INSTALLATION, COMMISSIONING AND PRELIMINARY MICROBIOLOGICAL AND OPERATIONAL INVESTIGATIONS OF FULL-SCALE SEPTIC TANK DIGESTION OF SEWAGE

By

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CONTENTS

ABSTRACT

This study investigated the commissioning and maintenance of a Pennells two-tank bioreactor system with specific reference to its application in rural areas of KwaZulu-Natal, South Africa to treat sewage and generate biogas. The septic tank configuration was installed in a community which lacked electricity and domestic waste disposal. An artificial wetland was constructed at the outlet of the system to facilitate further treatment.

Inefficient operation and maintenance of the system occurred due to various social/community-related problems which are typical of a field- and community-based project of this nature in a rural region of a Third World African country. These problems affected both maintenance and digester performance. The Pennells system was characterized by incomplete anaerobiosis which limited methanogenesis. Despite this, and attendant problems of low temperatures and elevated pH values, COD removal resulted.

Laboratory-scale batch cultures, in conjunction with fluorescence and scanning electron microscopy, were used to identify a suitable anaerobic digester sludge for inoculation purposes. Perturbation experiments with locally used detergents and toxic compounds demonstrated the inimical effects of these agents. In contrast, low concentrations of penicillin and tetracycline promoted methanogenesis. Further analysis with light, fluorescence and scanning electron microscopy identified the acidogens as the predominant bacterial species, whilst fluorescence microscopy confirmed the absence of methanogens in the bioreactor.

DECLARATION

I hereby certify that this research, unless specifically indicated in the text, is the result of my own investigations.

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INTRODUCTION

Most of South Africa is semi-arid and water has been identified as the country's most limited natural resource (Toerien and Hattingh, 1969). This dire situation is compounded by the pollution of existing water supplies (Louw, 1992). It has been estimated that approximately one third of the population of South Africa does not have access to safe water (Giacasso, 1996) and that 80-86% of the rural population does not have access to adequate sanitation facilities (S.A. Water Research Commission, 1993a; Lotter and Pitman, 1996).

In urban areas, sanitation can also be problematic. Estimates for 1990 showed that, out of the urban population of $\pm$ 25 million people: 4 million had minimal water supply provision; 7 million had minimal sanitation; and 13-14 million had good provision of water and sanitation (van Ryneveld, 1994). Only 58% of the urban population in KwaZulu-Natal have nominal access to water-borne sanitation. A large proportion (33%) of this population has access only to unimproved pit latrines as a large number of these people live in dense settlements where service provision is poor (S.A. Water Research Commission, 1992; 1993b).

In the Natal Umgeni catchment area, serious problems are being experienced with respect to the provision of adequate domestic waste disposal, potable water and electricity as well as sewage disposal. In general, lack of waste management in the rural regions often results in rivers being used as dumping facilities even though the water is subsequently used for drinking and household purposes, thereby exacerbating the problems of water-borne diseases (Taylor, Howgrave-Graham and Kruger, 1992; Taylor, Senior and Howgrave-Graham, 1993). Pressures to increase the quality of life and protect natural resources from pollution are mounting (van Rooyen and Slatter, 1996). The safe disposal of human excreta is, thus, of paramount importance for health and welfare and also for the social and environmental impacts that it can engender in the communities served (Blair Research Institute, World Health Organisation, 1992). In KwaZulu-Natal the above problems are compounded by a number of features: the population density (10% of the land area of South Africa is inhabited by 35% of the population); a high growth rate (S.A. Water

Research Commission, 1992); and much of the terrain is inaccessible making the supply of grid electricity practically impossible (S.A. Water Research Commission, 1992; Taylor *et al.*, 1992; Taylor *et al.*, 1993). Thus, it is clear that biogas technology should be considered to alleviate some of the problems with the objective of treating organic wastes, improving sanitation and providing an alternative energy source.

The methane gas produced during anaerobic digestion has an economic value and this has given rise to a technology called "biomethanation" (Nyns, 1990). During anaerobic digestion organic matter is mineralized to methane and carbon dioxide (Kalbermatten, Julius, Cunnerson and Mara, 1982). Anaerobic digestion was used extensively for treating domestic sewage prior to 1930 (Clausen and Gaddy, 1987). In recent years, increased fuel prices, depletion of fossil fuels (van Andel and Breure, 1984) and cheaper operating procedures have increased the attractiveness of this technology (Suthaker, Polprasert and Droste, 1991). The methane produced could provide a solution to the shortage of wood-fuel in rural areas (Marawanyika, 1986). Further beneficial uses include: odour reduction; and the production of compost and organic fertilizers (Pain, 1991). The principal requirement in African countries is that the technology and know how are made available to the inhabitants (Udo Ndebbio, Ekpo and Forje, 1989).

Against this background, this study was undertaken to establish whether a Pennells two-tank bioreactor could satisfy the dual objectives of sewage treatment and biogas production in rural KwaZulu-Natal.

CHAPTER 1

LITERATURE REVIEW

1.1 THE APPLICATION OF BIOGAS TECHNOLOGY WORLDWIDE

1.1.1 RURAL AREAS

In rural areas of developing countries sewage is traditionally land disposed (Wright, 1956). Night soil pail sanitation systems are employed in the majority of rural and developing black communities in South Africa since they are the most economical option (van der Westhuizen, 1990). A readily available supply of safe water and concomitant sanitation systems are essential (Kalbermatten *et al.*, 1982) although their sustainability within rural developing communities is problematic (Makhetha, 1990). Economic success of rural communities in developing countries requires integrated solutions to basic problems of water, food and energy supply. Imbalances caused by a rapidly increasing population have led to an increased energy demand and resulted in greater wood consumption in the short term, deforestation in the medium term and, in the long term, erosion of agricultural land, limitation of food production and reduction in water availability (Nyns, 1990). These problems can be solved by biogas technology and third world countries are particularly enthusiastic about this source of readily usable energy (Jiang, Steinsberger and Shih, 1987). Thus, biogas technology has good prospects in the rural areas of most Third World countries (Douthwaite, 1988; Hamad, El-Gammal, Mitry and El-Halwagi, 1989).

1.1.2 BIOGAS PRODUCTION IN AFRICA

For African and other undeveloped countries with no domestic oil production or those threatened by desertification, biomethanation offers potential (Section 1.2). Positive field developments include installation of prototype digesters in rural areas as demonstration units. This has been done in Burkina Faso, Zimbabwe and Madagascar (Rabezandrina, 1990).

South Africa is semi-arid with water the country's most limiting natural resource (Toerien

and Hattingh, 1969) determining its human population "carrying capacity" (Toerien and Maree, 1987). Although feasibility studies have shown that biomethanation is a viable process for purifying domestic wastewater and simultaneously producing energy, in South Africa it is almost non-existent (Williams and Eberhard, 1986). In KwaZulu-Natal high incidences of waterborne diseases and acute water shortages are experienced. About 80% of all illnesses in the region result from inadequate water supply and sanitation facilities (Rivett-Carnac, 1984). Biomethanation technology could, thus, be introduced in this region. Galvanised steel biogas systems, similar to those used in Lesotho and Swaziland, have been installed at Mdukutshani (Natal) (Williams and Eberhard, 1986). Various digester designs are also found in Botswana, Lesotho, Zimbabwe (Williams and Eberhard, 1986), Kenya, Malawi, Tanzania and Rwanda (Meynell, 1982).

Biogas technology can, therefore, be successful in the African rural context and can contribute considerably to local environment protection (Rabezandrina, 1990).

1.1.3 BIOGAS PRODUCTION IN THE REST OF THE WORLD

Biogas technology is well established in China where seven million plants have been established (Sinha and Kandpal, 1990; Nazir, 1991). The technology is also well developed in India (McGary and Stainforth, 1978; Kalbermatten et al., 1982) with a target of 12 million units by the year 2001 (Sinha and Kandpal, 1990). Digesters are also in operation in Korea, Philippines, Thailand, Taiwan, Turkey, New Zealand, Papau New Guinea and several west European countries as well as in Colorado (USA) (Nyns and Naveau, 1981; Nazir, 1991; Taşdemiroğlu, 1991).

1.2 USES OF BIOGAS AND SLUDGE

1.2.1 BIOGAS

The first known use of biogas was as fuel for street lighting in England in 1895 (Obias, 1988). Biogas was later used to run motor vehicles in Germany during World War II when fuel was in short supply (Obias, 1988). Biogas represents a low-cost household fuel for

cooking, heating and lighting (Weller and Willetts, 1977). It is particularly suitable for use in rural areas of developing countries (Gadre, Ranade and Godbole, 1986; Rabezandrina, 1990; Letcher, 1991) and *reduces the time and labour needed to collect firewood or transport coal* (McGary and Stainforth, 1978; Day, Chen, Anderson and Steinsberg, 1990). In India and Nepal, appropriate devices for biogas end use, such as adequate burners for stoves, membrane bulbs or lighting lamps and small biogas engines, are progressively being developed (Meynell, 1982; Obias, 1988). These must be made available to local markets at economic prices (Nyns, 1990).

On farms, biogas can be used for general heating and drying (Grundey, 1980; Ghaly and Ben-Hassan, 1989) as well as in gas lamp burners, refrigerators, digester tank heaters, vehicles/trucks and air compressors (Imhoff and Maskew Fair, 1956; Jiang, Liu and Zhong, 1989) and for electricity generation (Taber, 1976; Kottner, 1994). This final use requires, firstly, changing conventional equipment to burn the gas and, secondly, facilities for gas transport, clean-up, compression and storage (Walsh, Ross, Smith and Harper, 1989). Gas storage requirements may, however, be minimised by controlling production (Chayovan, Gerrish and Eastman, 1988). Biogas compression is possible with applications in dual fuel diesel-biogas engines (Jiang *et al.*, 1989). Fuel cell generators can also utilize biogas for electrification in remote rural areas of developing countries (Ascoli, Pandya and Redaelli, 1989).

Other uses of biogas include ice making and meat processing (Obias, 1988), and drying or incineration of sewage sludge, screenings and skimmings (Imhoff and Maskew Fair, 1956). It is also often distributed by pipeline (Walsh *et al.*, 1989).

1.2.2 SLUDGE

Dewatered anaerobically-digested sewage sludge can be used in the manufacture of clay bricks (Slim and Wakefield, 1991) while wet sludge may be used as a soil conditioner (Obias, 1988; Rabezandrina, 1990) and organic fertilizer (Rorick, Spahr and Bryant, 1980; Carrington, Harman and Pike, 1982). This is particularly advantageous on farms where large quantities are available on site (Lapp, Schulte, Kroeker, Sparling and Topnik, 1975;

Hashimoto, Chen, Varel and Prior, 1979; Skrinde, Fritz and Seabloom, 1981).

Sludge is also pelletized and sold as fertilizer in the Philippines and has the advantage of being no longer odorous but contains many nutrients including N, P, K, Ca, Mg, S and traces of Cu, Zn and Mn (Varel, Hashimoto and Chen, 1980; Korentajer, 1991). In addition, the protein content of dung is concentrated from about 8 to 15% (m/m) in digested slurry (Saxena, Nath and Srivastava, 1989). Instead of land spreading, sludge can be used for: algal (Sehgal, Kaur and Sehgal, 1991) and aquatic plant growth (Balasubramanian and Bai, 1992); as a hydroponics medium; and as animal (Merkel, 1981; Prior and Hashimoto, 1981) and fish feed supplements (Mahadevaswamy and Venkataraman, 1988; 1990; Ling, Den, Lu, Min, Wang and Yuan, 1993).

1.3 BIOGAS COMPOSITION

Biogas is mainly a mixture of methane and carbon dioxide (Mackie and Bryant, 1990). Methane is reported, variably, to comprise 40-45% (v/v) (Pandya, Ghosh and Rastogi, 1988) and 65-80% (v/v) of the total gas (Hamburg, 1989). These two gases often comprise some 90% (v/v) of the biogas (Rivett-Carnac, 1982; Hamburg, 1989). The specific substrate determines the methane to carbon dioxide ratio which is usually 2:1 (Weller and Willetts, 1977). The remaining components are traces of water vapour (2-5% v/v) (Hashimoto, Chen, Varel and Prior, 1980; Pandya et $al.$, 1988), hydrogen (1-10% v/v) (Rivett-Carnac, 1982; Obias, 1988), nitrogen (1-5% v/v), oxygen (0.5-1% v/v), carbon monoxide (0.1% v/v), ammonia ($<0.1\%$ v/v) (Obias, 1988), sulphonated compounds such as H_2S ($<0.1\%$ v/v) (Hashimoto et $al.$, 1980; Pandya et $al.$, 1988) and a few hydrocarbons (Röhr and Wimmerstedt, 1990).

About 90% of the available energy in the substrate is retained in the methane produced with a relatively low yield of microbial cells (Mackie and Bryant, 1990). The net amount of biogas energy generated within a digester is a function of the fraction of total waste available to the anaerobic bacteria and the amount of energy used in process operation (Jewell and Loehr, 1977).

The exact composition of biogas, however, depends on a large number of factors. These include the electron donor(s) (Hamburg, 1989; Drauschke and Neumann, 1992), catabolic products and various bactericide/bacteriostatic compounds present in the feed (Drauschke and Neumann, 1992). In addition, the ratio of carbon to nitrogen of the feed material (Rivett-Carnac, 1982; Pandya *et al.*, 1988), bioreactor retention time, pH (Hassan, Hassan and Smith, 1975) and mixing conditions all influence biogas composition (Pandya *et al.*, 1988). Furthermore, fermentation conditions within the digester (Hamburg, 1989), such as operating temperature (Pandya *et al.*, 1988; Hamburg, 1989), solids concentration, hydrogen ion concentration and absence of oxygen, all affect biogas composition (Hassan *et al.*, 1975) (Section 1.5).

1.4 THE MICROBIOLOGY OF ANAEROBIC DIGESTION

Anaerobic digestion requires diverse complex microbial populations in which stable interactions between various metabolic groups are critical for efficient waste conversion and maintenance of the methanogenic population (Zeikus, 1980; Williams and Shih, 1989). Anaerobic digestion occurs stepwise with interacting physiological groups mediating successive reactions. The products of one reaction are substrates for the next and, consequently, the concentration of any intermediate depends on a balance between its production (genesis) and degradation rate (trophy) (Boone, 1987). Despite its apparent simplicity, the process is microbiologically and biochemically very complex and depends on a finely balanced microbial community and careful operation (Holland, Knapp and Shoesmith, 1987). Figure 1 shows the biochemical stages in digestion while Figure 2 depicts the microbial groups involved in the digestion process. There are, however, a vast number and diversity of microorganisms which have been identified in anaerobic digesters (Howgrave-Graham, 1995).
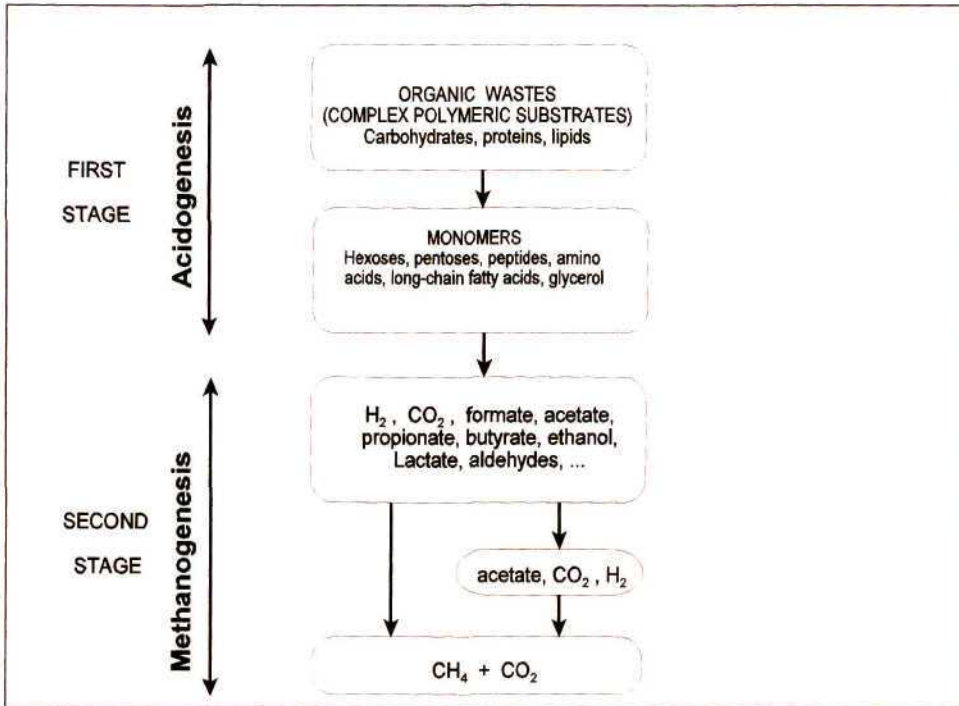
Figure 1: Simplified biochemical stages in biomethanization systems (Vriens, van Soest and Verachtert, 1990).
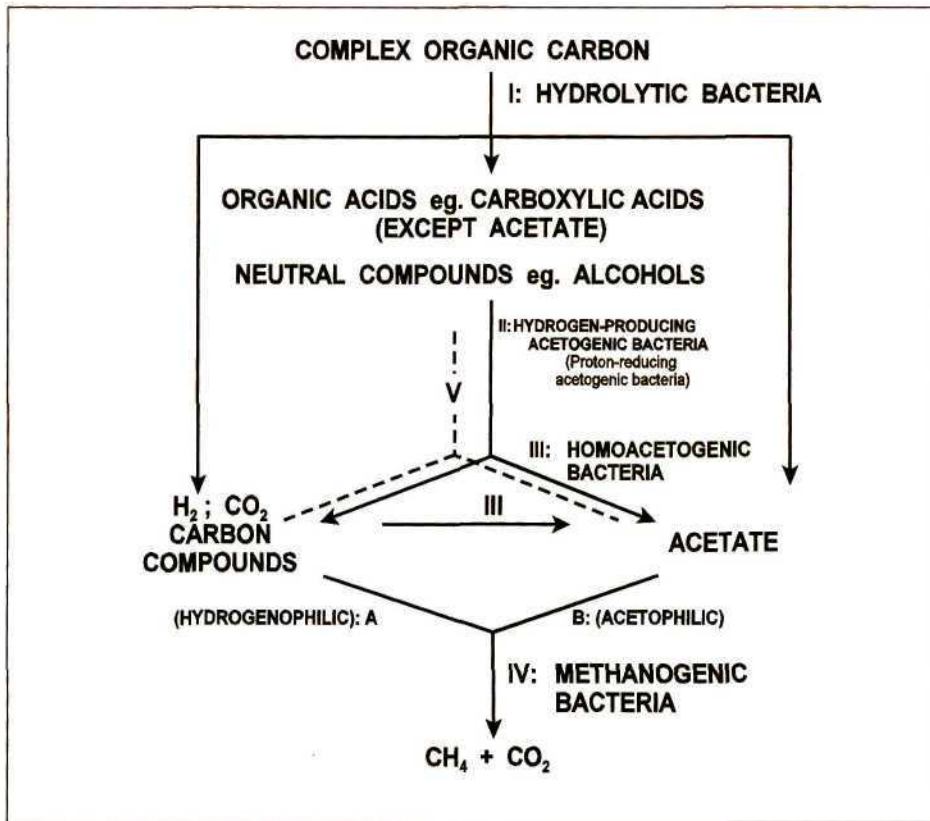
Figure 2: Anaerobic fermentation of organic compounds (Zeikus, 1980; Schink, 1988).

1.4.1 HYDROLYTIC BACTERIA

Group 1 in Figure 2 are the hydrolytic bacteria (Zeikus, 1982; Zoetemeyer, Van Den Heuwel and Cohen, 1982) including the acetogenic (Barnes and Fitzgerald, 1987; De Haast and Britz, 1987) and fermentative bacteria (Zeikus, 1980; Schink, 1988). The principal obligate anaerobes are *Clostridium, Bacteroides, Ruminococcus* and *Butyrivibrio* while the facultative anaerobes include *Escherichia coli* and *Bacillus* species (Schink, 1988; Vriens *et al.*, 1990). Although little is known about the quantitative importance of each species, for polysaccharide degradation, species of *Clostridium, Bacteroides* and, possibly, *Ruminoccocus* and *Bacillus* are likely to be important, while *Clostridium*, especially *Clostridium proteolyticum* (Jain and Zeikus, 1987), is central in protein and nucleic acid catabolism (Holland *et al.*, 1987). Thermophilic hydrolytic bacteria include *Clostridium* spp, the asporogenous *Thermoanaerobium* spp and other unidentified rods (Zeikus, 1980).

The microorganisms present vary according to the waste. For example, in cattle waste digesters most bacteria are hydrolytic and include cellulolytic and acidogenic species (Malik, Tauro and Dahiya, 1989). Cellulolytic bacteria are numerically dominant in cattle manure (Simankova and Nozhevnikova, 1991). Many are spore-forming rods such as *Sporolactobacillus* spp (Sharma and Hobson, 1986) or *Sarcina* spp (Sharma and Hobson, 1990). Other bacteria found in cattle waste include: various *Bacillus, Flavobacterium* and *Micrococcus* species; *S. aureus, Corynebacterium bovis, Pseudomonas aeruginosa, E. coli* and *Salmonella* spp (Taber, 1976); and *Bacteroides xylanolyticus* (Scholten-Koerselman, Houwaard, Janssen and Zehnder, 1986). *Streptococcus, Bacteroides, Peptostreptococcus, Eubacterium, Lactobacillus, Peptococcus* and *Clostridium* spp occur in piggery waste where the major end products are acetate, propionate, succinate, lactate and ethanol, either singly or in various combinations (Taber, 1976; Iannotti, Fischer and Sievers, 1982).

During hydrolysis, complex biopolymers, polysaccharides, lipids, proteins, amino acids, fatty acids and sugars are hydrolysed by extracellular enzymes such as cellulases, amylases, proteases and lipases (Barnes and Fitzgerald, 1987; Schink, 1988; Vriens *et al.*, 1990) to acetic acid, H_2, CO_2, single carbon compounds and organic acids larger than acetic acid, and/or a mixture of alcohols larger than methanol, fatty acids, succinate and

lactate (Weland and Chereminisoff, 1975; Smith, 1981; Vriens *et al.*, 1990). Subsequently, lactate may be catabolized by strains of *Clostridium propionicum*, *Propionibacterium* species, *Megasphaera elsdenii* and *Butyribacterium methylotrophicum* to propionic acid (Touzel, Samain, Albagnac and Morfaux, 1981).

1.4.2 HYDROGEN-PRODUCING ACETOGENIC BACTERIA

Most intermediates, except H_2, formate and acetate, organic acids larger than acetic (eg. butyric or propionic), and neutral compounds larger than methanol (eg. ethanol and propanol) are then converted to acetate, H_2 and CO_2 by organisms of Group 2 (Figure 2), the hydrogen-producing acetogenic bacteria (HPA) (Schink, 1988; Vriens *et al.*, 1990). These are also called the proton-reducing acetogenic bacteria (Zeikus, 1980; Schink, 1988). These obligate and facultative anaerobes depend, for thermodynamic reasons, upon efficient removal of fermentation products (Schink, 1988). In particular, they depend on the presence of hydrogen-removing bacteria (Table 1), such as methanogens (Schink, 1988; Vriens *et al.*, 1990) or sulphate reducers (Schink, 1988), to maintain a sufficiently low hydrogen partial pressure for substrate degradation. Solubilized hydrogen, whilst inhibitory in high concentrations, is an important intermediate in digestion (Mosey and Fernandes, 1989) since its limitation suppresses methanogenesis and sulphate reduction (Bryant, Campbell, Reddy and Crabill, 1977).

The importance of the HPA bacteria depends on substrate input and methanogenic activity of the sludge both of which regulate the flux of carbon and electrons through the reduced intermediate (Schink, 1988). Populations of 4.2×10^6 ml^{-1} of this group of bacteria (Table 1) have been recorded (Zeikus, 1980).

Table 1: Known obligate hydrogen-producing acetogenic bacteria.

Organism	In Co-culture with:	Source	Substrate	Reference
Clostridium bryantii	*Desulfovibrio* E70, *M. hungatei* M1h	Marine and fresh water sediments and digester sludge	Monocarboxylic fatty acids (to 11-carbon)	Schink (1988)
Desulfovibrio vulgaris	*Methanobacterium formicicum*	Digester sludge	Ethanol/ lactate	Bryant *et al.*(1977)
D. vulgaris and *D. desulfuricans*	*Methanobacterium* strain MOH	Digester sludge	Ethanol	Bryant *et al.*(1977)
Syntrophobacter wolinii	*Desulfovibrio* G11, or *M. hungatei* JF1 plus D. G11	Digester sludge	Propionic acid	McCarty and Smith (1987); Samain, Moletta, Dubourguier and Albagnac (1987); and Schink (1988)
Syntrophomonas wolfei	*Desulfovibrio* G11, *M. hungatei* JF1	Digester sludge, aquatic sediments and rumen	Monocarboxylic saturated fatty acids (14- to 8- carbon)	Schink (1988)
Syntrophus buswellii	*Desulfovibrio* G11, *M. hungatei* JF1 plus D. G11	Digester sludge, aquatic sediments	Benzoic acid	Schink (1988)
Unidentified Strain Gra 1 val	*Desulfovibrio* E70	Marine sediment	*Iso*valeric acid	Schink (1988)
Unidentified Strain Go 1 val	*Desulfovibrio* (Marburg)	Digester sludge	*Iso*valeric acid	Schink (1988)
Unidentified Strains SF-1 and NSF-2	*M. hungatei* or *Desulfovibrio* sp.	Digester sludge	Monocarboxylic saturated fatty acids (4- to 6- carbon)	Schink (1988)
Unidentified Strain BZ-2	*Desulfovibrio* PS-1 or *Methanospirillum* PM-1 plus *Desulfovibrio* PS-1	Digester sludge	Benzoic acid	Schink (1988)

Little is known about the physiology of hydrogen- and acetate-producing syntrophs since growth is slow and only occurs in co-culture with hydrogen-using bacteria (Beaty, McInerney and Wofford, 1987). The close symbiotic relationship between molecular hydrogen-producing and- consuming species is dependent on the energy available (McCarty and Smith, 1987). In digesters, sulphate-reducing bacteria such as *Desulfobulbus propionicus or D. elongatus* play limited roles in propionate degradation (Samain *et al.*, 1987). Propionate and butyrate metabolism may be the rate-limiting step in anaerobic digestion where high organic loading rates result in increased concentrations of these intermediates (Henson, Bordeaux and Smith, 1987).

1.4.3 HOMOACETOGENIC BACTERIA

Group 3 in Figure 2 are the homoacetogenic bacteria (hydrogen-consuming acetogenic bacteria) which catabolise single carbon or multi-carbon compounds to acetic acid (Zeikus, 1980; Schink, 1988). The importance of homoacetogenic bacteria is less obvious in methanogenic ecosystems, such as anaerobic digesters (Schink, 1988), with *Clostridium* and *Acetobacterium* the recognized genera (Zeikus, 1980). Homoacetogens are the vital link between hydrolysis/acidogenesis and methanogenesis in anaerobic ecosystems. Interspecies hydrogen transfer, with methanogens the hydrogen consumers, makes acetogenic dehydrogenations possible for both obligate and facultative proton-reducing bacteria. Hydrogenotrophs metabolise hydrogen and create the conditions for catabolic oxidations (Mah, Ward, Baresi and Glass, 1977; Dolfing, 1988). Complex interactions between fermentative acid-forming and methanogenic steps occur, with microorganisms having different generation times and the hydrogen molecule playing a critical role in the overall scheme, dictated by a narrow thermodynamic window. This is because the hydrogen concentration must be maintained to promote methanogenesis but must not hinder propionic acid degradation. Hydrogen-forming and-utilizing microorganisms must thus be closely associated for interspecies hydrogen transfer to occur (Aivasidis and Wandrey, 1990).

1.4.4 NITRATE-REDUCING BACTERIA

In the nitrogen cycle, ammonia is converted to nitrite (by *Nitrosomonas* spp.) and then to nitrate (by *Nitrobacter* spp.). These bacteria are chemolitotrophs (Poindexter, 1971; Cheremisinoff, 1994) and occur widely in soil, sewage and aquatic environments (Pelczar, Chan and Krieg, 1986).

Nitrate reduction (denitrification) is the reduction of nitrates to nitrites and nitrites to ammonia (Pelczar *et al.*, 1986) or nitrogen gas (Stolp, 1988; Cheremisinoff, 1994). This is accomplished by heterotrophic bacteria which derive their energy from organic chemicals through the reductions (Stolp, 1988; Cheremisinoff, 1994) under anoxic conditions (Eckenfelder and Grau, 1992; Pitt, 1996). The oxygen of the nitrate serves as an electron acceptor for electrons and hydrogen (Stolp, 1988). The nitrate-reducing bacteria include *Pseudomonas* spp., *Bacillus* spp., *Micrococcus* (*Micrococcus denitrificans*) (Lue-Hing, Zenz and Kuchenrither, 1992; Cheremisinoff, 1994), *Achromobacter* spp., *Spirillum itersonii*, *Alcaligenes* spp., *Thiobacillus* spp. (*T. denitrificans* and *T. thioparus*), *Azospirillum brasilense*, *Agrobacterium* spp., *Chromobacterium* spp., *Corynebacterium* spp., *Flavobacterium* spp., *Hydromicrobium* spp., *Vibrio* spp., *Moraxella* spp., *Enterobacter* spp., *Escherichia* spp., *Mycobacterium* spp., *Staphylococcus* spp., and *Clostridium* spp. (Pelczar *et al.*, 1986; Stolp, 1988; Lue-Hing *et al.*, 1992).

Denitrifying bacteria establish anaerobiosis by reducing the redox potential through the utilization of nitrate (Kulkarni and Godbole, 1990). Denitrification is enhanced by an abundance of organic matter, elevated temperatures and neutral/alkaline pH values (Pelczar *et al.*, 1986). Denitrifying bacteria can, however, inhibit methanogenesis when nitrate is available in the ecosystem (Kulkarni and Godbole, 1990). Nitrite and cadmium can be inhibitory to denitrification (Stolp, 1988).

1.4.5 SULPHATE-REDUCING BACTERIA

Some members of this group were introduced above (1.4.2) but their pivotal roles warrant further discussion. Methanogens and sulphate-reducing bacteria compete for electron

donors in anaerobic environments. In the presence of available sulphate, sulphate reduction generally dominates methanogenesis due to kinetic and thermodynamic advantages. Populations of 10^4 sulphate reducers ml^{-1} of sewage digester sludge have been recorded for both *Desulfotomaculum* and *Desulfovibrio* species (Zeikus, 1980; Dubourguier, Presnier and Albagnac, 1988).

Following sulphate depletion, sulphate-reducing bacteria facilitate methanogenesis by interspecies hydrogen transfer. Studies on interrelationships between different groups of bacteria have shown that the sulphate reducers reduce the initial sulphate content of fermenting slurry while the denitrifiers promote anaerobiosis by reducing the redox potential through nitrate utilization (Kulkarni and Godbole, 1990).

1.4.6 METHANOGENIC BACTERIA

The methanogenic bacteria (Group 4, Figure 2), catabolise one- and two-carbon compounds to methane and carbon dioxide (Zehnder and Stumm, 1988; Vriens *et al.*, 1990). They remove hydrogen produced by the HPA thus making the thermodynamic conditions favourable for growth of the HPA (Jarrell, Saulnier and Tey, 1987; Vriens *et al.*, 1990). Digesters are often "seeded" with methanogens since they take time to multiply (Boopathy, 1987).

These bacteria often display more microbiological diversity than the other trophic groups (Zeikus, 1980). Populations of 10^8 methanogens ml^{-1} occur in sewage digester sludge with four genera of H_2-oxidizing methanogens, *Methanospirillum*, *Methanosarcina*, *Methanococcus* and *Methanosaeta*, present in numbers between 10^6 and 10^8 ml^{-1} (Zeikus, 1980). The main hydrogenotrophic methanogen, *Methanospirillum hungatei*, has been observed in propionate- and butyrate-degrading associations enriched and isolated from sewage sludge and sediments (Fannin, Conrad, Srivastava, Jerger and Chynoweth, 1982; Dubourguier, Presnier, Samain and Albagnac, 1985). This bacterium oxidizes H_2 to H_2S or methane with sulphate or carbon dioxide the respective electron acceptors and acetate and carbon dioxide as the cell material precursors (Schink, 1988).

Strains of *Methanosarcina* and *Methanosaeta*, which use acetate as an energy source to form methane and carbon dioxide (Weigant and de Man, 1986; Tomei, Rouse, Maki and Mitchell, 1988), are important in waste treatment (Touzel and Albagnac, 1987). Acetate has been shown to account for $>$ two-thirds of the methane produced (Koster and Cramer, 1987; Touzel and Albagnac, 1987). Methanogenesis from acetate occurs via two mechanisms: a primary route where methane is derived from an intact methyl group; and a secondary pathway where methane is formed by coupling methyl group oxidation to reduction of the carboxyl group (Krzycki and Zeikus, 1987). Acetate degradation is the step which determines the stability of anaerobic digestion (Siegrist, Renggli and Gujer, 1993).

1.4.7 DIVERSE BACTERIAL SPECIES (GROUP 5)

Species in Group 5 (Figure 2) can reverse some of the energetically delicate reactions whereby fatty acids are oxidized to acetate and CO_2 in the presence of excess ethanol or H_2, eg. during butyrate formation from acetate and ethanol by *Clostridium kluyveri* (Schink, 1988). Propionate-forming hydrogenase-producing bacteria convert acetate or ethanol to propionate which acts as a hydrogen sink. These bacteria may be involved in ethanol degradation and may cooperate with homoacetogenic bacteria in propionate formation from one-carbon compounds (Schink, 1988).

1.5 THE EFFECTS OF PHYSICOCHEMICAL FACTORS ON DIGESTION

Numerous parameters, both physical and chemical, influence microbial populations and digester performance (Zeikus, 1980) and their monitoring is important to identify impending digester failure (Holland *et al.*, 1987). The main factors are pH, alkalinity, volatile fatty acid (VFA) concentration, nutrient availability and the presence of toxins. In addition, temperature, hydraulic retention time, influent substrate concentration and composition, degree of mixing and solids (organic) loading rate are important (Morris, Jewell and Caster, 1975; Weland and Cheremisinoff, 1975), as are redox potential (E_h), digester biomass content (Archer, 1983) and the partial pressure of hydrogen (pH_2) (Collins and Paskins, 1987).

Conditions within a digester determine whether there is a suitable habitat for a given microorganism. A physical or biological change may lead to an adaptive reaction by the associated microbiological population which may manifest as changes in bacterial metabolism, through alterations at the enzymic level, or growth or displacement of certain metabolically active members of the population (van Andel, Breure and Cohen, 1986). In general, the methanogens are the most sensitive to environmental condition changes (Pfeffer, 1980; Jarrell *et al.*, 1987) since the usual indication of impending process failure is the accumulation of acetate and, subsequently, propionate (Pfeffer, 1980; Vriens *et al.*, 1990).

A higher CO_2 percentage in the gas evolved and decreases in the pH, total gas production and COD removal efficiency are all indicators of digester failure (Vriens *et al.*, 1990). Other tests which can also be used to identify failure include tracer tests with lithium to ascertain flow patterns (Nilsson and Karlsson, 1990) and phenyl acetic acid concentrations which indicate metabolic activity and overall process stability (Iannotti, Mueller, Sievers, Georgacakis and Gerhardt, 1986).

The most important physicochemical factors affecting digester performance and microbial population composition are discussed below.

1.5.1 NUTRIENTS

Bacteria responsible for waste conversion and stabilization require nitrogen, phosphorus and other minerals in trace quantities for optimum growth (McCarty, 1964; Pfeffer, 1980). If these are absent or limiting (Pfeffer, 1980) they must be supplied since metal deficiencies limit anaerobic digester performance (Callander and Barford, 1983). The absence of a particular nutrient can preclude microorganisms with obligate requirements (Speece and Parkin, 1987). Nutrient requirements vary and are complex although the micronutrients nickel (Hassan Dar and Tandon, 1987; Raju, Devi and Nand, 1991), cobalt (White and Plaskett, 1981; Meynell, 1982) and iron (Raju *et al.*, 1991) are often required. Magnesium, sodium and potassium are also required (Loehr, 1977). The elements copper, manganese, calcium, cobalt, selenium, molybdenum and tungsten are needed in small

quantities (Meynell, 1982; Oremland, 1988; Portier and Palmer, 1989). Ideally, methanogenesis requires a carbon:nitrogen:phosphorus ratio of 200:5:1 (Vriens *et al.*, 1990). Some methanogens need growth factors such as fatty acids (acetate, 2-methylbutyrate), vitamins and complex nutrient supplements, whilst others are autotrophs (Holland *et al.*, 1987; Oremland, 1988). Coenzyme M is needed by *Methanobrevibacter ruminantium* while reduced organic (cysteine) and inorganic (sulphide) sulphur compounds, which are used as reducing agents in isolation procedures or are present in the wastewater, satisfy methanogen sulphur requirements (Oremland, 1988).

1.5.2 TEMPERATURE

Organic waste conversion rates in anaerobic digesters are slow in the psychrophilic temperature range. These then require lower space loadings (Vriens *et al.*, 1990) and, hence, longer hydraulic retention times (Pos, Eszes and Pavlicik, 1985; Vriens *et al.*, 1990). With mesophilic and thermophilic temperatures, bacterial activity (as indicated by the degree of acidification (Dinopoulou, Rudd and Lester, 1988), treatment efficiency (COD removal) and biogas production (Archer, 1983; Beba, 1988)), is high (Holland *et al.*, 1987) and the reaction rates are faster (Vriens *et al.*, 1990). Thus, a high degree of stabilization is effected by a short hydraulic retention time (Holland *et al.*, 1987). This promotion, however, must be balanced against increased digester heat requirements (Beba, 1988). The optimum temperature for methanogenesis is, generally, between 30 and 35°C although it can by higher (Holland *et al.*, 1987) and can be advantageous for some substrates (Weigant and de Man, 1986; Sandberg and Ahring, 1992). Sudden temperature changes are disadvantageous and may adversely affect methanogenesis (Holland *et al.*, 1987).

1.5.3 pH

Microorganisms generally grow within limited pH ranges (Sasahara, Kitamura and Ogawa, 1982). pH stability is thus crucial in anaerobic digestion since sudden changes may inhibit gas production and result in digester failure. If operated correctly, however, digesters may be self-stabilizing systems (Holland *et al.*, 1987). Heterophilic acetogenic bacteria are less

sensitive to low pH than methanogens but pH control is essential (McInery and Bryant, 1981; Zehnder, Ingvarsen and Marti, 1982) to maintain viability of the latter (Archer, 1983). pH 6.8 to 7.2 is the ideal range for anaerobic digester operation (Ross, Novella, Pitt, Lund, Thomson, King and Fawcett, 1992).

If acidogenesis proceeds too rapidly (eg. following shock loadings) compared with acetoclastic methanogenesis, organic acids and hydrogen accumulate (Archer, 1983), the pH falls (Zoetemeyer et al., 1982; Vriens et al., 1990), the methanogens are inhibited (Zoetemeyer et al., 1982) and the fermentation fails. Methanogenesis inhibition by acidification can be prevented by efficient pH control. Hence, under conditions of shock loading coupled to pH control, organic acid production (R_1) exceeds methanogenesis (R_2) and excess acids appear in the effluent until the system is once again balanced (Archer, 1983). Excess hydrogen inhibits the acetogens and is an indicator of an impending pH fall (Heyes and Hall, 1981; Vriens et al., 1990). A drop below pH 6.6 causes significant methanogen inhibition whilst at pH 6.2 the acid conditions effect "souring" (Pfeffer, 1980; Holland et al., 1987). Digester imbalances favouring rapid growth of non-methanogens (high feed rate or feed chemical composition imbalances) lead to low pH values and, thus, inhibition of methanogens. At pH values up to 6 fermentation by the butyric acid-type species predominates, in the pH rage of 6-7 fermentation by coliforms dominates, while at higher pH values acetic acid bacteria play a role (Zoetemeyer et al., 1982). In balancing tanks the effluent pH can decrease due to organic acid formation (Sasahara et al., 1982).

1.5.4 REDOX POTENTIAL AND THE PARTIAL PRESSURE OF HYDROGEN

For obligate anaerobes, such as methanogens, a few ppm of oxygen are inhibitory (McCarty, 1964; Pfeffer, 1980). This exposure lowers their adenylate charges and death results from an irreversible dissociation of the methanogen F_{420}-hydrogenase enzyme complex. In cells grown under an Fe^{2+} limitation, F_{420} degrades rapidly upon exposure to oxygen (Oremland, 1988) or any other highly oxidized material such as nitrites or nitrates (Pfeffer, 1980).

The redox potential (E_h) is affected by changes in pH by an alteration of the ionic

equilibrium (Archer, 1983). Methanogens need a low E_h for growth (Oremland, 1988) and the optimum value is $\leqslant -200mV$ (Mah and Smith, 1981; McInery and Bryant, 1981). A low tolerance to higher redox potentials is exhibited by methanogens in monoculture (Mah and Smith, 1981). Electron acceptors which create standard redox potentials higher than that for the CO_2/CH_4 couple, by diverting electrons from the reduction of carbon dioxide, oxygen, nitrate and sulphate, are all inhibitory to methanogenesis (Archer, 1983).

The partial pressure of hydrogen (pH_2) is a key factor in the anaerobic metabolism of carbohydrates, alcohols, fatty acids and xenobiotics (Bleicher and Winter, 1994). Low E_h systems, such as those involved in acidogenesis from pyruvate, are relatively unaffected by pH_2. High E_h reactions such as hydrogen production from NAD(P)H or acetogenesis from propionate or butyrate are only favourable at low pH_2 values (Archer, 1983). pH_2 increases cause the flow of electrons to shift from hydrogen production to fatty acid formation (Senior and Balba, 1984; Aragno, 1988). Methanogenesis from H_2/CO_2 becomes unfavourable at a pH_2 of 2.5×10^{-6} atmospheres while acetogenesis from H_2/CO_2 becomes unfavourable at a pH_2 of 2.5×10^{-5} atmospheres. A pH_2 rise is inhibitory to the production of more hydrogen and acetate from fatty acids so organic acids can only be removed if their concentrations relative to acetate are very high. However, since acetate removal is rate limiting, if it is prevented the fermentor fails due to acidogenesis. Fluctuations in pH_2 or fatty acid concentrations occur normally due to changes in the feed rate or composition and need not be harmful (Archer, 1983) unless these fluctuations become excessive.

1.5.5 CHEMICALS DETRIMENTAL TO THE MICROBIOLOGY OF ANAEROBIC DIGESTION

Methanogen viability is crucial to the digestion process and a fall in methane concentration may reflect inhibitors in the influent (Pfeffer, 1980; Vriens *et al.*, 1990). Many organic and inorganic substances are inhibitory to anaerobic digestion (Morris *et al.*, 1975; Fedler and Day, 1985) and their activities may vary according to the physicochemical conditions in the digester (Vriens *et al.*, 1990). However, only materials in solution are toxic (Pfeffer, 1980).

ORGANIC SUBSTANCES

Increased VFA concentrations, due to organic overloading, as a result of an increase in the influent concentration (Jarrell *et al.*, 1987; Vriens *et al.*, 1990), or volumetric overload, due to an increase in substrate throughput (Whitmore, Lloyd, Jones and Williams, 1987), cause digester failure. This can only be eliminated by cessation of feeding, acids neutralization, dissolved hydrogen concentration regulation via controlled substrate addition, and a recovery period (Holland *et al.*, 1987; Whitmore *et al.*, 1987).

Antibiotics, pesticides, disinfectants (Meynell, 1982), detergents and some animal feed additives (eg. monensin) may also cause toxicity (Strauch and Winterholder, 1985). In digesters where minerals are supplemented, the influent may unavoidably contain traces of toxic elements (Prior and Hashimoto, 1981).

Antibiotics which reduce methane production include chlortetracycline, bacitracin, lincomycin, oxytetracycline and virginramycin, while the degree of inhibition depends on the retention time (Fedler and Day, 1985). When animal manure is used as the substrate for biogas generation, drugs used for antimicrobial treatment generally do not reach the slurry as they are metabolized in animal organs and are excreted after inactivation. Thus, they do not adversely affect biogas production (Strauch and Winterholder, 1985).

Some synthetic detergents, particularly diphatic sulphonates (White and Plaskett, 1981), chloroform, chlorinated hydrocarbons, trichloroethane or pentachlorophenol (White and Plaskett, 1981; Meynell, 1982) and alkylbenzene sulphonate detergents (Holland *et al.*, 1987; Khalil, Whitmore, Gamel-El-Din, El-Bassel and Lloyd, 1989) are also inhibitory. Certain synthetic pyrenoids and non-ionic detergents can, depending on their concentration, actually increase gas production (Cohen, 1991).

Many disinfectants are toxic, especially those with a CS_2/chloroform base which may completely and irreversibly inhibit methanogenesis (Strauch and Winterholder, 1985; Vriens *et al.*, 1990). This results in hydrogen, acetate and propionate accumulation (Ukei, Ukei and Simogoh, 1988). Disinfectants which contain aldehydes, alcohols, phenols and

quaternary ammonium compounds are, however, only slightly toxic (Vriens *et al.*, 1990).

Formaldehyde (Jans, 1981; Lettinga, Roersma, Grin, de Zeeuw, Hulshof Pol, van Velsen, Hobma and Zeeman, 1982), iodopropane (Oremland, 1988) and low concentrations of acetylene (Sprott, Jarrell and Knowles, 1981) are toxic.

INORGANIC SUBSTANCES

Salts of sodium, potassium, calcium and magnesium exert inhibition with toxicity attributed to the cations (Loehr, 1977; DaSilva, 1981). The threshold concentration depends upon whether the metals act singly or in combination (Pfeffer, 1980; Holland *et al.*, 1987). Ammonia, particularly in the NH_3 form, is inhibitory in concentrations between 1500 and 3000mgl^{-1} total ammonia nitrogen and a pH > 7.4 (Morris *et al.*, 1975; Jarrell *et al.*, 1987; Vriens *et al.*, 1990). At higher concentrations the ammonia ion itself becomes toxic regardless of pH (Pfeffer, 1980; Holland *et al.*, 1987).

Other toxic non-metals include CN^-, CCl_4, $CHCl_3$, CH_2Cl_2 (Lettinga *et al.*, 1982; Dolfing and Tiedje, 1988), 2-bromoethanesulfonic acid and nitrogen oxides (Oremland, 1988). Heavy metals, including poorly soluble concentrations of copper, zinc, nickel, mercury and chromium salts (White and Plaskett, 1981; Meynell, 1982; Jarrell *et al.*, 1987), can be toxic. Heavy metal toxicity is determined by the degree of attraction to natural metal binding sites on and within the cell and is affected by environmental and nutritional factors since cells in nutrient-depleted environments are often more susceptible (Portier and Palmer, 1989). Toxic heavy metal and trace metal ions can be precipitated as sulphides and thus detoxified or removed by stripping (Lettinga *et al.*, 1982; Vriens *et al.*, 1990). Microorganisms can to some extent acclimate to inhibiting concentrations (Pfeffer, 1980; Lettinga *et al.*, 1982).

Molybdate is an effective inhibitor of acetogenesis by sulphate reducers (Clancy, Venkataraman and Lynd, 1991). Electron acceptors such as nitrate and sulphate inhibit methanogenesis in mixed microbial ecosystems by channelling electron flow to microorganisms such as denitrifiers and sulphate reducers which are thermodynamically

more efficient than the methanogens (Zehnder *et al.*, 1982; Oremland, 1988). Sulphides inhibit methanogenesis (Ukei *et al.*, 1988) by three proposed mechanisms: (i) sulphide formed during bacterial sulphate reduction is inhibitory to methanogens (Zehnder *et al.*, 1982; Cohen, 1991); (ii) the sulphide formed precipitates essential trace metals (Fe, Ni, Co, Mo) thus limiting their accessibility (Zehnder *et al.*, 1982; Oremland, 1988); and (iii) electrons from the oxidation of organic matter are used mostly for sulphate reduction which is thermodynamically more favourable than methanogenesis (Zehnder *et al.*, 1982). Sulphate ions themselves have no effect on methanogens in monoculture although sulphite and elemental sulphur strongly inhibit methanogenesis by monocultures of methanogens growing on hydrogen (Oremland, 1988). Soluble sulphide concentrations from 50 to 100 mgl^{-1} can be tolerated in anaerobic digestion with little or no acclimation whereas up to 200 mgl^{-1} can be tolerated with some acclimation (Pfeffer, 1980). Low sulphite concentrations can be attenuated by sulphate-reducing bacteria, by removal as hydrogen sulphide (Vriens *et al.*, 1990).

1.6 TYPES OF BIOGAS DIGESTERS

The various designs of digesters may be batch or continuous loading (Weller and Willetts, 1977; Mann and Williamson, 1982). For the purpose of this thesis, only a few of the more common types will be considered.

1.6.1 FACTORS TO CONSIDER IN DIGESTER DESIGN AND CONSTRUCTION

When selecting and designing sanitation systems there are numerous factors to consider for successful waste management (Lombard, 1994). These include pertinent topographical features, ground elevations, property lines, existing and proposed buildings and the proximity and situation of adjacent water bodies (Laak, 1986). In addition, climatic and on-site conditions, sociocultural factors typical of the community (Kalbermatten *et al.*, 1982; Nyirenda, 1994; Lombard, 1994), population size, current sanitation practices, the institutional framework (Kalbermatten *et al.*, 1982) and education levels of the public and professionals destined to manage and operate such systems are important (Lombard, 1994).

Envisaged gas plus sludge uses are also important (Meynell, 1982). The mixing facilities required to promote maximum contact between the microorganisms and substrate, insulation, biogas release from the liquid phase, and prevention of stratification and scum blanket formation must be catered for in any biogas digester design (Meynell, 1982; Rivett-Carnac, 1982).

In choosing a design the following must be considered: waste characteristics (Nyirenda, 1994), including volume and strength (Meynell, 1982); anticipated shifts in pH and temperature; hydraulic loads or wastewater flow fluctuations (Laak, 1986); the maximum biogas recovery projected from the available organic matter; and the optimum digester performance expected (to determine the minimum effective digester volume) (Rivett-Carnac, 1982). The availability of cheap, efficient, locally-produced equipment and materials (Nyirenda, 1994; Lombard, 1994), wage rates and fuel costs are also important considerations (Nyirenda, 1994).

Based upon the above criteria, many digester designs have been developed.

