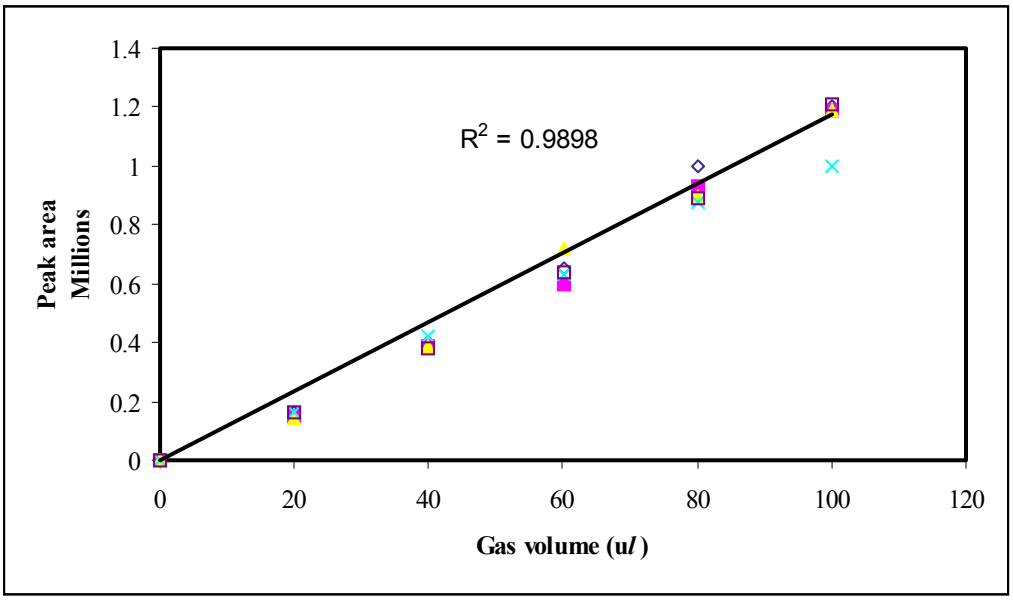

Figure C-3: Plot of the recorded peak areas of five replicates of increasing volumes of standard methane gas.

The GC gave an accurate reproduction for the five replicates of each successive volume. The plot of the trendline through the results had an R^2 value of 0.9898, which is very close to 1. Since the accurate measurement of methane gas was very important in this study, the experiment was repeated, this time with three replicates of each of the methane volumes.

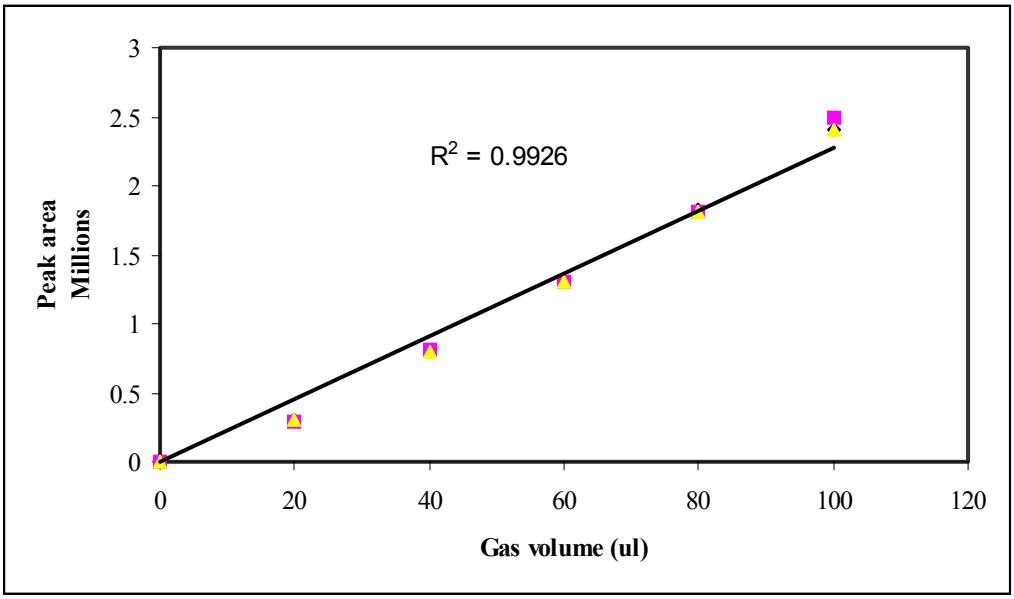

Figure C-4: Plot of the recorded peak areas of three replicates of increasing volumes of standard methane gas.

These results ascertained the accuracy of the GC method, with very similar peak areas for the replicates and a resultant trendline of R^2 value of 0.9926. A similar test was carried out for standard carbon dioxide (Fedgas). The degree of scatter was increased but the reproducibility was still good.

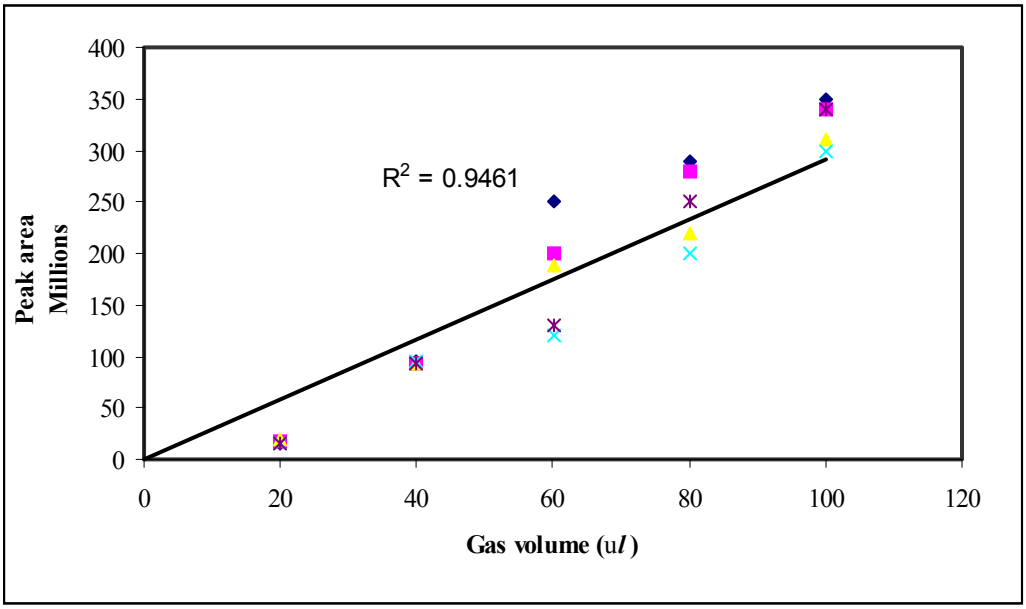

Figure C-5: Plot of the recorded peak areas of five replicates of increasing volumes of standard carbon dioxide gas.