1.6.2 THE MOST COMMON DIGESTER DESIGNS

FLOATING GAS HOLDER TYPES

The first main digester type used in India was the floating gas holder type (Gobar gas plant) (Figure 3) (Kalbermatten *et al.*, 1982; Williams and Eberhard, 1986). This is also known as the KVIC type (Khandi and Village Industries Commission) and Indian Agricultural Research Institute (IARI) design. This digester is simple to build, operate and maintain (Rivett-Carnac, 1982), it has two chambers, is rough plastered and is sealed with dung (Weller and Willetts, 1977).

There are vertical types for non-rocky areas and low water tables or horizontal types for rocky regions with a high water table (Letcher, 1991). Both are buried and, thus, semi-insulated. The configuration consists of a digester, gas holder, inlet and outlet assembly and water removal device (Singh and Gupta, 1990; Letcher, 1991) (Figure 3).
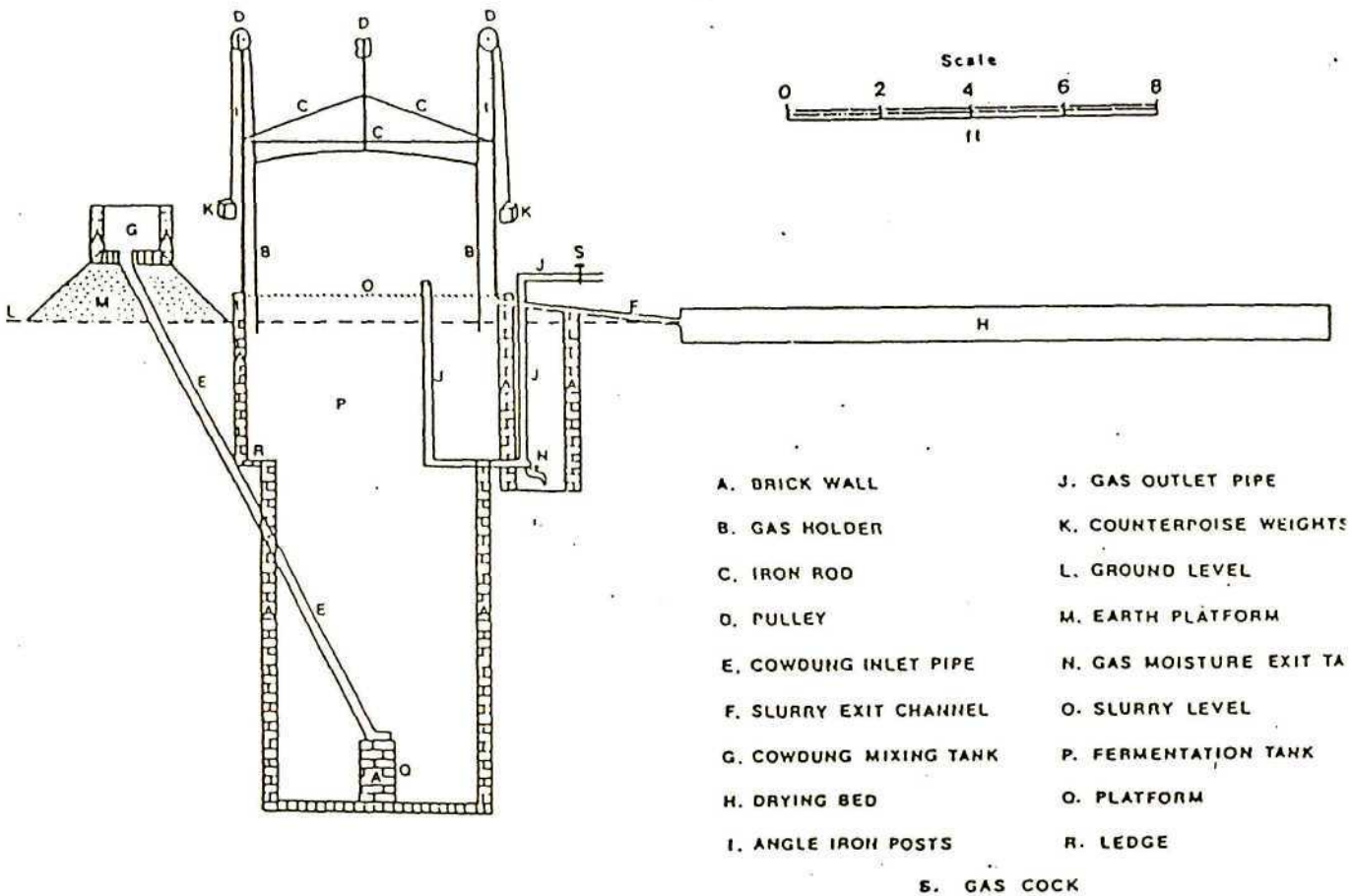
Figure 3: Design of Indian Gobar gas plant (Rivett-Carnac, 1982).

Floating gas holders are usually made from 16-gauge mild steel plate which has the disadvantage of corrosion (Letcher, 1991). The Ganesh and Sasse floating drum designs (Figure 4) (Day *et al.*, 1990; Letcher, 1991) can, however, be made of other materials such as fibre-reinforced plastics, high density polyethylene and butyl rubber (Letcher, 1991). The Sasse type has a floating cover which acts as a gas holder and rises and falls with gas production and usage, respectively, maintaining a near constant gas pressure, depending upon the gas holder lid weight (Day *et al.*, 1990).
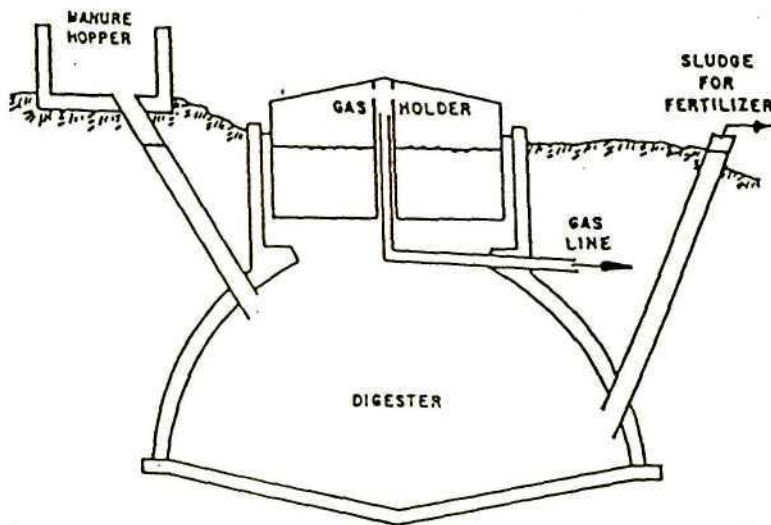
Figure 4: Diagram of the Sasse floating drum design (Day *et al.*, 1990).

The Ganesh is an adapted version of the floating gas holder type biogas digester with the digester portion made from an angle iron frame wrapped with a polyethylene sheet rather than masonry (Letcher, 1991). Installation costs are 30-40% less as transportation and installation costs are lower (Letcher, 1991).

Gas release is at constant pressure with the floating gas holder type digester and relatively less excavation work is required. The location of structural defects and repairs within the floating gas holder types are easier than in the fixed-dome types discussed below (Letcher, 1991).

FIXED-DOME DIGESTER TYPES

The second main group of Indian digester designs is the fixed-dome types such as the Janta biogas plant which is modelled on the Chinese fixed-dome design. Characteristically, a steel gas holder is not required and the digester and gas holder form an underground combined unit (Letcher, 1991).

The fixed-dome type has several advantages over the floating type. The capital investment

is lower, a steel gas holder is not required, the maintenance costs are minimized due to a lack of moving parts, and the temperature effects are minimised since it is below ground (Letcher, 1991).

Indian designs have the advantage of avoiding solids build up and they do not require mechanical mixing (Rivett-Carnac, 1982; Williams and Eberhard, 1986). By 1990, about 500 000 floating gas holders and fixed-dome-type digesters had been commissioned in India (Letcher, 1991).

CHINESE DIGESTER DESIGNS

Modified Chinese biogas plants (Figure 5) have the advantage of being low cost (Rivett-Carnac, 1982; Williams and Eberhard, 1986) due to the use of locally available materials and skilled expertise and labour within the community. These digesters have no moving parts and floating metal gas holders are not used thereby avoiding corrosion problems. A disadvantage is that, due to the fixed dome construction, they do not allow for gas storage, hence the gas must be regularly used (Rivett-Carnac, 1982). Other problems include gas leaks and drops in gas pressure which lead to erratic gas availability (Williams and Eberhard, 1986). In Chinese fixed-dome plants, the heat requirements are minimised by insulating the dome (Kishore, 1989).
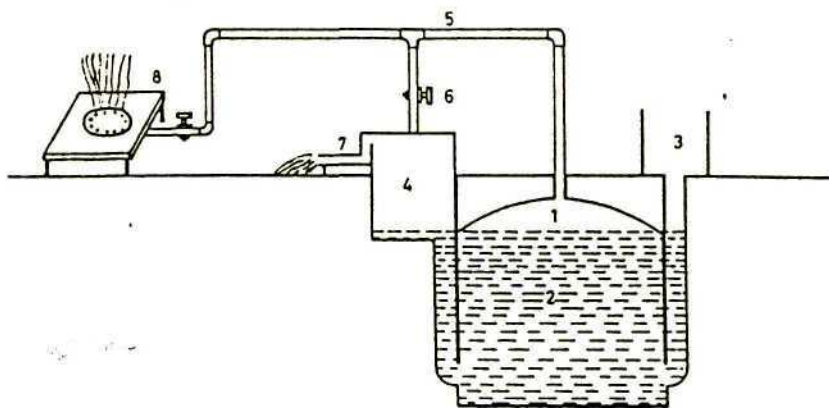
Figure 5: Modified Chinese type of biogas plant (Nazir, 1991). 1, fixed-dome gas holder; 2, digester; 3, mixing tank; 4, auxiliary chamber; 5, gas line; 6, water trap; 7, outlet; 8, stove.

Figures 6-8 show three distinct designs employed in China which incorporate different construction techniques. "Triple concrete" (Figures 7 and 8) in South Africa is stabilized earth made from lime mixed with sand and clay, cinders and clay or stone chips in varying proportions (Rivett-Carnac, 1982).
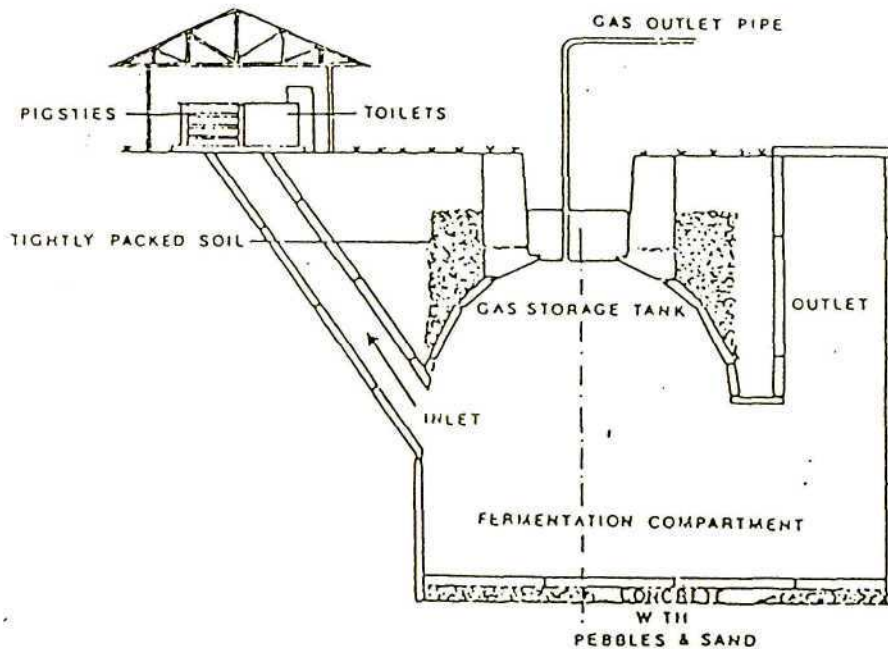
Figure 6: Circular pit Chinese digester design made of stone slabs (Rivett-Carnac, 1982).
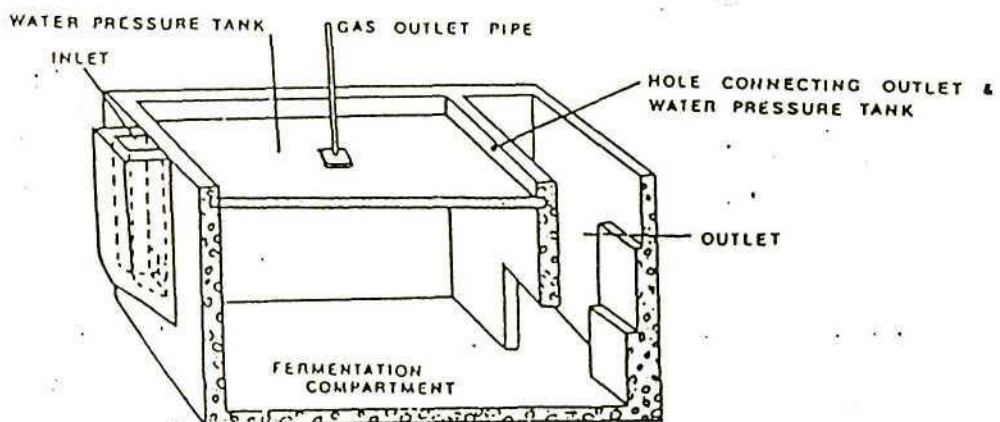
Figure 7: Rectangular triple concrete pit-type Chinese digester (Rivett-Carnac, 1982).
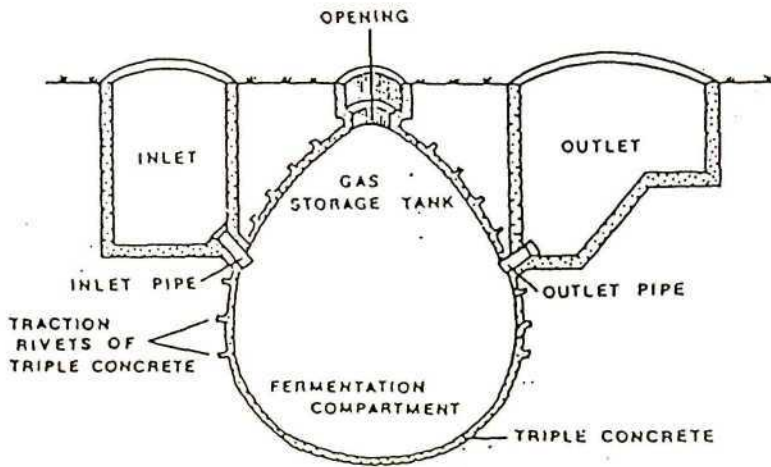
Figure 8: Hollowed out triple concrete pit Chinese digester (Rivett-Carnac, 1982).

Another digester design is the Fry type, or Plug-flow, where semi-continuous operation is preferable to batch feeding for a fairly constant gas production rate (Rivett-Carnac, 1982; Williams and Eberhard, 1986).

1.6.3 OTHER ANAEROBIC DIGESTER DESIGNS

There are many other developments in design, including a portable unit made from used bitumen drums, for treating buffalo dung. This unit was found to be durable and cheap, and easy to construct, operate and clean (Khatib, Usmani and Husain, 1989). A bamboo ring packed-bed digester was designed for rural areas of Thailand (Nazir, 1991). In Taiwan, a 0.55mm thick sausage-shaped haplon bag laminated with neoprene, reinforced with nylon and lined inside with rubber was developed with the biogas stored separately in a plastic bag (Langton, 1981; Nazir, 1991). Bag-type digesters have low cost, mass production capabilities and are easily transportable. They are placed in excavated holes and may be equipped with PVC inlet and outlet pipes and gas release (Langton, 1981).

In Taiwan, bag-type digesters with an 8-year life-span, made of strong, weather resistant red mud-plastic materials are used to digest hog manure. Light-weight, compact plastic digesters are also used. These are easy to operate, quick to assemble and are disposable after use (Langton, 1981). Some more complex digesters have waste heat recovery units

and solar water heating systems (Beba, 1988). A 25*l* unmixed anaerobic reactor has been used for biogas production and as a liquid-solid separator (Ghaly and Ben-Hassan, 1989). More advanced digesters include, for example, inexpensive water pressure digesters in China (Chen, 1982; Chen and Xiao, 1981) and a solids-concentrating (SOLCON super (+)) bioreactor developed for high-rate methane production (Srivastava, Biljetina, Isaacson and Hayes, 1989).

Developments in biomethanation have improved digester efficiency and economics. High load-rate digestion has been achieved at thermophilic temperatures, facilitating higher gas production from a lower digester volume, thus reducing construction costs. Plug-flow digesters using inexpensive construction materials have also been developed to lower costs (Jiang *et al.*, 1987).

It can, thus, be seen that the digestion process can be sustained by various digester designs and construction materials.

1.7 SEPTIC TANKS

Septic tanks, although not biogas digesters, are also used to provide a means of sanitation. They are used primarily for treating domestic sewage from single households (Langton, 1981). The system consists of the septic tank itself, usually with two components (Feachem and Cairncross, 1978; Perkins, 1989), and a final disposal system, usually subsoil percolation (Drews, 1985; Perkins, 1989). Raw domestic sewage enters the tank where the solids settle by gravitational forces and are partially digested or disposed of (Langton, 1981; Drews, 1985; A'Bear, 1991). Although some wastes are digested anaerobically (Feachem and Caincross, 1978), "anaerobic" is not an accurate description of the entire system (Perkins, 1989). No nutrients are added to aid digestion and the partially purified liquid is drained off, the gases are vented to the atmosphere and the accumulated solids must be periodically removed (Langton, 1981). The capacity of the septic tank must be sufficient to facilitate complete digestion of inflowing sewage. Blockages of the inlet, outlet and internal pipes often occur (Drews, 1985). During solids settling, three layers are formed in the tank: a bottom sludge layer; a floating scum layer on top; and a middle,

relatively clear, layer (Drews, 1985; Perkins, 1989). Since the digestion period is long, quiescent conditions are needed for the sedimentation tank.

The inlet tee inside the tank should be capped, to avoid flies and insects from breeding in the tank, and to reduce odours, thereby preventing a nuisance and potential health problems (Wolverton, 1989). Septic tank effluent is potentially as dangerous to public health as raw sewage (Drews, 1985; Clausen and Gaddy, 1987) since *Ascaris* ova and viruses may be present. Some reduction in faecal coliforms may, however, take place (Drews, 1985).

In South Africa, various types of latrine systems have been developed by the private firms Pennells Pty. (Ltd.), Norsechem (Pty.) Ltd. and Naschem, an environmental waste management consultant B.E. La Trobe, the Council for Scientific and Industrial Research (CSIR) and the University of Natal's Institute of Natural Resources.

1.8 WETLANDS

Natural and artificial wetlands are used to further aid wastewater purification after, for example, septic tank or biogas digester treatment (Hammer and Bastian, 1989; Mozes, 1992). By their intervention, water quality in river systems is effectively and reliably improved (Kadlec and Kadlec, 1979; Mitsch and Gosselink, 1986; Hammer, 1992). Wetlands may be regarded as transitional areas between terrestrial and aquatic systems (Cowardin, Carter, Golet and Laroe, 1979; Mitsch and Gosselink, 1986) and they account for about 6% of the global land area (Pearce and Turner, 1990).

Two artificial wetland types exist, namely horizontal and vertical flow systems (Bucksteeg, Muller, Mortl, Adelt, Scheer, Labitzky, Sendl, Hormann and Kastner, 1985; Wood and Rowley, 1987). Variations of these systems include the root zone method, the Max Planck Institute and Lelystad process (Wood and Rowley, 1987), and surface or subsurface flow and gravel or soil bed systems (Water Pollution Control, 1993). Stringent site selection and evaluation of land use, hydrology, geology and environmental/regulatory conditions are essential to optimize design, construction and long-term operation (Brodie, 1989), as are

the maintinance of optimal environmental conditions and substrate contact with the microbial species (Hammer and Bastian, 1989; Portier and Palmer, 1989).

Artificial wetlands are regarded as environmentally friendly, low technology, natural alternatives to conventional treatment systems (Rodgers, Rogers and Buzer, 1985; Trattner and Woods, 1989; Water Pollution Control, 1993), with applications in many situations (Bucksteeg et al., 1985; Wood and Hensman, 1989). They provide attractive green-space wildlife and fish habitats and are used as nature conservation, education and recreation areas (Trattner and Woods, 1989; Mozes, 1992). Artificial wetlands are ideal for rural areas (Wood and Hensman, 1989) and are particularly suitable for developing countries (Rodgers et al., 1985; Water Pollution Control, 1993). More than 60 are currently operating successfully in South Africa (Wood, 1996).

Artificial wetlands are relatively inexpensive to construct, operate and maintain (Hammer and Bastian, 1989). They possess hydrological, ecological and socioeconomic value (Trattner and Woods, 1989). They are relatively tolerant of fluctuating hydrological and contaminant loading rates and can be sited at the point of wastewater production (Trattner and Woods, 1989; Mozes, 1992). In addition, wetland aquatic plants provide a potential income to offset treatment costs through the production of compost and mulches (A. Wood, personal communication), the manufacture of pulp, paper and fibre products, and as building materials (Rodgers et al., 1985). They are also used as food for livestock (Rodgers et al., 1985; A. Wood, personal communication) and humans and for the production of energy by anaerobic digestion (Rodgers et al., 1985).

In summary, wetlands reduce/remove:
a) Coliforms, other pathogenic bacteria, enteric viruses, parasitic protozoa and worms (Rodgers et al., 1985; Gersberg, Gerhardt and Ives, 1989). This is accomplished by sedimentation, solar irradiation, the presence of toxic substances (Seidel, Federico, Milleson and Roosen, 1978; Rodgers, 1983) and detention while natural die-back occurs (Hemond and Benoit, 1988);
b) Organic matter (Rodgers et al., 1985; Gersberg et al., 1989) and nutrients (eg. phosphorus and nitrogen) (van der Valk, Davis, Baker and Beer, 1979; Adamus,

Clarain, Smith and Young, 1987; Begg, 1990). This is achieved by plant (DeLaune, Smith and Sarafyal, 1986) and animal accumulation, sedimentation, ammonia volatilization (Bailey, Zolteck, Hermann, Dolan and Tortora, 1985; Howard-Williams, 1985; Richardson and Marshall, 1986), photodegradation of pesticides (Zafiriou, Joussof-Dubien, Zepp and Zika, 1984), denitrification (Sather and Smith, 1984) and precipitation of phosphorus as insoluble iron, aluminium and calcium phosphate (Ponnamperuna, 1972; Nichols, 1983), or sediment deposition to which phosphorus is already adsorbed (Boto and Patrick, 1979). The removal rates vary (Hemond and Benoit, 1988; Hammer, 1992) and depend on the wetland type (Dolan, Bayley, Zoltek and Hermann, 1981; Richardson, 1985);

c) Heavy metals, by adsorption onto sediments, and precipitation as oxides, hydroxides, carbonates, phosphates and sulphides (Rodgers *et al.*, 1985). Plants accelerate mercury removal by emission into the atmosphere (Kozuchowski and Johnson, 1978; Hemond and Benoit, 1988). Removal efficiencies depend on the metal and the wetland type involved, with soil type (Vestergaard, 1979; Wieder and Lang, 1986) and redox potential also major factors (Gambrell and Patrick, 1988); and

d) Organic compounds such as halomethanes (Sleat and Robinson, 1983; Suflita, Robinson and Tiedge, 1983; Gambrell, Taylor, Reddy and Patrick, 1984).

It can, thus, be seen that natural and artificial wetlands offer many advantages.

1.9 BIOGAS GENERATION: PROBLEMS AND POSSIBLE SOLUTIONS

1.9.1 SUITABILITY OF INDIVIDUAL VERSUS COMMUNITY-SCALE BIOGAS PLANTS

Major problems associated with existing biogas plants include inadequate design and construction, together with poor maintenance and social acceptance (Day *et al.*, 1990). They are most suitable for communities rather than individual households as careful control, good operation and maintenance are needed (Feachem and Cairncross, 1978). It has been found that small-scale rural plants have been unsuccessful (Boshoff, 1992). Specific problems include inefficient digester management due to financial constraints. Also, the capital outlay for digester installation (Boshoff, 1992) and the maintenance costs

are problematic in low-income rural areas (Rivett-Carnac, 1982; Farinet, Forest and Saw, 1994). Thus, low-cost systems are essential (Jewell, Dellôrto, Fanfoni, Hayes, Leuschner and Sherman, 1981) as are government subsidies (Williams and Eberhard, 1986). Community-scale plants may require management by a permanent technical team drawn from the local population (Williams and Eberhard, 1986) rather than individuals with limited time and knowledge. These plants have the potential to generate electricity from excess gas. Also, adequate water supplies are more easily managed in communities rather than in individual scattered dwellings, and the collective confinement and management of cattle facilitate maximized dung utilization. Closely-knitted settlements also facilitate reticulation of piped gas, electricity and other services (Rivett-Carnac, 1982). In industrialized countries such as Denmark, commercialized centralized plants are overseen by a company board with regards to economic and legal agreements pertaining to plant construction, financing and supply and sale of biomass and energy (Christensen and Hjort-Gregersen, 1994; Tafdrup, 1994).

1.9.2 LIGNOCELLULOSE DEGRADATION

Lignocellulosic biomass is highly resistant to microbial attack (Rivard, Himmel and Grohmann, 1987) and, thus, has a slow degradation rate. Temperature, pH and bacterial culture composition influence the rate and extent of its hydrolysis (Tong and McCarty, 1991). It is for this reason that the occurrence of lignocellulose is considered a major factor limiting widespread use of biogas technology (Chandler, Jewell, Gosset, van Soest and Robertson, 1979; Jungersen and Ahring, 1994).

Lignocellulose can, however, be pretreated by microbiological and chemical means (Hobson, 1983; Sharma, Mishra, Sharma and Saini, 1988). Examples of microbiological methods include white-rot fungi (Muller and Trosch, 1986; Bisaria, Vasudevan and Bisaria, 1990) and rumen microorganisms (Behmel, Leupold and Vieweger, 1993). Chemical pretreatment includes liquefaction (Jungersen and Ahring, 1994) by temperature, pressure, gaseous sulphur dioxide, alkali hydroxide (Langton, 1981; Rivett-Carnac, 1982), acid (Langton, 1981; Rivard et al., 1987) and urine hydrolysis (Rivett-Carnac, 1982). Nutrient supplements and buffer additions enhance microbial degradation (Molnar and

Bartha, 1988). Physical methods include grinding, fractionation, size reduction and pre-incubation with water (Langton, 1981; Hobson, 1983). Instead of physical pretreatment, ensilage of certain feedstocks can be undertaken (Madhukara, Nand, Raju and Srilatha, 1993). Although various pretreatment methods exist, many are costly and, thus, impractical for use in small-scale rural biogas plants.

1.9.3 HYDROGEN SULPHIDE EVOLUTION

Hydrogen sulphide (H_2S) removal from biogas is often necessary due to its toxicity, smell and corrosive properties (Rivett-Carnac, 1982; Gadre, 1989). Removal is accomplished by physical sorption, chemical absorption, adsorption (Ashare, 1981), addition of iron filings (Rivett-Carnac, 1982), membrane separation (Bessarabor, Sanderson, Teplyakov, Beckman and Netrusov, 1994; Coetzer, 1994) and by the establishment of the families Chromatiaceae and Chlorobiaceae and some chemoautotrophic bacteria in the digester. The microbial approach is characterized by low capital costs, low energy requirements, direct conversion and minimal secondary waste generation (Gadre, 1989). Hydrogen sulphide may also be removed by intensive mass culture of microalgae (Conde, Moro, Travieso, Sanchez, Leiva, Dupeiron and Escobedo, 1993). As an alternative, the problem of H_2S can be obviated by careful operation and/or the installation of gas separators (Hamburg, 1989).

1.9.4 TEMPERATURE CONTROL

Anaerobic digestion is temperature sensitive and gas production declines at lower mesophilic temperatures (Kalbermatten *et al.*, 1982; Rivet-Carnac, 1982) (1.5.2) at which point uncontrollable and erratic process characteristics often result (Shelef, Kimchie and Grynberg, 1981). Longer hydraulic retention times can help to maintain efficiency when digesters are operated at lower temperatures (Pos *et al.*, 1985; Sanchez, Weiland and Traviesa, 1992). Insulation may be needed (Molnar and Bartha, 1988; Pandey, Misra, Singh and Singh, 1992) or the plants may be sited below ground to retain heat and maintain gas supply in periods of extended cold weather (Rivett-Carnac, 1982).

1.9.5 SCUM FORMATION

Scum, a mixture of animal hairs and skin particles, straw or wood shavings from animal bedding, feathers, fibre, undigested vegetable matter, fat, grease and/or generally anything that will float, is problematic in some plants but can be controlled by the use of skimmers (Meynell, 1982; Raman, Ranga Rao and Kishore, 1989). Scum interferes with gas production by plugging pipe outlets or reducing available digester capacity (Raman *et al.*, 1989).

1.9.6 SLUDGE USE

The ability of fermentation effluent/sludge to maintain crop yields and soil fertility, with no toxic or detrimental effects, is of major importance to digester sustainability (Ross, Tate, Speir, Stewart and Hewitt, 1989). Digester sludge residue characteristics are determined by the operating conditions, residence time and digester feed (Graetz and Reddy, 1988).

No conclusive evidence exists of intestinal pathogen inactivation during digestion although numbers are reported to be considerably reduced (Gadre *et al.*, 1986). In sludges, many pathogens occur. Principal among these are *Klebsiella, Staphylococcus, Salmonella, Mycobacterium* and fluorescent *Pseudomonas* spp. and *Clostridium perfringens* (Dudley, Guentzel, Ibarra, Moore and Sagik, 1980). *Salmonella* inactivation is dependent upon the frequency of feeding and sludge withdrawal, digester design and mixing characteristics (Carrington *et al.*, 1982). *Salmonella typhimurium*, in contrast, does not survive in a digester operated with a retention time >30 days (Gadre *et al.*, 1986). The potential problem of redistribution via the slurry is usually not problematic since minimal sludge is produced and this can be used to fertilize crops which are not eaten uncooked (Loehr, 1977).

1.9.7 SUBSTRATE AVAILABILITY AND MAINTENANCE OF THE CARBON:NITROGEN RATIO

A C:N ratio between 20:1 and 30:1 is needed to maximise biogas production (United Nations Environment Programme, 1981; Meynell, 1982; Rivett-Carnac, 1982). Elementally, farm manure and plant material are within this range (Brady, 1984) while bacteria have a ratio of between 4:1 and 5:1 (Donahue, Miller and Stickluma, 1985; Plaster, 1985). To maintain an optimum C:N ratio, nitrogen-rich livestock waste and domestic sewage, which provide nutrients for population maintenance, can be mixed with high C:N fibrous material such as grass, straw, paper and refuse. Supplements such as urea may be added to maintain a suitable C:N ratio (DaSilva, 1981; White and Plaskett, 1981). Excess N is problematic when it accumulates as ammonia which is bactericidal to methanogens (Meynell, 1982).

A major constraint of biogas generation is the low availability of dung to meet the energy requirements of small farm systems (Malik *et al.*, 1989). Time and effort are, therefore, required for its collection (Haugen and Lindley, 1988) for digester use (Rivett-Carnac, 1982; Rabezandrina, 1990). Also, dung may have a traditional use as an animal fodder and, thus, there is resistance to using it in any other way. This problem could be resolved by using locally available by-products (Day *et al.*, 1990) or alternative substrates to supplement existing sources of digester feed material (Rabezandrina, 1990). Such substances include residues from agriculture (Ashare and Buivid, 1981), forestry (Prasad, 1991; 1992) and industrial processes (Weiland, 1993), and food and market wastewaters and by-products (Ranade, Yeole and Godble, 1987; Nand, Devi, Viswanath, Deepak and Sarada, 1991; Nandy, Kaul, Pathe, Deshpande and Daryapurkar, 1992). Vinasse is just one example of the numerous possibilities (Souza, Fuzaro and Polegato, 1991; Borja, Martin, Luque and Duran, 1993; Vlissidis and Zouboulis, 1993). Multiple feedstocks may also be used and these have an advantage in batch systems since storage facilities and, hence, attendant decay rates (Curry and Deuermeyer, 1986) are reduced.

Manure from domestic animals (Ifeadi and Brown, 1975; Huang and Shih, 1981; Kanwar and Kalia, 1993), supplemented with various plant materials (Mallik, Singh and Ahmad,

1990), algae (Ramamoorthy and Sulochana, 1989) or even organic wastes (Wong and Cheung, 1989; Ahring, Angelidaki and Johansen, 1991), are suitable for biogas generation (Kumar, Jain and Chhonkar, 1987; Geeta, Gadees and Reddy, 1990). There is no shortage of manure since an estimated 2 billion tonnes are produced annually by farms in the U.S.A. (Varel, Isaacson and Bryant, 1977; Wohlt, Frobish, Davis, Bryant and Mackie, 1990).

Many types of plant materials can be used. These include seaweeds (Anjaneyulu, 1988; Anjaneyulu, Tarwadi and Mehta, 1989), green algae and aquatic plants (Reddy, 1988; Taheruzzaman and Kushari, 1989; Moorhead and Nordsted, 1993). Leaves (Sharma, Saini, Mishra and Sharma, 1987; 1989; Daniel, Mathiazhagan and Boominathan, 1990), crop residues (Kalia, Kumar, Jain and Joshi, 1992; Somayaji and Khanna, 1994), non-conventional herbaceous species (Mislevy, Gilreath, Prine and Dunavin, 1988), tall grasses (Prine, Dunavin, Brecke, Stanley, Mislevy, Karmbacher and Hensel, 1988; Rajasekaran, Vasil and Schank, 1988), roots (ÓHair, Snyder, White, Olson and Dunavin, 1988), woody crops (Rockwood and Prine, 1988) and weeds (Gunaseelan and Lakshman, 1990; Jagadeesh, Geeta and Reddy, 1990; Abbasi, Nipaney and Panholzer, 1991) can also be used although some pretreatment may be required (Kalia and Kanwar, 1990; Patel, Desai and Madamwar, 1993).

1.9.8 ECONOMIC, TECHNICAL AND SOCIAL PROBLEMS

Waste availability, digester operation (Taşdemiroğlu, 1988), economic analyses (Forster and Wase, 1989) in relation to returns on investment, environmental factors and cultural and social acceptability (Rivett-Carnac, 1982; Rabezandrina, 1990) are main factors preventing the adoption of biogas technology world wide.

The above factors must all be considered in relation to the local conditions. These include climatic conditions, water availability, feedstock, finance, construction material availability and skill of the constructors (Rabezandrina, 1990). If these are overlooked, biogas technology implementation in poor rural areas may become an irreversible failure (Nyns, 1990).

Most problems, especially in developing countries, are social (Rivett-Carnac, 1982; Day *et al.*, 1990). These include consumer resistance (Rivett-Carnac, 1982), reluctance of villagers to pay for alternative fuel technology when wood has been free (Rivett-Carnac, 1982; Williams and Eberhard, 1986), inconvenient gas availability, reduced food palatability, and not all available dung being placed in the digester (Rivett-Carnac, 1982). An example of consumer resistance is the rejection by the Zulu population of food cooked on biogas generated from human faeces (S. Sangweni, personal communication).

To obtain community acceptability the people must be involved in the planning process (Lombard, 1994; Matsoga, 1994). To realise this goal there must be:

a) Political and administrative desire to enlist participation of the community;

b) Availability of a willing and motivated community;

c) Availability of motivated and innovative staff;

d) Availability of an acceptable project, ranked well in the priorities of the community;

e) Creation of both short- and long-term benefits;

f) Involvement of, consultation with and participation by the community at the planning, execution and evaluation stages;

g) Maintenance or, preferably, improvement of the project tempo and quality;

h) Provision of facilities and infrastructure for back-up services and delivery; and

i) Cultivation of a spirit of ownership (Matsoga, 1994).

Thus, sociological and economic factors often relate to digester ownership and efforts to popularise and finance the project (Buchholz, 1988). Unfortunately, the economic viability of digester technology is often poor, particularly in farm-scale installations and results in long payback periods (Sims and Richards, 1990). Technical factors include the requirement for a labile waste since substrate costs dominate process economy (Buchholz, 1988). Also, the digester dimensions must reflect the biogas demands and local materials with minimal maintenance should be used (Rabezandrina, 1990). Education with well-planned training and demonstration programmes specific for rural areas (Taşdemiroğlu, 1988) and political will to implement biomethanation technology (Nyns, 1990) are required to encourage the adoption of biogas technology (Taşdemiroğlu, 1988). For technology adoption, a rational popularization structure is needed which also includes research and monitoring

organizations (Rabezandrina, 1990), maintenance services (Taşdemiroğlu, 1988; Nyns, 1990), and provision to train digester constructors. The costs of these programmes, however, must be within the reach of rural dwellers (Rabezandrina, 1990) or must be supported by government loans (Taşdemiroğlu, 1988). Technology popularization with only technical performance as a marketing criterion may fail. Examples of such failures include: Douala (Cameroon) where firewood was abundant; Tananarive (Madagascar) where the technology was too costly; and Zimbabwe where use of animal manure was rejected for cultural reasons (Rabezandrina, 1990).

1.9.9 OTHER PROBLEMS

Other problems also occur. Biogas lamps often overheat rooms (Rabezandrina, 1990), motor pumps fuelled by biogas have low outputs (10-12% efficiency) compared to, for example, diesel (20-25%) (Datong, 1989), gas burners are not always suitable for the slow cooking of some African foods (Rabezandrina, 1990) and biogas displaces the social focus of the open hearth fire and, attendant, wood smoke which is an effective insect repellent (Rivett-Carnac, 1982; Rabezandrina, 1990). Biogas production, although continuous, is often not used at night for periods of 10-12 hours thus gas storage is required (Datong, 1989).

1.10 CONCLUSIONS

Biomethanation is a promising technology and more and more countries around the world are adopting it (Nyns, 1990). It provides a stable source of clean energy (Rivett-Carnac, 1982; Day *et al.*, 1990; Letcher, 1991) and obviates deforestation and desertification (Bala and Satter, 1990; Batenin, 1990; Letcher, 1991). Disease control (Safley and Westerman, 1988; Tang and Wang, 1989), organic waste purification, fertilizer production (Batenin, 1990; Nyns, 1990) and improved sanitation make biogas technology ideal for resolving many waste management problems (Shelef *et al.*, 1981; Gadre *et al.*, 1986; Nyns, 1990). These advantages must, however, be viewed in the light of a number of potential limitations.

The technology, for example, is dependent on a finely balanced interacting microbial association and careful operation (Holland *et al.*, 1987). Thus, successful methane production is dependent on: reactor design; establishment of active, stable microbial associations; identification and control of the rate-limiting biochemical steps; and, possibly, genetic improvement of specific microorganisms to facilitate maximum methane yield (Gritzali, Shiralipour and Brown, 1988).

The feasibility of energy recovery from agricultural and domestic residues also depends on the amount of recoverable energy in the wastes relative to the total energy required for digester operation, the compatibility of the energy form, the availability of equipment and skill to maintain the process, and the costs of resultant energy utilization (Jewell and Loehr, 1977; Loehr, 1977).

Thus, biomethanation could obviate the inimical environmental impacts of some waste streams (Pantskhava, Varosyan, Tapaltsyan, Goldfard and Gyunter, 1989). Although the economic viability may be debatable, anaerobic digesters do provide benefits in relation to household energy supply, organic material and plant nutrient conservation, and sanitation (Hamburg, 1989). Successful exploitation, however, depends on a thorough understanding of the biochemical processes involved (Bala and Satter, 1990; Letcher and Kolbé, 1994), the specific economics of each system (Bala and Satter, 1990), careful management to maximise gas production and effective collection, transportation and storage (Letcher and Kolbé, 1994).

CHAPTER 2

MATERIALS AND METHODS

2.1 BIOREACTOR

The configuration, installation and commissioning of the two-tank bioreactor are detailed in Chapter 3.

2.1.1 SAMPLING PROCEDURE

Samples of sludge and supernatant were collected from the bioreactor sampling ports with a 50ml syringe via two graduated doweling rods joined in series with tubing.

Gas production in Tank 1 of the Pennells bioreactor was determined throughout the study by transferring the gas, via silicone rubber tubing and an inverted funnel, to, and displacement of, a solution which contained sodium chloride (20% m/v) and citric acid (0.5% m/v) (Dudley, Howgrave-Graham, Isherwood and Senior, 1993).

2.2 SLUDGES

The sludges used in this study were:
a) Sludge from the Pennells bioreactor from the sampling ports of Tank 1 outlet, Tank 2 inlet, Tank 2 and Tank 2 outlet;
b) Anaerobic digester sludge from Darvill Wastewater Purification Works;
c) Anaerobic digester sludge from Durban Wastewater Purification Works;
d) Cow manure; and
e) Upflow anaerobic sludge blanket granules (UASB) previously treating brewery wastewater.

The sludges were transported from the collection sites to the laboratory in completely filled screw-capped bottles.

2.3 LABORATORY CULTURES

2.3.1 CLOSED (BATCH) CULTURES

Artificial sewage (2.8) with the pH poised near neutral was decanted in 100ml aliquots into 160ml medical flats and autoclaved at 121^{0}C for 15min. After cooling, 25ml sludge were aseptically added to each. The medical flats were closed with butyl rubber stoppers and overgassed with OFN (Fedgas) for 5 min to create anaerobic conditions prior to attaching hypodermic needles and syringes (10ml) for gas collection. The cultures were incubated at 32^{0}C in a Heraeus-D-6540 Hanu incubator and periodically shaken.

2.3.2 OPEN (CONTINUOUS) CULTURES

For continuous cultivation, four 500ml Erlenmeyer flasks, each fitted with a rubber bung which contained two ports, one for the feed inlet and the other for gas collection, were used. Artificial sewage (2.8), with the pH adjusted to near neutral, plus a 20% (v/v) inoculum of sludge to a total volume of 125ml were added to each bioreactor.

The continuous cultures were held in a Heraeus-D-6450 Hanu incubator at 32^{0}C. From each bioreactor, a U-tube was inserted between the vessel and the effluent reservoir to minimise gas loss with the effluent. The system was designed to capture the evolved biogas by displacement of a citric acid (0.5% m/v)-acidified sodium chloride (20% m/v) solution. Each gas outlet port orifice was narrowed by insertion of a hypodermic needle to obtain smaller bubbles for easy observation (G.N. Tivchev, personal communication). Silicone rubber tubing was used in conjunction with an Ismatec IPC-24 peristaltic pump to draw feed into the bioreactors. The contents were mixed manually to model the conditions of the Pennells bioreactor.

The cultures were maintained as batch cultures first, until biogas was detected, at which stage flow was to be applied at 2.16mlh^{-1}.

2.4 ANALYSES

2.4.1 pH DETERMINATIONS

A Crison Micro pH 2 000 pH meter was used for all pH determinations.

2.4.2 TEMPERATURE MEASUREMENTS

Temperature measurements of the sludges and supernatants were made immediately after sampling at 0830. At each sampling time the ambient air temperature was also recorded.

2.4.3 CHEMICAL OXYGEN DEMAND

Reagents

1. Potassium dichromate solution, 0.25N.

$K_2Cr_2O_7$ (12.259g), previously dried at 103°C for a few hours, was dissolved in distilled water in a $1l$ volumetric flask and diluted to $1l$ with distilled water.

2. Ferrous ammonium sulphate solution (FAS titrant), 0.125N.

$Fe(NH_4)_2(SO_4)_26H_2O$ (40g) was dissolved in 400ml distilled water in a $1l$ volumetric flask. Sulphuric acid (20ml) was carefully added to this flask. The solution was allowed to cool and then diluted to $1l$ with distilled water.

Note: Since this solution was unstable it was standardized each time before use.

3. Ferroin indicator solution.

4. Sulphuric acid (19M).

Method

A Hach digester (model 45600) was used to facilitate COD determinations of the supernatant samples in the range of 0-1500ppm. The reagent vials were obtained from Holpro (Prospecton). Prior to digestion, the samples were homogenized at high speed for two minutes with a Thyristor Regler TR 50 ultraturrax to ensure uniform distribution of the suspended solids. The COD reactor was preheated to 150°C. After carefully opening the reagent vials, 2ml of sample were carefully pipetted into each while holding the vial

at an angle of 45°. Each vial was closed and inverted several times to mix the contents prior to placement in the digester block. Reagent blanks were also prepared in which the sample was replaced by 2ml of distilled water. The vials were heated (150°C) in the block in a fume cupboard for 2h before cooling, with periodic inversion while still warm (Hach Reactor Manual, 1994).

Each COD was then determined by titration (Hach Reactor Manual, 1994). Distilled water (1ml) was added to each cooled vial together with one drop of high-range ferroin indicator solution. The solutions were titrated with 0.125N ferrous ammonium sulphate until the sample colour changed from greenish-blue to orange-brown and the volume of titrant recorded. For ferrous ammonium sulphate standardization, 2ml of 0.25N potassium dichromate solution were pipetted into a clean empty vial. Sulphuric acid was added and gently mixed. Once cool, one drop of high-range ferroin indicator solution was added and the mixture titrated with 0.125N ferrous ammonium sulphate until the same colour change resulted (Hach Reactor Manual, 1994). The COD (mgl^{-1}) was then determined according to the following equation (Hach Reactor Manual, 1994):

$$COD\ (mgl^{-1}) = \frac{(A - B) \times 2\ 000}{C} \qquad (1)$$

where: A = ml ferrous ammonium sulphate used in titration of the blank;

    B = ml ferrous ammonium sulphate used in titration of the sample; and

    C = ml ferrous ammonium sulphate used in titration of the standard.

If the colour of the sample changed to brown when the ferroin indicator was added, the COD concentration was beyond the range of the reagent vials used and the assay was repeated with a diluted sample.

2.4.4 VOLATILE FATTY ACID (VFA) AND BICARBONATE ALKALINITY (BA) ANALYSES

Chemicals

Hydrochloric acid, 0.1N.

Sodium hydroxide, 0.1N.

Apparatus

Whatman No 40 filter paper.

Buchner funnel and flask.

Reflux condenser.

2x 25m*l* burettes.

50m*l* measuring cylinder.

Magnetic stirrer (Fried Electric).

pH meter.

Method

The method used was that of the Prospecton Brewery Effluent Pretreatment Plant Process Control Tests Manual (Hoffmann, 1986) which conforms to the South African Bureau of Standards (SABS) method.

The supernatant sample was filtered into a Buchner flask through Whatman No 40 filter paper. The filtrate (50m*l*) was transferred into a 100m*l* beaker, which was placed on a magnetic stirrer (Fried Electric), and titrated with 0.1N HCl to a pH of 3.0 and the volume of acid used recorded. At pH 3.0, bicarbonate alkalinity is converted to carbon dioxide and volatile fatty acids are mostly converted into the non-dissociated forms (Ross and Louw, 1987). The content of the beaker was then transferred into a flask, some boiling stones were added and the flask was connected to a reflux condenser. The liquid was boiled for 3 minutes to eliminate CO_2 (Ross and Louw, 1987) and then cooled for about 2 minutes. The liquid was returned to the beaker, immediately titrated with sodium hydroxide solution (0.1N) to a pH of 6.5 and the volume of alkali used recorded.

The calculations used to determine the overall VFA and BA concentrations were as follows (Hoffman, 1986):

$$\text{VFA (meg}l^{-1}) = \frac{[b \times 101 - a - 100] \times 100}{99.23 \qquad V} \qquad (2)$$

where a= ml acid titrated;

b= ml hydroxide titrated; and

V= sample volume

VFA (mgl^{-1}) as CH_3COOH = megl^{-1} x 60

BA (megl^{-1}) = $\dfrac{\text{(a-b) x 100}}{V}$ (3)

BA (mgl^{-1}) as $CaCO_3$ = megl^{-1} x 50

2.4.5 RIPLEY RATIO

The supernatant samples were filtered (Whatman No 40 filter paper) (Ross *et al.*, 1992) prior to titration. The two end points titration developed by Ripley, Boyle and Converse (1986) was then used. Bicarbonate alkalinity was estimated by titrating with 0.5M HCl to pH 5.75 (Ross *et al.*, 1992). The second titration for VFAs was from 5.75 to 4.3 (Ross *et al.*, 1992):

Ripley   VFA:alkalinity ratio = $\dfrac{\text{VFAs titrated}}{\text{alkalinity titrated}}$ (4)

2.4.6 METHANE

Methane concentrations of 50μl culture headspace samples were quantified with a Varian 3600 gas chromatograph, equipped with a flame ionization detector, in which the flow rate of the oxygen-free nitrogen (OFN) carrier gas was maintained at 20ml min^{-1}. The samples were injected directly into a glass column (length 1.45m, i.d. 3mm) packed with Poropak T (80/100 mesh). The injector, detector and column detectors were maintained at 110^{0}C, 200^{0}C and 35^{0}C, respectively. The methane concentrations were determined, after standard curve construction, by mean peak area comparison with methane standards (5-80% v/v) (Fedgas).

2.5 MICROSCOPY

2.5.1 LIGHT AND FLUORESCENCE MICROSCOPY

A Zeiss Axiophot microscope, fitted with epifluorescence equipment which consisted of a HBO 50 high pressure mercury short-arc lamp with a UVH 365 filter, was used for bacterial visualization. The exciter filters were BP 365/12, the dichromatic beam splitters were FT 395 and the barrier filters were LP397. The transmission peak wavelengths of the two excitation filters were 390-440nm and 450-490nm, respectively. The critical wavelengths of the dichromatic beam splitters were 460nm and 510nm. The transmission peaks of the barrier filters were 470nm and 520nm.

2.5.2 SCANNING ELECTRON MICROSCOPY

Sludge and supernatant samples were filtered (0.4μm) prior to fixation with 3% (v/v) glutaraldehyde. After washing twice with 0.05M cacodylate buffer the preparations were dehydrated stepwise in a series of ethanol dilutions to 100% ethanol and then critical point dried in a Hitachi HCP-2 critical point drier (CPD). The samples were mounted on stubs, sputtercoated with gold/palladium and viewed with a Hitachi S-570 scanning electron microscope.

2.5.3 ENERGY DISPERSIVE X-RAY MICROANALYSIS

Drops of sample for Energy Dispersive X-Ray (EDX) Microanalysis were placed on a carbon stub and dried under an electric lamp. Energy dispersive X-ray microanalyses were made at 20KV accelerating voltage with a Link eXL II EDX machine attached to a Hitachi S-570 scanning electron microscope.

2.6 BACTERIAL ISOLATION

2.6.1 SULPHATE-REDUCING BACTERIA

Postgate's medium (Postgate, 1984) which contained lactate as the electron donor and carbon source, was used to isolate sulphate-reducing bacteria.

Table 2: Postgate's medium for the isolation of *Desulfovibrio* and *Desulphotomaculum* spp.

Solution 1

COMPONENTS	MASS/VOLUME
Distilled water	980ml
K_2HPO_4	0.5g
NH_4Cl	1.0g
Na_2SO_4	1.0g
$CaCl_2.2H_2O$	0.1g
$MgSO_4.7H_2O$	2.0g
70% (m/v) Sodium lactate	3.5g
Yeast extract	1.0g
Agar	15.0g

Solution 2

COMPONENTS	MASS/VOLUME
Distilled water	10.0ml
$FeSO_4.7H_2O$	0.5g

Solution 3

COMPONENTS	MASS/VOLUME
Distilled water	10.0ml
Ascorbic acid	0.1g
Na-thioglycolate	0.1g

Preparation:

The three solutions were sterilized by autoclaving in an STMN-Y222 autoclave at 121^0C for 15min. Immediately after sterilization and mixing the medium was overgassed with a mixture of 10% (v/v) CO_2 and 5% (v/v) H_2 in N_2 and the plates poured (50°C) under the same gas atmosphere.

After inoculation, the plates were incubated at 32°C in a Forma Scientific anaerobic chamber (model 1024) under the same atmosphere.

2.6.2 BACTEROIDES

The bacteria were isolated from sludge samples with Hungate's medium (Macy, 1981).

Hungate's Habitats Stimulating Medium for Rumen Bacteroides

The following stock solutions were prepared:

Table 3: Mineral solution A.

COMPONENTS	CONCENTRATION (gl^{-1})
NaCl	6.0
KH_2PO_4	3.0
$(NH_4)_2SO_4$	3.0
$MgSO_4$ (anhydrous)	0.6
$CaCl_2$ (anhydrous)	0.6

Mineral solution B contained (gl^{-1}):

K_2HPO_4,   3.0.

Resazurin solution:

0.1% (m/v) solution in distilled water.

Cysteine-HCl solution:

3.0g of cysteine HCl were dissolved in 100ml deoxygenated distilled water (boiled 2 min and cooled under a stream of CO_2 or N_2) in a round-bottom flask and autoclaved at 121^{0}C for 15min.

Substrate solution:

10% (m/v) solution of glucose was prepared in deoxygenated (N_2) distilled water.

Rumen fluid:

Rumen contents were collected from cows via a stomach tube or a fistula. The contents were strained through several layers of cheesecloth to remove large pieces of plant material. The rumen fluid was then centrifuged (16 000xg) and the supernatant used for

medium preparation.

Preparation:

To prepare Hungate's medium, the following were combined (for 1*l* of medium):

Mineral solution A   167m*l*

Mineral solution B   167m*l*

Resazurin   1m*l*

Distilled water   398m*l*

(65m*l* "extra" H_2O were added, as approximately 10% of the medium volume was lost during boiling, as discussed below).

Agar was added ($\leqslant 2\%$ (m/v) if ion-agar was used) and the medium was boiled for approximately 2 min to remove oxygen (or until the agar was dissolved). A stream of oxygen-free carbon dioxide was then passed into the flask via a port in the Schott bottle and the medium was allowed to cool to 45-47°C. Once cooled, 333m*l* of rumen fluid were added. Sterile cysteine ($0.1\text{m}l/10\text{m}l^{-1}$), $NaHCO_3$ ($0.5\text{m}l/10\text{m}l^{-1}$), and substrate solution ($0.4\text{m}l/10\text{m}l^{-1}$) were added before use.

2.6.3 ACETOGENIC BACTERIA

The bacteria were isolated by inoculation onto a selective culture medium (Hespell and Bryant, 1981) for the isolation of *Butyrivibrio, Succinomonas, Lachnospira* and *Selenomonas* spp. The plates were incubated (32°C) in a Forma Scientific anaerobic chamber as described above (2.6.1).

Growth Medium for *BUTYRIVIBRIO, SUCCINOMONAS, LACHNOSPIRA, AND SELENOMONAS* spp.

Table 4: Basic mineral solution.

COMPONENTS	MASS (g)
KH_2PO_4	18.0
NaCl	18.0
$CaCl_2$	0.4
$MgCl_2.6H_2O$	0.4
$MnCl_2.6H_2O$	0.2
$CoCl_2.6H_2O$	0.2

Preparation:

The above ingredients were dissolved in distilled water and diluted to a final volume of 1l.

Table 5: Cysteine-sulphide reducing mixture.

COMPONENTS	MASS (g)
L-cysteine.HCl	2.5
$Na_2S.9H_2O$	2.5

Preparation:

The L-cysteine.HCl was dissolved in distilled water and the pH adjusted to 10.0 with NaOH (0.1N). The $Na_2S.9H_2O$ was added and, after dissolution, the solution was diluted to a final volume of 100ml with distilled water. The mixture was equilibrated under OFN prior to autoclaving at 121°C for 15min.

Table 6: Resazurin solution.

COMPONENTS	MASS/VOLUME
Resazurin	100mg
Distilled water	100ml

Table 7: Na_2CO_3 solution.

COMPONENTS	MASS/VOLUME
Na_2CO_3	0.02g
Distilled water	100ml

Preparation:

This solution was equilibrated with, and dispensed under, O_2-free CO_2 after sterilization by autoclaving at 121°C for 15min.

Clarified rumen fluid

Rumen contents of cows were collected via a stomach tube or fistula. The contents were filtered through two layers of cheesecloth and the filtrate centrifuged (16 000xg, 30 min, 15-22°C) to remove the smaller plant particles and microorganisms. The filtration and centrifugation were done shortly after collection. The resultant supernatant fluid was autoclaved at 121^0C for 15min after dispensing into screw-cap bottles. The clarified rumen fluid was stored refrigerated (4^0C).

Table 8: Mineral solution S.

COMPONENTS	MASS (g)
KH_2PO_4	12
$(NH_4)_2SO_4$	6
NaCl	12
$MgSO_4.7H_2O$	2.5
$CaCl_2.2H_2O$	1.6

Preparation:

The above ingredients were dissolved in distilled water and diluted to a final volume of 1*l*.

The growth medium can be prepared either with or without the addition of rumen fluid although in this study clarified rumen fluid was used.

Table 9: Growth medium for *Butyrivibrio*, *Succinomonas*, *Lachnospira* and *Selenomonas* spp.

Components	Medium with clarified rumen fluid
Rumen fluid	40ml
Glucose	0.05g
Cellobiose	0.05g
Soluble starch	0.05g
Xylose	0.05g
Basic mineral solution	5ml
Mineral solution S	4.1ml
Resazurin	0.1ml
$(NH_4)_2SO_4$	0.05g
Agar	2.0g
Distilled water	69ml
Na_2CO_3 solution	5.0ml
Cysteine-sulphide solution	1.0ml

Preparation:

All of the medium ingredients, with the exception the reducing agents, Na_2CO_3 and the heat labile components were dissolved in distilled water and the solution poised at pH 6.5 with NaOH (0.1N). The medium was diluted to volume (minus the total volume of ingredients to be added after autoclaving) and placed into a modified Schott bottle. A stream (ca. 500 ml/min^{-1}) of OFN was passed through the bottle while the medium was raised to boiling point for 2-3 minutes over a bunsen. For agar-containing medium, the medium was first heated to near boiling prior to gassing the vessel and boiling. The medium was then autoclaved at 121°C for 15min and the bottle allowed to cool to $\leq$ 55°C (or 47°C when agar was present). The vessel was continually flushed with OFN. The sterile oxygen-free solutions of cysteine-sulphide, Na_2CO_3 or other ingredients were then added to the medium under anaerobic conditions prior to dispensing in a Forma Scientific anaerobic chamber flushed with 10% (v/v) CO_2 and 5% (v/v) H_2 in N_2.

2.6.4 BUTYRIC ACID BACTERIA

The bacteria were isolated from sludge samples by inoculation onto Reinforced Clostridial Medium (RCM) (Oxoid) and incubation in a Forma Scientific anaerobic chamber at 32°C.

2.6.5 LACTIC ACID BACTERIA

The bacteria were isolated from sludge samples using Rogosa agar plus 1% (m/v) fructose and 0.4% (m/v) potassium sorbate (to suppress yeasts and catalase positive organisms) (Howgrave-Graham, Wallis and Steyn, 1991). The plates were incubated at 32°C in an anaerobic jar which contained an Anaerocult A 13829 envelope, in a Heraeus-D-6450 Hanau incubator.

Table 10: Rogosa agar (pH = 5.4 ±0.2).

COMPONENTS	CONCENTRATION (gl^{-1})
Tryptose	10.0
Yeast	5.0
Dextrose	10.0
Arabinose	5.0
Sucrose	5.0
Sodium acetate	15.0
Ammonium citrate	2.0
KH_2PO_4	6.0
Magnesium sulphate	0.57
Manganese sulphate	0.12
Ferrous sulphate	0.03
Agar	15.0

Preparation:

The components were dissolved in 1l distilled water. Tween 80 (1g) was added to the solution which was then heated to boiling point with stirring. Glacial acetic acid (1.32ml), fructose (1% m/v) and potassium sorbate (0.4% m/v) were added and the solution heated to 90 to 100°C. The solution was then cooled ($\leqslant$40°C) for direct inoculation or to 45°C for plate counts.

2.6.6 PROPIONIC ACID BACTERIA

The bacteria were isolated from sludge samples by inoculation onto modified yeast extract and lactate medium (Britz, 1975) with incubation at 32°C in the absence (anaerobic jar plus an Anaerocult A 13829 envelope) and presence of oxygen.

Table 11: Britz medium (pH = 7.2).

COMPONENTS	CONCENTRATION (gl^{-1})
Yeast extract	5.0
60% (m/v) Sodium lactate	20.0
Tween 80	1.0
Cysteine HCl	0.5
Agar	15.0

Preparation:

The above components were dissolved in distilled water and diluted to a final volume of $1l$. The solution was then autoclaved for 15 minutes at 121°C.

2.6.7 ENTEROBACTERIA

The bacteria were isolated from sludge with Eosin Methylene Blue (EMB) (Oxoid) and MacConkey agars (Oxoid) which were incubated aerobically and anaerobically at 32°C in a Heraeus-D-6450 Hanau incubator. Other enterobacteria were isolated on: *Salmonella-Shigella* (SS) agar (Biolab), Brilliant green agar (BGA) (Oxoid), *Staphylococcus* 110 agar (Difco) and *Enterococcus* selective agar (Biolab).

2.7 BACTERIAL IDENTIFICATION

2.7.1 ANTIBODY PROBES

Supernatant samples were sent to the Wadsworth Centre (Albany, New York State), where antibody probes were used (R. Silver, personal communication) to identify selected methanogens. Preparation of the samples and reference microorganisms was as follows:

Cultures (5m*l*) were grown to an absorbance of $\geqslant 0.4$ at 660nm (1 cm cuvette). The samples were then centrifuged at 15 000xg for 20 minutes at 4°C. Each supernatant was removed with a Pasteur pipette and discarded, while the pellet was resuspended with a newly made fixative solution of 4m*l* formalin (37% m/v in water) plus 96m*l* of 0.85% (m/v) NaCl to an absorbance of $\geqslant 0.7$ at 660nm (1cm cuvette). The samples were mixed gently with a Pasteur pipette to avoid formation of air bubbles. The formalinized cells were placed in leak-proof glass tubes at 4 °C for shipment.

2.7.2 PANEL OF REFERENCE MICROORGANISMS FOR ANTIGENIC FINGERPRINTING

The reference microorganisms used are listed below in the order prescribed by the antigenic finger printing method (the number preceding each bacterium defines its position in the fingerprint):

1, *Methanobrevibacter smithii* PS; 2, *Methanobacterium formicicum* MF; 4, *Methanobacterium bryantii* MoH; 5, *Methanobacterium bryantii* MoHG; 6, *Methanosarcina barkeri* R1M3; 7, *Methanospirillum hungatei* JF1; 8, *Methanobrevibacter ruminantium* M1; 9, *Methanobrevibacter arboriphilus* DH1; 10, *Methanobrevibacter smithii* AL1; 14, *Methanococcus voltae* PS; 18, *Methanosarcina mazei* S6; 21, *Methanobrevibacter arboriphilus* AZ; 22, *Methanobrevibacter arboriphilus* DC; 23, *Methanomicrobium mobile* BP; 30, *Methanosaeta soehngenii* OPFIKON; and 33, *Methanocorpusulum parvum* XII. The non-methanogens used as references were the syntrophs: 1, *Syntrophomonas wolfei*; 2, *Syntrophospora bryantii;* and 4, *Desulfobulus propionicus.*

2.7.3 BIOCHEMICAL TESTS

The following biochemical tests were used in this study: catalase; hydrogen sulphide production; growth in Koser's citrate medium; indole production from tryptophan; litmus milk test; nitrate reduction to nitrite or nitrogen gas; methyl red and Voges-Proskauer tests; urea hydrolysis; gelatin hydrolysis; starch hydrolysis; Kovac's oxidase test; acid and

gas production from carbohydrates; and utilization of organic acids (Collins, 1967; Wheelis and Segel, 1979; Collins, Lyne and Grange, 1989).

2.8 SYNTHETIC/ARTIFICIAL SEWAGE

Table 12: Synthetic/artificial sewage.

COMPONENTS	COMPOSITION (gl^{-1})
$Al_2(SO_4)_316H_2O$	0.02
K_2HPO_4	0.10
Sodium acetate	0.20
Starch	0.40
$NaHCO_3$	0.50
Urea	0.12
NaCl	0.12
KCl	0.028
$CaCl_2$	0.028
$MgSO_4$	0.02
Nutrient broth powder (Oxoid)	0.20

Note: The theoretical COD of the artificial sewage is 2 000mgl^{-1}

CHAPTER 3

SEPTIC TANK CONFIGURATION, INSTALLATION AND COMMISSIONING

3.1 INTRODUCTION

Howard and Posnik (1994) reported that one in five South Africans has sub-standard living conditions. It is also documented that one in every four people (10 millions) lacks access to potable water whilst half the population (19 millions) lack access to adequate sanitation (S.A. Water Research Commission, 1993b). For rural communities, a possible solution to the latter is the use of septic tanks particularly if the generated biogas can be harnessed as an energy source.

The site chosen for installation of the septic tank was at Foxhill, Pietermaritzburg. The bioreactor was designed to serve 8-10 permanent residents plus nightly visitors of ≥ 10 people. The community is housed in clustered small dwellings in a farmyard and was chosen as typical of the KwaZulu-Natal rural situation since it lacked adequate sanitation and electricity. The community was optimistic about the development once the digester system and the implications of replacing paraffin with biogas had been explained to the residents (E. Kruger, personal communication). This acceptance was probably at odds with the results of a survey made later by the Water Research Commission (1993b) which showed that 74% of rural community residents did not like the system.

An initial bag-type digester, constructed from PVC sheeting, was chosen as this was thought to be an easier and cheaper option than the Indian and Chinese digesters described in 1.6. This bag digester was eventually replaced by a Pennells (Pty) Ltd two-tank system constructed of low density polyethylene for reasons explained in 3.2.

3.2 BAG DIGESTER

The underground bag-type digester was located at a central point near the community dwellings and next to an old farmyard shed which was allocated to house the cooking

facility. The bag digester was 2m wide x 1m deep and was made of PVC sheeting with polystyrene insulation. A solar water heater was planned for eventual placement on top of the digester to heat the bioreactor.

3.3 PENNELLS TWO-TANK BIOREACTOR

This bioreactor consisted of two linked subterranean tanks. The toilet bowl and cistern were attached to the first tank (Figure 9 and Plates 1A-1G). The tanks were made of LP 105/06 linear low density polyethylene which, according to the Pennells Group of Companies, has the following advantages: high rigidity; good low temperature impact strength; excellent environmental crack resistance; broad spectrum chemical resistance; and good weatherability (ultraviolet light stabilized). Both the toilet and tanks were made by the process of rotational moulding which is characterized by: its success over a wide range of conditions; its outstanding mouldability and product definition; and its good thermal stability (G. Pennells, personal communication). The toilet was a low flush system which utilized $0.75l$ water compared with the $16l$ used in water-borne sewage systems. The toilet itself was housed in a metal hut. The above-ground sample ports of the bioreactor were located at: the outlet of Tank 1; the inlet to Tank 2; the middle of Tank 2; and the outlet of Tank 2 (Figure 9). The cost of the toilet system and hut housing in 1993 was R 1 200 but could, according to the installers, be produced at a lower cost (H. Gambon, personal communication). Each tank had a capacity of 1 250l, and was 1 430mm long, 1 140mm wide and 880mm deep, and had a mass of 38kg. Provisions for gas collection and storage were not provided in this configuration (H. Gambon, personal communication) as it was not a true anaerobic digester (G. Pennells, personal communication). These modifications were planned for addition after the system was functioning optimally.

3.4 ARTIFICIAL WETLAND

At the effluent port of the second tank an artificial wetland, for further water purification, was constructed and planted with *Canna flaccida* (cannas) and *Colocasia esculenta* (elephant ears) which have been used in other wetlands (Wolverton, 1987; Guntenspergen, Stearns and Kadlec, 1989). Both of these ornamental plants colonise soil percolation

systems situated at the outlets of septic tanks (Drews, 1985). The plants have the advantage that their roots penetrate into the discharged wastewater and add oxygen thus increasing biological activity (Wolverton, 1989).

The artificial wetland constructed was 2.7m long, 900mm wide and 250mm deep at the outlet of the second tank but tapered to 150mm deep at the end of the construction (Figure 10 and Plates 1D-1G). The lining was PVC and was covered with rocks, gravel and river sand and, finally, topsoil.
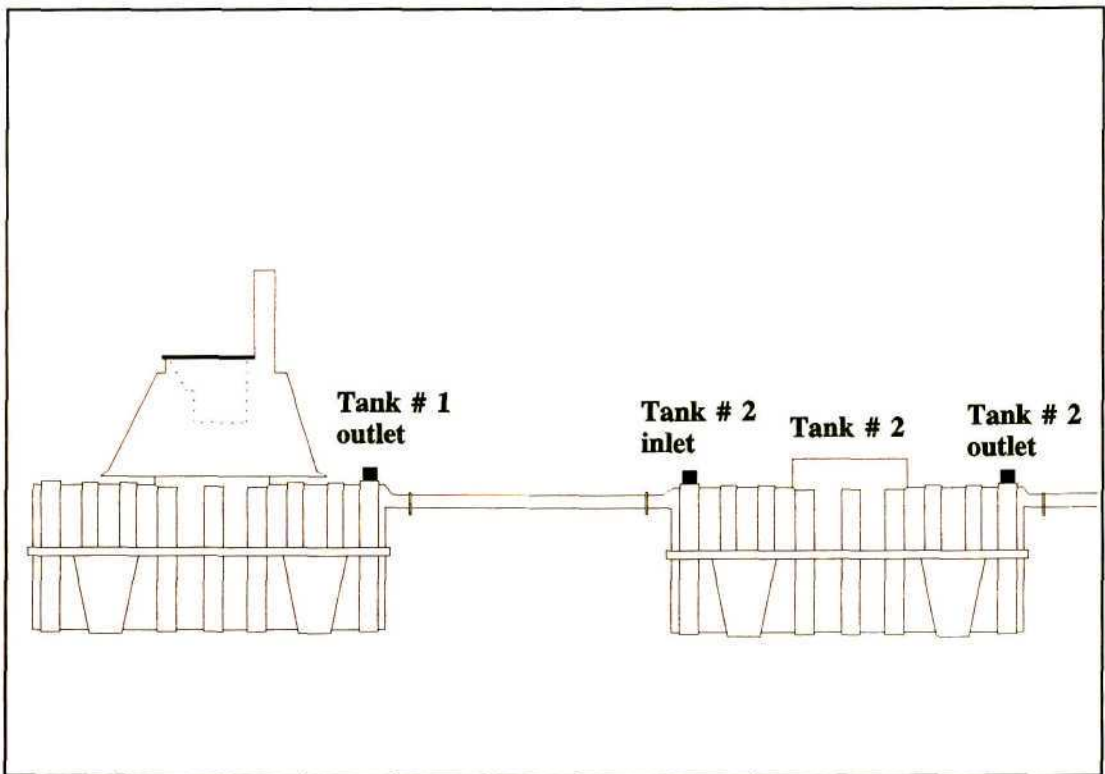
Figure 9: Rural bioreactor configuration installed at Foxhill.

Plates 1A-1G: Photographs showing the design and installation of the
                   bioreactor configuration and artificial wetland.

1A: Two-tank bioreactor configuration. The tank on the left is Tank 1 with the
       toilet attached and the outlet sample port visible. Tank 2 is the one on the
       truck.
1B: Installation of Tank 1.
1C: Installation of the hut over the toilet.
1D: Trench construction for the artificial wetland. The installed system is seen
       with the hut and sample ports visible.
1E: The artificial wetland, lined with PVC and covered with gravel.
1F: *Canna flaccida* (cannas) and *Colocasia esculenta* (elephant ears) planting.
1G: Installed bioreactor configuration and wetland.
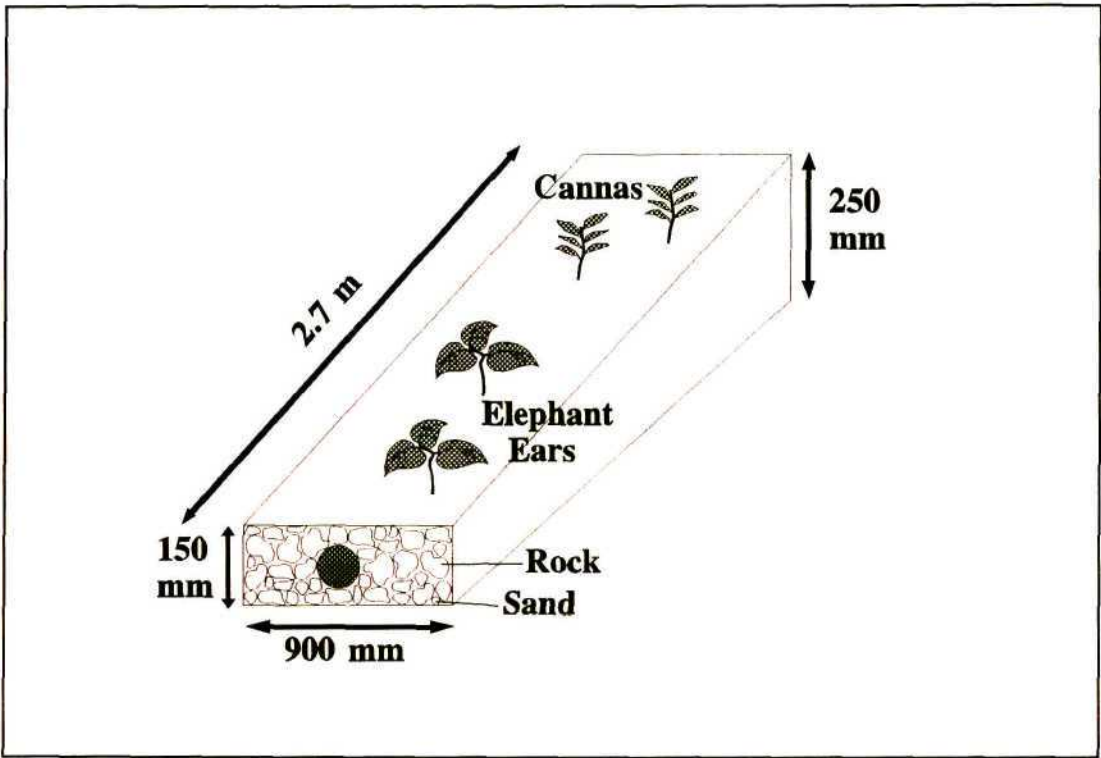
Figure 10: Diagram of the artificial wetland.

3.5 "START-UP" AND BIOREACTOR OPERATION

For start up, the tanks were filled with water to facilitate development of a suitable microbial population under the selection pressure of toilet flushings. As no gas production was detected (4.2.4) 50l of anaerobic digester sludge from Darvill Wastewater Purification Works were inoculated into Tank 2 via the central sample port to provide an active methanogenic population for biogas production. Since this procedure failed to effect biogas production for 24 weeks, the bioreactor was then inoculated with approximately 10kg of cow dung.

To facilitate an optimum C:N ratio, manure, grass, straw, etc. were added as required. In addition, the residents were instructed not to dispose inimical materials such as disinfectants.

3.5.1 SLUDGES TESTED

Different digester sludges were examined to determine which sludge exhibited optimal biogas production. This was important to identify the best sludge for digester seeding and start-up of rural bioreactors.

Standard syringe batch cultures (Viitasalo, 1981; Ishihara, Toyama and Yonaha, 1988) were used to evaluate various sludges and combinations of sludges (Table 13) for their biogas productions.

Table 13: Sludges and sludge mixes tested for biogas production.

Syringe Number	Sludge Sample
1	20ml anaerobic digester sludge from Darvill Wastewater Purification Works ("Darvill Sludge")
2	10ml "Darvill Sludge" plus 10ml Upflow Anaerobic Sludge Blanket (UASB) digester granules, previously treating brewery wastewater
3	20ml sludge from the Pennells bioreactor a few days after inoculation with "Darvill Sludge"
4	10ml sludge from the Pennells bioreactor plus 10ml "Darvill Sludge"
5	10ml sludge from the Pennells bioreactor plus 10ml artificial sewage (2.8)

The anaerobic digester sludge from Darvill Wastewater Purification Works (Syringe 1) evolved a high percentage of methane (Figure 11) and was, thus, thought to be a good inoculum for the Penells bioreactor. Unfortunately, the volume of methane evolved was much lower than the corresponding volume obtained with "Darvill Sludge" plus UASB digester granules (Syringe 2). Also the addition of "Darvill Sludge" to the Pennells bioreactor did not result in methanogensis.

Together with a biogas volume increase, Syringe 2 was characterized by a higher

percentage methane content. These results were in agreement with those of Taylor and Howgrave-Graham (1992) who found that a mixture of UASB granules (selected for good settling characteristics and high methanogenic activity) and "Darvill Sludge" (selected for high hydrolytic activity) was ideal for efficient anaerobic mineralization of raw sewage. However, despite this efficacy, UASB granules are unsuitable for rural digester inoculation (6.2.1).

The sludge of Syringe 3 proved to be particularly inactive and reflected the state of the Pennells bioreactor which proved inimical to the inoculated Darvill Sludge. Confirmation of the inactivity of the Pennells bioreactor sludge was found with Syringe 5.
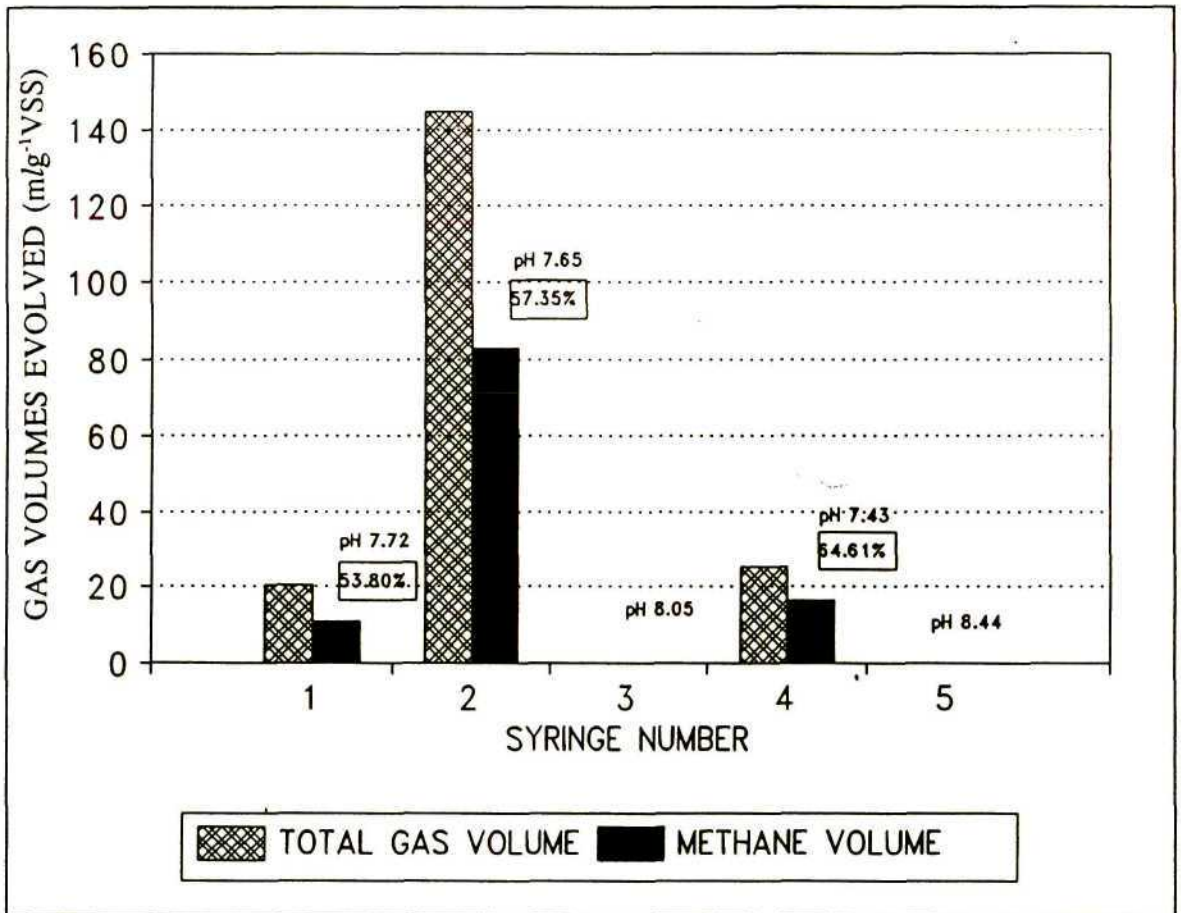
Figure 11: Volumes of gas produced by the various sludges tested during 2 weeks incubation at 32°C.

Note: The numbers recorded in the boxes are the percentages of biogas methane .

The only major pH changes were evident with Syringes 3 and 5 where increased values were recorded despite very low biogas generation activity.

3.6 DISCUSSION AND RECOMMENDATIONS

A bag-type digester was initially chosen due to its low cost (R 840) (1.6.3). Also, it lent itself to solar heating. Since this digester was not rigid, it allowed variations in shape and, due to its horizontal design, only a shallow hole was required (E. Kruger, personal communication). Unfortunately, this digester was found to be inappropriate due to installation problems encountered through the high shale content of the soil which either tore or punctured the bag.

The seeding of the Pennells (Pty) Ltd two-tank bioreactor was planned to reduce the lag phase of biogas production (Boopathy, 1987). Seeding involves the mixing of the waste under study with sludge which has already been digested anaerobically and, therefore, contains the required bacteria (Meynell, 1982). Thus, digested sludge from an anaerobic digester usually constitutes ideal seed material (DaSilva, 1981).

By digesting domestic sewage with manure, the catabolic processes are often more efficient due to synergistic effects. In addition, solid waste mixed with slurries is easier to handle and nutrient utilization and recycling costs are reduced (Tafdrup, 1994).

Despite efforts to establish active catabolic populations in the bioreactor, biogas production was not detected (4.2.4), possibly due to the incomplete anaerobiosis and, hence, inhibition of the methanogens (McCarty, 1964; Pfeffer, 1980). This was, perhaps, not surprising since the two-tank bioreactor used was not a true anaerobic digester but rather a septic tank or aqua-privy system (G. Pennells, personal communication; G.N Tivchev, personal communication).

Despite the lack of biogas production and exploitation the bioreactor did provide an improved means of sewage disposal for the residents. Together with the lack of biogas, frequent blockages also resulted. Similar systems with lower capacity installed by the

Pennells Group of Companies have also experienced blockage problems (G. Pennells, personal communication). The first blockage was between the toilet flushing system and Tank 1 and was caused by a plastic drink container which had to be removed by dismantling the entire toilet pan. Before this container was discovered it was thought that the blockage was due to either the low water volume of the low flush system or the design of the cone-shaped structure beneath the pan which was used as an anti-splash device. The latter, however, had not proved problematic in other Pennells systems. It has been documented that aqua-privy systems and septic tanks often fail due to low water additions < 1.5l (Kalbermatten *et al.*, 1982; Mann and Williamson, 1982) which result in losses of water seals by evaporation, concomitant bad smells and zero effluent discharge (Mann and Williamson, 1982). Where breakages in water seals result, these systems are not recommended as viable sanitation solutions (Kalbermatten *et al.*, 1982).

Following the first blockage, other blockage problems occurred regularly due to the residents forcing or flushing items down the toilet. Such objects included soft drink bottles, plastic piping, the author's data sheets, newspaper, leaves and corrugated cardboard. These last four items were used as toilet "paper". In Africa, maize cobs, leaves, newspapers and stones are common materials used for anal cleaning (S.A. Water Research Commission, 1993a). The blockages occurred despite constant efforts to teach the residents good practices.

Such community-based problems have been reported for other social upliftment projects (B. La Trobe, personal communication; G. Pryce Lewis, personal communication; G. Pennells, personal communication).

Many factors have been implicated in the failure of private sewage disposal systems, especially in rural areas. Principal among these are: low capacity; poor construction; lack of adequate maintenance (Wright, 1956); and non-involvement of the user community (Kalbermatten *et al.*, 1982; Muyiboi, 1992), especially in the planning and implementation processes (Muyiboi, 1992). Septic tanks will only function properly if designed and operated correctly (McKinney, 1962).

Studies undertaken by the S.A. Water Research Commission (1993b) on the Pennells tank systems and other low flush, on-site, anaerobic digesters/aqua-privies, raised concerns in relation to their community acceptance, their capacity to function effectively from a technical point of view and the organizational infrastructure required to manage them. The study showed that 74% of the people did not like the systems (S.A. Water Research Commission, 1993b). A complaint common to that found with the system installed at Foxhill related to blockages especially at the pedestal (or inlet) to the septic tank. This, therefore, showed that development of adequate specifications for these bioreactors, together with proper supervision of construction and an education programme related to their proper use and maintenance, are prerequisites before they will be widely accepted in South Africa (S.A. Water Research Commission, 1993b).

In addition to the above problems, grazing on wetland plants by farm animals occurred. To eliminate possible further problems, the bioreactor sampling points and the artificial wetland were isolated by a fence.

Other problems did, however, occur since the throughflow could not be accurately determined due to the irregular use of the toilet. The farm labourers, for example, did not return from the fields to use the toilet and more people used the facility in the evenings and at weekends when the users increased due to the arrival of visitors. These problems appear to be common since previous studies have not identified beneficial effects from improved sanitation because although latrines were built they were not kept clean and were not used by the children and adults working in the fields (A'Bear, 1991).

Unfortunately, the efficacy of the artificial wetland could not be determined as there was no final effluent emanating from the outlet. This was unfortunate especially since there is concern that widespread use of on-site sanitation systems may cause subsurface migration of contaminants and, ultimately, disease transmission and environmental degradation. The principal contaminants are biological (viruses, bacteria, protozoa and helminths) and chemical (primarily nitrogen and phosphorus) (Fourie and van Ryneveld, 1995). It was unfortunate that samples could not be taken and analyzed as artificial wetland wastewater treatment is recognized for its potential as a low technology, environmentally-friendly,

cost-effective option (Mozes, 1992; Water Pollution Control, 1993). Wetland treatment technology has considerable potential as both a primary treatment for wastewaters emanating from rural communities (Wood and Hensman, 1989) and a secondary wastewater purification following digestion (1.8).

Various modifications to the two-tank Pennells system could facilitate improved digestion. Strict anaerobiosis by making the tanks gas tight would facilitate biogas production. Gas exploitation facilities should also be added. Next, tank developments to eliminate dead spaces and, thus, decrease the hydraulic retention time (Meynell, 1982) should be considered.

Mixing is important (Ward, 1982) but was impractical in the system under study. It promotes contact between the microorganisms and the organic material (Morris *et al.*, 1975; Loehr, 1977; White and Plasket, 1981) and, thus, minimizes the short-circuiting of the raw feed (Ross *et al.*, 1992) and, hence, the rate of digestion is increased (White and Plasket, 1981). In addition, a uniform temperature is maintained and dispersion and dilution of toxic substances and potential metabolic inhibitors, such as volatile acids (Loehr, 1977; Forster, 1985; Ross *et al.*, 1992), are promoted. Through mixing, disintegration of coarser organic particles results thus increasing the net surface area and degradation rate (Loehr, 1977). Grit settlement and scum formation are also reduced (Forster, 1985; Forster and Senior, 1987; Ross *et al.*, 1992). Unfortunately, in the absence of appropriate training few of the above modifications would have any real impact.

Lombard (1994) and Matsoga (1994) both documented that communities' needs are often neglected in waste management. The individual community must be involved in the planning process to ensure appropriate and sustainable waste management through corporate commitment (S.A. Water Research Commission, 1993a; Lombard, 1994). Unfortunately, in the study reported here, although some of the community were involved in the construction stage, problems still occurred. The organizational capabilities of communities vary considerably and in some situations could limit the success of the biotechnology (S.A. Water Research Commission, 1993a).

Possible solutions to improve the chances of success include more sensitive/appropriate education, possibly reinforced by pictorial instructions strategically placed in the installation. Alternatively, the community could be asked to pay for removing blockages or pay for initial construction costs. Perhaps, with financial involvement, more care would then be taken. To effectively implement biomethanation requires: awareness; demonstrations; incentives; and education and training (Ward, 1982). A holistic approach to sanitation is, thus, a possible solution (S.A. Water Research Commission, 1993a).

Many digester designs (including septic tanks) are constantly being developed (1.7). The specific design must, however, reflect the needs of the individual community. Sophisticated systems, for example, often fail due to a lack of skilled manpower and financial resources to maintain the installation (S.A. Water Research Commission, 1993a; Lombard, 1994). More subsidies in conjunction with different bioreactor configurations are necessary for rural people of the lower socio-economic strata (Biswas, 1989). Appropriate technologies which have proved successful in other countries need to be tested and, possibly, modified for local conditions (S.A. Water Research Commission, 1993a). Increased biogas use in developing countries hinges on the economic and social benefits of the technology (Rybczynski, Polprasert and McGarry, 1978) and public acceptance through better training (S.A. Water Research Commission, 1993a).

The Pennells low density polyethylene tanks, although placed below ground, lacked insulation and this could prove problematic in winter when reduced catabolic activity may result.

To design suitable digesters for use in rural communities, collaboration is vital between manufacturers, scientists and users. Not only microbiological but also financial, economic and, especially, social aspects must be considered.

CHAPTER 4

OPERATIONAL INVESTIGATIONS OF
THE PENNELLS TWO-TANK BIOREACTOR

4.1 INTRODUCTION

The ease of bioreactor operation and the degree of control and monitoring required are governed by such factors as the plant design, type of waste, envisaged loading rates and operator experience (Holland *et al.*, 1987).

No single analytical criterion is sufficiently sensitive to facilitate reliable forecasting of incipient overloading or digester failure, particularly in the presence of high organic loading rates (Ross and Louw, 1987). Thus, effective monitoring requires determination of a combination of physical, chemical and biological indices and, notably, interrelationships such as that between organic loading rate and biomass retention (Ross and Louw, 1987). The main changes which are indicative of digester/septic tank perturbation, in typical order of increasing response time, are (Ross *et al.*, 1992):

a) An increase in the percentage of carbon dioxide in the gas produced;

b) A decrease in gas production;

c) An increase in VFA:alkalinity ratio;

d) Production of a malodorous sludge;

e) A pH decrease;

f) An increase in the solids content of the supernatant;

g) A decrease in the volatile solids reduction; and

h) A foam increase.

These changes can be caused by hydraulic or organic loading, mixing, heating or toxicity problems. The digestion process is also affected by the sludge composition, method of sludge addition, solids retention time and the presence or absence of anaerobic conditions (Ross *et al.*, 1992).

The efficacy of the two-tank bioreactor was assessed by monitoring the following key analytical criteria: biogas production; pH; chemical oxygen demand; volatile fatty acids (VFA) concentrations and bicarbonate alkalinity; and VFA:total alkalinity ratio. In addition, key bioreactor parameters such as liquid/sludge depth and temperature were monitored.

4.2 EXPERIMENTAL: RESULTS AND DISCUSSION

4.2.1 LIQUID/SLUDGE DEPTH

The total depth of liquid in the two tanks of the bioreactor were measured at each sampling port to identify any blockages. Liquid depths in the bioreactor were measured at four points ("Tank 1 outlet", "Tank 2 inlet", "Tank 2" and "Tank 2 outlet"), before and after inoculation, with a graduated sampling rod (2.1.1).

It is documented that the type of fermentation in small rural biogas digesters is semi-continuous (United Nations Environment Programme, 1981). Hence, once commissioned, the bioreactor was designed to function in this semi-continuous mode with both tanks permanently full. This was, however, not the case (Figure 12).

"Tank 1 outlet" recorded the two highest liquid/sludge depths on days 60 and 137. These could have been due to blockage problems experienced between Tanks 1 and 2 (3.6). "Tank 1 outlet" depths were, with few exceptions, near constant, and were higher than "Tank 2 inlet" and the other two measuring points which again indicated partial blockage.

The input of sewage and the discharge/removal of digested sludge must not effect sudden variations and, hence, digester performance (Degrémont, 1991). Once both tanks were full, constant liquid/sludge depths were expected due to the natural flow between the tanks.

Since both tanks were characterized by a gas headspace above the liquid/sludge surface an initial fully anoxic environment was unlikely. The bioreactor is, therefore, better described as a septic tank system rather than a true anaerobic digester.

Figure 12: Changes in the depth of liquid in all sample ports of the bioreactor with time.
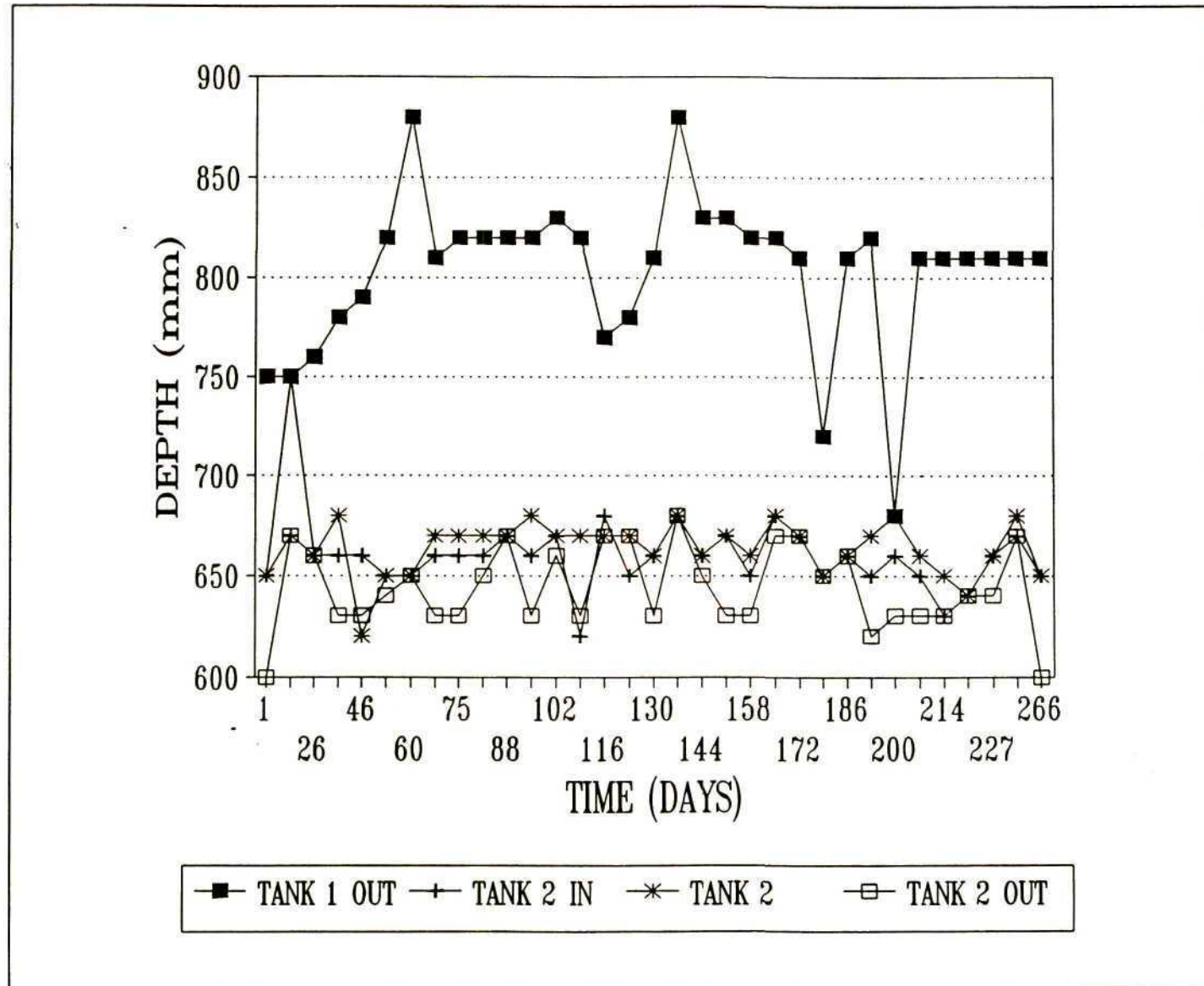
Although both the total and volatile suspended solids were analysed to determine the masses of sludge in the two tanks the results were of only limited value due to the floccose nature of the sludge which just coated the bottom surface of the tanks and the inherent variabilities of the assay results (American Public Health Association, 1955; 1980; Salkinoja-Salonen and Hughes, 1982).

4.2.2 TEMPERATURE

Temperature is an important operational parameter in the fermentation process. It is documented that as the temperature increases the reaction rates increase and more efficient operation results with lower retention times (Vriens et al., 1990; Ross et al., 1992). Below specific limits, the higher the temperature, the higher the gas production (United Nations Environment Programme, 1981). High temperatures also facilitate fat and grease liquefaction and, thus, promote catabolism (Ross et al., 1992). The degree of acidification, rate of product formation and treatment efficiency (COD removal) all increase with increasing temperature (Archer, 1983; Dinopolou et al., 1988). It must be stressed, however, that the effect of temperature is limited to the rate and not the extent of digestion of the raw materials (United Nations Environment Programme, 1981).

To optimise anaerobic digestion, the temperature should be constantly monitored and should be maintained within a specific range (Ross et al., 1992). The recommended mesophilic optimum temperature is 35°C (Ross and Louw, 1987) although a temperature range of 32 to 37°C is acceptable for full-scale systems equipped with heating facilities (Ross et al., 1992). The Pennells two-tank bioreactor was not heated and had no provisions for insulation other than burial. This is a limitation as insulation is often required to maintain an optimum temperature (Molnar and Bartha, 1988; Pandey et al., 1992). In this study, the temperatures of the sludge and supernatant at the four sample ports of the bioreactor were recorded (2.4.2) at regular intervals (Figures 13 and 14).

Figure 13: Changes of the bioreactor sludge and ambient air temperatures with time.
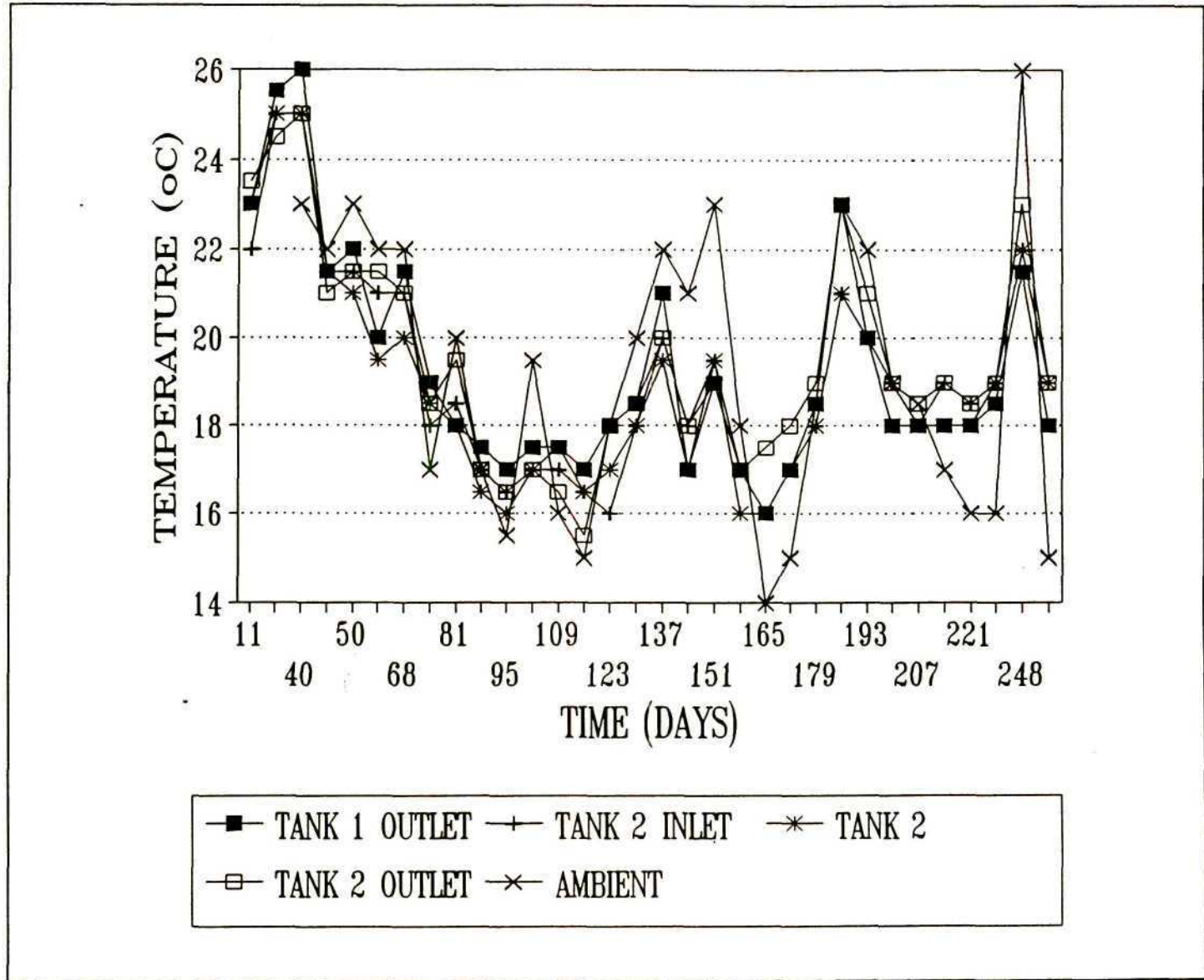
Figure 14: Changes in the supernatant and ambient air temperatures of the Pennells two-tank bioreactor with time.
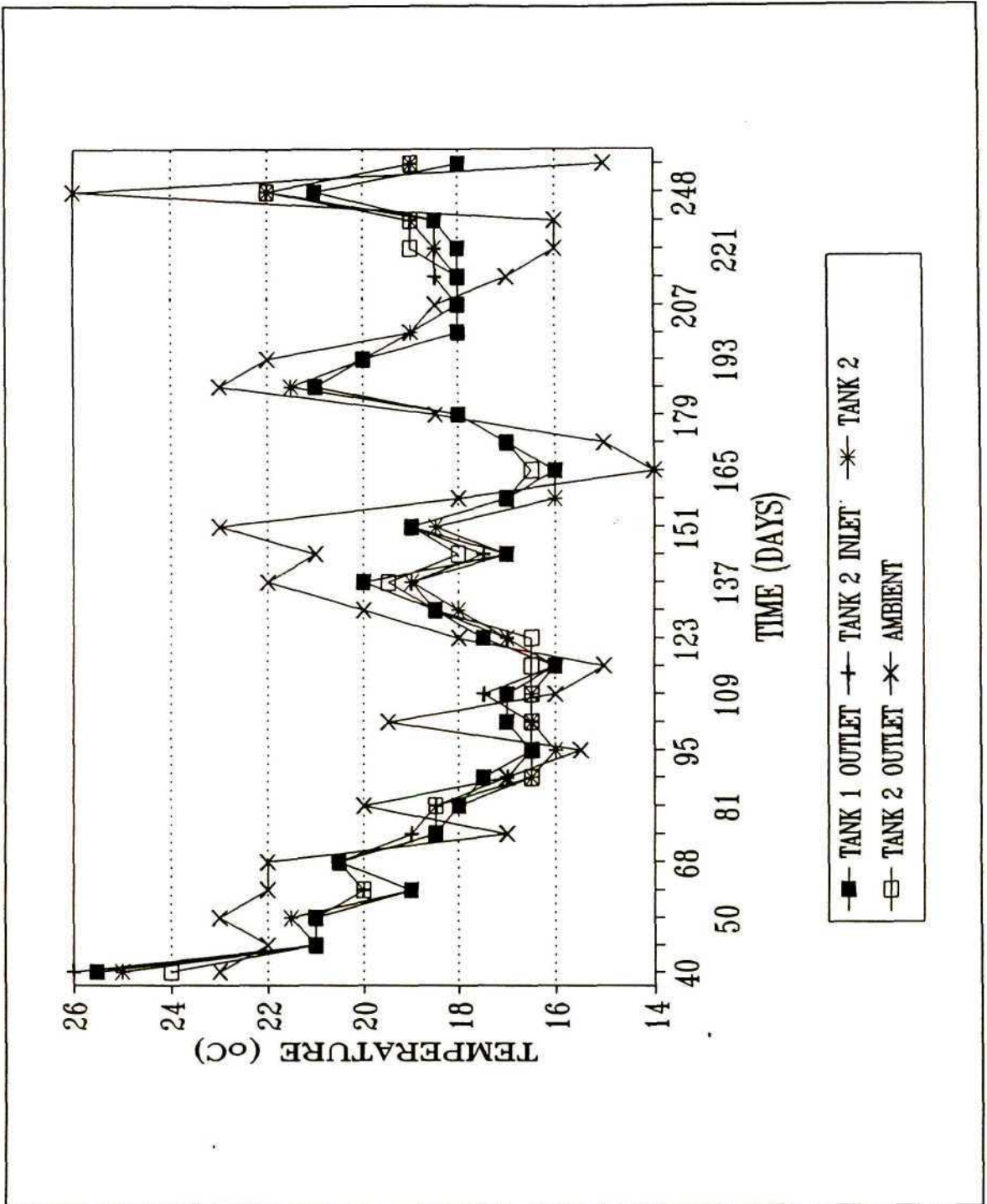
Figures 13 and 14 show that both the sludge and supernatant temperatures at all four sample ports varied with time. The sludge temperatures were generally warmer than those of the supernatant but were often <25°C. Bacterial conversion rates in this psychrophilic range (Holland *et al.*, 1987), which is often dominated by the homoacetogens (Nozhevnikova and Kotsyurbenko, 1994), would, thus, be slower than in mesophilic or

thermophilic conditions (Beba, 1988; Safley and Westerman, 1992; Thom and Banks, 1992) with temperatures <15°C necessitating retention times >30 days (Ross *et al.*, 1992). In addition to rate limitations, stratifications and solids accumulations also often occur in unheated (and unstirred) digesters and result in stagnant regions (Jash and Ghosh, 1990).

Methanogenesis is adversely affected by sudden changes in temperature >2 to 3°C (Holland *et al.*, 1987; Ross *et al.*, 1992). Although sudden temperature changes did not occur in the Pennells system, biogas was not produced (4.2.4) probably due to a lack or inhibition of an active catabolic population.

In rural biogas systems where no heating is present, the digester temperatures fluctuate in response to changes in the ambient air temperature (United Nations Environment Programme, 1981). For example, temperatures in rural biogas digesters in China vary between 22 and 26°C in the summer but fall to 13 to 15°C in the winter (United Nations Environment Programme, 1981) while the comparable range for septic tank effluent is between 6 and 13°C (Laak, 1986). The Pennells two-tank bioreactor recorded higher temperatures than those of Laak (1986) but the temperatures were slightly lower than the summer-time temperatures recorded from rural biogas digesters in China.

The ambient air temperatures recorded did not take into account the wind chill factor which was often substantial. The temperatures fluctuated according to, and within, the seasons depending upon meteorological factors such as cloud cover, rain, etc. In general, the ambient air temperatures correlated with season with days 40-68 (autumn), days 75-130 (winter) and days 150-248 (spring).

A comparison of the sludge and supernatant temperatures with the ambient air temperatures showed an interesting phenomenon. When the ambient air temperatures were high, the sludge and supernatant temperatures were lower and when the ambient air temperatures fell those within the tanks were higher. To maintain temperature, digesters can be located facing the sun, sheltered from wind or, preferably, covered (United Nations Environment Programme, 1981). Unfortunately, provisions of insulation and temperature

control incur major costs (Williams and Eberhard, 1986).

4.2.3 CHEMICAL OXYGEN DEMAND

Chemical oxygen demand (COD) is a widely used analytical criterion for digester performance. It is a measure of the oxygen equivalent of the organic materials present in the wastewater subject to oxidation by a strong chemical oxidant (American Public Health Association, 1980; Slatter and Alborough, 1992). This includes certain oxidizable mineral salts (sulphides, sulphites, etc.) and most organic compounds. Few nitrogen compounds and hydrocarbons escape the powerful oxidizing agent (Degrémont, 1991). When wastewater contains only labile molecules and no toxic matter, the COD test results provide a good estimate of the biochemical oxygen demand (BOD), organic carbon or organic matter content values (American Public Health Association, 1980; Hach Reactor Manual, 1994).

Chemical oxygen demand measurements were made on supernatants taken from the outlet ports of both tanks (2.4.3). Unfortunately, no sample could be taken from the terminal point of the artificial wetland (3.6). The percentage COD removals of Tanks 1 and 2 were estimated by assaying the supernatants:

$$\frac{COD \ (mgl^{-1}) \ \text{Tank 1 outlet} - COD \ (mgl^{-1}) \ \text{Tank 2 outlet}}{COD \ (mgl^{-1}) \ \text{Tank 1 outlet}} \times 100 \qquad (5)$$

Supernatant samples from the outlet of Tank 1 contained, predominantly, poorly digested sewage and this manifested as high COD values (Figure 15). The COD values also fluctuated substantially during the study in comparison with the equivalent values of "Tank 2 outlet".
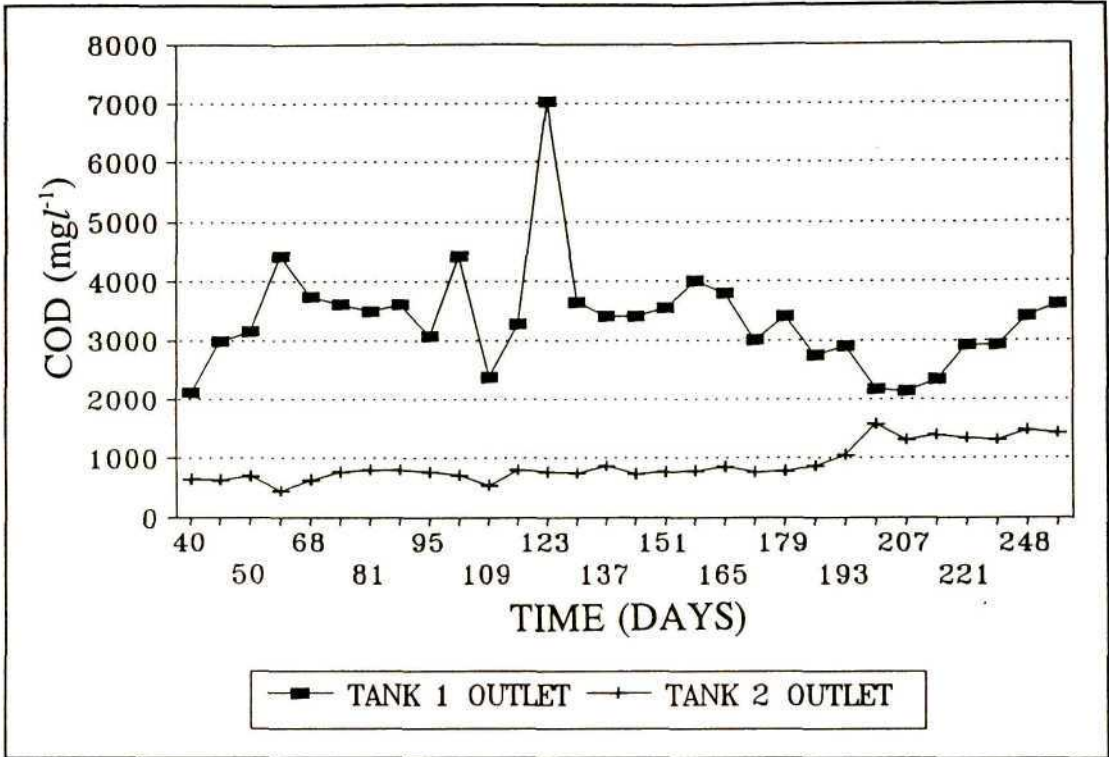
Figure 15: Changes in the CODs of the Pennells bioreactor supernatants during the 250 days study period.
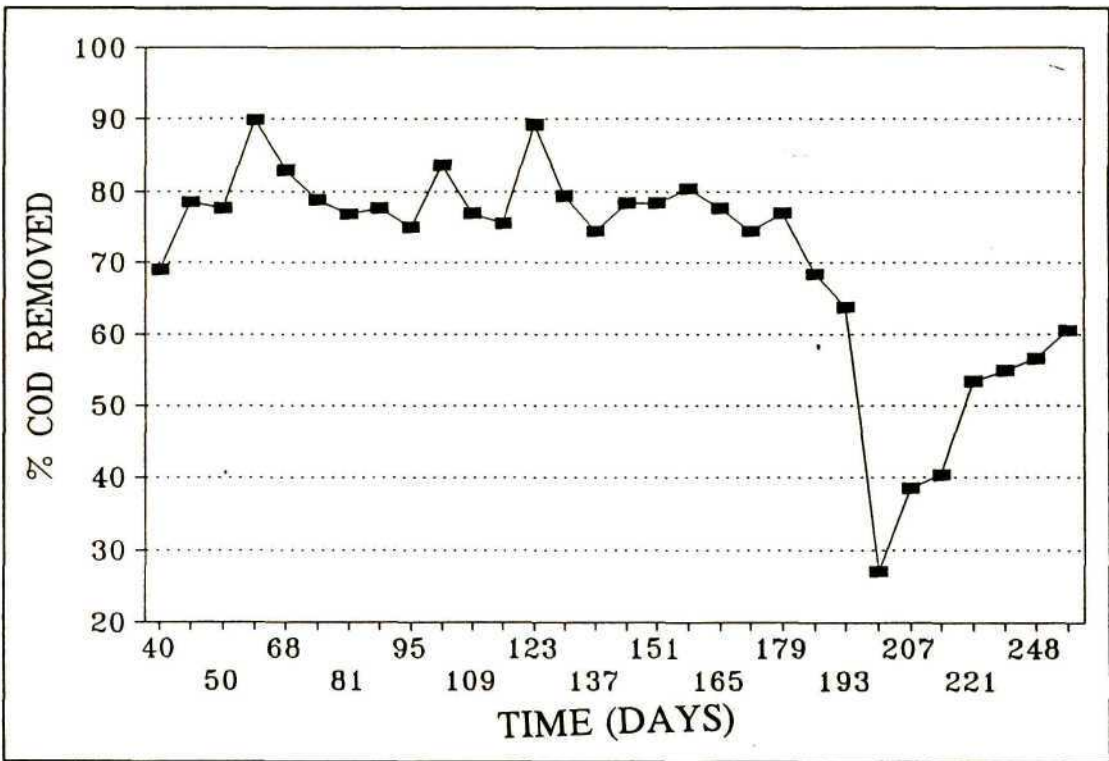
Figure 16: Percentage CODs removed by Tanks 1 and 2 during the 250 days study period.

Laak (1986) documented that the average pollutant COD is 210g per capita per day. Of this, the average faeces and urine produced are 120g faeces per capita per day, where 100g faeces has a COD of 30g, and $1.1l$ urine per capita per day, where $1l$ of urine has a COD of 20g. Unfortunately, toilet use for the bioreactor could not be accurately determined. The COD of septic tank effluent is documented to be between 150 and $720mgl^{-1}$ (Laak, 1986), while Meynell (1982) found that the equivalent value for raw sewage was between 300 and $600mgl^{-1}$. The supernatant COD values of "Tank 1" were substantially higher than the above septic tank results. "Tank 2 outlet" values were also slightly higher than the documented values. The high readings for Tank 1 were possibly due to the low flush mode of operation which limited dilution.

Despite this lack of dilution, COD removal by Tank 2 was obtained (Figures 15 and 16). The COD removal did, however, fall dramatically after day 179 before recovering. The addition of cow manure (3.5) on days 179 and 186 could possibly have effected this fall through organic overloading. Aqua-privies are documented to effect a 50-75% COD removal (S.A. Water Research Commission, 1993a). A comparable treatment efficiency for the bioreactor under study could not, however, be determined since the design militated against influent sampling.

Despite the COD removal, no biogas was detected. This could have been because the organic molecules in wastewater undergo limited anaerobic digestion in septic tanks (Atlas and Bartha, 1987) although some CO_2 should have been evolved. In the absence of methanogenesis, stabilization of organic matter is dominated by hydrolysis (McKinney, 1962) with microbial biomass the COD sink.

No COD measurements were taken at the outlet of the artificial wetland due to the lack of supernatant. This was disappointing since the capacity of the wetland to attenuate COD could not be estimated. With artificial wetlands, the COD/BOD associated with the settleable solids component of a wastewater are removed by sedimentation. The colloidal and soluble COD/BOD remaining are removed as a result of microbial intervention by free-living (water column) and surface-attached (sediments, roots, stems) species (Wood and Pybus, 1993). Artificial wetlands are documented to remove 60-80% BOD (Brix, 1987).

4.2.4 BIOGAS PRODUCTION

Sludges used in the batch cultures were taken from below the outlet of Tank 1 and the sample port of Tank 2. For continuous cultures the four sludge inocula were: Tank 1 sludge, Tank 2 sludge, anaerobic digester sludge from Darvill Sewage Works and anaerobic digester sludge from Durban Wastewater Purification Works (2.2).

Throughout the study, no methane production (2.4.6) was detected in the two-stage bioreactor. Similarly, the laboratory batch and continuous cultures (2.3) did not generate gas. For all these cultivation systems the sludges were characterized by an absence of fluorescence (2.5.1) which indicated a destruction of the bacterial methanogenic population. For the two-tank bioreactor a possible cause was a lack of anaerobiosis (3.6) (Pfeffer, 1980; Jarrell *et al.*, 1987; Vriens *et al.*, 1990) and/or absence of mixing which minimized contact between the organic matter and the microorganisms and the dispersion of metabolic inhibitors (Loehr, 1977; White and Plaskett, 1981; Degrémont, 1991).

For the laboratory cultures, the artificial sewage used could have effected an inhibition of biogas production through a pH increase from near neutral to between 8 and 9.7. This could possibly be explained by ammonia production from the urea which increased the pH. The optimum pH for anaerobic digestion is between 6.8 and 7.2 while pH values >8 have been reported to be bactericidal (Ross *et al.*, 1992).

4.2.5 pH

One of the most important requirements for an effective digestion process is maintenance of the pH between 6.8 and 7.2 as a pH which is too high or too low can prove bacteriostatic/bactericidal or effect acid accumulation (Ross *et al.*, 1992). Thus, pH is used as an indicator of digester overload (Archer, 1988) (1.5.3). pH in itself is not entirely satisfactory as an indicator of stable digester performance due to its slow response time at the onset of digester upset (Schellenbach, 1981; Ross *et al.*, 1992). This is because by the time the pH has decreased the alkalinity has already declined (Ross and Louw, 1987). pH measurements should, therefore, be made in conjunction with some form of VFA and

alkalinity monitoring (Ross *et al.*, 1992).

In sewage treatment facilities both the influent and digested sludges should be routinely monitored for pH. Primary feed sludges are tested to ensure that septic conditions do not develop in the primary settling tanks which, in turn, would upset the thickening process. The pH of primary sludge should remain above 5.0. Digested sludge is tested to ensure that the conditions in the digester are satisfactory for the methanogens. In a stable digester the pH should be between 6.8 and 7.2. If the pH drops below 6.8 or goes above 8.0 then this is a definite indication of process upset and, possible, failure (Ross *et al.*, 1992).

pH measurements of the sludge (commencing from day 1) and liquid supernatant (commencing from day 40) from "Tank 1 outlet", "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" were taken at regular intervals (Figures 17 and 18) (2.4.1). The pH readings were taken immediately after sampling to minimise errors due to carbon dioxide loss during storage (Ross *et al.*, 1992).

Day 1 pH values were those before "Darvill Sludge" (pH 7.34) addition and day 11 were those after addition (3.5). The high sludge pH values temporarily decreased to more neutral values after inoculation with "Darvill Sludge". Addition of cow manure on days 179 and 186 to Tank 2 resulted in slightly decreased supernatant pH values but marked decreases in "Tank 2" and "Tank 2 outlet" sludges.

In general, the pH values of both the sludge and, particularly, the supernatant (Figures 17 and 18) were high and could have proved bactericidal (Ross *et al.*, 1992).

Figure 17: Changes in the sludge pH sampled at different ports of the two-tank bioreactor with time.
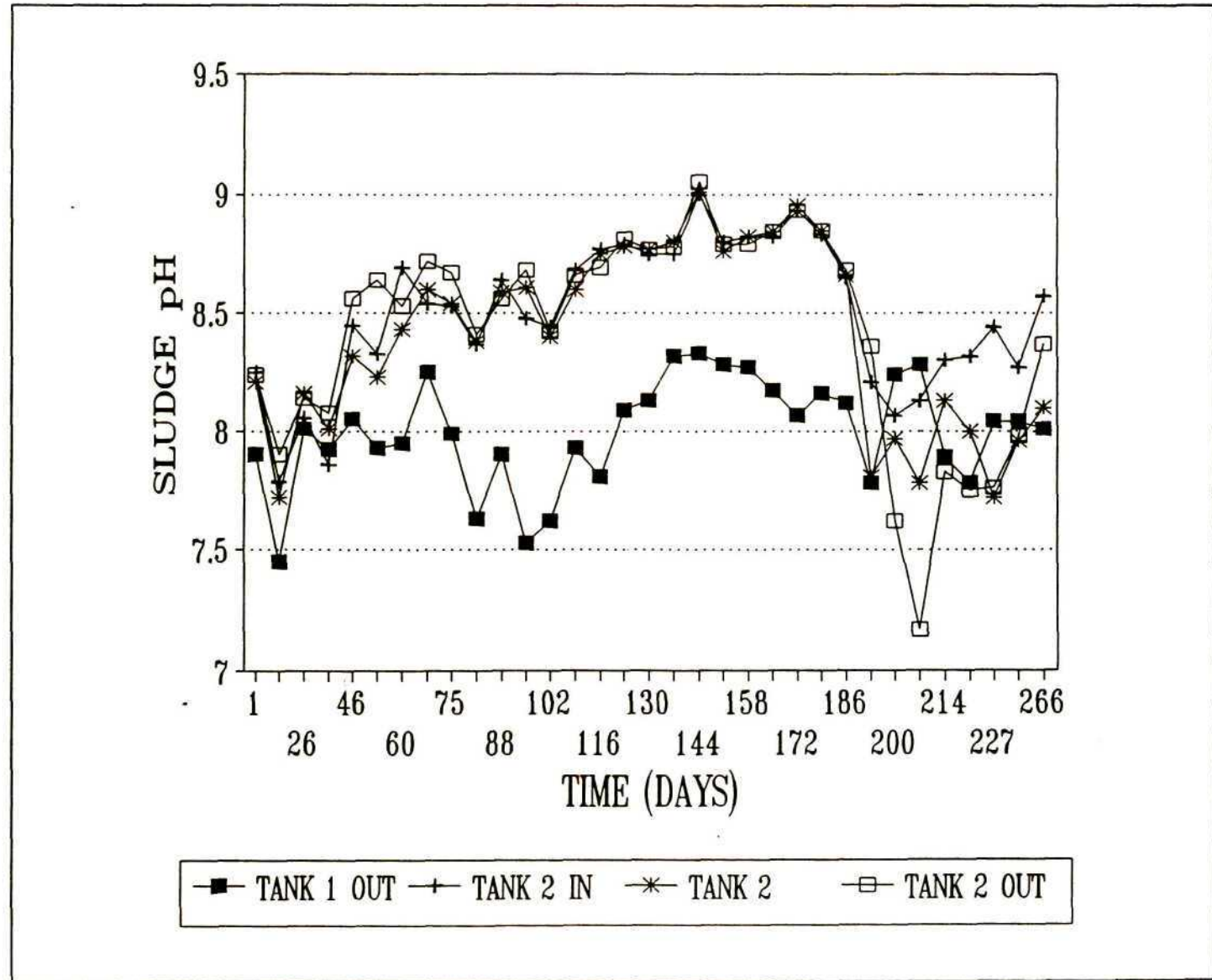
Figure 18: Changes in the supernatant pH sampled at the different ports of the Pennells two-tank bioreactor with time.
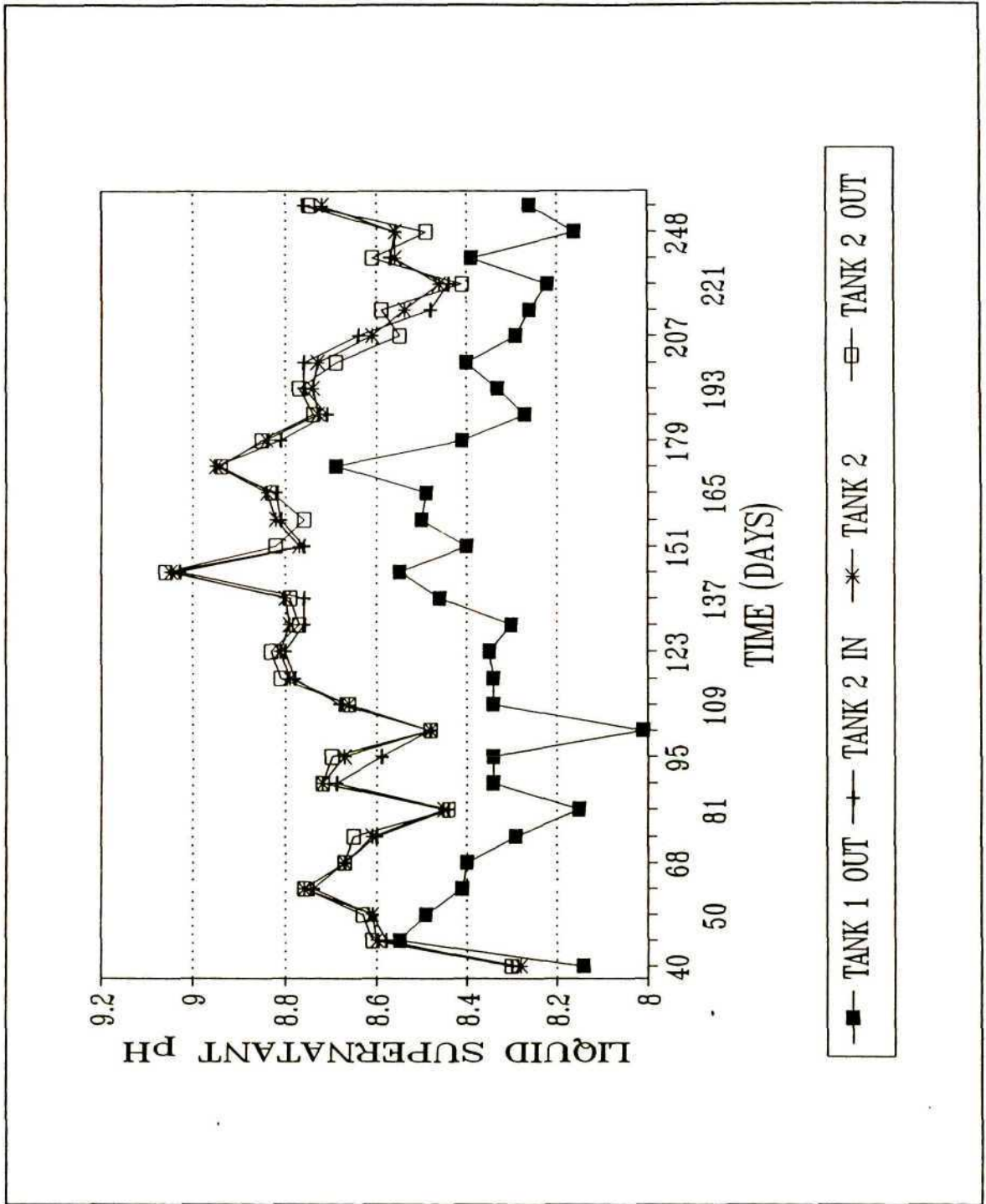
Typically, the pH of effluent from septic tanks is between 7.1 and 8.3 (Laak, 1986). Thus, the elevated pH values recorded could have perhaps been due to addition of substances by the residents since living and eating habits have an effect on the sanitation system (S.A. Water Research Commission, 1993a).

The sludge and supernatant pH values recorded, generally, followed an erratic pattern, especially after day 186. The pH values of "Tank 1 outlet", typically, were lower than "Tank 2" possibly due to blockage and increased residence time in the first tank. Extremely high pH values ($\pm$ pH 9.0) were recorded on day 144. Possible reasons for this could be protein degradation and/or urea hydrolysis (Kroeker, Schulte, Sparling and Lapp, 1979). In addition, although less likely, CO_2 could have been lost from the samples (Meynell, 1982; Ross *et al.*, 1992) which, in turn, would have altered the bicarbonate alkalinity (Meynell, 1982).

The presence of ammonium ions increases the bicarbonate alkalinity and pH thereby increasing the buffering capacity (Rivett-Carnac, 1982). Urea, which occurs in the urine of man, is decomposed by numerous microorganisms (notably *Micrococcus ureae*, *Enterobacter ureae* and *Sarcina ureae*) with the liberation of ammonia (Frobisher, 1962). In biogas plants this effect is overcome by adding more carbon, for example, in the form of cow manure to adjust the C:N ratio and, in turn, the pH.

From the results of this study it can be concluded that the elevated pH in the Pennells two-tank bioreactor was unfavourable for efficient digester operation and biogas production as it exceeded the optimal documented pH (Ross *et al.*, 1992). Perhaps insufficient VFAs were produced (Figure 18) or, less likely, acidotrophy greatly exceeded acidogenesis. Sludge pH is controlled by the residual VFA concentration and the alkalinity of the digester (Ross *et al.*, 1992). pH is also a function of the fraction of the carbon dioxide in the digester gas. Since low VFA concentration changes occur before pH changes are noted, the VFA content of the reactor should also be measured (Ward, 1982). Although a constant feed pH should promote improved digester operation this was impractical in the bioreactor under study. Addition of cow manure and supplementary organics through community participation would possibly have improved the efficiency of the system by lowering the pH.

4.2.6 VOLATILE FATTY ACIDS AND BICARBONATE ALKALINITY

During start-up, the aim is to quickly reach a stable fermentation with a VFA:alkalinity

ratio <0.3 and a near-neutral pH. This ratio gives a direct indication of the acidity/alkalinity balance of the sludge and a warning of impending upset (4.2.7). The VFA and alkalinity concentrations should, therefore, be monitored and the ratio between the two calculated (Ross *et al.*, 1992).

The alkalinity of the sludge is important as it equates to the ability of the digester to neutralize the VFAs formed. It usually consists of bicarbonate, carbonate and hydroxide moieties (Hashimoto *et al.*, 1979; American Public Health Association, 1980; Ross *et al.*, 1992) but may include contributions from borates, phosphates or silicates if they are present (American Public Health Association, 1955; 1980). The alkalinity, therefore, buffers the system and controls the anaerobic digestion process stability (Jarrell *et al.*, 1987). The ability to neutralize acid at a specific pH is called the buffering capacity of the liquid at that pH and is estimated by measuring the alkalinity (Ross *et al.*, 1992). The total alkalinity is divided into VFA alkalinity and bicarbonate (or partial) alkalinity (BA) (Ross *et al.*, 1992).

Raw domestic wastewater has an alkalinity < or only slightly > than that of the water supply (American Public Health Association, 1955; 1980). Alkalinity, thus, varies with the locality in which the water is found (American Public Health Association, 1955). In a digester, the range of alkalinity reflects the nature of the influent and the bioreactor design and operation (Schellenbach, 1981). Stable operating anaerobic digesters, typically, have supernatant alkalinities in the range of 2 000 to 4 000 mg $CaCO_3$ (American Public Health Association, 1980).

Alkalinity and pH buffering capacity in the digester are chiefly a function of the bicarbonate concentration and should be within 10 to 50mM for stable operation (Ward, 1982; Hawkes, Guwy, Hawkes and Rozzi, 1994). Cation availability is also important since it may determine the alkalinity and pH of the digester system. Nitrogenous compounds, for example, may be decomposed and reduced to ammonia. A proportion of the alkalinity is metabolized by the process organisms while the remainder combines with liberated carbon dioxide to produce stable inorganic compounds, such as NH_4HCO_3, which act as buffers. Thus, the ammonium ion concentration plays an important role in

determining the BA or buffering capacity of the digester system (Ross and Louw, 1987).

In the presence of high ammonium concentrations, buffering of the digestion process is effected by weak acids and acid salts (Hashimoto *et al.*, 1979). The common ones found in anaerobic fermentations are sulphides and short-chain fatty acids (propionic and acetic) (Moosbrugger, Wentzel, Ekama and Marais, 1993).

Monitoring of the VFA concentrations and alkalinity is important even though there is no clear simple practical method to determine these with reasonable accuracy (Moosbrugger *et al.*, 1993).

In this study, volatile fatty acid and bicarbonate alkalinity analyses of liquid supernatant (Degrémont, 1991) from the four sample points of the bioreactor were made (2.4.4) (Figures 19 and 20) but were not initiated until day 60 due to the absence of gas production.
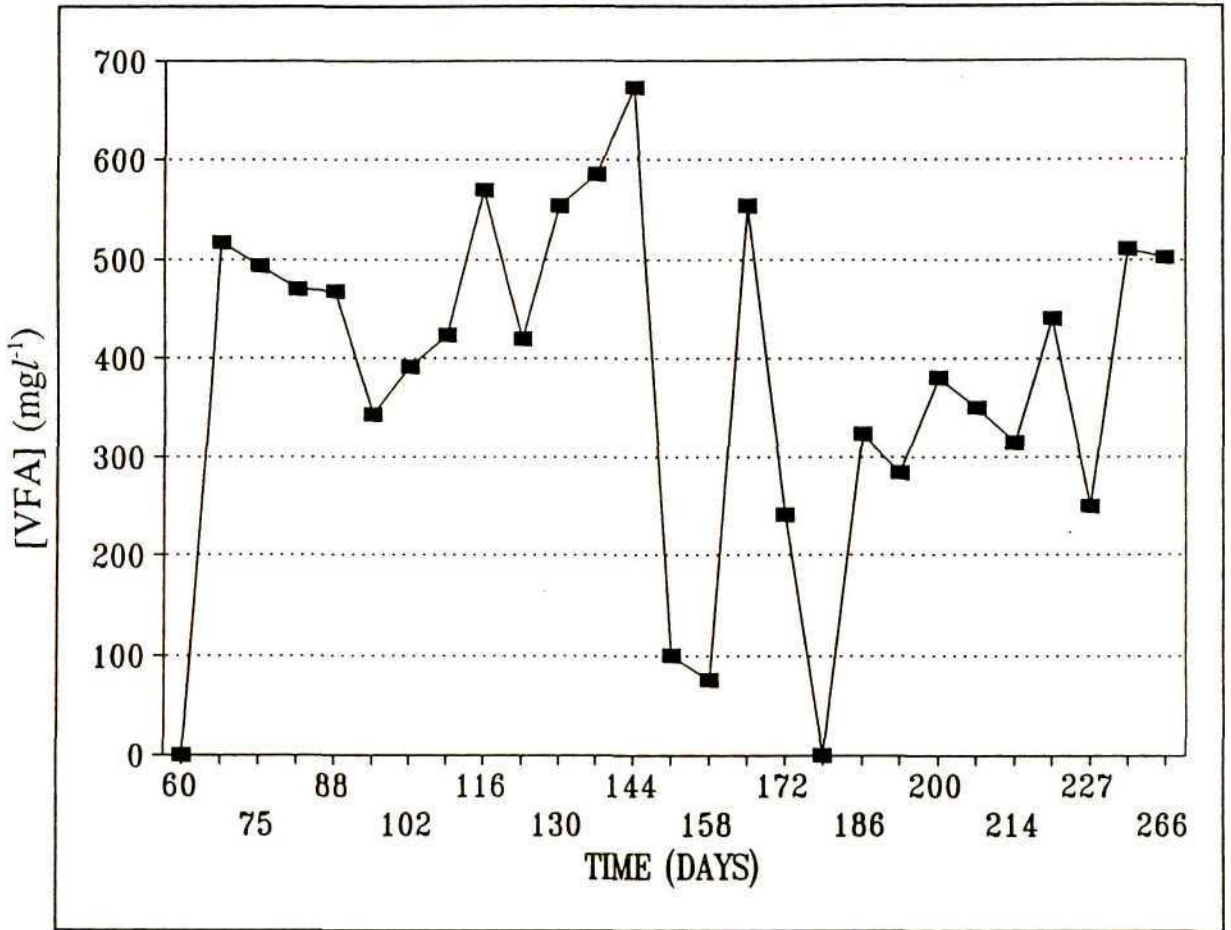
Figure 19: VFA as CH₃COOH (mg*l*⁻¹) concentrations of "Tank 1 outlet" supernatant during the 250 days study period.

Figure 20: Changes in bicarbonate alkalinity concentrations of supernatant sampled at the different ports of the Pennells bioreactor with time.
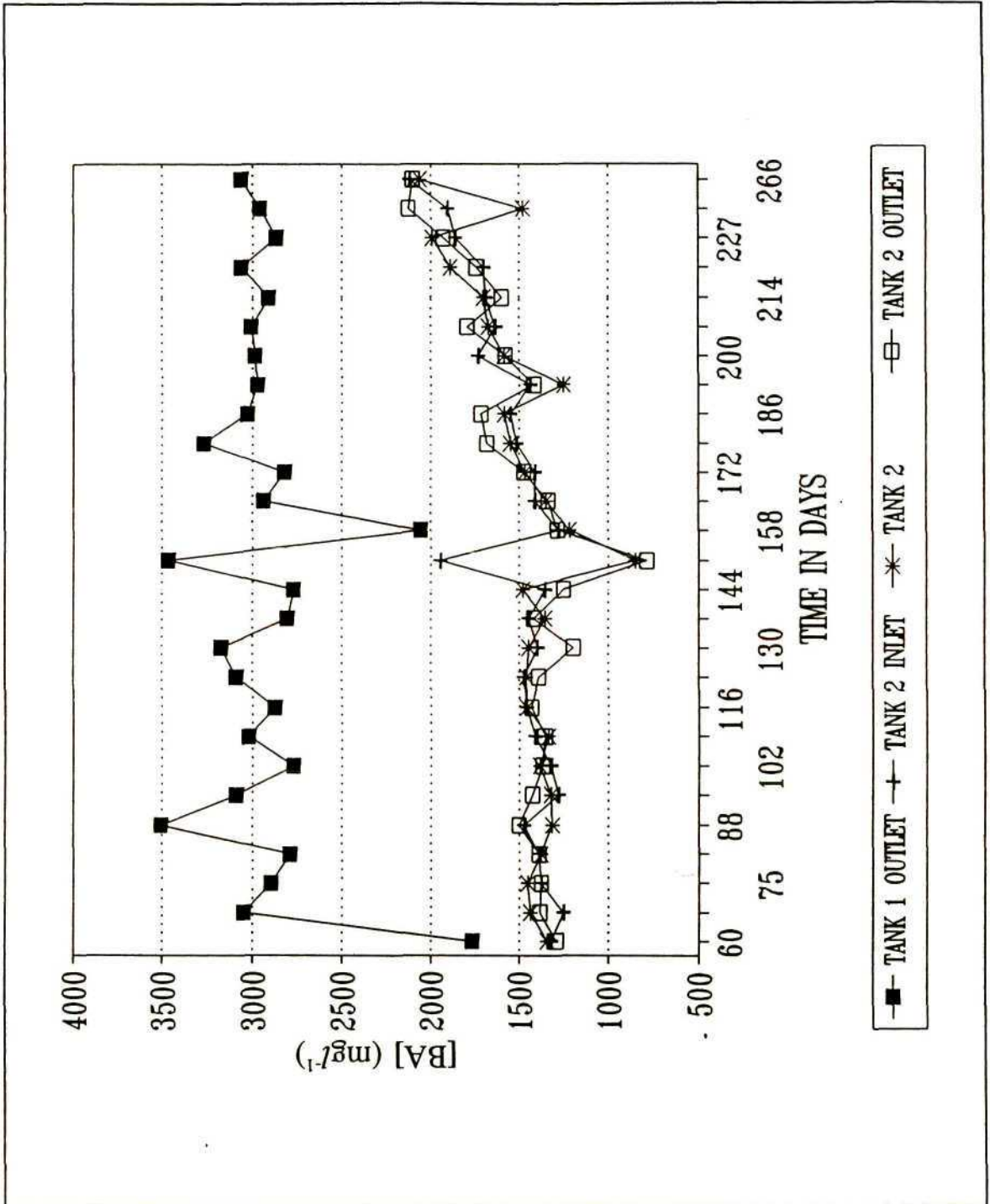
Typically, supernatant VFA concentrations in digesters can vary between 200 and 400mgl^{-1} (Meynell, 1982) while the equivalent range for sludge is between 50 and 300mgl^{-1} (Ross *et al.*, 1992). Rivett-Carnac (1982) documented that VFA concentrations >300mgl^{-1} may be inhibitory to biogas production. The VFA concentrations for the Pennells bioreactor were below optimum values indicating that insufficient VFAs were present, especially in

"Tank 2" (Figure 19). The "Tank 1 outlet" HAc concentrations were somewhat elevated while the equivalent concentrations for "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" were usually around 0. On day 75 and after day 193 concentration increases ($100mgl^{-1}$) were, however, apparent. The low HAc concentrations of Tank 2 correlated with high pH values.

During anaerobic treatment pH decreases may be mediated by acid concentration increases due to uncoupling of the acidogenic and methanogenic phases, sub-optimal pH_2 and the presence of toxins or inhibitory substances which affect the methanogens. An increase in the VFA concentration should increase the COD and reduce the gas production and methane content (Moosbrugger *et al.*, 1993) as noted in Tank 1. In contrast, in Tank 2 the pH was high, the HAc concentration was low and no gas was detected although COD removal did continue.

A substantial HAc concentration reduction was noted in "Tank 1 outlet" on day 158 and coincided with uncharacteristically high ammonia evolution.

Increased VFA concentrations (as with "Tank 1 outlet") become inhibitory (Ross *et al.*, 1992) and indicate an upset digester (United Nations Environment Programme, 1981). An accumulation of HAc, as noted in "Tank 1 outlet" samples, can often be caused by numerous factors including: a lack of methanogens to produce gas (United Nations Environment Programme, 1981; Meyer and Oellermann, 1994); the organic loading being too high; no fresh feed; and the presence of too much acid in the feed (United Nations Environment Programme, 1981). All the factors, except the last, applied to the bioreactor under study. Despite the slightly increased HAc concentration in Tank 1, the pH did not decrease substantially although the pH values of "Tank 1 outlet" supernatant samples were generally lower than "Tank 2" samples.

An unbalanced digester as indicated by VFA accumulation and/or inhibited gas production, can also be caused by other operational aspects such as under-loading, poor mixing or the presence of inhibitory/toxic material (Forster, 1985). Poor mixing was evident in the bioreactor under study (3.6) whilst toxic substances may have been added. Volatile fatty

acids in a balanced fermentation are mineralized at the same rate at which they are formed. With an organic overload, excess VFAs may decrease the alkalinity/buffering capacity (Ross and Louw, 1987) and pH, and, thus, lower gas production (Ross and Louw, 1987; Whitmore *et al.*, 1987; Ross *et al.*, 1992). In the Pennells bioreactor, however, instead of a reduced pH and concomitant reduced biogas production, there was an increased pH in addition to a lack of biogas production. In Tank 2 an organic overload was not the cause of the inhibition.

Recorded bicarbonate alkalinity concentrations were initially in the 1 000 to 2 000 mgl^{-1} range for "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" samples, while those for "Tank 1 outlet" were considerably higher. In general, digester total alkalinity concentrations reflect the nature of the influent, the mechanical design and the operation techniques (Schellenbach, 1981). Rivett-Carnac (1982) documented that a total alkalinity < 1 000mgl^{-1} was inhibitory to biogas production while a normal concentration was between 2 000 and 4 000mgl^{-1} (American Public Health Association, 1980). Laak (1986) recorded a septic tank alkalinity of approximately 400mgl^{-1} $CaCO_3$. The BA concentrations recorded in this study were only part of the total alkalinity (Ross *et al.*, 1992) but were generally higher than the values reported by Rivett-Carnac (1982) probably due to limited acid for neutralization (Ross *et al.*, 1992). The BA concentrations of "Tank 1 outlet" were generally higher than at the other sampling points and corresponded to elevated HAc concentrations. A probable explanation was the presence of NH_4^+ which adds alkalinity to the system (Ross and Louw, 1987). In the relationship between VFA concentration, alkalinity and pH, a BA deficit occurs at a pH < 6.8. Thus, a digester pH should be held within the range of 6.8 to 7.2 (Ross and Louw, 1987).

The VFA concentration is the best indicator of digester problems particularly when monitored in conjunction with pH and total alkalinity (Ross *et al.*, 1992).

4.2.7 VOLATILE FATTY ACIDS:TOTAL ALKALINITY RATIO

The VFA:total alkalinity ratio of feed sludge is a control indicator which should be routinely monitored to give a direct indication of the acidity/alkalinity balance. This

empirical relationship gives an adequate measure of the state of the anaerobic digestion process and can identify an impending upset. The total alkalinity is determined by titrating the supernatant from the prevailing digester pH to 4.3 with 0.5M HCl. By titrating to pH 4.3, the alkalinity of buffering substances in the liquor, with the exception of bicarbonate (obtained by titrating to 5.75), is estimated (Ross *et al.*, 1992). Volatile fatty acid and total alkalinity concentration changes are the first to result when the digestion process is perturbated (Ross *et al.*, 1992). As such, their determinations are an important aspect of digester operation and maintenance.

The Ripley ratio gives a measure of the state of the anaerobic digestion process. Since the concentrations of VFAs rise and the alkalinity falls when a problem is developing, the ratio will change faster than the individual values and thus warn of an impending upset. A low ratio indicates good operating conditions whilst an increasing ratio indicates an upset digester, due to the loss of buffering capacity within the reactor. Optimum digestion occurs when the ratio is <0.3 (Ross *et al.*, 1992).

The method employed to determine the VFA:alkalinity ratio (2.4.5) was a modified version than that used by Hoffmann (1986).

Figure 21 shows that the Pennells bioreactor operated somewhat erratically. Up to day 116 the fermentation was characterized by a poor acidity/alkalinity balance. Subsequently, the ratio for "Tank 2" stabilized around 0.2-0.3 until day 200 after which a loss of buffering capacity was apparent. The Ripley ratio values for "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" were slightly lower than those for "Tank 1 outlet" which emphasised differences between the two tanks. "Tank 1 outlet" values were generally >0.3 although an extremely high value of 0.76 was recorded on day 176. This coincided with a tank blockage. After day 200 Ripley ratio increases reflected VFA and BA concentration increases following cow manure supplementation (Figures 19 and 20).
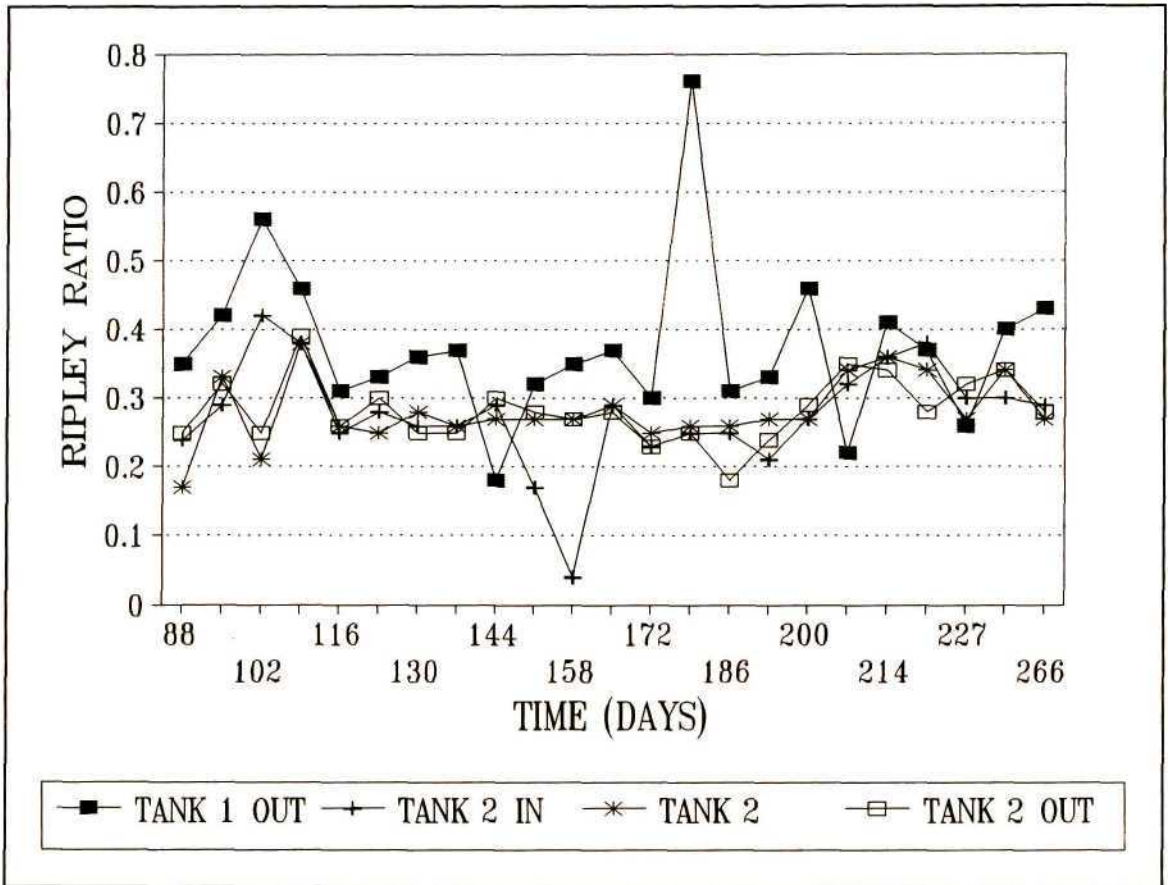
Figure 21: Changes in the Ripley ratio of samples taken from the different ports of the Pennells bioreactor with time.

A low Ripley ratio of 0.04 was recorded for "Tank 2 inlet" on day 158 which indicated that the digester was then probably functioning more optimally with good buffering capacity. At this point, an odour of ammonia was apparent.

Since alkalinity is determined by acid titration to a particular pH value, there is a direct correlation between the pH and the relative concentrations of acids and bases within the bioreactor. In essence, the alkalinity present during digestion is the summation of an internal titration and/or neutralization of the product acids and bases. High VFA concentration accumulations tend to decrease the alkalinity and depress the pH as the buffering capacity is exhausted (Pohland and Gosh, 1971). This was not apparent in the bioreactor under study.

It thus appeared that "stable" conditions were generally not obtained in the Pennells two-tank bioreactor particularly in Tank 1 where the VFA:alkalinity ratios were often above 0.3. Tank 2, however, more closely approximated to 0.3 which indicated more stable conditions. Unfortunately, high pH values were still recorded which indicated inherent problems.

4.3 CONCLUSION

The results of this study showed that the fermentations in the two tanks of the bioreactor varied.

No biogas was produced which was indicative of incomplete mineralization (Ross and Louw, 1987). At this stage it was unclear whether the prevalent conditions were bacteriostatic/bactericidal to the methanogens.

Tank 1 was frequently blocked as indicated by the high CODs which were, in part, also due to the low flush mode of operation. Both Tank 1 and Tank 2 recorded high pH values which were inhibitory to methanogenesis. A general lack of buffering prevailed in both tanks as evident by the VFA, BA and VFA:alkalinity ratio results. The HAc concentrations in Tank 1 were generally higher than those in Tank 2 but still below optimum. Operational investigation results of Tank 1 were generally more erratic than those for Tank 2 thus emphasising the differences in fermentations between the two tanks. It, thus, appeared that two tanks were better than one. In septic tanks an interior baffle is often used to divide the tank to limit the discharge of solids in the effluent from the bioreactor (Tchobanaglous and Burton, 1991).

The Pennells two-tank bioreactor was, thus, generally erratic in performance. Of significance was the fact that although biogas production was not evident, a COD removal resulted which suggested that the bioreactor functioned more as a septic tank than an anaerobic digester. The primary function of a septic tank is to condition the incoming raw sewage so that solids largely separate from the water (Drews, 1985). In this regard the Pennells bioreactor served this function. Septic tanks have specific disadvantages,

however, since they do not remove non-settleable solids (Laak and Crates, 1978) thus, essentially, being only a primary treatment system (Tchobanaglous and Burton, 1991) where the final effluent is unsuitable for open discharge (Laak and Crates, 1978). An artificial wetland could, however, obviate this problem.

Education, digester design and operation and socio-economic factors all play important roles in the operation of bioreactors (3.6) which, in turn, will effect efficiency and efficacy of such systems. In particular, a lack of total community involvement hampered operation of the bioreactor (3.6). This was evidenced by blockages. The lack of adequate maintenance (Wright, 1956) and non-involvement of the user community (Kalbermatten *et al.*, 1982; Muyiboi, 1992) will cause failure of such systems. The Pennells two-tank bioreactor despite its limitations did provide the community with a sanitation system and, as such, prevented inimical environment impacts of raw sewage. In this regard, the installation of the Pennells bioreactor, in essence as a septic tank, was a positive improvement. The lack of methane production, however, was unfortunate as the benefits of an alternative energy source were not realised. Future installation of such bioreactors, however, would require alterations and/or additions (3.6) to promote community acceptance and bioreactor functioning since blockage problems appear common with such systems (S. A. Water Research Commission, 1993b). Education and community involvement must be implemented (3.6) to ensure successful operation.

CHAPTER 5

INVESTIGATION OF THE EFFECTS OF KEY PERTURBANTS ON ANAEROBIC DIGESTION

5.1 INTRODUCTION

Various analytical criteria can be used to monitor digester performance (Chapter 4). Of these, gas production, particularly methanogenesis, is often used to assess digester efficiency (Degrémont, 1991) and to provide an immediate indication of the intensity of the digestion process (Mata-Alvarez, Mitz-Viturtia and Torres, 1986; Ross and Louw, 1987; Ross *et al.*, 1992). Two methods are often used to measure biogas production. The first determines the volume of biogas produced in relation to the mass of volatile suspended solids (VSS) passed through the digester and is termed the "bioefficiency" of the reactor (Pos *et al.*, 1985). The second monitors the volume of biogas produced in relation to the working volume of the digester and this is known as the "economic efficiency" of the digester (Pos *et al.*, 1985). The volume of gas produced kg^{-1} VSS destroyed appears to be the most often used and sensitive process control indicator of microbial activity and the progress of sludge digestion. It has been shown that, in general, $1m^3$ biogas is produced kg^{-1} VSS catabolized in 20 days at 35^0C (Ross *et al.*, 1992).

The carbon dioxide percentage (v/v) of the gas is often the first indication of whether the fermentation is unbalanced and the acidogens are more active than the methanogens (Schellenbach, 1981). This can indicate whether problems are developing within the digester such as during organic overload conditions (Ross *et al.*, 1992). A reduction in the ratio of methane to carbon dioxide often indicates a reduction in methanogenic activity and, possibly, impending process failure (Pos *et al.*, 1985). When starting up bioreactors of this type, the methane content of the produced biogas can vary. Initially, the carbon dioxide concentration is greater than the methane concentration although, with time, there should be an increase in the methane content (G.N. Tivchev, personal communication). Also, more methane can be produced in response to higher carbon dioxide concentrations or in carbon dioxide rich environments (Sato and Ochi, 1994). Further, the composition of the produced biogas and, thus, the volume of methane also depends on the composition

of the substrate, as well as the operational conditions of the bioreactor (Degrémont, 1991).

The experimental approach used in this study was adapted from Viitasalo (1981) and Ishihara *et al.* (1988). Total gas, methane production and methane percentage (%v/v) were the analytical criteria and the perturbations examined were heavy metals, antibiotics, various detergents and an antiseptic.

There are many toxic substances in wastewaters which can inhibit microorganisms (1.5). Inhibition can be either permanent or temporary. For example, initial suppression of methanogenesis by metals can be relieved by precipitation (Lettinga *et al.*, 1982; Capone, Reese and Krine, 1983), complexation or transformation of the metal (possibly by methylation) with subsequent promotion resulting from sustained inhibition of competing organisms (eg. sulphate-reducing bacteria) (Capone *et al.*, 1983). In addition, microorganisms can remove heavy metals from solution, firstly, by metabolism-dependent uptake into cells and, secondly, by binding metal ions to extracelluar material or the cell wall (Portier and Palmer, 1989).

Although heavy metal solutions such as $CuCl_2$, $Pb(NO_3)_2$ and $CdCl_2$ would not normally perturbate a Pennells bioreactor in a rural situation, they were chosen as model molecules to evaluate worst case scenarios. Together with direct perturbation, heavy metals can also be added indirectly to bioreactors through, for example, animal faeces (Loehr, 1977). Conventional animal feeds may contain traces of toxic elements which are derived from the environment as a result of mineral supplementation. The heavy metals lead, inorganic mercury and cadmium appear to be absorbed poorly by animals and, thus, the potential exists that they could be present in higher concentrations in wastes compared with conventional feed ingredients (Prior and Hashimoto, 1981).

The "detergents" tested in this study were: a bleaching agent which contained the active ingredient sodium hypochlorite; a household cleaner which contained active ammonium; and a soap bar which contained brine, water, acid oil, fat and caustic soda. These were chosen as they are used by the residents.

Penicillin and tetracycline were also examined. These antibiotics inhibit a wide range of microorganisms (Laskin, 1967; Blackwood and English, 1977; Russell and Quesnel, 1983) and are often prescribed by the local doctor. Antibiotics are also administered to animals and thus enter digesters via manure (Ortega, Rojas and Perez-Gil, 1984). Finally, an antiseptic, which contained chloroxylenol, was examined for its bacteriostatic activity.

5.2 EXPERIMENTAL

5.2.1 SYRINGE BATCH CULTURES

Sealed duplicate calibrated syringes (50ml), each containing a 20ml total working volume of sludge (Darvill) and test perturbant (Table 14), were incubated at 32°C in a Heraeus-D-6450 Hanau incubator. After 2 weeks incubation the sludge pH values were measured and gas volumes recorded.

5.2.2 PERTURBANTS

Table 14: Various perturbants tested for their inhibitory effects on biogas production from Darvill Sludge.

Heavy Metals and Final Concentrations (mgl^{-1})

$CuCl_2$	$Pb(NO_3)_2$	$CdCl_2$
0 (control)	0 (control)	0 (control)
25	25	25
50	50	50
100	100	100
150	150	150

"Detergents" and Final Concentrations (% v/v)

Bleach (Jik)	Household Cleaner (Handy Andy)	Soap Bar (Sunlight) *1	Antiseptic with Chloroxylenol (Dettol)
0 (control)	0 (control)	0 (control)	0 (control)
0.025	5	10	0.05
0.05	10	25	0.25
0.1	25	50	0.325
	50	75	5

Antibiotics and Final Concentrations (mgl^{-1})

Penicillin	Tetracycline*2
0 (Control)	0 (Control)
50 (x units)	15.62
100 ($2x$ units)	31.24
250 ($5x$ units)	39.65
500 ($10x$ units)	78.1

*1 43g bar dissolved in 1l water

*2 Standard solution prepared by dissolving 15.62mg in 50% (v/v) ethanol

5.3 RESULTS AND DISCUSSION

Laboratory batch culture studies provide useful information of bioreactor responses to perturbant molecules. In turn, these results could be used to compile a detailed Code of Practice for bioreactor operation in a specific community. The laboratory batch culture approach does, however, have some limitations. Principal among these are the constant optimum conditions applied which contrast the *in situ* situation where frequent changes in environmental conditions make interpretation of results difficult. Care must, therefore, be taken in extrapolating laboratory results (Parkes, 1982).

Digestion and the resultant production of biogas require diverse complex microbial populations with stable interactions between various metabolic groups critical for efficient anaerobic mineralization of organic wastes (Williams and Shih, 1989). Perturbation studies must, therefore, be made on balanced fermentations. It was for this reason that the "Darvill Sludge" was used in the study rather than the Pennells bioreactor sludge which was not characterized by a balanced methanogenic fermentation.

Another disadvantage of the batch culture approach adopted was the lack of mixing which in digesters has been reported to reduce the effects of toxic substances by promoting rapid dispersion and dilution (Ross *et al.*, 1992) (3.6). The Pennells bioreactor was also characterized by limited mixing.

The Pennells bioreactor was, essentially, operated in a fed-batch mode rather than as a batch culture as per the laboratory studies. In addition, the batch cultures lacked accuracy with gas volume analysis, therefore more elaborate systems may be required (Salkinoja-Salonen and Hughes, 1982) or the incubation time should have been increased.

Environmental factors influence the rate and efficacy of microbial destruction. Temperature, pH, time and organic material also influence the rate and efficiency of antimicrobial action. Organic matter can reduce the efficiency of the antimicrobial agents by inactivating them or protecting the microorganisms. The physiological state of the bacterial cells may also influence their susceptibility to antimicrobial agents. For example, agents which interfere with metabolism are more effective against young actively metabolizing cells (Pelczar *et al.*, 1986; Degrémont, 1991). It is, however, possible that bacteria can acclimatize to the presence of toxins/inhibitors and thus lower the toxicity thresholds (Degrémont, 1991).

5.3.1 HEAVY METALS

Heavy metals, especially copper, chromium, nickel, zinc, mercury and lead, play significant roles in anaerobic digestion inhibition and toxicity since most heavy metals, in sufficient concentrations, are toxic to bacteria (Ahring and Westermann, 1983). The

antimicrobial action of heavy metals results from: the metal combining with, and the inactivation of, cellular proteins (Pelczar and Reid, 1965; Pelczar *et al.*, 1986); attraction and blockage of certain enzymatic sites; enzyme denaturation; and cellular membrane permeability change (Degrémont, 1991; Tchobanoglous and Bruton, 1991). High concentrations of salts of heavy metals such as mercury, copper and silver coagulate cytoplasmic proteins and result in damage or death of the cell. Salts of heavy metals are also precipitants and, in high concentrations, could cause cell death (Pelczar *et al.*, 1986). Heavy metal toxicity depends, to a large extent, upon the various chemical forms which the metals may assume under the anaerobic, temperature and pH conditions of the digester (Ahring and Westermann, 1983). Toxicity is also determined by the degree of attraction to natural metal binding sites on and within the cell. This, in turn, is affected by environmental and nutritional factors as cells in nutrient depleted environments are often more susceptible (Portier and Palmer, 1989) while precipitated heavy metals show little toxicity to biological systems (Ahring and Westermann, 1983). There are four responses of digesters to heavy metals, namely, no response, inhibition, failure and inhibition and recovery (Alkan, Anderson and Ince, 1996).

COPPER CHLORIDE (CuCl₂)

From Figure 22 it can be seen that even low concentrations of copper inhibited biogas generation. These results were, thus, in agreement with those of Jarrell *et al.*(1987), who found strains of methanogens sensitive to concentrations of copper in the range of 1-5 mgl^{-1}, and the United Nations Environmental Programme (1981) and Tchobanoglous and Bruton (1991), who documented that 100 mgl^{-1} copper was inhibitory to microorganisms. For divalent metals such as Cu, Cd and Zn, the toxicity of free metal ions is thought to be > than that of complexes (Hunt and Wilson, 1986). Copper could, possibly, be added to rural bioreactors via the manures of animals raised on nutrient-supplemented feeds. Copper also occurs in natural waters, wastewaters and industrial effluents as soluble copper salts or as precipitated copper compounds on suspended solids (Hach Water Analysis Handbook, 1983). In addition, biological degradation of certain organic compounds, such as cyanide and humic acids, may cause the release of toxic metals from soluble complexes (Tchobanoglous and Bruton, 1991).
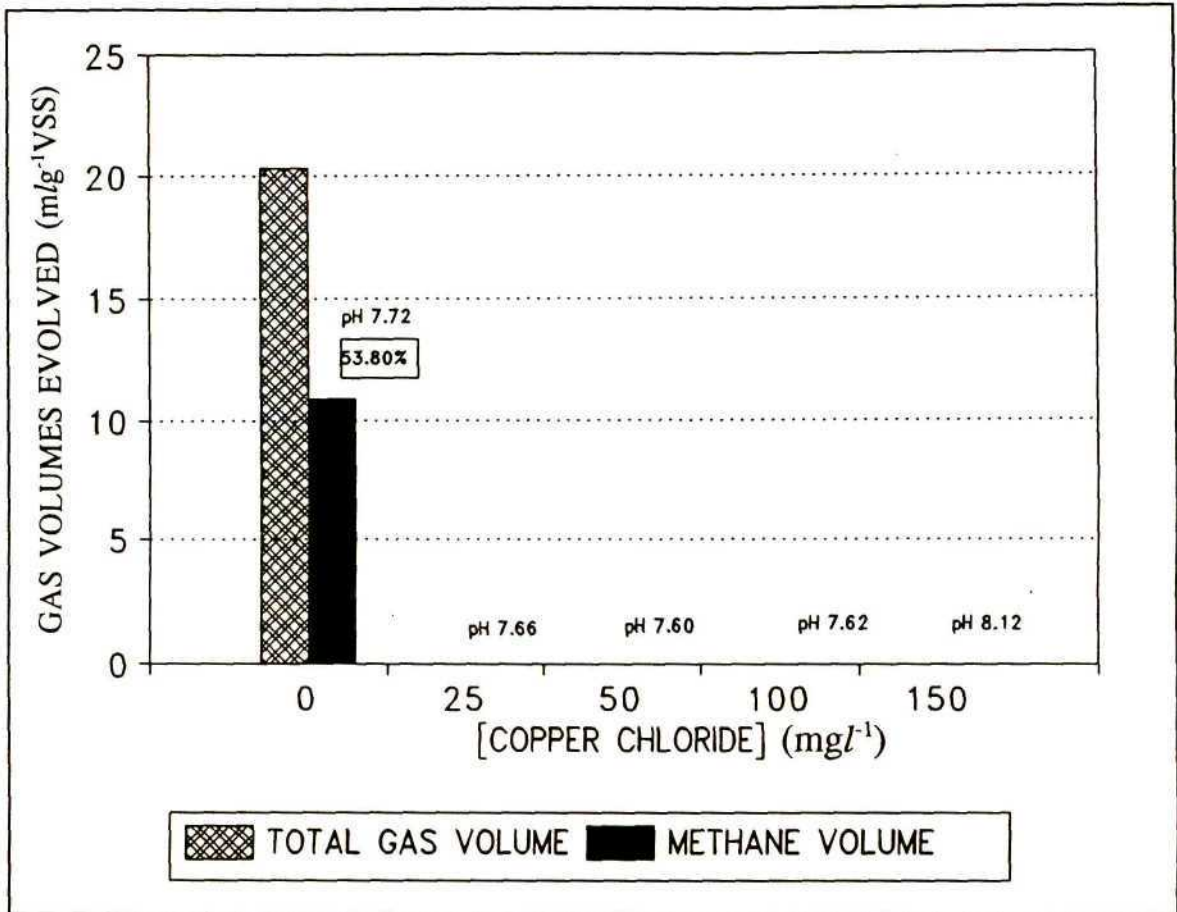
Figure 22: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant copper chloride.

Note: The number recorded in the box is the percentage of biogas methane.

LEAD NITRATE (Pb(NO₃)₂)

Perhaps surprisingly, the presence of lead nitrate, particularly in low concentrations, promoted the generation of biogas (Figure 23) possibly due to nitrate reduction lowering the redox potential to a level favourable for methanogenesis. Concentrations of lead nitrate $>50mgl^{-1}$ did, however, effect fermentation balance changes to the detriment of methane.
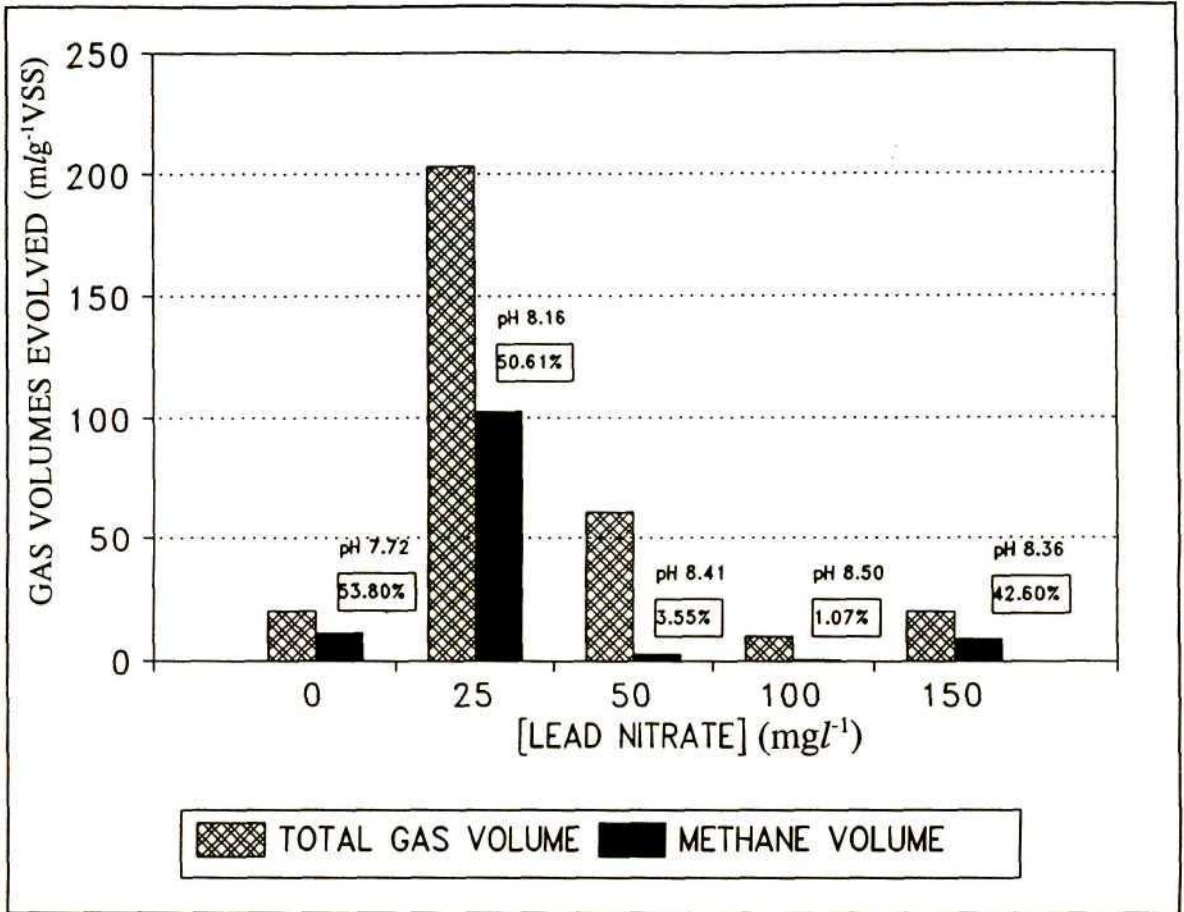
Figure 23: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the
presence of perturbant lead nitrate.

Note: The numbers recorded in the boxes are the percentages of biogas methane.

With $50 mgl^{-1}$ $Pb(NO_3)_2$ although biogas production was promoted, the methane volume fell compared with the control. A further decrease in methane volume was effected by 100 mgl^{-1} $Pb(NO_3)_2$ (Figure 23) and could, in part, have also been due to the elevated pH (8.5) which is known to increase toxicity (Portier and Palmer, 1989). Although not a factor in this study, it is documented that heavy metals present as precipitates may be solubilized by a change in pH causing a decrease in biological process efficiency (Tchobanoglous and Bruton, 1991).

Although the syringes were only monitored for two weeks it is possible that with time the methanogenic cultures could have acclimated to the presence of lead (Capone *et al.*, 1983). Acclimation to a metal such as lead may entail a variety of processes such as enzyme

induction, tolerance development, changes in metabolism and even methylation (Ahring and Westermann, 1983). In addition, the degree of inhibition can vary according to the type of compounds (organic and inorganic) present and whether the compounds are present individually or in combination with other substances (Fedler and Day, 1985). Lead also has a tendency to be precipitated by a large number of substances (Hach Water Analysis Handbook, 1983).

Despite acclimation, however, the denitrifying bacteria should have out-competed the methanogens for hydrogen and methanogensis should have been depressed even if none of the concentrations of lead nitrate was toxic.

From the results of this study it was apparent that concentrations of Pb (as $Pb(NO_3)_2$) $>25mgl^{-1}$ were inhibitory to the methanogens.

CADMIUM CHLORIDE ($CdCl_2$)

Reduced gas and methane evolutions were effected by the four concentrations of cadmium examined (Figure 24). The pH value recorded for the $25mgl^{-1}$ perturbated culture was high (9.10) and this could have lowered the bacteriostatic/bactericidal activity of the heavy metal through possible precipitation which is promoted by high/low pH regimes (Portier and Palmer, 1989).

Cadmium is a concern with land application of wastewater sludges as it can accumulate in plants to concentrations which are toxic to humans and animals (Tchobanoglous and Bruton, 1991).

In rural bioreactors, a possible source of cadmium could be from added plant matter or, more likely, via nickel/cadmium batteries disposed in the septic tank. The presence of cadmium in water can also be due to deterioration of galvanized plumbing (Hach Water Analysis Handbook, 1983).

Figure 24: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant cadmium chloride.

Note: The numbers recorded in the boxes are the percentages of biogas methane.

5.3.2 "DETERGENTS"

BLEACH (JIC)

Figure 25 shows that the active ingredient of the bleaching agent used, sodium hypochlorite, was inhibitory to the methanogens once the concentration exceeded 0.025% (v/v).
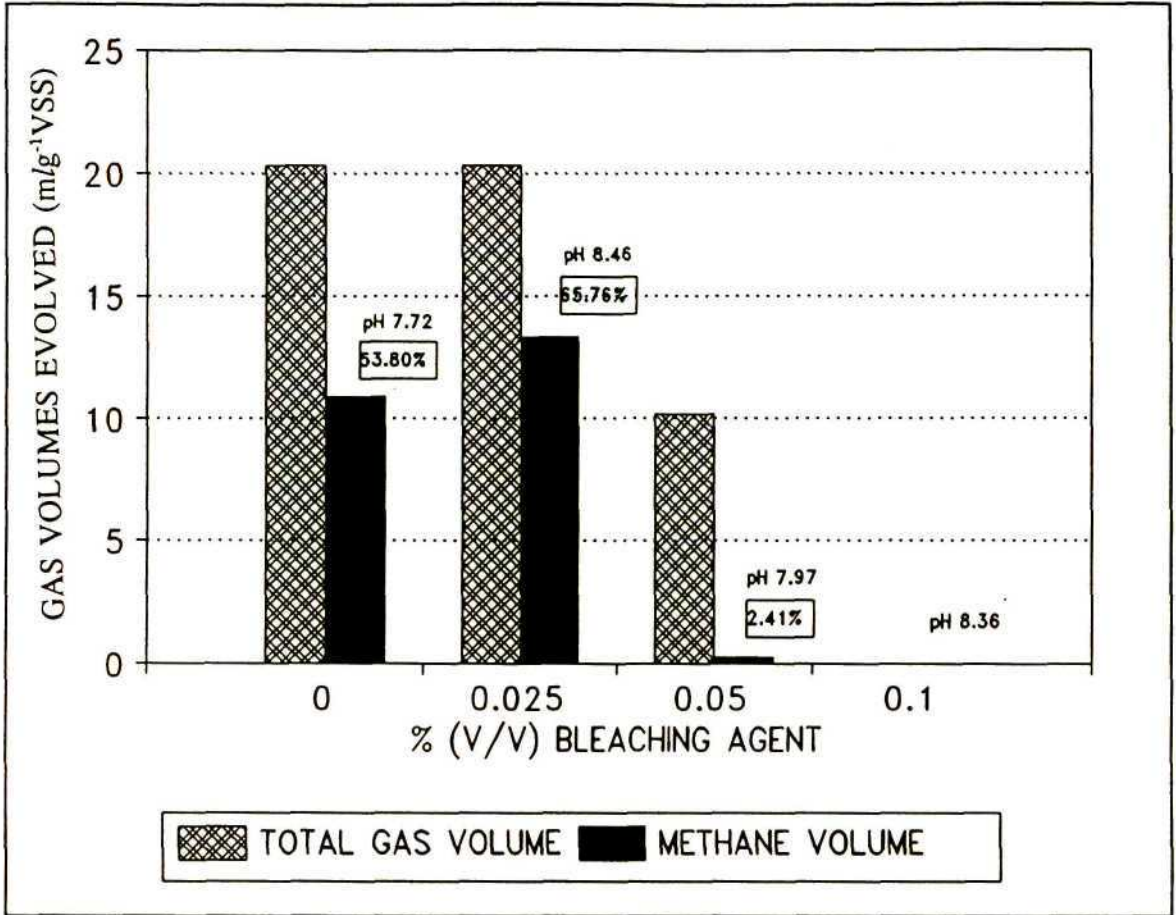
Figure 25: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the
presence of perturbant bleach.

Note: The numbers recorded in the boxes are the percentages of biogas methane.

With higher concentrations (1.25, 2.5, 5, 10, 20, 30, 40 and 50% v/v) of the bleaching
agent, which were accompanied by elevated pH values, in addition to biogas production
inhibition, the sludge became floccular, cream coloured and foamed probably due to
oxidation. Methanogens are sensitive to detergents (Meynell, 1982). Cohen (1991) found
that bleaching agents which contained H_2O_2 inhibited methanogens while reversible gas
production was apparent with sodium metabisulphite which did not adversely effect
methanogenesis in the long term. The resistance of some methanogens, particularly Group
III methanogens, to detergents has been attributed to the presence of cell wall proteins
which resist solubilization (Stanier, Ingraham, Wheelis and Painter, 1987).

HOUSEHOLD CLEANER (HANDY ANDY)

From Figure 26 it can be seen that all concentrations of the household cleaner used proved inhibitory to biogas production. Together with this inhibition, the sludge changed to white and floccose. After two weeks exposure to 25 and 50% (v/v) household cleaner, the sludge had a yellow tinge and settled to form a thin layer which possibly indicated sludge destruction. The household cleaner also had an effect on the sludge pH which increased as the perturbant concentration was increased (Figure 26). For example, the pH increased from an initial value of 7.16 to final pH values of 9.45 and 10.12 with the addition of 25 and 50% (v/v) household cleaner, respectively probably due to the presence of ammonium.
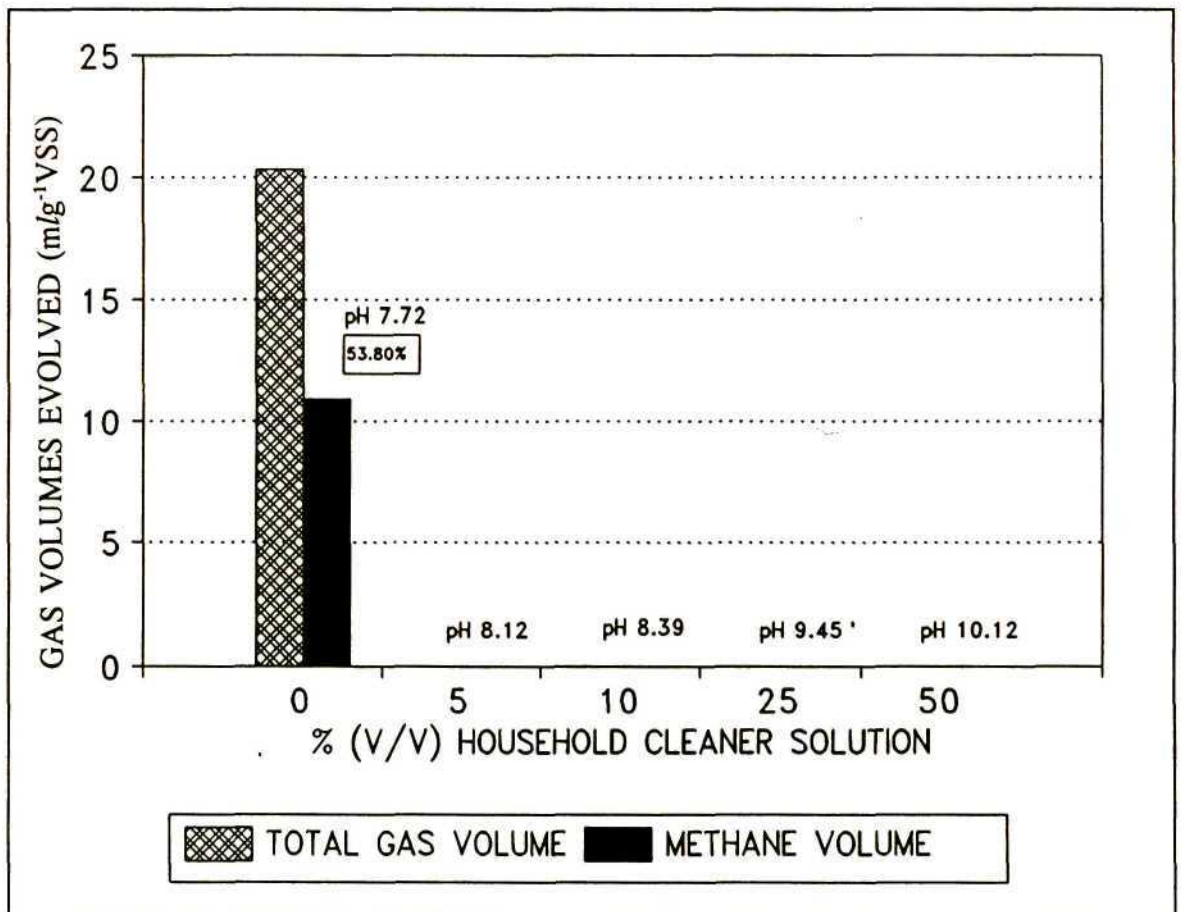
Figure 26: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant household cleaner (with ammonium).

Note: The number recorded in the box is the percentage of biogas methane.

Although methanogens utilize ammonium as a nitrogen source (Jones, Nagle and Whitman, 1987; Hajarnis and Ranade, 1994) high concentrations can be inhibitory (De Baere, Devocht, Van Assche and Verstraete, 1984). Different methanogen strains show variations in tolerance to ammonium (Hobson and Shaw, 1976; Jarrell *et al.*, 1987) due to differences in the pseudomurein layer of the cell wall (Jones *et al.*, 1987). Sprott and Patel (1986) reported that excess ammonium causes inhibition at Ca^{2+} and Mg^{2+} sites on the external face of the cytoplasmic membrane, with eventual loss of methanogenic activity. Depending on the initial ammonium concentration, a large biomass of inhibited cells should recover gradually when transferred to a medium which contains the optimum concentration of ammonium due to the efflux of excess accumulated ammonium at the K^+/H^+ antiporter sites (Hajarnis and Ranade, 1994).

SOAP SOLUTION (SUNLIGHT SOAP)

In the presence of soap solution, with caustic soda as the main active ingredient, methanogenesis was inhibited (Figure 27). Although methane production was inhibited by 10% (v/v) soap solution, a substantial increase in biogas generation was evident in comparison with the control. This could possibly be explained by the selective toxicity of the perturbant. With higher soap solution concentrations, however, all biogas production was inhibited. The inhibitory effect of the soap solution was possibly pH mediated. Under highly alkaline conditions, some microbial cell components may be hydrolysed or enzymes may be denatured. The pH of the environment affects the microorganisms and microbial enzymes directly and also influences the dissociation of many molecules which indirectly influence the microorganisms. The pH determines the solubility of CO_2, the availability of essential nutrients, such as ammonium and phosphate, and the mobility of toxic heavy metals such as copper (Atlas and Bartha, 1987).
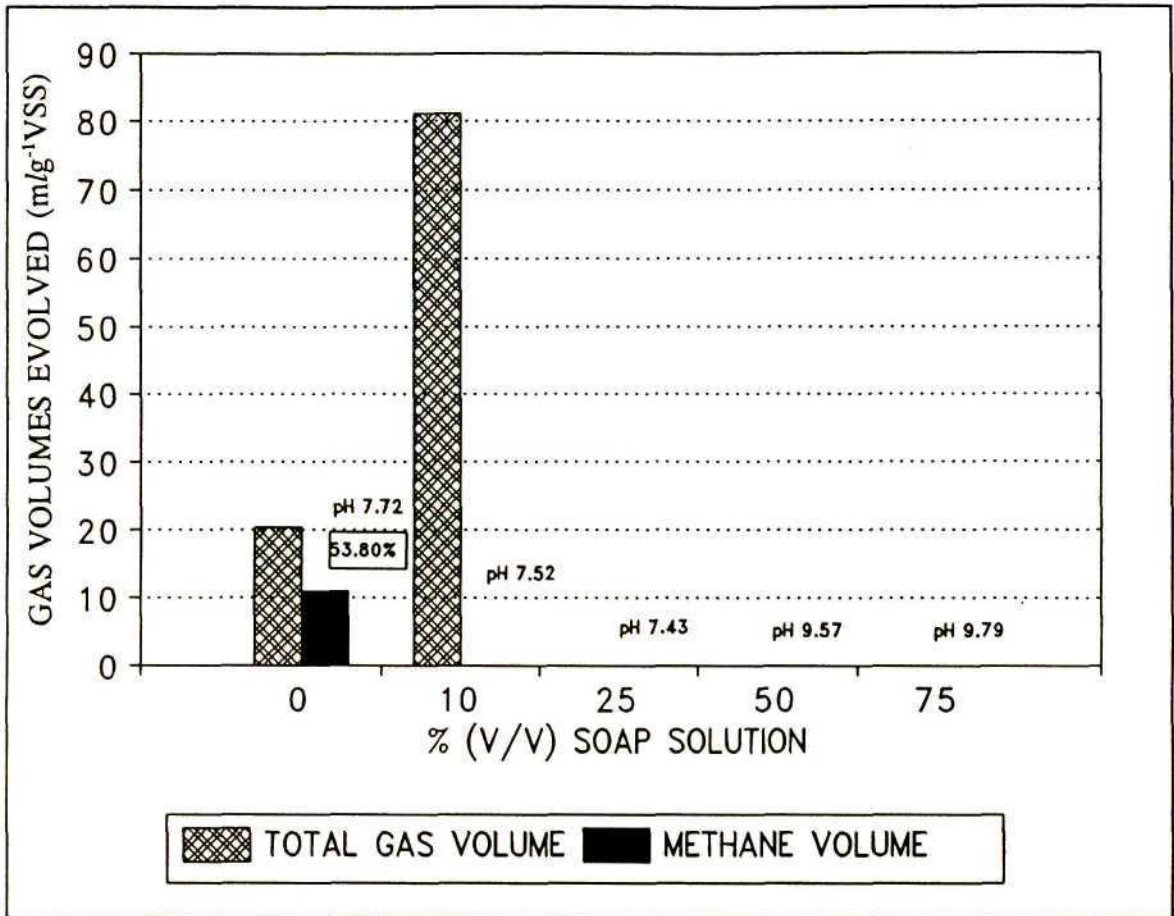
Figure 27: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant soap solution.

Note: The number recorded in the box is the percentage of biogas methane.

5.3.3 ANTISEPTIC (DETTOL)

The antiseptic, which contained chloroxylenol, even in low concentrations proved bacteriostatic/bactericidal to the microorganisms (Figure 28) and the elevated pH (8.17) was a possible causal factor.
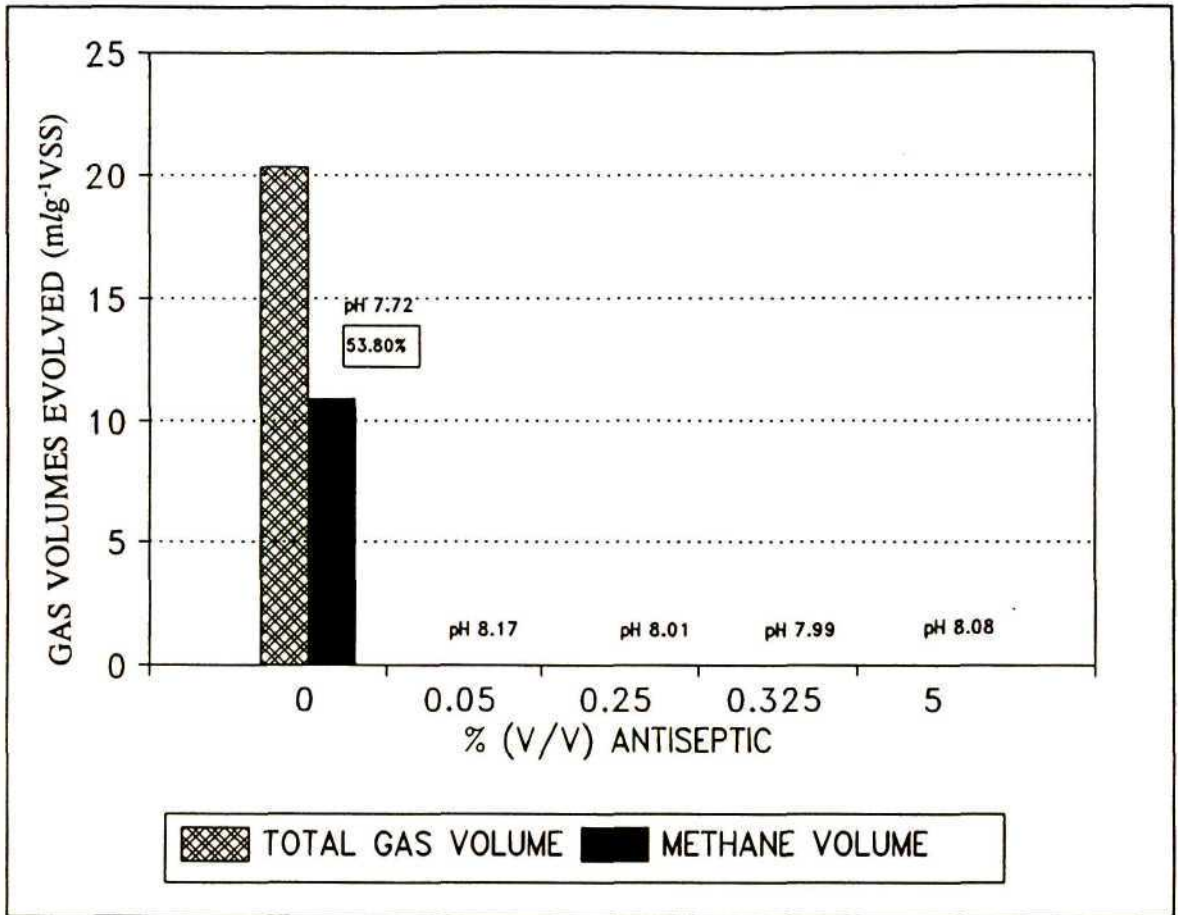
Figure 28: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant antiseptic.

Note: The number recorded in the box is the percentage of biogas methane.

In the presence of 5% (v/v) antiseptic the sludge became white and floccular. In addition, the elevated pH could have inhibited methanogenesis.

Chloroxylenol has a high degree of interaction with non-ionic surfactants such as the preservative cetomacrogol 1000 where bactericidal activity is slightly greater than with the same amount of compound alone (Dempsey, 1988). The inhibitory action of chloroxylenol is enhanced by certain compounds (e.g. ethylenediaminetetraacetic acid) through chelation of essential bridging cations and consequent destabilization of the outer membrane barrier (Denyer and King, 1988).

5.3.4 ANTIBIOTICS

PENICILLIN

From Figure 29 it can be seen that penicillin concentrations $>50mgl^{-1}$ proved inhibitory to biogas generation.
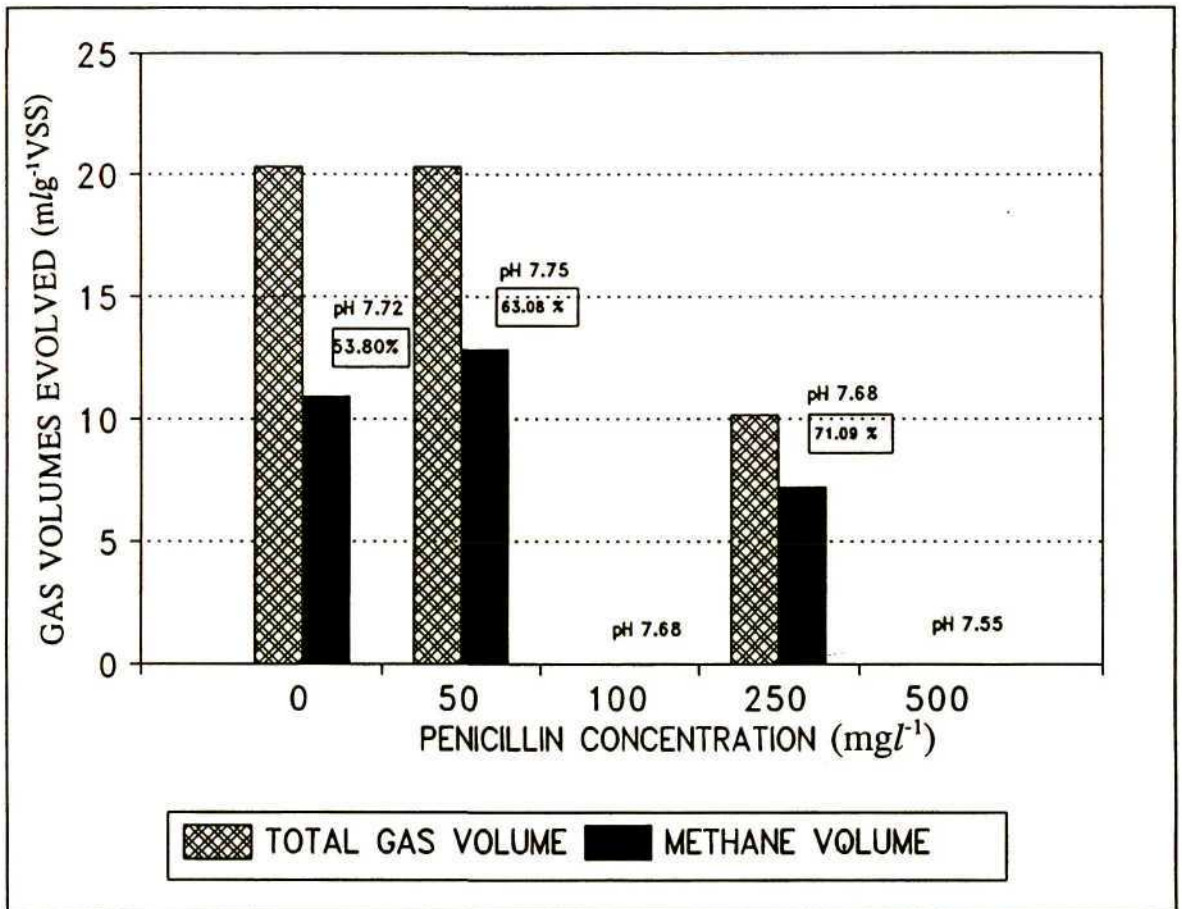
Figure 29: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant penicillin.

Note: The numbers recorded in the boxes are the percentages of biogas methane.

In the presence of $50mgl^{-1}$ penicillin methanogenesis was stimulated. The change in fermentation balance recorded was possibly due to selective inhibition. The phenomenon of selective inhibition was reported by Watson-Craik and Senior (1989) who found enhanced methanogenesis in the presence of inhibited sulphate reduction by perturbated

phenol. Similarly, Daneel, Percival and Senior (1997) found selective inhibition of nitrate-reducers by liquid petroleum gas perturbation. A structured bioassay test is thus needed to distinguish perturbation effects on specific physiological groups (Daneel *et al.*, 1997) particularly with respect to interspecies hydrogen transfer which plays a pivotal role in regulating fermentation balances (Large, 1983; Aragno, 1988).

Penicillin inhibits synthesis of the bacterial cell wall component peptidoglycan in Gram positive bacteria. Since the Archaebacteria (Õmura, 1981; Russell and Quesnel, 1983; Stanier *et al.*, 1987) have a different cell wall composition this could confer resistance. Penicillin, as an antibiotic, is only effective during active microbial growth (Flynn and Godzeski, 1967). Methanogens obtain energy for growth by oxidation of compounds such as H_2 or formate and utilize the electrons generated to reduce CO_2 with the formation of methane (Pelczar *et al.*, 1986; Atlas and Bartha, 1987). In so doing they make conditions favourable for the growth of the hydrogen-producing acetogenic bacteria (Vriens *et al.*, 1990). Thus, the test perturbant could have had no effect on the methanogens but inhibited the acetogens which provided key metabolic precursors for methanongenesis.

Environmental factors influence the rate, as well as the efficiency, of microbial destruction and these will be considered in greater detail later (5.4). Although unlikely, resistance to penicillin concentrations $<50mgl^{-1}$ could have been effected by the production of penicillinases which convert penicillin to inactive penicilloic acid or 6-amino-penicillanic acid plus a side chain (Pelczar and Reid, 1965; Pelczar *et al.*, 1986). Secondly, if the culture medium had had a high osmotic pressure, sphaeroplasts may have been produced. Finally, non-lethal penicillin concentrations could have facilitated the accumulation of compounds which are peptidoglycan precursors (Pelzcar *et al.*, 1986).

Although acidic pH values can promote the bactericidal effect of penicillin (Williams and Drasar, 1979), pH was unlikely to have played a role in this study since the values ranged between 7.55 and 7.68.

If penicillin is prescribed to a member of the community as a general antibiotic, it would also be introduced into the bioreactor in a diluted form as the human body breaks down

and absorbs most of the antibiotic. Generally, 250 mgl^{-1} penicillin is prescribed of which approximately 3% is excreted in the urine. In contrast, when animal manure is used as a substrate for biogas production, drugs used for antimicrobial treatment are not usually detected in the slurry as they are rapidly metabolised by the animal and excreted after inactivation. As such, they should not adversely affect biogas production (Strauch and Winterholder, 1985). However, antibiotic absorption by animals is sometimes incomplete and at least a portion of the amount ingested is excreted (Ortega *et al.*, 1984).

TETRACYCLINE

Antibiotics which limit methane production include bacitracin, linomycin, oxytetracycline, virginromycin and chlortetracycline (Fedler and Day, 1985). Tetracycline was chosen for this study since it is a readily available broad spectrum antibiotic and is often prescribed. Chlortetracycline and tetracycline are both tetracyclines, with chlortetracycline containing an additional chlorine derivative (Laskin, 1967; Blackwood and English, 1977; Russell and Quesnel, 1983). Chlortetracycline is more active than tetracycline against *Streptococcus*, *Staphylococcus* and *Pneumococcus* spp. (Laskin, 1967).

Tetracycline final concentrations < 39.65mgl^{-1} stimulated gas production (Figure 30). This could be explained by the fact that the antibiotic *per se* possibly served as a substrate. Higher gas volumes in response to perturbations have been documented by Alkan *et al.* (1996). Alternatively, the tetracycline selectively inhibited the species competing with methanogens for common electron donors.
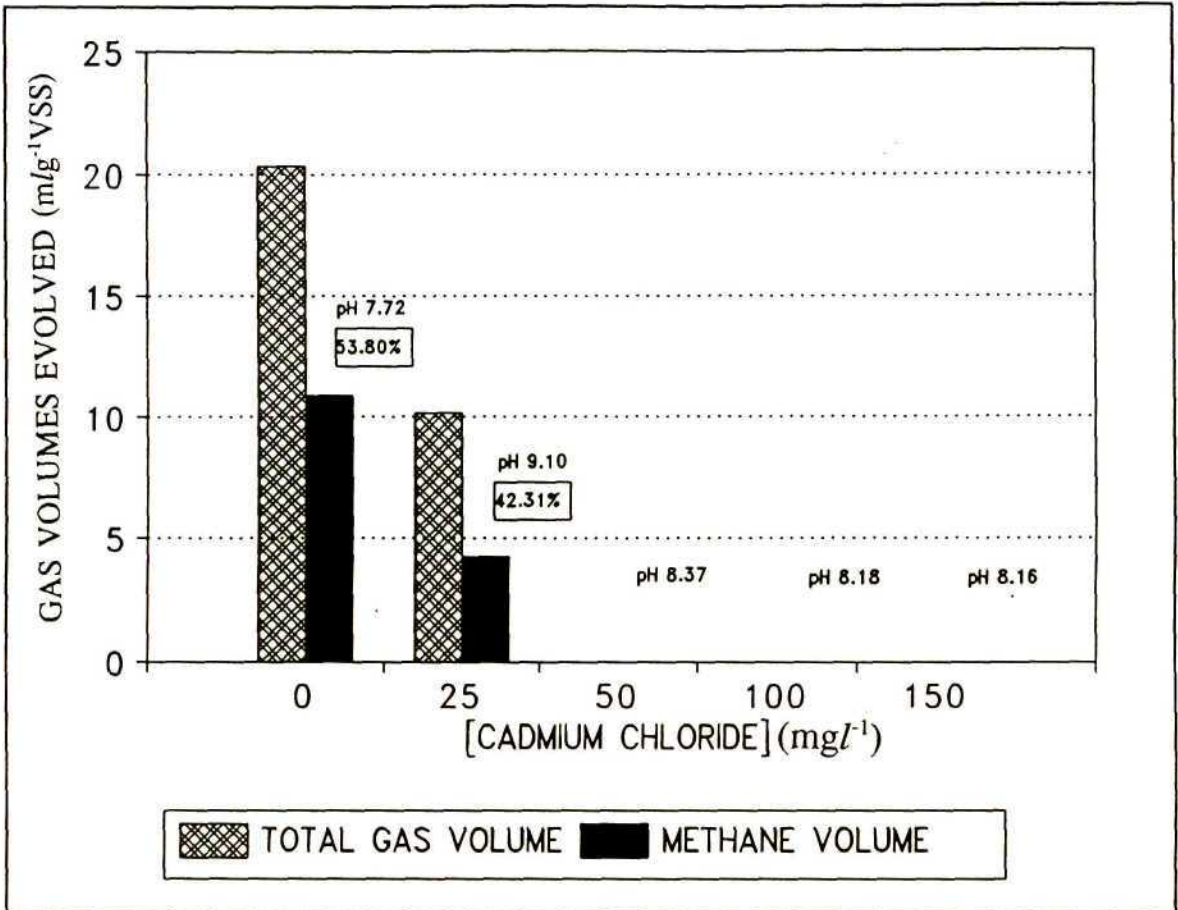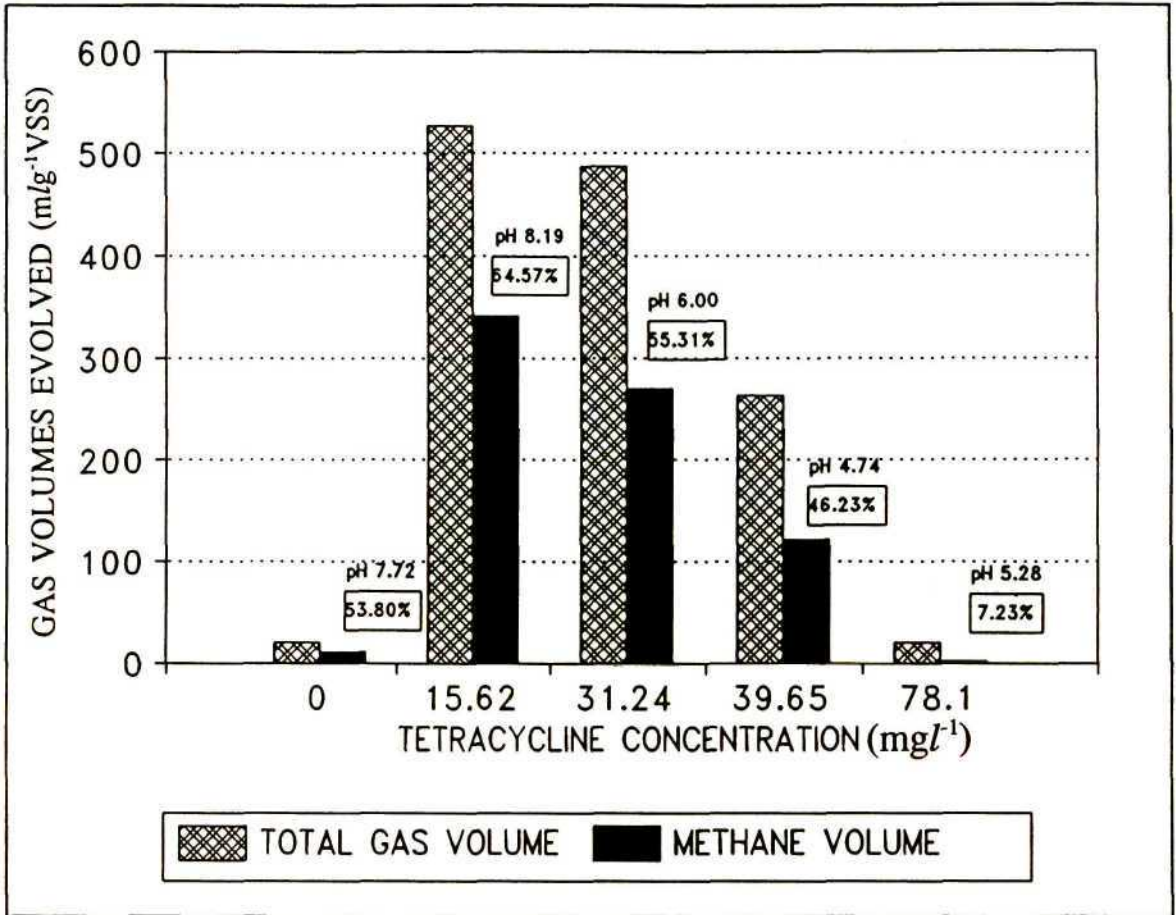
Figure 30: Gas evolved by Darvill sludge during 2 weeks incubation at 32°C in the presence of perturbant tetracycline.

Note: The numbers recorded in the boxes are the percentages of biogas methane.

Tetracycline inhibits protein synthesis (Laskin, 1967; Ōmura, 1981; Russell and Quesnel, 1983) and acts at the level of the 30S ribosomal unit (Russell and Quesnel, 1983). The tetracycline group of antibiotics also inhibits translation in both prokaryotic and eukaryotic systems (Vázquez, 1981). Alternatively, tetracycline can inhibit binding of aminoacyl tRNAs to ribosomes (Pelczar et al., 1986; Stanier et al., 1987). Methanogens, as Archaebacteria, lack ribothymine in tRNA and use methionyl tRNA (Stanier et al., 1987). Tetracycline is bacteriostatic in action (Pelczar et al., 1986). The principal mechanisms of antibiotic resistance include (Pelczar et al., 1986):

a) Competitive inhibition between essential metabolites and a metabolic analogue (drug);

b) Development of an alternative metabolic pathway which bypasses some reaction that would normally be inhibited;

c) Production of an enzyme altered in such a way that it functions on behalf of the cell but is not affected by the drug;

d) Synthesis of excess enzymes over the amount that can be inactivated by the antibiotic or drug;

e) Inability of the drug to penetrate the cell due to some alteration of the cell membrane; and

f) Alteration of ribosomal protein structure.

In addition, the nature of the environment influences antibiotic efficiency.

Although all the concentrations of antibiotic examined promoted biogas generation the highest promotion was effected by 15.62 mgl^{-1} while the results with 78.1mgl^{-1} were comparable with the control. At this highest concentration, however, methanogenesis was inhibited compared with the control. With the exception of 15.62mgl^{-1} tetracycline the antibiotic effected significant pH changes. Nevertheless, biogas generation was promoted despite the depressed pH values.

It was concluded that if the residents were prescribed the antibiotic tetracycline, it should not adversely effect the microbial population of the bioreactor and may promote biogas generation. Also, it is expected that the antibiotic concentrations would be significantly diluted in the bioreactor. As with penicillin, however, approximately 3% of the antibiotic is generally excreted.

5.4 CONCLUSION

The community habits (including disposal of substances into the bioreactor) influence the wastewater composition. It is documented that living and eating habits of communities have an effect on the sanitation system as previously discussed (3.6). The detergents tested, all of which are used by the community, were found to be toxic to the methanogens, even in low concentrations. The household cleaner with active ammonium, which is frequently used by the residents, inhibited methanogenesis by increasing the pH and destroying the sludge. Likewise, the soap, which is regularly used by the resident community for washing dishes and clothing, could, particularly in high concentrations, be

inhibitory. The antiseptic, which contained chloroxylenol, was inhibitory to the microorganisms even in low concentrations and should not, therefore, be allowed to enter the bioreactor. Unfortunately, empty antiseptic and detergent bottles were often found near the Pennells bioreactor.

From the investigation of perturbation effects it was evident that a range of locally used substances were inhibitory to methanogenesis and, thus, the overall functioning and efficacy of the bioreactor. A preliminary Code Of Practice must, therefore, be developed to identify to the community which products may/may not be disposed in the bioreactor. This information should be reinforced by pictorial demonstrations (3.6). In particular, the Code Of Practice should be specific to the specific community.

From this study it was evident that selective bactericidal/bacteriostatic inhibition of specific metabolic groups of bacteria could have occurred. This emphasised the need for an anaerobic bioassay test to assess the impacts of specific perturbants on anaerobic digestion which has been highlighted by a number of authors (Blum and Speece, 1991; United Kingdom Department of the Environment, 1994). Current tests based on rates and product concentrations of acidogenesis and methanogenesis are somewhat limited (Battersby and Wilson, 1989; Azhar and Stuckey, 1994). The general use of methanogens has, however, been justified on the basis of their sensitivity (Jarrel *et al.*, 1987). Increasing evidence shows that other key members of interacting associations may be more sensitive to specific perturbants than methanogens and thus different physiological groups should be used for assay purposes. Daneel *et al.* (1997), for example, emphasised the need for a structured anaerobic bioassay which differentiated the effects of perturbants on specific physiological groups of interacting microbial associations.

CHAPTER 6

MICROBIAL CHARACTERIZATION BY LIGHT AND SCANNING ELECTRON MICROSCOPY OF DIGESTER GRANULES AND SLUDGES AND SPECIES COMPLEMENT CHANGES FOLLOWING INOCULATION

6.1 INTRODUCTION

Microscopy (light and scanning electron) analyses of various sludges were made to determine the morphologies of the constituent species and monitor changes in the microbial populations. Both microscopy types were thus employed to characterize: Upflow Anaerobic Sludge Blanket (UASB) digester granules; "Darvill Sludge"; Pennells bioreactor sludge; and Durban Wastewater Purification Works Sewage sludge and monitor species complement changes following inoculation of laboratory continuous cultures and the Pennells bioreactor. Microscope examination included bright field (of Gram stained cultures), interference phase, fluorescence and scanning electron.

With interference phase microscopy an increase in the contrast of cells or intracellular structures that differ slightly in refractive index from their surroundings is obtained (Stanier *et al.*, 1987).

Fluorescence microscopy can be used to: determine the methanogen morphotypes which autofluoresce as a result of coenzyme F_{420}; detect and quantify methanogens in microbial associations; and determine methanogen population segregation and changes within bacterial associations such as those present in UASB digester granules (Taylor and Howgrave-Graham, 1993). Coenzyme F_{420}, a 5-deazaflavin analogue, plays a key metabolic role in methanogenic bacteria as it acts as an electron carrier in both anabolic and catabolic redox reactions (Dolfing and Mulder, 1985; Heine-Dobbernack, Schobeth and Sahm, 1988). In its oxidized state F_{420} fluoresces blue-green and, thus, may be used to indicate the presence (Heine-Dobbernack *et al.*, 1988) but not the activity (Dolfing and Mulder, 1985) of methanogens. Methanogens are the only fluorescent bacteria found in anaerobic methanogenic environments (Heine-Dobbernack *et al.*, 1988). For this reason, fluorescence microscopy is a useful analytical tool to determine the presence of

methanogens within bioreactors.

With scanning electron microscopy (SEM), images are formed by a focused beam of electrons which is scanned across the surface of the specimen. Signals produced by the electron beam/specimen interaction are then collected to form an image. Scanning electron microscopy is advantageous as, compared with light microscopy, it gives a greater magnification at high resolution and has a substantial increase in the depth of field, with easily interpreted three-dimensional images (Bruton, 1994). Electron microscope studies can be employed to observe population changes and give an indication of the microorganisms present which, in turn, can aid in microbial identification.

6.2 EXPERIMENTAL: RESULTS AND DISCUSSION

6.2.1 MICROBIAL CHARACTERIZATION OF GRANULES AND SLUDGES

UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) DIGESTER GRANULES

Upflow anaerobic sludge blanket (UASB) digester granules, treating brewery wastewater, were viewed by fluorescence (2.5.1) and electron (2.5.2) microscopy to assess whether sufficient methanogenic populations were present to seed the Pennells bioreactor and, hence, rural digesters in general.

The granules contained numerous fluorescent rods and filaments arranged in bands as previously reported (Taylor and Howgrave-Graham, 1992;1993). The granules had fluorescent centres while the exteriors were separated by darker regions. The phenomenon of fluorescent bands was previously observed by MacLeod, Guiot and Costerton (1990) in granules from a sucrose-fed reactor and could have been due to the presence of a substrate gradient (Macario, Earle, Chynoweth and de MaCario, 1989). The fluorescent central region detected in the present study correlated with the layered bacterial aggregates observed by MacLeod *et al.* (1990). The positions of these fluorescent bands do, however, vary according to the hydraulic loading rate applied to the digester (Taylor, Bandu and Howgrave-Graham, 1991; Taylor and Howgrave-Graham, 1993).

The fluorescence microscopy images were not photographed due to the rapid fading of the epifluorescence by coenzyme F_{420} exposure to oxygen and ultraviolet light (UV). Oxygen irreversibly dissociates the F_{420} hydrogenase enzyme complex (Heine-Dobbernack *et al.*, 1988; Oremland, 1988). This occurs concomitantly with the formation of two derivatives (F_{390-A} and F_{390-G}) which are absent in cells protected from oxygen (Heine-Dobbernack *et al.*, 1988).

The UASB granules had an outer layer of, predominantly, filaments (Plate 2A) while the centre consisted of square-ended rods, resembling *Methanosaeta* spp., in extracellular polymer (Plate 2B). This dense surface population and microbial distribution confirmed the results of previous studies (Taylor *et al.*, 1991; Taylor and Howgrave-Graham, 1992;1993). The layering detected with SEM was similar to that reported by MacLeod *et al.* (1990).

Despite the dense microbial distribution and fluorescent methanogenic biomass it was decided not to seed the Pennells bioreactor with UASB granules as these are not readily available to rural communities. In addition, these granules are specific to UASB digesters which are quite different in their mode of operation to that of the rural bioreactor.

Plate 2A: Scanning electron micrograph of a granule surface.

Plate 2B: Central region of the granule shown in Plate 2A, with honeycomb-
              shaped extracellular polymer.

A

2 μm

B

10 μm
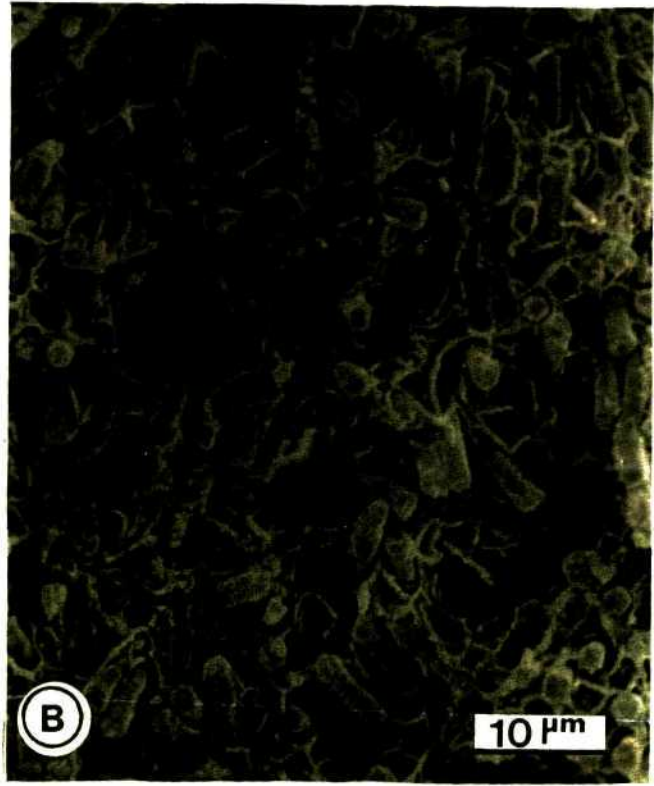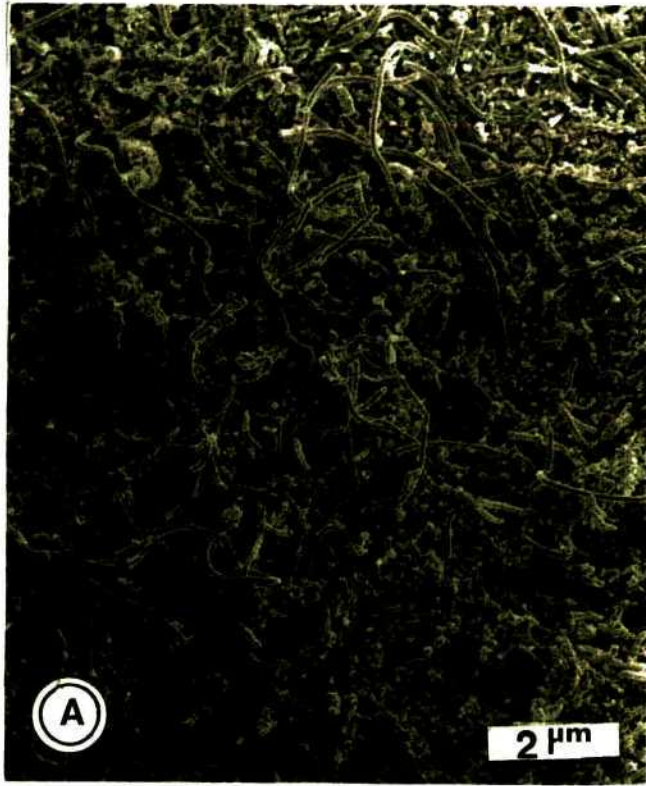
ANAEROBIC DIGESTER SLUDGE FROM DARVILL WASTEWATER PURIFICATION WORKS ("DARVILL SLUDGE")

"Darvill Sludge" (2.2; Table 13) was viewed by fluorescence (2.5.1) and electron (2.5.2) microscopy to assess whether sufficient methanogens were present to seed the Pennells bioreactor and, hence, rural digesters in general.

The "Darvill Sludge" contained many Gram-positive bacteria and was characterized by densely packed associations (Plates 3A and 3B) with the bacteria adhering to surfaces (Plate 3C). Fluorescent bacteria, particularly rods, were present together with some filaments. The percentage of fluorescent cells was, however, fewer than in some UASB digester granules (Taylor and Howgrave-Graham, 1992). *Methanobacterium*-like, *Methanocorpusulum parvum*-like and *Methanosaeta soehngenii*-like cells were present. As with UASB digester granules, the fluorescence microscopy images were not photographed.

The "Darvill Sludge", in contrast to the UASB granules, was floccular in nature and had poor settling characteristics. It contained numerous morphotypes (Plates 3A, 3B and 3D) including filaments, rods, cocci, vibrios and, possibly, spirochaete-like bacteria. Although quantification analyses were not made, it appeared that fewer cells were present than in the UASB granules (Taylor and Howgrave-Graham, 1992). The microbial cell density of the "Darvill Sludge" (Plate 3B) was, however, higher than the Pennells bioreactor sludge (Plates 4A and 4B). In addition, the "Darvill Sludge" contained much extracellular polymer (Plate 3E).

Due to the presence of fluorescent biomass plus a more substantial microbial inoculum, especially when compared to the Pennells bioreactor sludge, "Darvill Sludge" should be a good sludge inoculum for the rural bioreactor.

Combinations of UASB digester granules and anaerobic digester sludge from Darvill Wastewater Purification Works could also be suitable seed materials for the Pennells bioreactor. This was supported in laboratory batch culture studies when biogas generation was promoted (3.5.1).

Plate 3A: Scanning electron micrograph of "Darvill Sludge" (R = rod; C = coccus; F = filament).

Plate 3B: Scanning electron micrograph of "Darvill Sludge" (R = rod; C = coccus; F = filament).

Plate 3C: Scanning electron micrograph showing microorganisms adhering to surfaces in "Darvill Sludge".

Plate 3D: Scanning electron micrograph of "Darvill Sludge" containing a range of morphotypes including possible spirochaete-like bacteria (S).

Plate 3E: Scanning electron micrograph of "Darvill Sludge" showing extracellular polymer (E) between thicker fibres.
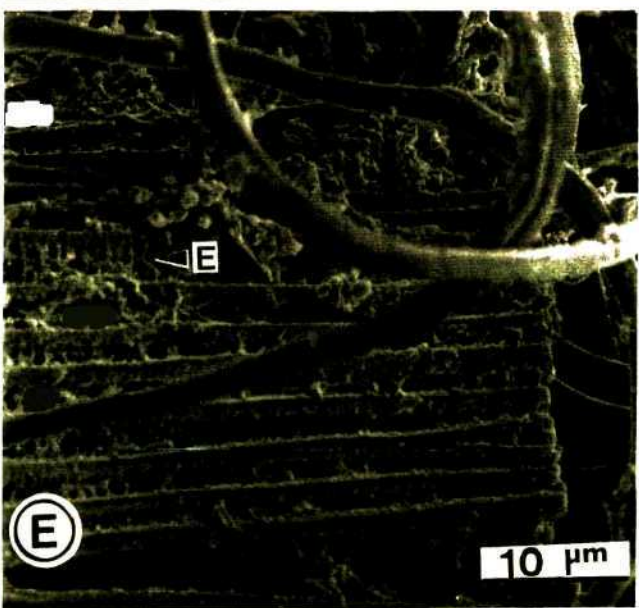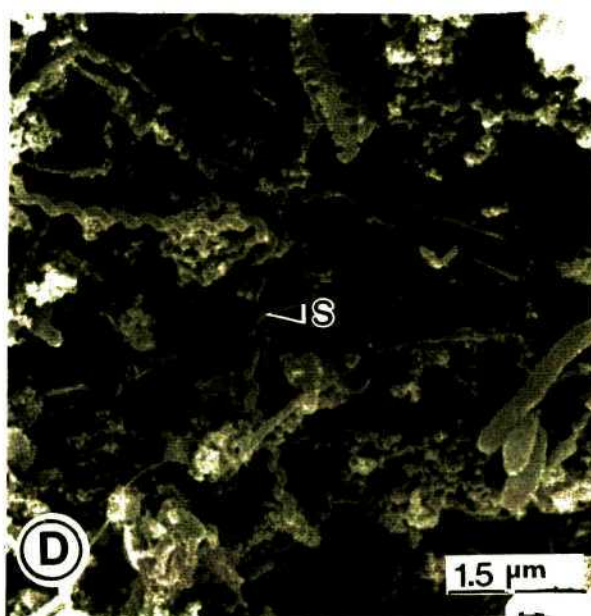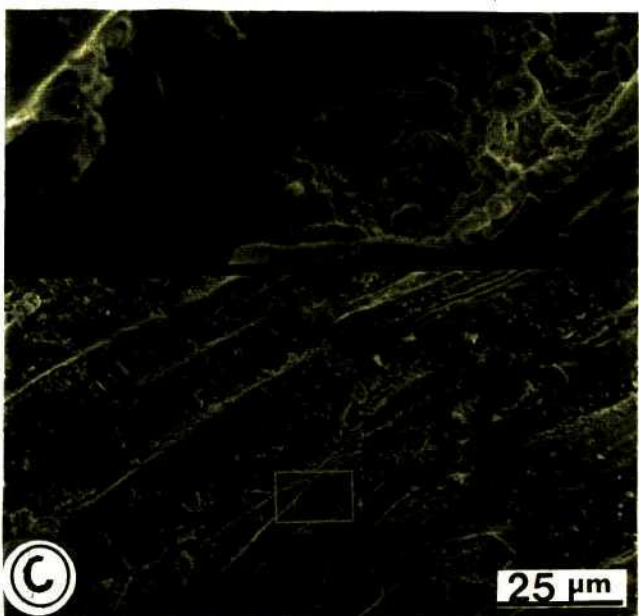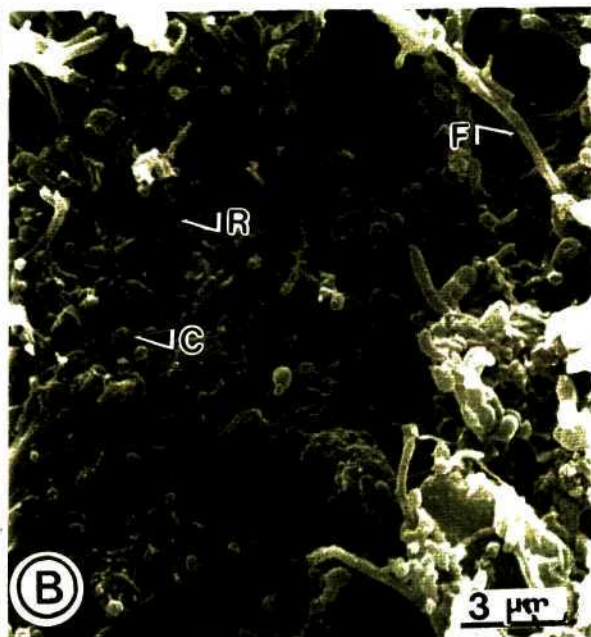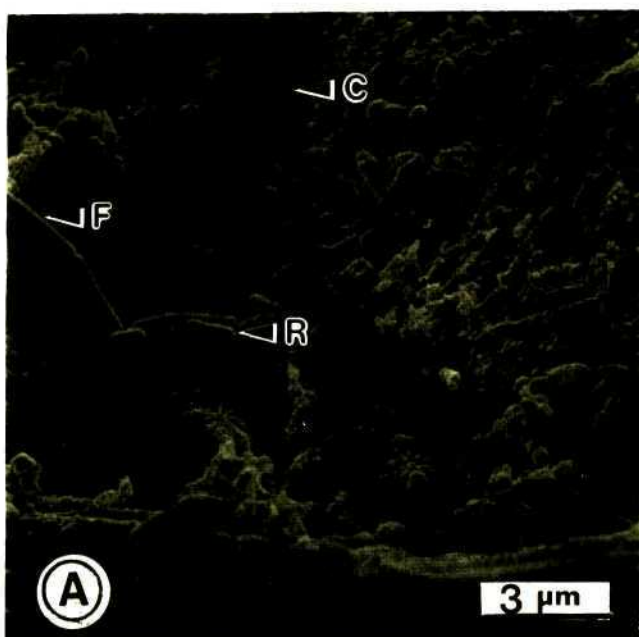
Plates 4A and 4B: Scanning electron micrographs of bacteria present in "Tank 2" sludge of the Pennells bioreactor. Note the spiral-shaped bacterium with polar flagellum (S) and filter-paper pores (F).
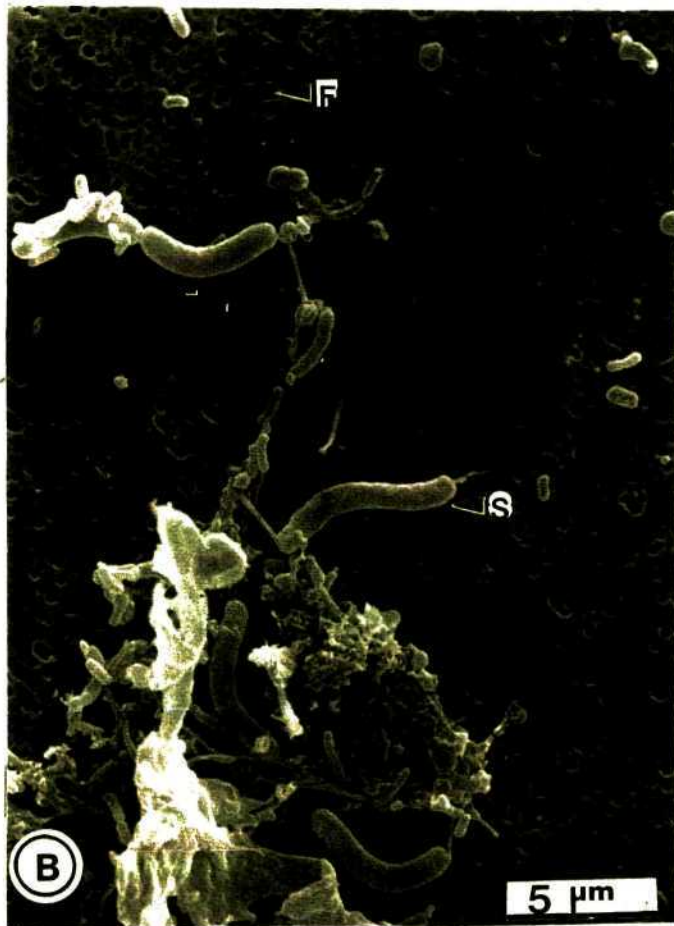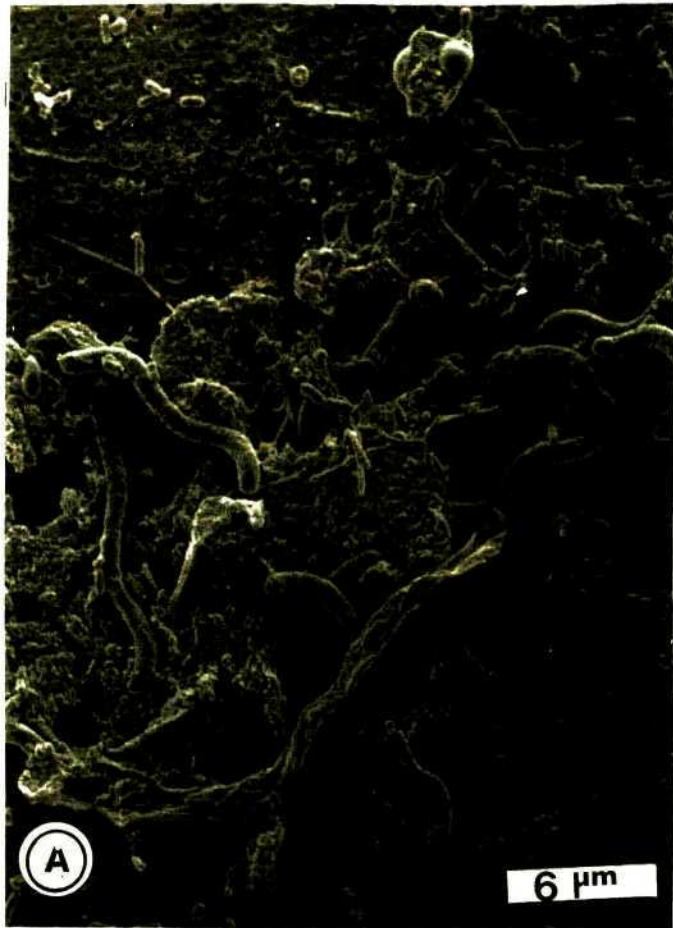
PENNELLS BIOREACTOR SLUDGE PRIOR TO INOCULATION

Electron microscopy (2.5.2) (of sludge from "Tank 2" and "Tank 2 outlet" sample ports) plus both bright-field (of Gram-stained sludge) and interference-phase microscopy (2.5.1) were employed to examine samples to aid in microbial characterization.

In contrast to the "Darvill Sludge" (Plates 3A and 3B), sludge sampled from the Pennells bioreactor was less dense in its constituency with fewer morphotypes present (Plates 4A-4D). This could possibly have been due to unfavourable conditions within the Pennells bioreactor which, in turn, could have effected poor biogas production. The Pennells bioreactor sludge contained a mixture of Gram-positive and Gram-negative bacteria. Many Gram-negative, predominantly, rod-shaped bacteria were evident of which some were slightly curved. A few cocci and larger spiral-shaped bacteria with flagella at one (monotrichous) or, predominantly, both poles (amphitrichous) (Plate 4C) were evident. In "Tank 2" sludge what appeared to be a "water flea" was also evident. Its presence could have been indicative of aerobic conditions. Some spiral-shaped bacteria were also present (Plates 4B and 4C). Few fluorescent cells were visualized possibly indicating a lack of methanogens.

Scanning electron microscopy of filtered sludge sampled from "Tank 2" and "Tank 2 outlet" ports also revealed the presence of microbial flocs (Plate 4D).

Plate 4C: Scanning electron micrograph of bacteria present in "Tank 2" sludge of the Pennells bioreactor. Note the spiral-shaped bacterium with polar flagellum (S) and filter-paper pores (F).

Plate 4D: Scanning electron micrograph of a floc from the same sludge.

C   4.3 µm

D   3 µm
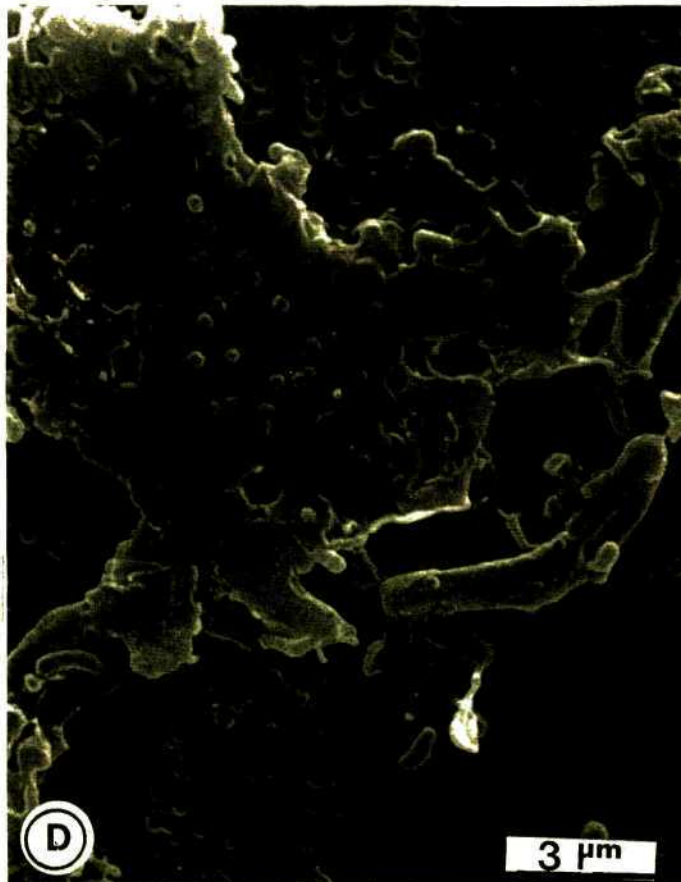
DURBAN WASTEWATER PURIFICATION WORKS SLUDGE

Scanning electron microscopy was used to examine "Durban Sludge" (2.2) which was only one of the sludge types used in the laboratory continuous culture (2.3.2).

The sludge contained, predominantly, cocci and rods (Plate 5) with extracellular polymers. The polymers were probably artifacts as the extracellular polymer components of bacterial biofilms are reported to contain 99% (v/v) water (Costerton and Irvin, 1981). The presence of water results in biofilm collapse during standard SEM dehydration procedures and usually leaves strands between the cells (Costerton and Irvin, 1981). Howgrave-Graham and Wallis (1991) examined UASB granules treating brewery wastewater and only observed these strands if acetone, ethanol or 2,2-dimethoxypropane dehydration was made on stored refrigerated material.

The extracellular polymer shown in Plate 5 could possibly have been proteinaceous in nature as glutaraldehyde strongly fixes proteins but has limited lipid-or carbohydrate-fixing capacity (Hayat, 1981). Proteinaceous polymer would, thus, be preserved while lipid and carbohydrate would be washed out during dehydration (Howgrave-Graham and Wallis, 1991). The extracellular polymer strands seen in Plate 5 differed from the honeycomb-shaped polymer seen with the UASB digester granules (Plate 2B); a phenomenon previously reported by Taylor and Howgrave-Graham (1992; 1993). The same observation was made by Howgrave-Graham and Wallis (1994) but only when the granules were frozen in liquid nitrogen and sectioned with a cooled razor prior to fixation.

Plate 5: Scanning electron micrograph of anaerobic digester sludge from Durban Wastewater Purification Works showing the morphotypes present and extracellular polymer strands.

2 µm
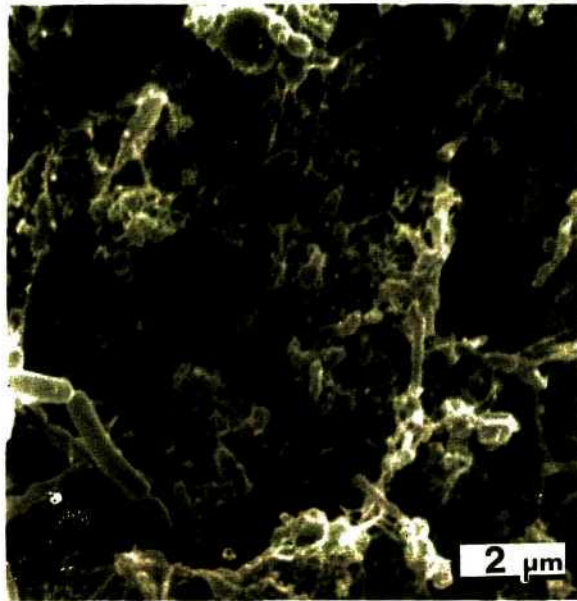
6.2.2 SPECIES COMPLEMENT CHANGES FOLLOWING INOCULATION

"DARVILL SLUDGE"
LABORATORY CONTINUOUS CULTURE

Electron (2.5.2) and light (2.5.1) microscopy were utilized to examine sludges from the laboratory continuous cultures.

Scanning electron microscopy of sludge from one of the laboratory-scale culture vessels (2.3.2) which contained artificial sewage and "Darvill Sludge" revealed extracellular polymer strands (Plate 6) similar to those in Plate 5 but with fewer cells and morphotypes visible.

Comparison of this sludge (Plate 6) with "Darvill Sludge" (Plates 3A-3D) showed changes in the sludge following inoculation. These could have been in response to the different selection pressures applied to the laboratory culture. Different selection pressures do occur in laboratory situations and effect different enrichments and isolations (Parkes, 1982). These, in turn, result in differences in biogas production.

Plate 6:   Scanning electron micrograph of sludge from the laboratory
             continuous culture experiment (2.3.2) (containing artificial sewage
             and "Darvill Sludge") following inoculation with "Darvill Sludge".
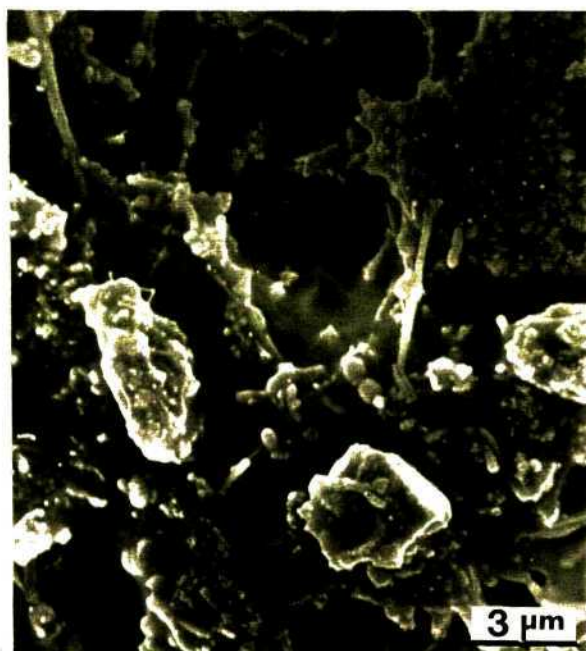
3 μm

"DARVILL SLUDGE"
PENNELLS BIOREACTOR

Scanning electron microscopy was used to examine sludge from the Pennells bioreactor one week after inoculation with "Darvill Sludge".

Following inoculation, "Tank 1 outlet" sludge contained a mixture of plant debris and a densely aggregated microbial population (Plate 7A). Much leaf material was present in the "Tank 2 inlet" and "Tank 1 outlet" sludges probably as a result of the residents using plant material as a toilet paper substitute (3.6). "Tank 2 inlet" sludge contained microbial morphotypes/aggregates (Plate 7B) similar to those in "Tank 1 outlet" which, together with the debris, resembled the sludge from "Tank 1" (Plate 7A).

The "Tank 2" sludge samples also contained dense microbial aggregates of various morphotypes including cocci, rods and filaments (Plate 7C). Many of the morphotypes resembled those recorded with the "Darvill Sludge" inoculum. The sludge sampled at "Tank 2 outlet" was also similar (Plate 7D).

By comparing Plates 4A-4D with Plates 7C and 7D it can be concluded that the microbial populations differed. For example, the spiral-shaped bacteria detected in "Tank 2" before "Darvill Sludge" inoculation (Plates 4A-4D) were not subsequently detected, possibly, due to displacement or dilution. Despite the increased range of morphotypes (Plates 7C and 7D) no biogas generation resulted (3.5.1).

"Darvill Sludge" alone was, however, added to the bioreactor to provide an active catabolic population to enhance biogas production. This approach was also used by DaSilva (1981) who documented that sludge from wastewater treatment works is ideal seed material.

Plate 7A: Scanning electron micrograph of sludge sampled from "Tank 1 outlet" after "Darvill Sludge" inoculation.

Plate 7B: Scanning electron micrograph of "Tank 2 inlet" sludge after "Darvill Sludge" inoculation.

Plate 7C: Scanning electron micrograph of microbial populations of "Tank 2" sludge after "Darvill Sludge" inoculation (R = rod; C = coccus; F = filament).

Plate 7D: Scanning electron micrograph of "Tank 2 outlet" sludge after "Darvill Sludge" inoculation (R = rod; C = coccus; F = filament).
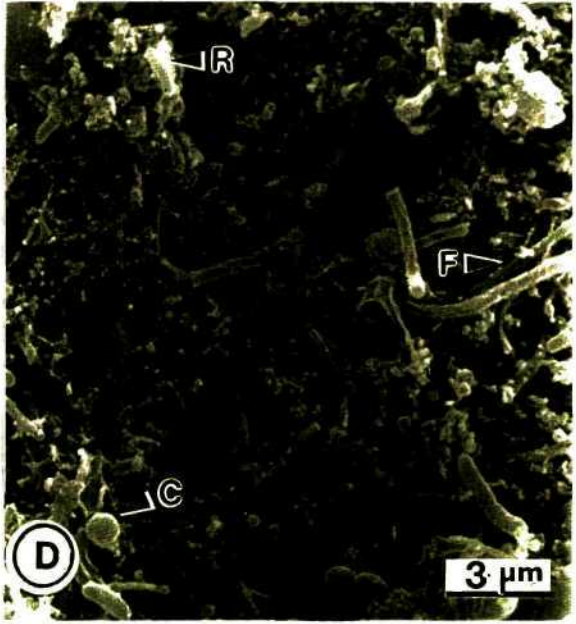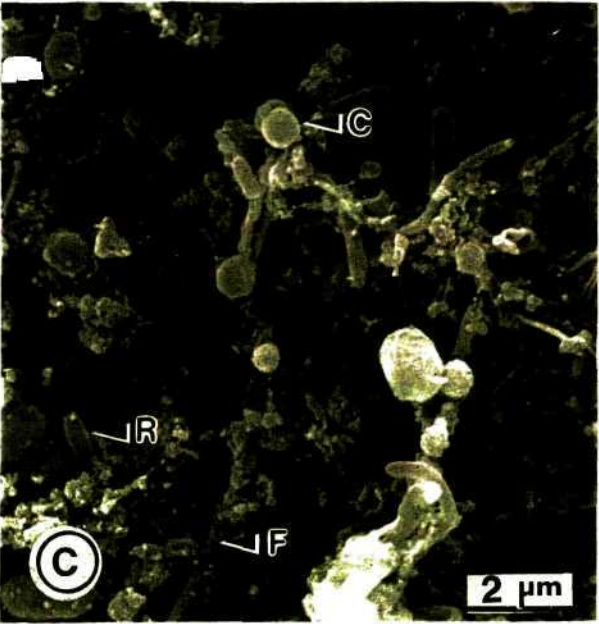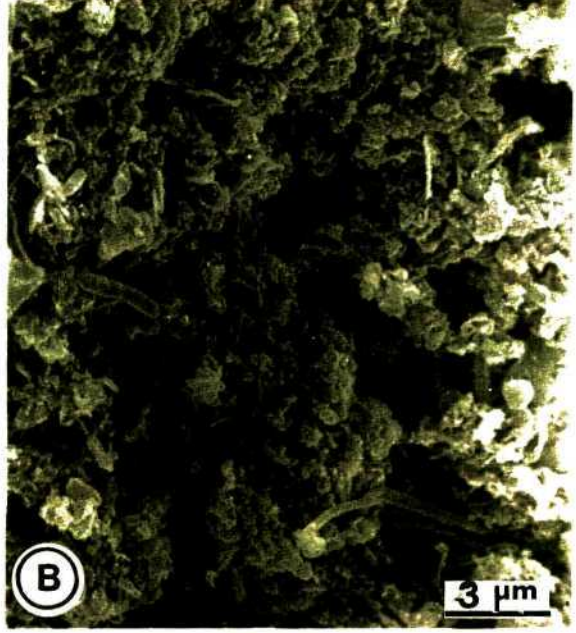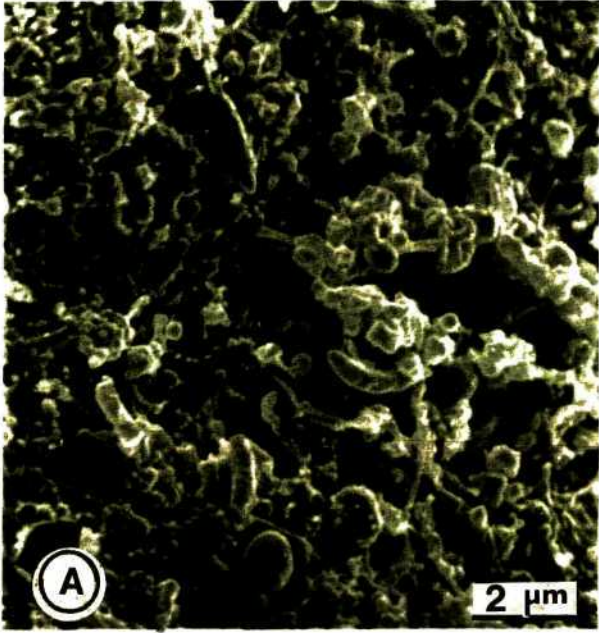
COW MANURE

PENNELLS BIOREACTOR

Scanning electron microscopy was used to examine sludge from the Pennells bioreactor to determine any visible population changes after addition of cow manure to "Tank 2".

Sludge sampled from "Tank 2" after the addition of cow manure contained a diverse microbial population (Plate 8A). Although quantitative analyses were not made, the sludge appeared to have a sparser microbial distribution than that noted after "Darvill Sludge" addition (Plate 7C). Extracellular polymer strands were also evident (Plate 8A).

"Tank 2 outlet" sludge before addition of cow manure contained few microorganisms and consisted primarily of debris (Plate 8B). Following cow manure inoculation, the density of the sludge increased (Plate 8C) and was characterized by the presence of extracellular polymers and debris. After cow manure addition, "Tank 2 outlet" sludge contained spiral-shaped bacteria (Plate 8C).

The added cow manure thus changed the species complement although, as noted with "Darvill Sludge" addition, these changes were possibly not mainained.

Plate 8A: Scanning electron micrograph of "Tank 2" sludge after addition of cow manure (S = possible spirochaete-like bacterium).

Plate 8B: Scanning electron micrograph of "Tank 2 outlet" sludge before cow manure addition.

Plate 8C: Scanning electron micrograph of "Tank 2 outlet" sludge after addition of cow manure (S = possible spirochaete-like bacterium).
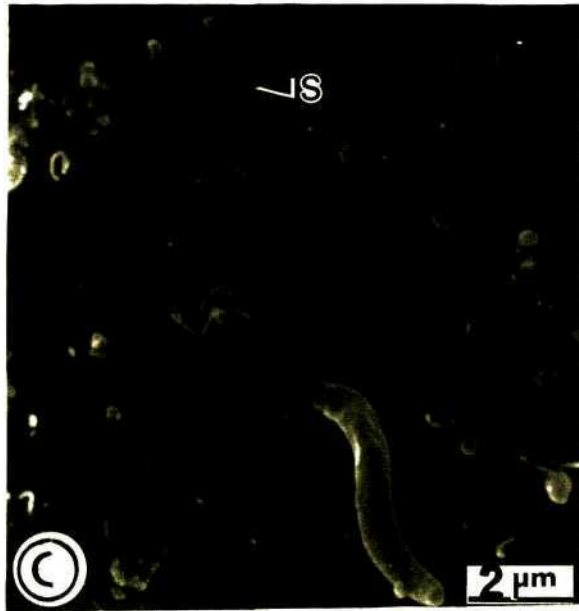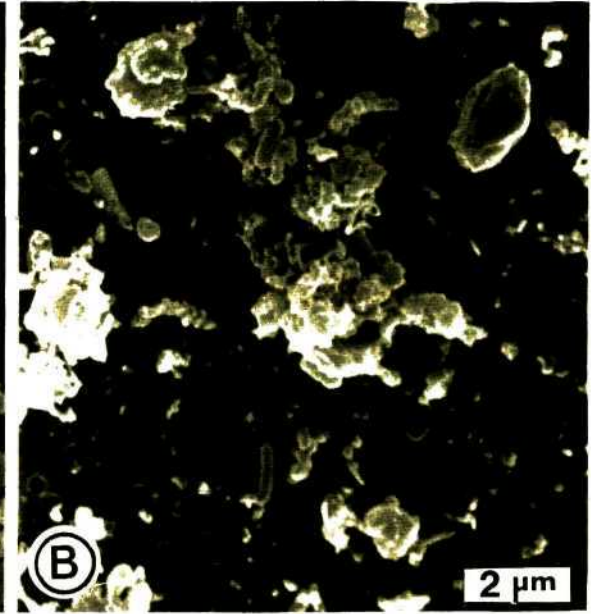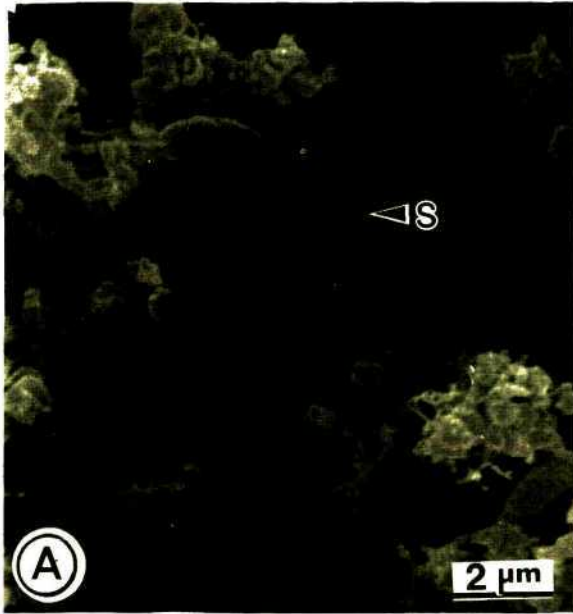
6.3 CONCLUSIONS

Light and electron microscopy proved useful methods for the selection of suitable sludges for bioreactor inoculations. In particular, the presence of methanogens was readily detected. This, in turn, lent itself to selecting a sludge for inoculation which could be substantiated by further biogas production experimentation (3.5.1). From microscopy analyses, "Darvill Sludge", UASB digester granules or a combination of the two would be suitable seed material. "Darvill Sludge", however, as discussed, should be better seed material to facilitate the establishment of a catabolic population. Continued addition of cow manure could also prove advantageous.

Sludge changes following Pennells bioreactor inoculation were clearly evident. Various additions to and inoculations of the Pennells bioreactor affected the discrete microbial populations. Darvill sludge, for example, added various morphotypes and resulted in a higher population density.

From the microscopy studies it became evident that the population densities within the Pennells bioreactor declined with time due to unfavourable operating conditions and a range of perturbations and this was confirmed by the biogas production results (3.5.1).

CHAPTER 7

MICROANALYSIS OF THE METAL CONTENTS OF THE DIFFERENT
SLUDGES BY ENERGY DISPERSIVE X-RAY

7.1 INTRODUCTION

Sewage is composed primarily of water together with organic and inorganic components. The organic fraction is generally classified as nitrogenous (urea, proteins, amines and amino acids) and non-nitrogenous (carbohydrates, fats and soaps) fractions. Some of these molecules are semi-recalcitrant but are catabolized as the sewage "ages". The inorganic components include ions such as sodium, potassium, ammonium, calcium, magnesium, iron (Murray, 1987), bicarbonate, sulphate, chloride and phosphate (McKinney, 1962; Pelczar and Reid, 1965; Pelczar et al., 1986). Sewage also contains sulphide, silica (Tchobanoglous and Bruton, 1991) and the metals cadmium, zinc, nickel (Arnold, 1979; Beckett, Phil, Davis and Brindley, 1979; Bradley, 1981), copper and boron (Bradley, 1981). Heavy metals (Tyagi and Couillard, 1989) characterize the sludges of wastewater purification works treating industrial effluents (Ross et al., 1992). The concentrations of heavy metals in wastewater sludges vary (Table 15) and high levels may limit sludge application to land (Tchobanoglous and Bruton, 1991).

In domestic wastewater, other groups of contaminants may be found including (Fourie and van Ryneveld, 1995):
-refractory organics:

-surfactants (eg. detergents), particularly the non-ionic variety. These are considered potentially problematic because they are less biodegradable than their anionic counterparts;

-pesticides and agricultural chemicals;

-cleaning solvents (eg. benzene, toluene and carbon tetrachloride). These originate from sources such as toilet bowl cleaners, paint brush cleaners and stove and oven cleaners;

-organics produced by processing of natural organics (eg. trihalomethanes);

-mineral oils (eg. engine oil, PCB's); and

-toxic inorganic ions.

The above are not generally present in domestic wastewater particularly from low-income communities but may occur where inappropriate disposal practices exist (Fourie and van Ryneveld, 1995), as was found in this study.

Table 15: Typical metal content of municipal wastewater sludge.

Metal	Dry sludge, mgkg^{-1} (Range)	Dry sludge, mgkg^{-1} (Median)
Arsenic	1.1-230	10
Cadmium	1-3 410	10
Chromium	10-99 000	500
Cobalt	11.3-2 490	30
Copper	84-17 000	800
Iron	1 000-154 000	17 000
Lead	13-26 000	500
Manganese	32-9 870	260
Mercury	0.6-56	6
Molybdenum	0.1-214	4
Nickel	2-5 300	80
Selenium	1.7-17.2	5
Tin	2.6-329	14
Zinc	101-49 000	1 700

7.1.1 SLUDGE ANALYSIS

For metal analysis the electron beam/specimen interaction in the SEM produces an X-ray signal which, with the aid of an X-ray spectrometer, can be collected to provide information on the elemental composition of the specimen (Bruton, 1994). According to Bruton (1994) the important features of the technique include:

a) The detection of elements. The technique is, however, incapable of distinguishing between ionic and non-ionic species;

b) Since electrons and X-rays are strongly absorbed by air molecules, all samples are

analysed under relatively high vacuum. This has serious implications for the preparation of "wet" specimens;

c) Elements are detected and measured *in situ* with a spatial resolution ranging from about 10 nanometres to a few micrometres;

d) Valence electrons participate in chemical bonding whilst inner-shell electrons are involved in transitions that frequently result in X-ray emissions. Thus, X-ray detection is, generally, independent of the chemical state of the elements;

e) X-ray microanalysis does not distinguish between "free" and various "bound" elemental states;

f) The composition of the organic matrix itself cannot be determined since the major constituents are elements of low atomic number (carbon, hydrogen, nitrogen and oxygen); and

g) The technique is non-destructive.

Characteristic and Continuum X-rays are produced when an incident electron enters the sample material. Each element produces a discrete and fairly easily characterized spectrum. It is this characteristic X-ray signal which is collected and used to give information on the nature and position of the elements within the specimen (Bruton, 1994).

Element results are digitised and stored in a multichannel analyzer which is able to produce, as an output, a complete X-ray spectrum. This spectrum is displayed on a cathode ray (television) tube where the energy is on the horizontal axis and the number of photons counted per energy level on the vertical axis (Bruton, 1994).

Energy Dispersive X-ray Microanalysis (EDX) allows the ratios of the various elements present to be determined. In contrast, X-ray spectroscopy may be used to determine the local concentration of individual inorganic elements (Fang and Yan, 1995). Energy dispersive X-ray microanalysis was used in this study to qualitatively determine which elements were present within the various sludges (Hobbs, Griffiths and Hann, 1986) and, thus, if any inhibitory elements were present which militated against biogas production in the Pennells bioreactor. In addition, elemental changes in response to specific selection pressures were also evaluated.

7.2 EXPERIMENTAL: RESULTS AND DISCUSSION

Various sludge samples were analysed by EDX (2.5.3) (Table 16).

Table 16: Sludges and supernatants analysed by EDX.

Sludge	Day(s) of Sampling
Sludge from the laboratory continuous culture (Darvill Sludge plus Artificial Sewage)	-
Darvill Wastewater Purification Works Sewage Sludge	Initial sample
Darvill Wastewater Purification Works Sewage Sludge	Sampled 6 months later
Durban Wastewater Purification Works Sewage Sludge	-
"Tank 1 outlet", "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" sludges	50 and 109
"Tank 1 outlet" and "Tank 2 outlet" supernatants	50 and 109
"Tank 1 outlet" sludge	After cow manure addition
"Tank 2" sludge	After cow manure addition
"Tank 2 outlet" sludge	After cow manure addition

When analyzing results of the percentage metal occurrence it must be recognised that the estimates are percentages of the detectable elements present. This is because the composition of the organic matrix itself cannot be determined by this method since the major constituents are elements of low atomic mass (carbon, hydrogen, nitrogen and oxygen) (Bruton, 1994).

7.2.1 SLUDGE FROM THE LABORATORY CONTINUOUS CULTURE (2.3.2)

Elemental analysis was made on this sludge to determine any possible elemental limitation

(Callander and Barford, 1983).
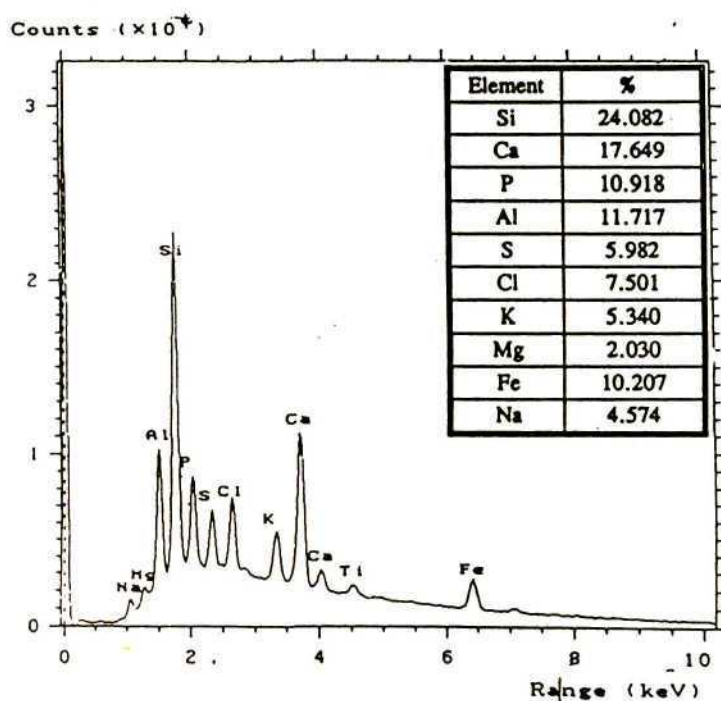
Figure 31: EDX spectrum for sludge from laboratory continuous culture inoculated with "Darvill Sludge".

Two calcium peaks and a small titanium peak were detected (Figure 31) together with the elements silicon, sulphur and iron. The presence of silicon in the culture could have been due to diatoms or sand/grit in the inoculum. Although iron was detected by EDX, gas production did not occur. It is, however, documented that iron is needed to stimulate acetate conversion to methane (Raju *et al.*, 1991). If essential elements are not supplied in the medium then the integrity of the sludge can only be retained by recycling.

The absence of a particular element can preclude those microorganisms with obligate requirements (Speece and Parkin, 1987). The lack of nickel, for example, is disadvantageous. The coenzyme F_{430} of methanogens (Staley, Bryant, Pfenning and Holt, 1989; Degrémont, 1991) contains nickel which is indispensable to the growth of these organisms (Degrémont, 1991). The undetection of this element in "Darvill Sludge" (Figure 31) could, possibly, explain the lack of methanogenesis in both the laboratory cultures and the Pennells bioreactor following inoculation. Nickel was also absent from the anaerobic digester sludges from Durban and Darvill Waste Water Purification Works (7.2.2). This

essential element could possibly have precipitated out as the hydroxide, especially under alkaline conditions (Moody, 1969), or sulphide thus preventing its detection.

7.2.2 DARVILL AND DURBAN WASTEWATER PURIFICATION WORKS SLUDGES

The EDX metal profiles of the various sludges show that the percentage ratios of elements varied (Figures 32-34). This was expected since sludge composition can vary between waste treatment plants and with time in response to the specific selection pressures applied.

Silicon appeared to be the dominant element relative to the others detected. This was probably due to the presence of sand/grit in the samples. Alternatively, biological groups such as diatoms and other algae have cell walls which are heavily impregnated with silica (Stanier *et al.*, 1987) and diatoms had been detected in "Darvill Sludge" although no scanning electron micrographs were taken.

Titanium was present in two of the sludge samples. Titanium is not toxic to bacteria and could have originated from added sand/soil.

Element	%
Mg	0.973
Al	17.341
Si	32.068
P	10.375
S	4.964
Cl	2.249
K	4.506
Ca	10.371
Fe	15.401
Ti	1.768
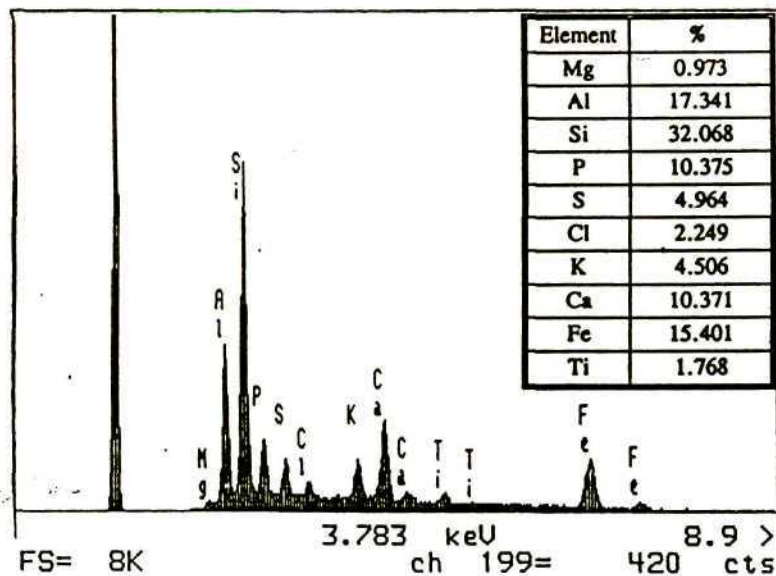
Figure 32: EDX spectrum of "Darvill Sludge".
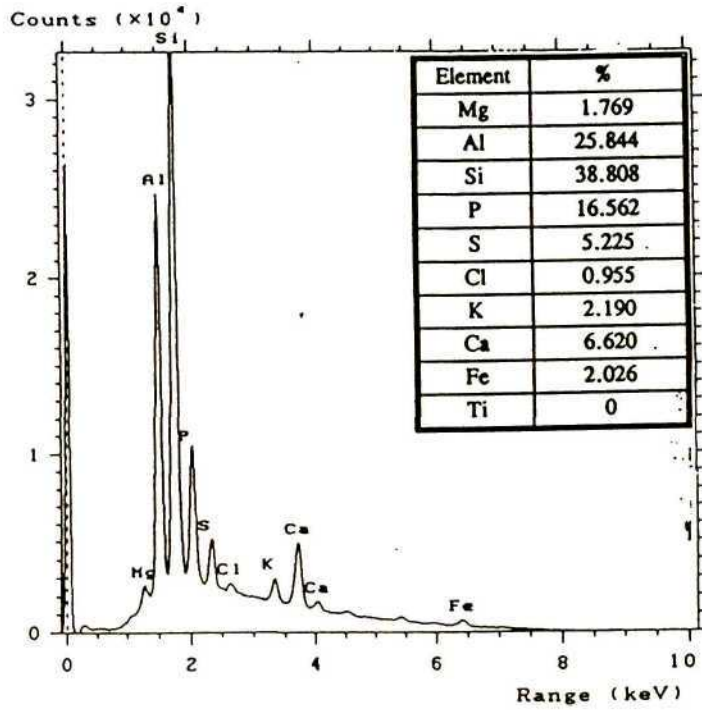
Figure 33: EDX spectrum of "Darvill Sludge" sampled 6 months later.
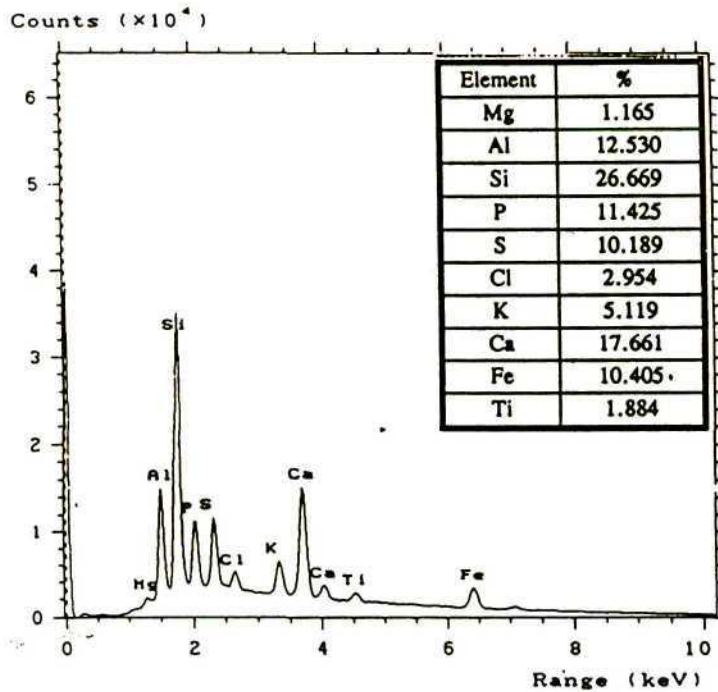
Figure 34: EDX spectrum of anaerobic digester sludge from Durban Wastewater
Purification Works.

7.2.3 SLUDGES AND SUPERNATANTS FROM THE PENNELLS BIOREACTOR (DAYS 50 AND 109)

Marked differences in the elemental ratios of the sludges from each sample point were recorded (Figures 35-40). Again, nickel was absent from all samples and indicated a possible limitation to methanogenesis. The higher ratios of titanium and silicon in "Tank 2 inlet" and "Tank 2" sludges (Figures 36 and 37) could have been due to the addition of "Darvill Sludge". The element sulphur was present in a higher concentration in "Tank 1 outlet" sludge than in the other three sludge samples (Figures 35-38). The presence of this element could, possibly, have inhibited the methanogens by facilitating the sulphate-reducing bacteria which outcompete the methanogens for hydrogen and acetate (Zeikus, 1980; Dubourguier *et al.*, 1988).

Element	%
Na	4.070
Mg	2.628
Al	3.682
Si	8.828
P	13.561
S	14.210
Cl	15.029
K	10.419
Ca	22.175
Fe	5.402
Ti	0
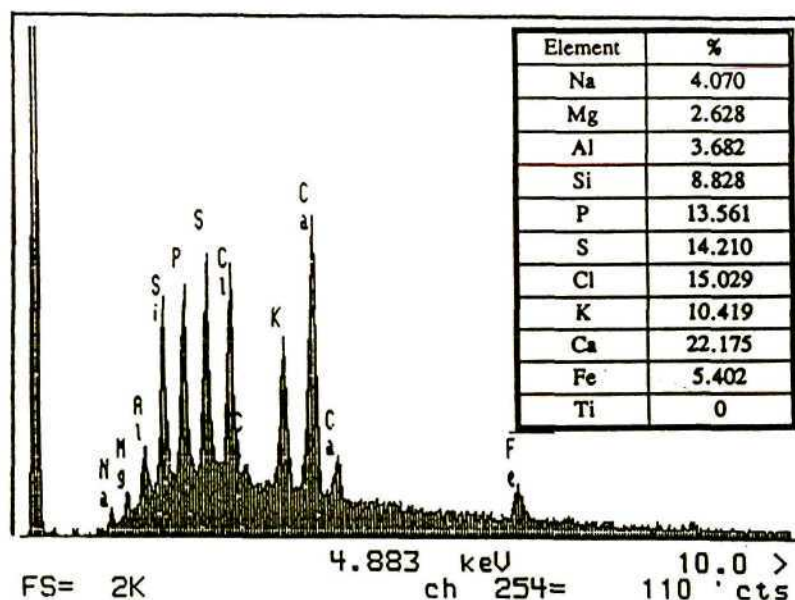
Figure 35: EDX spectrum of sludge sampled at "Tank 1 outlet" (Day 50).

Element	%
Na	0
Mg	1.829
Al	15.416
Si	27.819
P	12.889
S	8.361
Cl	1.589
K	3.244
Ca	12.433
Fe	15.008
Ti	1.425

Figure 36: EDX spectrum of sludge sampled at "Tank 2 inlet" (Day 50).

Element	%
Na	0
Mg	1.642
Al	16.607
Si	29.070
P	13.845
S	6.789
Cl	2.490
K	3.530
Ca	12.356
Fe	12.419
Ti	1.263

Figure 37: EDX spectrum of sludge sampled from "Tank 2" (Day 50).
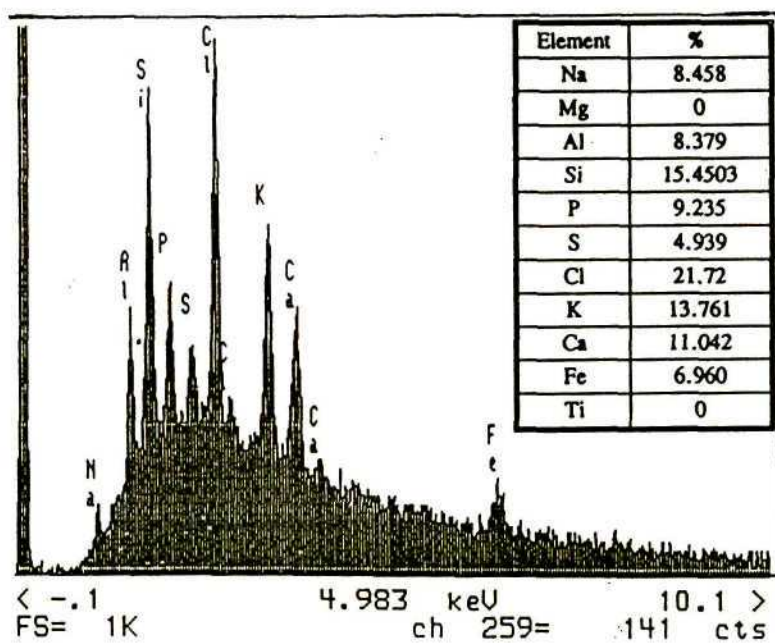
Figure 38: EDX spectrum of sludge sampled at "Tank 2 outlet" (Day 50).

Element	%
Na	8.458
Mg	0
Al	8.379
Si	15.4503
P	9.235
S	4.939
Cl	21.72
K	13.761
Ca	11.042
Fe	6.960
Ti	0

< -.1   4.983   keV   10.1 >
FS=   1K   ch 259=   141   cts
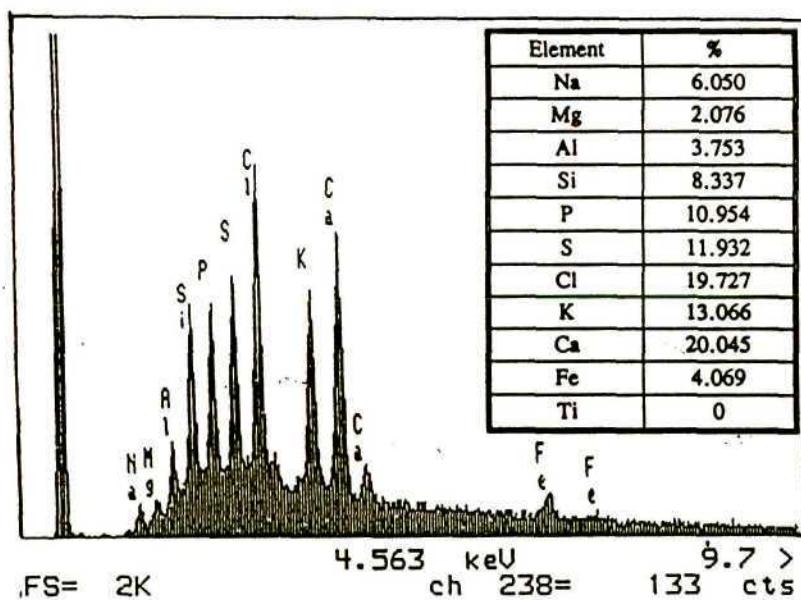
Figure 39: EDX spectrum of sludge sampled at "Tank 1 outlet" (Day 109).

Element	%
Na	6.050
Mg	2.076
Al	3.753
Si	8.337
P	10.954
S	11.932
Cl	19.727
K	13.066
Ca	20.045
Fe	4.069
Ti	0

                4.563   keV   9.7 >
,FS=   2K   ch 238=   133   cts

Element	%*
Na	0
Mg	0
Al	15.431
Si	29.351
P	10.281
S	7.474
Cl	2.029
K	4.023
Ca	12.891
Fe	17.028
Ti	1.504
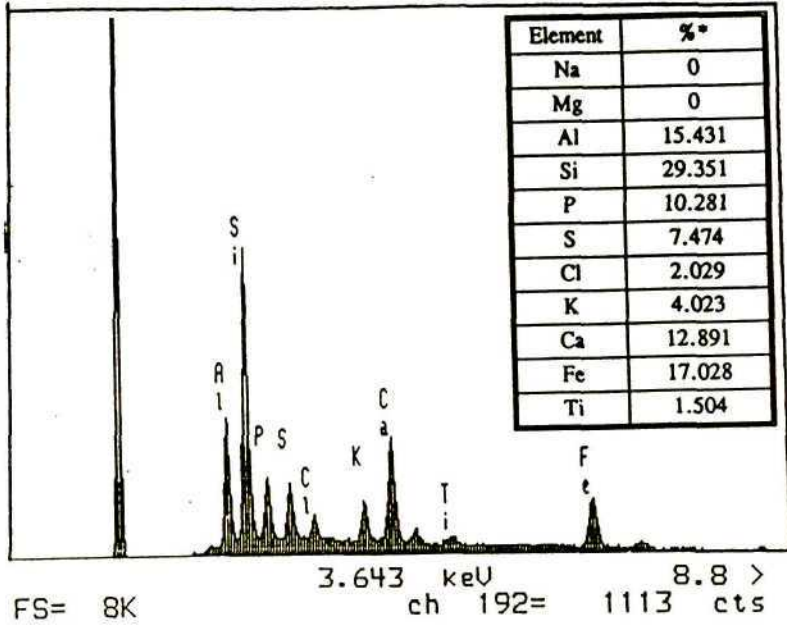
Figure 40: EDX spectrum of sludge sampled from "Tank 2" (Day 109).

KEY: * = All the values for "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" were the same and are thus tabulated together.

Figures 35-38 and 41-42 show that the ratios of elements varied between the sludges and supernatant samples on day 50, especially with respect to the elements aluminium, iron and titanium.

Element	%
Na	16.187
Mg	2.227
Si	1.934
P	7.159
S	6.794
Cl	31.529
K	25.613
Ca	8.591
Al	0
Fe	0
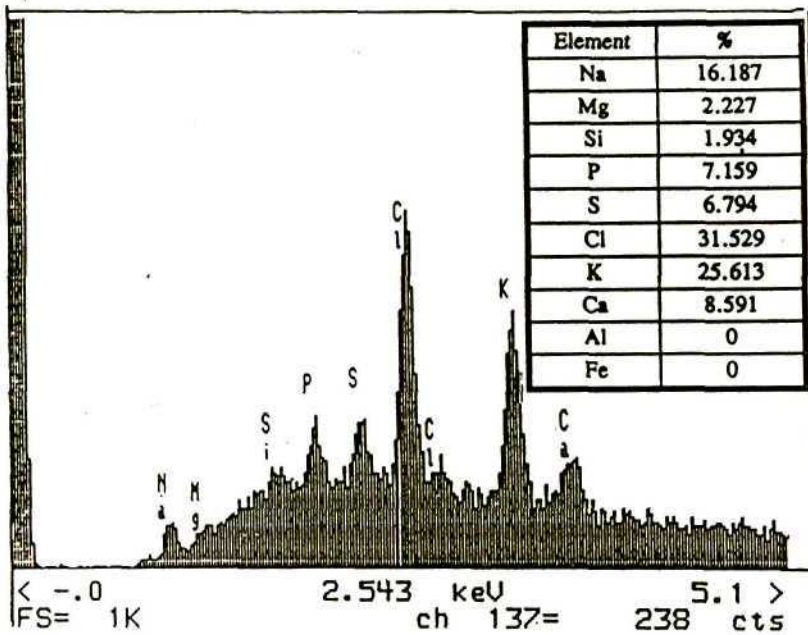
Figure 41: EDX spectrum of "Tank 1 outlet" supernatant (Day 50).

Element	%
Na	9.135
Mg	3.707
Si	6.928
P	13.058
S	4.002
Cl	24.411
K	15.471
Ca	15.919
Al	3.417
Fe	3.966
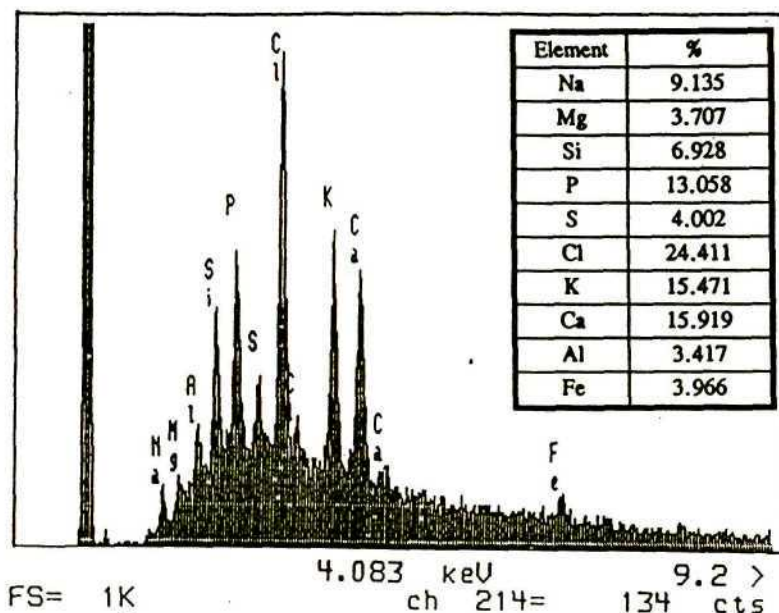
Figure 42: EDX spectrum of "Tank 2 outlet" supernatant (Day 50).

Silicon appeared to be present in lower percentages in the supernatants than in the sludges possibly due to gravity sedimentation. Lower sulphur percentages were also present in the supernatants due, possibly, to sulphate-reducing bacterial activity and consolidation of the precipitated sulphide in the sludges. The presence of sulphide in solution is also pH dependent. The presence of chlorine was possibly indicative of community use of "detergents".

From Figure 40 it can be seen that, unlike the elemental ratios recorded on day 50, the results obtained for "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" sludges on day 109 were the same.

There were, however, marked differences between the results of "Tank 1 outlet" sludge and the other three sludges. In particular, silicon was more concentrated in "Tank 2" sludge than in "Tank 1 outlet" sludge probably due to the addition of "Darvill Sludge" to Tank 2. As with the Day 50 samples, sulphur was again present, especially in the "Tank 1 outlet" sludge sample. Titanium was present in the sludge samples from "Tank 2 inlet", "Tank 2" and "Tank 2 outlet" but not in the sludge from "Tank 1 outlet" possibly due to addition to Tank 2 of "Darvill Sludge" which contained the element titanium (7.2.2). Sodium and magnesium were, however, present in "Tank 1 outlet" sludge but not in "Tank

2" sludge. Chlorine was a major element in "Tank 1" sludge probably due to the flushing of "detergent"-containing waters.

Figures 43 and 44 show the EDX spectra of supernatants sampled on day 109.

Element	%
Na	13.661
Si	1.596
P	5.115
S	5.959
Cl	36.306
K	29.996
Ca	7.400
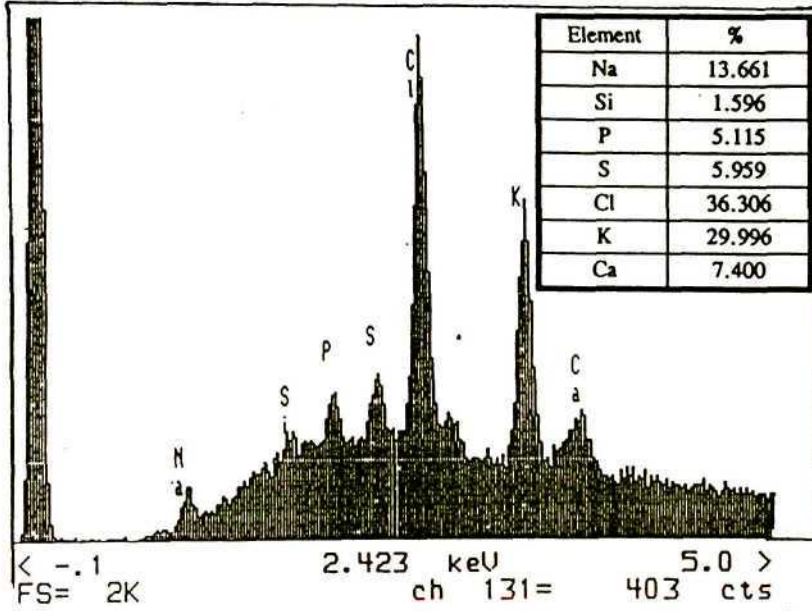
Figure 43: EDX spectrum of "Tank 1 outlet" supernatant (Day 109).

Element	%
Na	16.241
Si	1.876
P	5.279
S	4.909
Cl	36.136
K	24.565
Ca	11.014
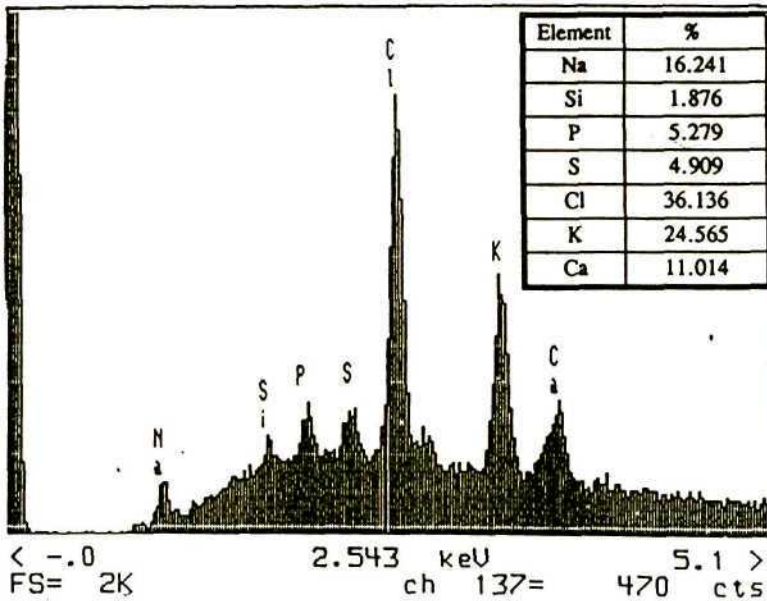
Figure 44: EDX spectrum of "Tank 2 outlet" supernatant (Day 109).

Differences were apparent between the sludge and liquid supernatant samples from "Tank 1 outlet" and "Tank 2 outlet" on days 50 and 109. Magnesium was present in "Tank 1 outlet" sludge but not in "Tank 1 outlet" supernatant and "Tank 2 outlet" sludge and

supernatant (Figures 43 and 44). Similarly, sodium was detected in "Tank 2 outlet" supernatant but not in the sludge. Aluminium and iron were found in "Tank 1 outlet" and "Tank 2 outlet" sludges but not in the equivalent supernatants. Titanium was present in "Tank 2 outlet" sludge but not in "Tank 1 outlet" and "Tank 2 outlet" supernatants and "Tank 1 outlet" sludge.

Relatively speaking, all the supernatant samples tested had lower ratios of silicon than the equivalent sludges. Chlorine and potassium were the dominant elements in the supernatants. The variations in the elements detected were probably due to additions by the residents.

7.2.4 SLUDGE SAMPLES FROM THE PENNELLS TWO-TANK BIOREACTOR AFTER ADDITION OF COW MANURE

These analyses were made on sludge sampled from "Tank 1 outlet", "Tank 2" and "Tank 2 outlet" one week after addition of the cow manure. From Figures 45 to 47 it can be seen that the elements silicon, calcium, phosphorus, aluminium, sulphur, chlorine, potassium, magnesium, iron and sodium were present in the sludges but in different ratios. Titanium, however, was not detected in the sludge samples.
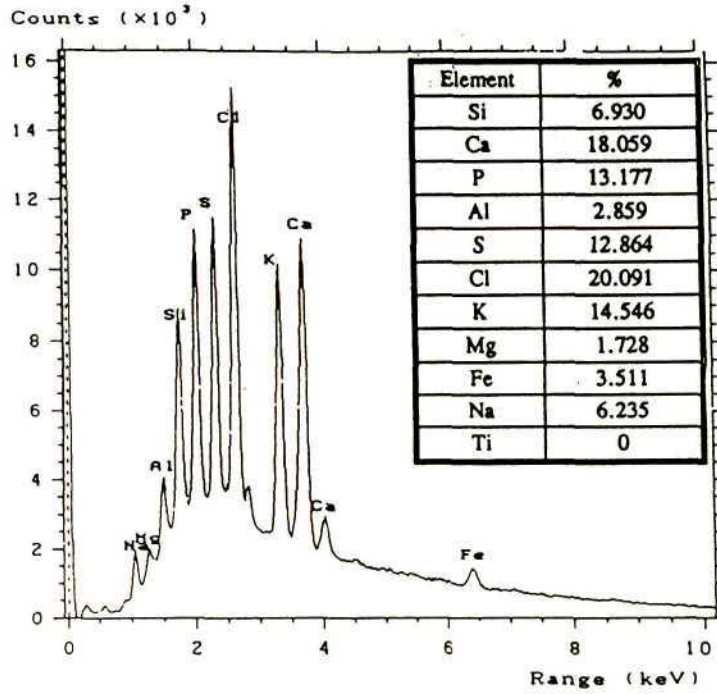
Figure 45: EDX spectrum of sludge sampled at "Tank 1 outlet" after addition of cow manure.

Element	%
Si	6.930
Ca	18.059
P	13.177
Al	2.859
S	12.864
Cl	20.091
K	14.546
Mg	1.728
Fe	3.511
Na	6.235
Ti	0
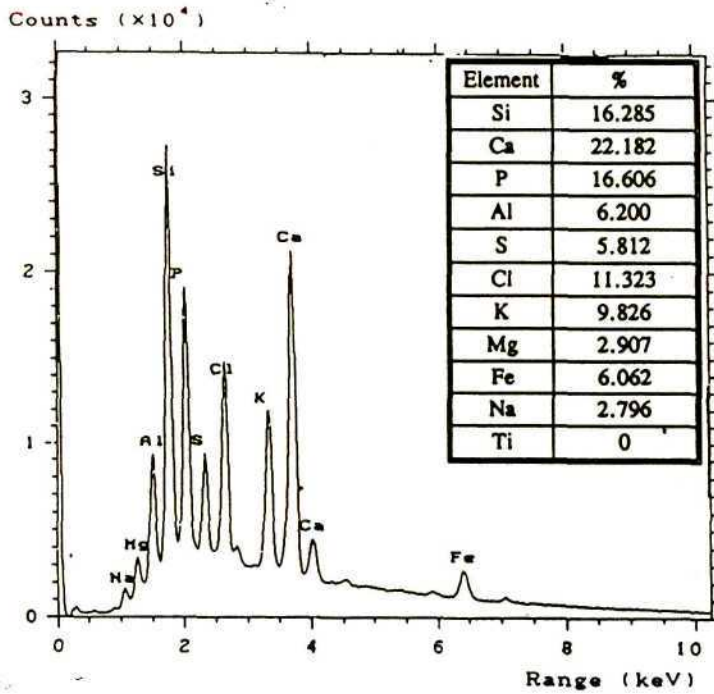
Figure 46: EDX spectrum of sludge sampled from "Tank 2" after addition of cow manure.

Element	%
Si	16.285
Ca	22.182
P	16.606
Al	6.200
S	5.812
Cl	11.323
K	9.826
Mg	2.907
Fe	6.062
Na	2.796
Ti	0

Counts $(\times 10^4)$

Element	%
Si	26.637
Ca	18.009
P	11.172
Al	2.122
S	6.411
Cl	14.159
K	12.965
Mg	2.375
Fe	2.205
Na	3.944
Ti	0

Range (keV)
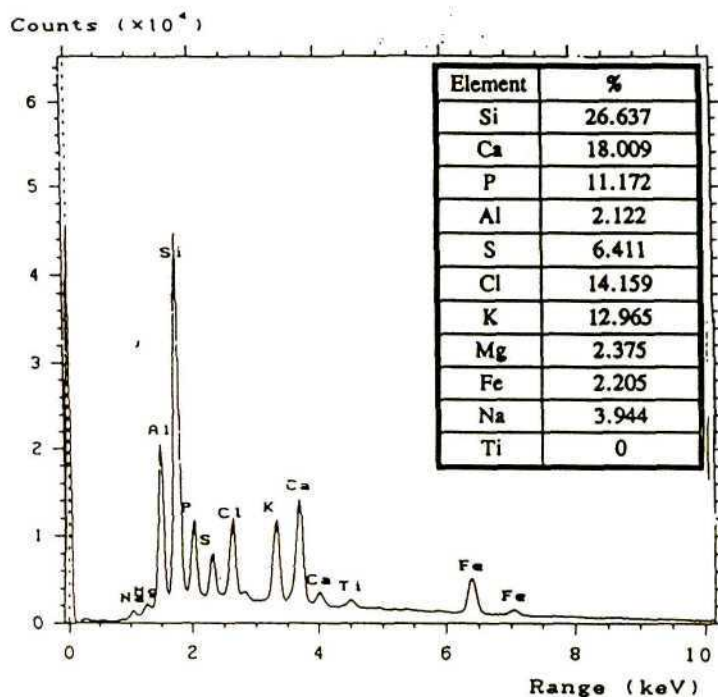
Figure 47: EDX spectrum of sludge sampled at "Tank 2 outlet" after addition of cow manure.

7.3 CONCLUSIONS

Energy Dispersive X-ray Microanalysis allows ratios of elements to be determined. From this study it was evident that the ratios of elements within samples varied considerably. The elemental compositions and ratios of the various elements detected should effect the microbial populations present as specific elements are required by the microorganisms for growth and metabolism. Elemental analysis is useful to identify suitable sludges, that do not contain toxic or inhibitory heavy metals, for inoculation of bioreactors. Energy Dispersive X-ray Microanalysis can also identify inhibitory elements and thus explain bioreactor failure. Its use, however, for a rural community bioreactor may not be justified on a cost basis.

Elemental composition changes are common in anaerobic bioreactors. For example, with changes in the pH-alkalinity-carbon dioxide balance, calcium carbonate may precipitate and thus reduce the calcium concentration. Iron and manganese are readily soluble in their lower oxidation states but relatively insoluble in their higher oxidation states. Therefore,

these cations may precipitate out depending upon the redox potential. It is also documented that changes in pH may produce secondary changes in certain inorganic constituents such as iron and manganese (American Public Health Association, 1980). This could explain the differences in the elemental compositions noted between the sludge and supernatant samples.

Energy Dispersive X-ray Microanalysis is a useful tool to qualitatively determine the presence of key elements (Hiam, Hann, Furr and Russel, 1995). This is particularly important since there has been concern that widespread use of on-site sanitation schemes may cause subsurface migration of contaminants (Fourie and van Ryneveld, 1995). This, however, should not be the case with the Pennells system as it was enclosed in PVC. Placing sludge on land provides essential micronutrients (Zn, Cu, Mo and Mn) but contamination by highly toxic organic constituents of sludge and the accumulation of toxic heavy metals can occur. Contaminants originating from on-site sanitation systems are microbiological and chemical (nitrogen and phosphorus in the form of nitrates and phosphates, respectively) in nature (Fourie and van Ryneveld, 1995). Energy Dispersive X-ray Microanalysis would thus facilitate the detection of phosphate and thus predict possible environmental impact potential.

A limitation of EDX is that it can not detect carbon and nitrogen. From this study, it was concluded that EDX should be made in conjunction with either atomic absorption spectrophotometry or X-ray fluorescence spectroscopy which are quantitative methods for elemental analysis. The method of EDX, however, has been used by Fang and Yan (1995) to determine the local concentrations of individual organic elements within anaerobic granules. The need to use EDX in conjunction with a more quantitative method was highlighted by Hiam *et al.* (1995) who found EDX to be a useful tool to determine the qualitative and quantitative effects of chlorexidine diacetate on yeasts but concluded that their quantitative data needed to be interpreted with caution and further work on methodologies was required to validate the results. The use of sophisticated equipment, however, would not be financially viable for rural community bioreactors.

CHAPTER 8

ISOLATION AND IDENTIFICATION OF MICROORGANISMS FROM DARVILL SLUDGE AND SLUDGE FROM THE PENNELLS BIOREACTOR

8.1. INTRODUCTION

Wastewater represents one of the most complex microbiological environments (Pelczar *et al.*, 1986), as discussed in Chapter 1. Numerous bacteria have been identified in anaerobic digesters as listed by Howgrave-Graham (1995).

Faecal coliform bacteria have been considered useful indicators of faecal contamination in water quality control and are more numerous than faecal streptococci in domestic sewage (American Public Health Association, 1985). Some examples of the latter microorganisms include: *Streptococcus faecalis, S. durans, S. faecium, S. bovis* and *S. equinus* (Anon, 1978). The species complement of sewage may change during its digestion. For example, facultative types (*Enterobacter, Alcaligenes, Escherichia, Pseudomonas* spp., etc.) predominate during the initial stages. These are succeeded by methane producers, eg. *Methanobacterium, Methanosarcina* and *Methanococcus* spp. (Pelczar *et al.*, 1986). The fermentation pattern of anaerobic digestion can also be manipulated by selective inoculation (Verstraete, Baere and Rozzi, 1981).

Wastewater sludges, in addition to sewage, contain organisms which are pathogenic to humans (Burge, Colacicco and Cramer, 1981; Snowdon, Cliver and Converse, 1988). These are present in high numbers in fresh manure and slurry (Bendixen, 1994). The microorganisms include viruses (Berg, 1978), bacteria and cysts of protozoa such as *Entamoeba* spp. and *Giardia* spp. together with ova of intestinal helminths, such as *Taenia* spp. (tapeworm), *Trichuris* spp. (whipworm) and *Ascaris* spp. (round worm) (Burge *et al.*, 1981; Ross *et al.*, 1992). The most important enteric pathogens of human sewage are shown in Table 17 (Burge, 1983).

Table 17: Major pathogens found in sewage and the diseases associated with these pathogens (Burge, 1983).

ORGANISM	DISEASE
Helminthic parasites (intestinal worms)	
Ascaris lumbricoides	Ascariasis
Ancylostoma duodenale	Hookworm infection
Necator americanus	Hookworm infection
Taenia saginata (beef tapeworm)	Taeniasis
Trichuris trichiura (whipworm)	Trichuriasis
Protozoa	
Entamoeba histolytica	Amoebic dysentery
Bacteria	
Salmonella spp.	Salmonellosis
Shigella spp.	Shigellosis
Mycobacterium tuberculosis	Pulmonary tuberculosis
Viruses	
Polio virus	Poliomyelitis
Coxsackie virus	Aseptic meningitis, gastroenteritis*
Echo virus	Aseptic meningitis**
Reo virus	Mild respiratory infection, gastroenteritis
Adeno virus	Acute respiratory infection***
Hepatitis virus	Infectious hepatitis

KEY:

*   Two of the diseases caused by several serotypes of this virus. Diseases caused range from trivial to lethal.

**   Diseases caused are similar to those of coxsackie virus.

***   Other diseases include pharyngitis and infant pneumonia.

There is a random distribution of pathogens in faeces (Collins and Lyne, 1984; Collins *et al.*, 1989). Gerhardt (1988) showed that faecal coliforms were present in the lowest numbers. Likely pathogens in faeces include: *Salmonella* spp., *Shigella* spp., *Vibrio* spp. (Collins and Lyne, 1984; Ross *et al.*, 1992), enteropathogenic *E. coli, C. perfringens* (both heat resistant and non-heat resistant), *Staphylococcus aureus, Bacillus cereus, B. subtilis, Campylobacter* spp., *Yersinia* spp., *Aeromonas* spp., *P. aeruginosa, Clostridium botulinum* and *C. difficile.* Commensals include coliform bacilli, *Proteus* spp., *Clostridium* spp., *Bacteroides* spp. and *Pseudomonas* spp. (Collins and Lyne, 1984; Collins *et al.*, 1989), *Giardia lamblia, Dientomoeba fragilis, Isospora belli* and *I. hominis* (Anon, 1978).

Effluents and sludges are generally monitored to ensure that the specific treatment reduces the numbers of pathogens (such as *Salmonella, Listeria* and *Campylobacter* spp.) which can survive in both water and soil if the sludge is land spread (Collins *et al.*, 1989).

Over 100 different virus types are known to be excreted in human faeces (Gersberg *et al.*, 1989). Viruses present in sludge usually include those causing infectious hepatitis, poliomyelitis, sore throats, gastroenteritis and HIV (Pelczar *et al.*, 1986; Ross *et al.*, 1992). The last is fairly stable in wastewater for up to 12 hours (Casson, Sorber, Palmer, Enrico and Gupta, 1992).

Sewage harbours several bacteriophages including a high proportion of somatic coliphages and F-specific phages (Dhillon, Dhillon, Chau and Tsang, 1976; Havelaar and Pot-Hogeboom, 1988; Havelaar, Pot-Hogeboom, Furuse, Pot and Hormann, 1990). Anaerobic enteric bacteria infected by phages include: *Bacteroides* spp. eg. *B. fragilis* (Boothe, van Tassell, Johnson and Wilkins, 1979; Kai, Wantanabe, Furuse and Osawa, 1985; Tartera and Jofre, 1987); *Bifidobacterium* spp. (Youssef, Muller-Buethow and Hanel, 1970); *Fusobacterium* spp. (Tamada, Harasawa and Shingo, 1985) and clostridia (Taylor and Gruha, 1974; Sell, Scharberg and Fekety, 1983; Maeda, Tshii, Tamaka, Mikami and Arai, 1986). Phages of *Bacteroides fragilis* and *Bifidobacterium brevi* occur exclusively in human faeces (Havelaar, 1991; Ito, Ohno and Tanoka, 1992).

Little information is available on the long term fate of biological indicators of pollution,

such as total coliform bacteria, in wetlands (Wood and Pybus, 1993). Coliforms, pathogenic bacteria, enteric viruses, parasitic protozoa and worms are, however, removed by wetlands (Rodgers et al., 1985; Gersberg et al., 1989).

The specific objective of this part of the research programme was to isolate predominant microbial species (2.6) characteristic of the four-stage model of metabolism together with pathogens known to occur in sewage. The objective was to, thus, characterize the species complement of different sludges since total populations, as well as the distribution of physiological types, are subject to wide fluctuations particularly in response to wastewater composition variations (Pelczar et al., 1986). Microorganisms were identified (2.7) on the basis of their morphological and biochemical characteristics by a number of standard tests (2.7.3). The sludges used were as per Table 13 (Syringes 1 and 3). Together with these, enterobacteria were also isolated from laboratory batch cultures (2.3.1). Although three cultures of each isolate from each sludge were made, biochemical tests were made on only one monoculture of each isolate from each sludge.

In a separate study, the bacteriophage titres were determined by L. Gozo by enumerating the plaque-forming units (p.f.u) (ie. number of phage particles able to cause lysis) at high phage dilution of the sludge/supernatant of the Pennells tanks. L. Gozo (1993) also determined the time-course effect of temperature on inactivation of the bacteriophages to give an indication of the efficacy of the digestion process to eliminate pathogens within the bioreactor.

8.2 EXPERIMENTAL: RESULTS AND DISCUSSION

8.2.1 *BACTEROIDES*

Isolated colonies on the Hungate's Habitats Stimulating medium (2.6.2) were small, circular to slightly irregular, entire, convex and smooth, and grey/white in colour. Table 18 shows the results of the various biochemical tests made. *Bacteroides* species are chemoorganotrophs and metabolize carbohydrates or peptone (Buchanan and Gibbons, 1974).

Table 18: Biochemical tests results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor with medium selective for *Bacteroides* spp.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	-	-
H$_2$S Production	+	+	+
Indole Production	+	+	+
Nitrate	Reduced	Reduced	Reduced
Litmus Milk	Rennet reaction	Rennet reaction	Rennet reaction
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+ (adonitol and mannitol producing a weak reaction)	+ (adonitol and mannitol producing a weak reaction)	+ (adonitol and mannitol producing a weak reaction)
Gram Character	-ve	-ve	-ve
Cell Morphology	Uniform to pleomorphic rods	Uniform to pleomorphic rods	Uniform to pleomorphic rods

The results of Table 18 show that *Bacteroides* spp. were probably present in all the sludge samples. *Bacteroides* spp. have been identified in anaerobic digesters as listed by Howgrave-Graham (1995). They are dominant members of the normal intestinal microbiota of humans and other animals (Atlas and Bartha, 1987) thus explaining their presence within the sludges. Table 18 indicates that, with the exception of the isolates from "Darvill Sludge", catalase was not produced. Buchanan and Gibbons (1974) reported that *Bacteroides* spp. usually do not produce catalase although it can be produced in trace amounts.

Common *Bacteroides* spp. in anaerobic digesters include: *B. fragilis* which is an opportunistic pathogen which is capable of indole production and strong fermentation of carbohydrates; and *B. putredinis* which effects positive indole production but negative or weak carbohydrate fermentation (Buchanan and Gibbons, 1974). *Bacteroides putredinis* is also known to produce catalase in trace amounts (Buchanan and Gibbons, 1974).

Bacteroides fragilis, B. putredinis and *B. furcosus* have similar colony morphologies and catabolic test results to those listed in Table 18 but they do not produce indole.

8.2.2 ACETOGENIC BACTERIA

When cultured on selective medium (2.6.3) the colonies appeared smooth, entire, approximately 2mm in diameter and were translucent with a light tan colour. Table 19 shows the biochemical test results.

Table 19: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor with medium selective for acetogenic bacteria.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	-	-	-
H₂S Production	+	+	+
Indole Production	+	+	+
Urease Production	+	+	+
Nitrate	Reduced	Reduced	Reduced
Litmus Milk	Rennet reaction	Rennet reaction	Rennet reaction
Methyl Red	+	+	+
Voges-Proskauer	+	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+ (no gas from lactose and maltose)	+ (no gas from lactose and maltose)	+ (no gas from lactose and maltose)
Tartrate Succinate Propionate	- - weak +	- - weak +	- - weak +
Gram Character	-ve	-ve	-ve
Cell Morphology	Slightly curved to rod shaped	Slightly curved to rod shaped	Slightly curved to rod shaped

No bacteria morphologically resembling *Lachnospira* and *Selenomonas* were observed. Microscope examination and the biochemical test results indicated that the isolates were all possibly *Butyrivibrio* species. Members of the genus *Butyrivibrio* are biochemically versatile (Buchanan and Gibbons, 1974). In this study the isolates produced H_2S although, according to Buchanan and Gibbons (1974), H_2S production is variable. *Butyrivibrio* species have been isolated in granular sludge from a domestic sewage UASB-reactor (Novaes, Rech, Fiqueirido and Giaj-Cevra, 1988).

8.2.3 BUTYRIC ACID BACTERIA

When cultured on agar-set selective medium, the isolates were turbid, white to slightly yellow in colour, while in liquid culture the meniscus appeared foamy, possibly due to gas production via fermentation. The results of the Gram stains and biochemical tests (Table 20) indicated that *Clostridium* spp. were, possibly, present.

With antigenic fingerprinting, *Clostridium populeti* and *Desulfobulbus propionicus* were identified in the "Darvill Sludge" but not in the Pennells bioreactor sludge which probably indicated bacterial inactivation following inoculation.

Clostridium perfringens C1051 has been isolated from human faeces (Allison and MacFarlane, 1989) and may be used as an indicator of human faecal pollution of water (Frobisher, 1962). Cellulolytic anaerobic cattle dung digesting sludge is documented to contain *Clostridium cellobioparum* and *C. populeti* (Kelkar, Gadre and Ranade, 1990). Clostridia, especially *C. proteolyticum* (Jain and Zeikus, 1987), are important in protein and nucleic acid degradation (Holland *et al.*, 1987). Britz, Spangenberg and Venter (1994) found *C. bifermentans* to be the dominant anaerobic species isolated from a digester treating sewage works sludge. Various *Clostridium* species have been identified in anaerobic digesters as listed by Howgrave-Graham (1995).

Table 20: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludge from both tanks of the Pennells bioreactor with medium selective for butyric acid bacteria.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	-	-
H_2S Production	+	+	+
Indole Production	+	+	+
Urease Production	-	-	-
Litmus Milk	Rennet reaction	Rennet reaction	Rennet reaction
Methyl Red	+	+	+
Voges-Proskauer	+	+	+
Adonitol, trehalose, mannose, rhamnose, D-xylose, L-arabinose, D- glucose, D-fructose, D-galactose, lactose, raffinose, mannitol, sorbitol, glycerol, sucrose & maltose	+ (-adonitol)	+ (-adonitol)	+ (-adonitol)
Tartrate & succinate, Propionate	- - +	- - +	- - +
Gram Character	+ve*	+ve*	+ve*
Cell Morphology	Rod shaped	Rod shaped	Rod shaped

KEY:

* = Clostridia are documented to be Gram-positive in the early stages of growth but become Gram-variable later (Buchanan and Gibbons, 1974).

8.2.4 LACTIC ACID BACTERIA

The colonies isolated were creamy white in colour and mucoid in appearance. According to Buchanan and Gibbons (1974), extracellular polysaccharide is known to be produced when lactic acid bacteria are cultured in a sucrose-containing medium. Lactic acid bacteria metabolism is documented to be fermentative where at least half the carbon end products of carbohydrate metabolism are lactate (Buchanan and Gibbons, 1974). The isolates were not positively identified but their biochemical characteristics approximated to lactobacilli (Table 21).

Table 21: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor with medium selective for lactic acid bacteria.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	-	-	-
H₂S Production	-	-	-
Indole Production	-	-	-
Urease Production	-	-	-
Nitrate	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite
Methyl Red	-	-	-
Voges-Proskaeur	+	+	+
Oxidase	-	-	-
Growth on Koser's Citrate Medium[1]	-	-	-
Trehalose, mannose, rhamnose & D-xylose	Variable	Variable	Variable
D-fructose	+	+	+
L-arabinose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose, lactose	–	-	-
Tartrate, succinate Propionate	- Variable reaction	- Variable reaction	- Variable reaction
Gram Character	+ve	+ve	+ve
Cell Morphology	Straight to curved rod, to coccobacilli	Straight to curved rod, to coccobacilli	Straight to curved rod, to coccobacilli

KEY:[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

8.2.5 PROPIONIC ACID BACTERIA

On the selective medium, the colonies were white in colour and were often small with a

"feathery" appearance. Table 22 shows the biochemical test results.

Table 22: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor with medium selective for propionic acid bacteria.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+	+
H$_2$S Production	+	+	+
Urease Production	-	-	-
Nitrate	Not Reduced	Not Reduced	Not Reduced
Voges-Proskaeur	+	+	+
Growth on Koser's Citrate Medium[1]	+	+	+
Oxidase	+	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+	+	+
Tartrate	-	-	-
Succinate	+	+	+
Propionate	+	+	+
Gram Character	+ve	+ve	+ve
Cell Morphology	Pleomorphic rods	Pleomorphic rods	Pleomorphic rods

KEY: [1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

From the biochemical tests (Table 22) it appeared that all the isolates were possibly *Propionibacterium avidum*. This bacterium is anaerobic to aerotolerant and is able to grow on the surface of agar-set medium incubated aerobically (Buchanan and Gibbons, 1974; Holt, Krieg, Sneath, Staley and Williams, 1994). After several transfers this bacterium usually grows exceedingly well on the surface of aerobic agar-set plates (Buchanan and Gibbons, 1974) as was observed in this study. *Propionibacterium avidum* has also been isolated from faeces (Buchanan and Gibbons, 1974).

8.2.6 ENTEROBACTERIA

Numerous colonies developed on the Eosin Methylene Blue (EMB) and MacConkey agar (2.6.7) plates. On the latter, pH changes, due to acid production, were evidenced by a colour change from red to yellow. It is known that acid is produced by enterobacteria from the fermentation of glucose, other carbohydrates and alcohols (Buchanan and Gibbons, 1974). On EMB plates, the characteristic metallic green sheen, in the presence of reflected light, of *Escherichia coli* was evident with dark purple centres visualized with transmitted light. *Escherichia coli* was also clearly evident as pink/red colonies on the SS agar (2.6.7) and green/yellow colonies on the BGA (2.6.7). Lactose/sucrose fermenters (which include *E. coli*) are distinguished on BGA by the yellow to greenish-yellow colonies surrounded by yellow-green coloration of the agar, with non prolific growth (Bridson, 1990). *Escherichia coli* from the "Darvill Sludge" and sludges from the Pennells bioreactor and from the laboratory batch culture (containing "Darvill Sludge") was further characterized by API 20E identification strips and additional biochemical tests (Table 23).

Table 23: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor and laboratory batch culture with medium selective for *E. coli*.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate	RESULT Laboratory batch culture
Catalase	+	+	+	+
H₂S Production	-	-	-	-
Indole Production	+	+	+	+
Urease Production	-	-	-	-
Nitrate	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite
Methyl Red	+	+	+	+
Voges-Proskauer	-	-	-	-
Oxidase	-	-	-	-
Adonitol, trehalose, rhamnose, L-arabinose, D-glucose, D-fructose, mannitol, sorbitol, maltose, lactose	+ (-adonitol)	+ (- adonitol)	+ (-adonitol)	+ (- adonitol)
Gram Character	-ve	-ve	-ve	-ve
Cell Morphology	Rod	Rod	Rod	Rod

Klebsiella species were isolated on Enterobacteria Selective Agar (2.6.7) and identified with API 20E identification strips and additional biochemical tests (Tables 24-26). Tentative identification showed *Klebsiella pneumoniae*, *Klebsiella ozaena*, a subspecies of *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates.

Table 24: Biochemical test results of monocultures isolated from "Darvill Sludge" with medium selective for *Klebsiella pneumoniae*.

TEST	RESULT "Darvill" isolate
Catalase	+
H_2S Production	-
Indole Production	+
Urease Production	+
Nitrate	Reduced to nitrite
Methyl Red	-
Voges-Proskauer	+
Growth on Koser's Citrate Medium[1]	+
Oxidase	-
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-galactose, raffinose, adonitol, sorbitol, glycerol, sucrose, maltose, lactose	+
fructose & mannitol	-
Tartrate, succinate	-
Propionate	+
Gram Character	-ve
Cell Morphology	Rod

KEY:

[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

Table 25: Biochemical test results of monocultures isolated from "Darvill Sludge" and "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor with medium selective for *Klebsiella ozaena*.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+	+
H$_2$S Production	-	-	-
Urease Production	+	+	+
Nitrate	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite
Methyl Red	+	+	+
Voges-Proskauer	-	-	-
Oxidase	-	-	-
D-xylose, L-arabinose, D-glucose, mannitol, sorbitol, sucrose, maltose, lactose	+	+	+
Gram Character	-ve	-ve	-ve
Cell Morphology	Rod	Rod	Rod

Table 26: Biochemical test results of monocultures isolated from "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor and laboratory batch cultures with medium selective for *Klebsiella oxytoca*.

TEST	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate	RESULT Laboratory batch culture
Catalase	+	+	+
H$_2$S Production	-	-	-
Indole Production	+	+	+
Urease Production	+	+	+
Nitrate	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite
Litmus Milk	Acid formation	Acid formation	Acid formation
Methyl Red	-	-	-
Voges-Proskauer	+	+	+
Oxidase	-	-	-
Growth on Koser's Citrate Medium[1]	+	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+	+	+
Tartrate, succinate & propionate	-	-	-
Gram Character	-ve	-ve	-ve
Cell Morphology	Rod	Rod	Rod

KEY:

[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

According to Holt *et al.* (1994), *K. pneumoniae* has a polysaccharide capsule which gives rise to large mucoid colonies, similar to those found in this study. Britz *et al.* (1994) isolated 12 strains of *K. pneumoniae* from a digester treating sewage sludge. *Klebsiella pneumoniae* and *K. oxytoca* are normally found in the intestinal tract of man and animals (Krieg and Holt, 1984). Various *Klebsiella* spp. have been identified in anaerobic digesters

as listed by Howgrave-Graham (1995).

Hafnia alvei (formerly *Enterobacter alvei*) (Krieg and Holt, 1984) was isolated from both tanks of the Pennells bioreactor and preliminarily identified by API 20E identification strips and biochemical tests (Table 27).

Table 27: Biochemical test results of monocultures isolated from "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor with medium selective for *Hafnia alvei*.

TEST	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+
H$_2$S Production	-	-
Indole Production	-	-
Urease Production	-	-
Nitrate	Reduced	Reduced
Methyl Red	-	-
Voges-Proskauer	+	+
Oxidase	-	-
Litmus Milk	Alkaline reaction	Alkaline reaction
Growth on Koser's Citrate Medium[1]	+	+
β-galactosidase	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, glycerol, sucrose, maltose	+	+
Adonitol, sorbitol & lactose	-	-
Tartrate, succinate & propionate	-	-
Gram Character	-ve	-ve
Cell Morphology	Rod	Rod

KEY:[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

Acinetobacter lwoffii, or a *Moraxella* species, was isolated from the "Darvill Sludge" and the sludges from the Pennells bioreactor and preliminarily identified with API 20E identification strips. This organism was Gram-negative and catalase positive. Both *Acinetobacter lwoffii* and *Moraxella* species have been isolated from anaerobic digesters (Britz *et al.*, 1994).

Aeromonas caviae was isolated from "Tank 1 outlet" and the laboratory batch culture experiment and identified with an API 20E identification strip. According to Krieg and Holt (1984), *Aeromonas caviae* is found in sewage. Aeromonad species previously isolated from faeces, especially in patients with diarrhoea, include *A. caviae, A. sobria* and *A. hydrophila* (Gray and Griffiths, 1990).

The denitrifying bacterium *Sphingobacterium multivorum* (Holmes, 1991; Holt *et al.*, 1994) was isolated from sludges from the Pennells bioreactor and tentatively identified with API 20E identification strips in conjunction with biochemical tests (Table 28).

Table 28: Biochemical test results of monocultures isolated from "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor with medium selective for *Sphingobacterium multivorum*.

TEST	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+
H$_2$S Production	-	-
Indole Production	-	-
Urease Production	-	-
Nitrate	Not reduced	Not reduced
Litmus Milk	Decolorization	Decolorization
Methyl Red	+	+
Voges-Proskauer	+	+
Oxidase	+	+
Growth on Koser's Citrate Medium[1]	-	-
Trehalose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, sucrose, maltose, lactose	+	+
mannose, mannitol & sorbitol	-	-
Tartrate & propionate	+	+
Succinate	-	-
Gram Character	-ve	-ve
Cell Morphology	Rod	Rod

KEY:

[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

Organisms in Group C of *Flavobacterium* spp. are now termed *Sphingobacterium* spp. (Yabuuchi, Kaneko, Yano, Moss and Miyoshi, 1983). *Sphingobacterium multivorum* is documented to produce a pale yellow pigment specifically when grown on blood agar (Krieg and Holt, 1984; Balows, Truper, Dworkin, Horder and Schleifer, 1991).

Citrobacter freundii was isolated and tentatively identified (Table 29).

Table 29: Biochemical test results of monocultures isolated from "Darvill Sludge", and "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor and laboratory cultures inoculated with "Darvill Sludge" with medium selective for *Citrobacter freundii*.

TEST	RESULT "Darvill Sludge" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate	RESULT Laboratory batch culture
Catalase	+	+	+	+
H$_2$S Production	+	+	+	+
Indole Production	-	-	-	-
Urease Production	+	+	+	+
Nitrate	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite	Reduced to nitrite
Methyl Red	+	+	+	+
Voges-Proskauer	-	-	-	-
Oxidase	-	-	-	-
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+ (-raffinose)	+ (-raffinose)	+ (-raffinose)	+ (-raffinose)
Gram Character	-ve	-ve	-ve	-ve
Cell Morphology	Rod	Rod	Rod	Rod

The morphology of *Citrobacter freundii* resembles *E. coli* (Krieg and Holt, 1984). This bacterium has been isolated from human faeces (Buchanan and Gibbons, 1974) and from anaerobic digesters, as listed by Howgrave-Graham (1995), and a laboratory digester treating sewage sludge (Britz *et al.*, 1994).

Kluyvera ascorbata/cryocresc was isolated from the Pennells bioreactor but not from "Darvill Sludge" and identified by API 20E identification strips and other biochemical tests (Table 30) . *Kluyvera ascorbata* occurs in sewage, food and soil (Krieg and Holt, 1984).

Table 30: Biochemical test results of monocultures isolated from "Tank 1 outlet" and "Tank 2" sludges from the Pennells bioreactor with medium selective for *Kluyvera ascorbata*.

TEST	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+
Indole Production	-	-
Nitrate	Not reduced	Not reduced
Litmus Milk	Acid formation	Acid formation
Growth on Koser's Citrate Medium[1]	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+	+
Tartrate, succinate & propionate	+	+
Gram Character	-ve	-ve
Cell Morphology	Rod	Rod

KEY:

[1] = For critical work or in case of doubt at least 3 serial subcultures had to be made in the medium before a definite positive was recorded.

Gram-negative rods of a *Salmonella* species were isolated from the batch system inoculated with "Darvill Sludge". These bacteria were clearly evident on the BGA as red colonies surrounded by a bright red medium (Bridson, 1990).

Staphylococci were isolated from Pennells bioreactor sludges on *Staphylococcus* 110 selective agar. The colonies were initially cream/white in colour with orange-tinged edges. *Staphylococcus* 110 medium facilitates the isolation of pathogenic staphylococci based on salt tolerance, orange pigmentation, mannitol fermentation and gelatin liquefaction (Bridson, 1990). Gram stains revealed Gram-positive cocci.

Gram-positive spherical-shaped bacteria were isolated on Enterococcus Selective Agar from the batch culture inoculated with "Darvill Sludge". The colonies were red in colour and darkened slightly.

From the laboratory batch culture inoculated with "Darvill Sludge", pink mucoid colonies with dark centres were evident on MacConkey agar and pH changes were noted in the surrounding medium. Biochemically, these colonies resembled the genus *Enterobacter*. *Enterobacter aerogenes*, for example, is a Gram-negative rod which occurs in the faeces of man and other animals, soil, water and dairy products (Buchanan and Gibbons, 1974; Krieg and Holt, 1984; Pelczar *et al.*, 1986).

8.2.7 SULPHATE-REDUCING BACTERIA

The selective sulphate reducer isolation medium turned black and black colonies of sulphate-reducing bacteria were observed. Table 31 shows the biochemical test results.

Table 31: Biochemical test results of monocultures isolated from "Darvill Sludge" and sludges from "Tank 1 outlet" and "Tank 2" of the Pennells bioreactor with medium selective for *Desulfovibrio* and *Desulfotomaculum* spp.

TEST	RESULT "Darvill" isolate	RESULT "Tank 1 outlet" isolate	RESULT "Tank 2" isolate
Catalase	+	+	+
H$_2$S Production	+	+	+
Indole Production	+	+	+
Urease Production	-	-	-
Nitrate	Not reduced	Not reduced	Not reduced
Litmus Milk	Rennet reaction	Rennet reaction	Rennet reaction
Methyl Red	+	+	+
Voges-Proskauer	+	+	+
Trehalose, mannose, rhamnose, D-xylose, L-arabinose, D-glucose, D-fructose, D-galactose, raffinose, mannitol, adonitol, sorbitol, glycerol, sucrose, maltose & lactose	+ (no gas from adonitol or glycerol)	+ (no gas from adonitol or glycerol)	+ (no gas from adonitol or glycerol)
Tartrate, succinate & propionate	+	+	+
Gram Character	-ve	-ve	-ve
Cell Morphology	Curved to spiral shaped	Curved to spiral shaped	Curved to spiral shaped

Comparisons of the isolates with light and electron microscopy studies (6.2.1) supported the possible presence of *Desulfovibrio* spp.; possibly *Desulfovibrio gigas*, a spiral bacterium with polar tufts of flagella (lophotrichous arrangement) (Plates 4A-4C). Cells of *Desulfovibrio gigas* often occur in chains appearing as spirilla (Krieg and Holt, 1984). Sulphate-reducing bacteria, predominantly of the genus *Desulfovibrio*, have been isolated from cattle faeces (Carli, Diker and Eyigar, 1995). Thus, the addition of cow manure could have inoculated these bacteria into the bioreactor. Microbial population changes were noted after the addition of cow manure (6.2.2).

8.2.8 ARCHAEBACTERIA

R. Silver identified *Methanocorpusulum parvum*, *Methanosaeta soehngenii* OPFIKON and *Methanobacterium bryantii* in the "Darvill Sludge". These were not present in "Tank 1 outlet" sludge but were present in "Tank 2", after "Darvill Sludge" inoculation of the Pennells bioreactor (R. Silver, personal communication) possibly indicating a lack of anaerobiosis and/or other bactericidal/bacteriostatic conditions for the methanogens. The methanogens thus appeared to have been displaced with time. Acetate degradation by the acetoclastic methanogens is the step which confers stability to the anaerobic digestion process (Siegrist *et al.*, 1993). If optimum conditions within the Pennells bioreactor were maintained a stable methanogenic fermentation should have resulted.

8.2.9 BACTERIOPHAGES

Various bacteriophages including those parasitic to *E. coli* B were detected in different sludge samples (Gozo, 1993). Table 32 shows the phage titres.

Gozo (1993) documented that bacteriophage growth was affected by temperature, with these effects varying within the two tanks of the Pennells bioreactor. In laboratory experiments with higher temperatures of 37 and 50°C no survival occurred although the bacteriophages were capable of replicating at 4 and 25°C (Gozo, 1993). This indicates that the phages had possibly acclimatised to the cooler temperatures (4.2.2) within the Pennells bioreactor.

Table 32: Sludge phage titres.

Sample Point	Dilution	Phage Titre (p.f.u ml^{-1})
Tank 1 Outlet	10^0	*
Tank 1 Outlet	10^{-2}	1×10^5
Tank 1 Outlet	10^{-4}	5×10^6
Tank 2 Inlet	10^0	Lysis
Tank 2 Inlet	10^{-2}	*
Tank 2 Inlet	10^{-4}	2.4×10^7
Tank 2	10^0	*
Tank 2	10^{-2}	8×10^4
Tank 2	10^{-4}	2×10^6
Tank 2 Outlet	10^0	*
Tank 2 Outlet	10^{-2}	8×10^4
Tank 2 Outlet	10^{-4}	1×10^6

KEY: * = Close to confluent lysis.

8.3 DISCUSSION

The bacterial populations within both tanks of the Pennells bioreactor were found to be heterogeneous and specific physiological groups of bacteria were isolated (Table 33).

Table 33: Summary of bacterial populations isolated.

Bacteroides
Butyrivibrio
Clostridium spp
Lactic acid bacteria
Propionic acid bacteria
Enterobacteria

Although various species were preliminarily identified, many of these could have originated from the "Darvill Sludge" which was added just prior to the isolations. The bacterial populations of sewage vary from place to place (Murray, 1987) and reflect the operating conditions, influent substrate (Meynell, 1982; Britz *et al.*, 1994) and specific

perturbations (5.3). The inoculum is also important and can change the fermentation balance (Verstraete *et al.*, 1981). Thus, inoculations of "Darvill Sludge" and cow manure could have effected such changes. Some of the species inoculated could have, in turn, been displaced from the bioreactor in response to the specific selection pressures. This was noted by microscopy (6.2.1 and 6.2.2). The methods of enrichment and isolation must also be questioned since they were specific for fast growing species to the possible exclusion of slower growing bacteria (Parkes, 1982).

Many of the bacteria isolated, such as *Escherichia coli* and *Klebsiella* spp., were pathogens. Generally, there is a random distribution of pathogens in faeces (Collins and Lyne, 1984; Collins *et al.*, 1989) although these species constitute a limited percentage of the bacterial mass of faeces (Degrémont, 1991). The presence of pathogens from the community justified the use of the bioreactor to minimize entry into the water source and thus prevent possible disease.

The influent composition determines species dominance (especially the hydrolytic and acidogenic species (Zeikus, 1982)) and, hence, diversity in the stabilization process unless the environment changes (Britz *et al.*, 1994). Such changes were noted in the bioreactor under study particularly in relation to inoculations (6.2.2) and perturbant additions (5.3).

In the Pennells bioreactor various acidogens were present and were provisionally identified. Principal among these were *Clostridium* spp., *Bacteroides* spp., *Butyrivibrio* spp., sulphate-reducing bacteria and *E. coli*. The methanogens, however, were generally absent or inactive. It is documented that the acidogenic population is generally greater and that its behaviour affects the subsequent methanogenic phase. The acidogens are rate limiting in terms of providing the methanogens with a suitable carbon source (Britz *et al.*, 1994).

The Pennells bioreactor sludges contained phages (Gozo, 1993) which possibly reflected a lack of anaerobiosis since phages and viruses are, generally, inactive in anaerobic digestion processes. The source of the bacteriophages was not confirmed since the "Darvill Sludge" was not titrated.

SUMMARY

The main objective of this study was to commission and maintain a Pennells two-tank bioreactor in a rural community which lacked electricity and a domestic waste treatment facility and to determine whether the bioreactor could satisfy the dual objectives of sewage treatment and biogas production in rural KwaZulu-Natal.

Unfortunately, the system suffered as a result of inefficient operation and maintenance, largely due to social/community-related problems which are typical of rural regions of Third World countries. In addition, it was unfortunate that the artificial wetland could not be evaluated as a secondary means of purification as these systems are documented to be efficient in this regard.

During operation, problems were clearly apparent. In particular, the bioreactor suffered from a lack of complete anaerobiosis thus, possibly, inhibiting the methanogens. Although depressed temperatures, unfavourable volatile fatty acid:alkalinity ratios and high pH values were recorded, COD removals still resulted. As such, the Pennells bioreactor equated an aqua-privy/septic tank rather than an anaerobic digester. The efficacy of the bioreactor as a sanitation facility was, thus, confirmed although little evidence was gained of pathogen destruction. The provision of a sanitation system in an area lacking in sewage treatment was nevertheless advantageous, especially with regards to concerns over environmental pollution. Unfortunately, the second role of biogas generation was not fulfilled.

Other problems were also experienced including: blockages; and possible destruction/inhibition of the microbial populations over time and with respect to perturbants and erratic use of the facility. Some of the problems could possibly be eliminated by: maintenance of strict anaerobiosis; mixing in the tanks; provision of insulation; and, perhaps more importantly, education/training. Sensitive education combined with pictorial instruction are essential.

Together with an appropriate bioreactor, many factors are involved in effective gas

generation. These include community involvement (Nhlapo, 1996) linked to sound education. It is imperative that the community members should, at all times, be involved in the local planning, organization and bioreactor installation (Blair Research Institute, World Health Organization, 1992; S.A. Water Research Commission, 1993a;b; Jackson, 1996). Unfortunately, despite particular emphasis on community involvement in the installation and operation of the bioreactor, social problems still prevailed.

The tools of light, fluorescence and scanning electron microscopy employed in this study were useful to visualize microbial morphotypes within the bioreactor and to show population changes both with time and in response to inoculations. Energy Dispersive X-ray Microanalysis qualitatively identified the elements present within the various sludges although, as discussed, this analysis should be made in conjunction with a quantitative method such as atomic absorption spectroscopy. Both these approaches are, however, unrealistic in a rural situation.

With respect to community participation, the population should be encouraged to maintain the bioreactor through, for example, addition of cow manure and grass. Simple monitoring should, however, be undertaken by a local support laboratory.

Laboratory-scale batch cultures proved effective to assess various perturbations of the microbial populations with particular respect to biogas production. The results identified the inhibitory nature of some locally used detergents and toxic compounds. Since these compounds should not be discharged through the bioreactor, their effective disposal could prove problematic. In contrast, antibiotics, such as penicillin and tetracycline, especially in low concentrations, stimulated gas production. Laboratory-scale batch cultures highlighted the need for a simple anaerobic bioassay to assess the impacts of perturbants on specific physiological groups of the operative microbial associations. A preliminary Code of Practice should thus be constructed whereby locally used commodities such as antiseptics, soap, bleaching agents and household cleaners should not be discharged through the Pennells bioreactor. The Code of Practice should once again be reinforced with education/training specific to the community.

REFERENCES

A'Bear, D.R. 1991. *Umgeni Water. Rural Areas Water and Sanitation Plan. Volume 1 and 2.* pp 1-356. Umgeni Water : South Africa.

Abbasi, S.A., Nipaney, P.C. and Panholzer, M.B. 1991. Biogas production from the aquatic weed Pistia (*Pistia stratiates*). *Bioresource Technology*, **37**: 211-214.

Adamus, P.R., Clarain, E.J., Smith, D.R. and Young, R.E. 1987. *Wetland Evaluation Technique (WET). Volume 2: Technical Report Y-87.* pp 1-206. US Army Corps of Engineers : Washington D.C.

Ahring, B.K. and Westermann, P. 1983. Toxicity of heavy metals to thermophilic anaerobic digestion. *European Journal of Applied Microbial Biotechnology*, **17**: 365-370.

Ahring, B.K., Angelidaki, I. and Johansen, K. 1991. Anaerobic treatment of manure together with industrial waste. Proceedings of the Sixth International Symposium on Anaerobic Digestion, Sáo Paulo, Brazil. pp 309-317.

Aivasidis, A. and Wandrey, C. 1990. Development and scale-up of a high-rate biogas process for treatment of organically polluted effluents. *Annals New York Academy of Sciences*, **589**: 599-615.

Alkan, U., Anderson, G.K. and Ince, O. 1996. Toxicity of trivalent chromium in the anaerobic digestion process. *Water Research*, **30**: 731-741.

Allison, C. and MacFarlane, G.T. 1989. Protease production by *Clostridium perfringens* in batch and continuous culture. *Letters in Applied Microbiology*, **9**: 45-48.

American Public Health Association. 1955. *Standard Methods for the Examination of Water, Sewage and Industrial Wastes.* Tenth Edition. pp 221-279. American Public Health Association : New York.

American Public Health Association. 1980. *Standard Methods for the Examination of Water and Wastewater.* Fifteenth Edition. pp 24-525. American Public Health Association : Washington D.C.

American Public Health Association. 1985. *Standard Methods for the Examination of Water and Wastewater.* Sixteenth Edition. pp 20-46. American Public Health Association : Washington D.C.

Anjaneyulu, K. 1988. Bioconversion of seaweed to biogas: a kinetic study. Proceedings of the Indian National Science Academy. *Biological Sciences*, **54**: 277-281.

Anjaneyulu, K., Tarwadi, S.J. and Mehta, D.J. 1989. Anaerobic digestion of seaweed for biogas : A kinetic evaluation. *Journal of Chemical Technology and Biotechnology*, **45**: 5-14.

Anon. 1978. *Methods for Microbiological Analysis of Water, Wastewater and Sediments*. pp 11.5, 11.6, 11-29, IVIK and IVII. Inland Water Directorate, Scientific Operations Division, Canada Centre for Inland Waters : Ontario, Canada.

Aragno, M. 1988. The landfill ecosystem : look inside a black box. The landfill : reactor and final storage. Swiss Workshop on Land Disposal of Solid Waste, Gernzee. pp 15-38. *Cited by*: Daneel, R.A., Percival, L.J. and Senior, E. 1997.

Archer, D.B. 1983. The microbiological basis of process control in methanogenic fermentations of soluble wastes. *Enzyme Microbiological Technology*, **5**: 162-170.

Archer, D.B. 1988. Report on the microbiological aspects of granulation. *In*: Lettinga, G., Zehnder, A.J.B., Grotehuis, J.T.C. and Hulshoff Pol, L.W. (eds). *Granular Anaerobic Sludge. Microbiology and Technology*. Proceedings of the Gasmat Workshop. pp 107-112. Pudoc Wageningen : Wageningen, The Netherlands.

Arnold, J.L. 1979. Sewage chemistry for civil engineers. *Water Pollution Control*, **78**: 364-370.

Ascoli, A., Pandya, J.D. and Redaelli, G. 1989. Electrical characterization of a 2.5kW phosphoric acid fuel cell stack operating on simulated reformed biogas. *Energy*, **14**: 875-878.

Ashare, E. 1981. Analysis of systems for purification of fuel gas. *In*: Wise, D.L. *Fuel Gas Production From Biomass*. Volume 2. pp 1-22. CRC Press : Boca Raton.

Ashare, E. and Buivid, M.G. 1981. Feasibility of anaerobic digestion of agricultural crop residues for production of methane. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 2. pp 23-46. CRC Press : Boca Raton.

Atlas, R.M. and Bartha, R. 1987. *Microbial Ecology : Fundamentals and Applications*. Second Edition. pp 374-402. The Benjamin Cummings Publishing Company : California.

Azhar, N.G. and Stuckey, D. 1994. The influence of chemical structure on the anaerobic catabolism of refractory compounds : A case study of instant coffee wastes. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 264-273.

Bailey, S.E., Zolteck, J., Hermann, A.J., Dolan, T.J. and Tortora, L. 1985. Experimental manipulation of nutrients and water in a freshwater marsh : effects on biomass, decomposition and nutrient accumulation. *Limnology and Oceanography*, **30**: 500-572.

Bala, B.K. and Satter, M.A. 1990. Kinetic and economic considerations of biogas production systems. *Biological Wastes*, **34**: 21-38.

Balasubramanian, P.R. and Bai, R.K. 1992. Biogas plant slurry utilization through *Wolffia* aquatic plant culture. *Environmental Ecology*, **10**: 247-251.

Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. 1991. *The Prokaryotes. A Handbook on the Biology of Bacteria; Ecophysiology; Isolation; Identification and Appreciations*. Second Edition. pp 3620-3630. Springer-Verlag : New York.

Barnes, D. and Fitzgerald, P.A. 1987. Anaerobic wastewater treatment processes. *In*: Forster, C.F. and Wase, D.A. (eds) *Environmental Biotechnology*. pp 57-113. Ellis Horwood : Chichester.

Batenin, V. 1990. Alternative power sources in use in the USSR. *Ambio*, **19**: 220-224.

Battersby, N.S. and Wilson, V. 1989. Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge. *Applied and Environmental Microbiology*, **55**: 433-439.

Beaty, P.S., McInerney, M.J. and Wofford, N.Q. 1987. Energetics of H_2 producing syntrophic bacteria. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 67-70. Noyes Data Corporation : New Jersey.

Beba, A. 1988. Analysis of solar heated biogas fermenter. *Solar Energy*, **40**: 281-287.

Beckett, P.H.T., Phil, D., Davis, R.D. and Brindley, P. 1979. The disposal of sewage sludge onto farmland: the scope of the problem of toxic elements. *Water Pollution Control*, **78**: 419-439.

Begg, G.W. 1990. The wetlands of Natal (Part 4): Policy proposals for the wetlands of Natal and KwaZulu. *Natal Town and Regional Planning Report*, **75**: 86.

Behmel, U., Leupold, G. and Vieweger, S. 1993. Production of biogas from vegetable wastes. I. Optimized hydrolysis of the lignocellulose components in spent grain. *Chemie Mikrobiologie Technologie der Lebensmittel*, **15**: 558-61.

Bendixen, H.J. 1994. Safeguards against pathogens in Danish biogas plants. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 629-638.

Berg, G. 1978. *Indicators of Viruses in Water and Food*. pp 334-348. Ann Arbor Science : Michigan.

Bessarabor, D.G., Sanderson, R.D., Teplyakov, W., Beckman, N. and Netrusov, A.I. 1994. New liquid membrane system for biogas separation. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 185-188.

Bisaria, R., Vasudevan, P. and Bisaria, V.S. 1990. Utilization of spent agro-residues from mushroom cultivation for biogas production. *Applied Microbiology and Biotechnology*, **33**: 607-609.

Biswas, D. 1989. Low cost biogas technology. *Environment and Ecology*, **7**: 1009-1011.

Blackwood, R.K. and English, A.R. 1977. Structure-activity relationships in the tetracycline series. *In*: Perlman, D. (ed) *Structure-Activity Relationships Among the Semisynthetic Antibiotics*. pp 397-426. Academic Press : New York.

Blair Research Institute, World Health Organization. 1992. *Appropriate Sanitation for Very Low Income Communities*. Volume 1: Background reader for a workshop held in Harare, Zimbabwe, November 1991. pp 1-36.

Bleicher, K. and Winter, J. 1994. Formate production and utilization by methanogens and by sewage sludge consortia - interference with the concept of interspecies formate transfer. *Applied Microbiology and Biotechnology*, **40**: 910-915.

Blum, J.W. and Speece, R.E. 1991. A database of chemical toxicity to environmental bacteria and its use in interspecies comparisons and correlations. *Research Journal Water Pollution Control Federation*, **63**: 198-207.

Boone, D.R. 1987. Thermodynamics of catabolic reactions in the anaerobic digester. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 85-96. Noyes Data Corporation : New Jersey.

Boopathy, R. 1987. Inoculum source for anaerobic fermentation
of coffee pulp. *Applied Microbiology and Biotechnology*, **26**: 588-594.

Boothe, S.J., van Tassell, R.L., Johnson, J.L. and Wilkins, T.D. 1979. Bacteriophages of *Bacteroides*. *Review of Infectious Diseases*, **1**: 325-334.

Borja, R., Martin, A., Luque, M. and Duran, M.M. 1993. Kinetic study of anaerobic digestion of wine distillery wastewater. *Process Biochemistry*, **28 (2)**: 83-90.

Boshoff, W.H. 1992. Methane production limitations. *Rēsource*, **5**: 1.

Boto, K.G. and Patrick, W.H. 1979. Role of wetlands in removal of suspended sediments. *In*: Greeson, P.E., Clark, J.R. and Clark, J.L. (eds) *Wetland Function and Values: The State of Our Understanding*. pp 479-489. American Water Resources Association : Minneopolis.

Bradley, R.M. 1981. Disposal of septic tank contents in Cyprus. *Water Pollution Control*, **80**: 131-138.

Brady, N.Y. 1984. *The Nature and Properties of Soil*. Ninth Edition. pp 632-639. Macmillan : New York.

Bridson, E.Y. 1990. *The Oxoid Manual*. Sixth Edition. pp 1-235. Unipath Limited : Hampshire.

Britz, T.J. 1975. 'n Studie van *Propionibacterium* spp uit suiwelprodukte. MSc Thesis, Universiteit van Pretoria.

Britz, T.J., Spangenberg, G. and Venter, C.A. 1994. Acidogenic microbial species diversity in anaerobic digesters treating different substrates. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 74-79.

Brix, H. 1987. Treatment of wastewater in the rhizosphere of wetland plants - the root zone method. *Water Science and Technology*, **19**: 107-118.

Brodie, G.A. 1989. Selection and evaluation of sites for constructed wastewater treatment wetlands. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 307-317. Lewis Publishers : Chelsea, Michigan.

Bruton, A. 1994. *Scanning Electron Microscopy - A Practical Handbook for Postgraduate Students*. pp 1-44. Electron Microscope Unit : Pietermaritzburg, South Africa.

Bryant, M.P., Campbell, L.L., Reddy, C.A. and Crabill, M.R. 1977. Growth of Desulfovibrio in lactate or ethanol low in sulphate in association with hydrogen utilizing methanogenic bacteria. *Applied and Environmental Microbiology*, **33**: 1162-1169.

Buchanan, R.E. and Gibbons, N.E. 1974. *Bergeys Manual of Determinative Bacteriology*. Eighth Edition. pp 1-1146. Williams and Wilkins : Baltimore.

Buchholz, K. 1988. Biotechnological advances in the production of alcohol and biogas from agricultural products. *Zucker Industrie*, **113**: 681-683.

Bucksteeg, K., Muller, H.H., Mortl, R., Adelt, H., Scheer, G., Labitzky, W., Sendl, H., Hormann, O. and Kastner, P. 1985. Initial experience with 12 wetland plant treatment systems. *Korrespondenz Abwasser*, **32**: 376-385.

Burge, W.D. 1983. Monitoring pathogen destruction. *BioCycle*, **24**: 48-53.

Burge, W.D., Colacicco, D. and Cramer, W.N. 1981. Criteria for achieving pathogen destruction during composting. *Journal of Water Pollution Control*, **53**: 1683-1690.

Callander, I.J. and Barford, J.P. 1983. Precipitation, chelation and the availability of metals as nutrients in anaerobic digestion: methodology. *Biotechnology and Bioengineering*, **25**: 1947-1957.

Capone, D.G., Reese, D.D. and Kiene, R.P. 1983. Effects of metals on methanogenesis, sulphate reduction, carbon dioxide evolution and microbial biomass in anoxic salt marsh sediments. *Applied and Environmental Microbiology*, **45**: 1586-1591.

Carli, T., Diker, K.S. and Eyigar, A. 1995. Sulphate-reducing bacteria in bovine faeces. *Letters in Applied Microbiology*, **21**: 228-229.

Carrington, E.G., Harman, S.A. and Pike, E.B. 1982. Inactivation of Salmonellas during anaerobic digestion of sewage sludge. *Journal of Applied Bacteriology*, **53**: 331-334.

Casson, L.W., Sorber, C.A., Palmer, R.H., Enrico, A. and Gupta, P. 1992. HIV survivability in wastewater. *Water Environment Research*, **64**: 213-215.

Chandler, J.A., Jewell, W.J., Gosset, J.M., van Soest, P.J. and Robertson, J.B. 1979. Predicting methane fermentation biodegradability. Proceedings of the Second Symposium on Biotechnology in Energy Production and Conservation, Tennessee. pp 1-26.

Chayovan, S.S., Gerrish, J.B. and Eastman, J.A. 1988. Biogas production from dairy manure: The effects of temperature perturbations. *Biological Wastes*, **25**: 1-16.

Chen, R.C. 1982. Building rural digesters. *In*: Hughes, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L. (eds). *Anaerobic Digestion 1981, Proceedings of the Second International Symposium on Anaerobic Digestion*. pp 293-314. Elsevier Biomedical Press : Amsterdam.

Chen, R.C. and Xiao, Z.P. 1981. The use of water pressure digesters in China. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 1. pp 147-154. CRC Press : Boca Raton.

Cheremisinoff, P.N. 1994. *Biomanagement of Wastewater and Wastes*. pp 80-87. Prentice Hall : New Jersey.

Christensen, J. and Hjort-Gregersen, K. 1994. The commercialization of biogas production. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 102-103.

Clancy, P.B., Venkataraman, N. and Lynd, L.R. 1991. Biochemical inhibitor of sulphate reduction in batch and continuous anaerobic digesters. Proceedings of the Sixth International Symposium on Anaerobic Digestion, São Paulo, Brazil. pp 43-61.

Clausen, E.C. and Gaddy, J.L. 1987. High solids digestion of MSW to methane by anaerobic digestion. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 283-302. Noyes Data Corporation : New Jersey.

Coetzer, F.W.C. 1994. Recovery and utilization of biogas. Proceedings of the All-Africa Congress, Sommerset West. pp 406-415.

Cohen, A. 1991. Effects of some industrial chemicals on methanogenic activity measured by sequential automated methanometry (SAM). Proceedings of the Sixth International Symposium on Anaerobic Digestion, São Paulo, Brazil. pp 11-20.

Collins, C.H. 1967. *Microbiological Methods*. Second edition. pp 134-142. Butterworths : Oxford.

Collins, C.H. and Lyne, P.M. 1984. *Microbiological Methods*. Fifth Edition. pp 151-152. Butterworths : Oxford.

Collins, C.H., Lyne, P.M. and Grange, J.M. 1989. *Microbiological Methods*. Sixth Edition. pp 58-60; 146-147. Butterworths : Oxford.

Collins, L.J. and Paskins, A.R. 1987. Measurement of trace concentrations of hydrogen in biogas from anaerobic digesters using an exhaled hydrogen monitor. *Water Research*, **21**: 1567-1572.

Conde, J.L., Moro, L.E., Travieso, L., Sanchez, E.P., Leiva, A., Dupeiron, R. and Escobedo, R. 1993. Biogas purification process using intensive microalgae cultures. *Biotechnology Letters*, **15**: 317-320.

Costerton, J.W. and Irvin, R.T. 1981. The bacterial glycocalyx in nature and disease. *Annual Review of Microbiology*, **35**: 299-324.

Cowardin, L.M., Carter, V., Golet, F.C. and Laroe, E.T. 1979. Classification of Wetlands and Deepwater Habitats of the United States. US Department of Interior, Fish and Wildlife Services Report FWS/UBS. pp 79-81.

Curry, G.L. and Deuermeyer, B.L. 1986. Optimal scheduling of multiple feedstock batch biogas production systems. *IIE Transactions*, **18**: 367-373.

Daneel, R.A., Percival, L.J. and Senior, E. 1997. Liquid petroleum gas (LPG) perturbation of an anaerobic interacting microbial association maintained in a multi-stage chemostat. *Letters in Applied Microbiology*, **25**: 157-161.

Daniel, T., Mathiazhagan, A. and Boominathan, K. 1990. Evaluation of *Gliricidia maculata, Ipomea cornea* and paddy straw as alternative feedstocks for the production of biogas. *Journal of Ecological Biology*, **2**: 113-121.

DaSilva, E.J. 1981. Biofuels and their generation from biomass- an overview. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 1. pp 53-71. CRC Press : Boca Raton.

Datong, Z. 1989. An analysis of domestic biogas storage installations in China. *Biomass*, **20**: 61-67.

Day, D.L., Chen, T.H., Anderson, J.C. and Steinberg, M.P. 1990. Biogas plants for small farms in Kenya. *Biomass*, **21**: 83-99.

De Baere, L.A., Devocht, M., Van Assche, P. and Verstraete, W. 1984. Influence of high NaCl and NH₄Cl salt levels on methanogenic associations. *Water Research*, **18**: 543-548.

Degrémont. 1991. *Water Treatment Handbook*. Volumes 1 and 2. Sixth Edition. pp 287-939. Lavoisier Publishing : New Jersey.

De Haast, J. and Britz, T.J. 1987. Characterisation of some anaerobic bacteria from the liquid phase of a mesophilic anaerobic digester fed with a prefermented cheese whey substrate. *Microbial Ecology*, **14**: 167-177.

DeLaune, R.D., Smith, C.J. and Sarafyal, M.N. 1986. Nitrogen cycling in a freshwater marsh of *Panicum hemitomon* on the deltaic plain of the Mississippi river. *Journal of Ecology*, **74**: 249-256.

Dempsey, G. 1988. The effects of container materials and multiple phase formulation components on the activity of antimicrobial agents. *In*: Bloomfield, S.F., Baird, R., Leak, R.E. and Leech, R. (eds). *Microbial Quality Assurance in Pharmaceuticals, Cosmetics and Toiletries*. pp 94-103. Ellis Horwood : Chichester.

Denyer, S.P. and King, R. 1988. Development of preservative systems. *In*: Bloomfield, S.F., Baird, R., Leak, R.E. and Leech, R. (eds). *Microbial Quality Assurance in Pharmaceuticals, Cosmetics and Toiletries*. pp 156-170. Ellis Horwood : Chichester.

Dhillon, T.S., Dhillon, E.K.S., Chau, H.C. and Tsang, A.H.C. 1976. Studies on bacteriophage distribution : virulent and temperate bacteriophage content of mammalian species. *Applied and Environmental Microbiology*, **32**: 68-74.

Dinopoulou, G., Rudd, T. and Lester, J.N. 1988. Anaerobic acidogenesis of a complex wastewater: I. The influence of operational parameters on reactor performance. *Biotechnology and Bioengineering*, **31**: 958-968.

Dolan, T.J., Bayley, S.E., Zoltek, J. and Hermann, A. J. 1981. Phosphorus dynamics of a Florida freshwater marsh receiving treated wastewater. *Journal of Applied Ecology*, **18**: 205-219.

Dolfing, J. 1988. Acetogenesis. *In*: Zehnder, A.J.B. (ed) *Biology of Anaerobic Microorganisms*. pp 417-468. John Wiley and Sons : New York.

Dolfing, J. and Mulder, J.W. 1985. Comparison of methane production rate and coenzyme F_{420} content of methanogenic consortia in anaerobic granular sludge. *Applied and Environmental Microbiology*, **49**: 1142-1145.

Dolfing, J. and Tiedje, J.M. 1988. Acetate inhibition of methanogenic, syntrophic benzoate degradation. *Applied and Environmental Microbiology*, **54**: 1871-1873.

Donahue, R.L., Miller, R.W. and Stickluma, J.C. 1985. *Soils, an Introduction to Soils and Plant Growth*. Fifth Edition. pp 144-149. Prentice Hall : New Jersey.

Douthwaite, R. 1988. Biogas digesters in India. *BioCycle*, **29**: 52-53.

Drauschke, G. and Neumann, W. 1992. Investigations on influence of toxic substances on microbial methane production from cattle wastes. *Zentralblatt Füer Mikrobiology*, **47**: 308-318.

Drews, R.J.L.C. 1985. *A Guide to the Use of Septic Tank Systems in South Africa*. pp 1-32. Council for Scientific and Industrial Research : Pretoria.

Dubourguier, H.C., Presnier, G. and Albagnac, G. 1988. Structure and microbial activities of granular anaerobic sludge. *In*: Lettinga, G., Zehnder, A.J.B., Grotehuis, J.T.C. and Hulshoff Pol, L.W. (eds). *Granular Anaerobic Sludge; Microbiology and Technology*. Proceedings of Gasmat Workshop. pp 18-33. Pudoc Wageningen : Wageningen, The Netherlands.

Dubourguier, H.C., Presnier, G., Samain, E. and Albagnac, G. 1985. Granular methanogenic sludge : microbial and structural analysis. *In*: Palz, W., Coombs, J. and Hall, P.O. (eds). *Energy from Biomass. Third EC Conference*. pp 542-546. Elsevier Applied Science Publishers : London.

Dudley, B.T., Howgrave-Graham, A.R., Isherwood, H.A. and Senior, E. 1993. Laboratory-scale UASB digesters (with/without conditioning tank and recycle): Efficacy to treat increased hydraulic loads. *Water SA*, **19**: 313-319.

Dudley, D.J., Guentzel, M.N., Ibarra, M.J., Moore, B.E. and Sagik, B.P. 1980. Enumeration of potentially pathogenic bacteria from sewage sludge. *Applied and Environmental Microbiology*, **39**: 118-126.

Eckenfelder, W.W. and Grau, P. 1992. *Activated Sludge Process Design and Control: Theory and Practice*. Volume 1. pp 11-16. Technomic Publishing Co : Lancaster.

Fang, H.H.P. and Yan, L. 1995. X-ray analysis of anaerobic granules. *Biotechnology Techniques*, **9**: 513-518.

Fannin, K.F., Conrad, J.R., Srivastava, V.J., Jerger, D.E. and Chynoweth, D.P. 1982. Anaerobic process. *Journal Water Pollution Control Federation*, **54**: 612-623.

Farinet, J.L., Forest, F. and Sow, O.S. 1994. Substitution of firewood using a small scale rural anaerobic system - a case study in Senegal. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 592-600.

Feachem, R. and Cairncross, S. 1978. *Small Excreta Disposal Systems*. pp 14, 29 and 59. The Ross Institute of Tropical Hygiene : London.

Fedler, C.B. and Day, D.L. 1985. Anaerobic digestion of swine manure containing an antibiotic inhibitor. *In*: American Society of Agricultural Engineers. *Agricultural Waste Utilization and Management*. Proceedings of the Fifth International Symposium on Organic Wastes. pp 523- 530. American Society of Agricultural Engineers : St Joseph Michigan.

Flynn, E.H. and Godzeski, C.W. 1967. Penicillins and cephalosporins. *In*: Gottlieb, D. and Shaw, P.D. (eds) *Antibiotics. Volume II: Mechanism of Action*. pp 1-19. Springer-Verlag : Berlin.

Forster, C.F. 1985. *Cambridge Studies in Biotechnology*. Part 2. pp 212-217. Cambridge University Press : Cambridge.

Forster, C.F. and Senior, E. 1987. Solids. *In*: Forster, C.F. and Wase, D.A.J. (eds) *Environmental Biotechnology*. pp 176-233. Ellis Horwood : Chichester.

Forster, C.F. and Wase, D.A.J. 1989. *Anaerobic Digestion*. Part 2. pp 1-7. University of Birmingham : Department of Chemical Engineering, Birmingham.

Fourie, A.B. and van Ryneveld, M.B. 1995. The fate in the subsurface of contaminants associated with on-site sanitation : A review. *Water SA*, **21**: 101-111.

Frobisher, M. 1962. *Fundamentals of Microbiology*. Seventh Edition. pp 421-510. WB Saunders Co : Philadelphia.

Gadre, R.V. 1989. Removal of hydrogen sulfide from biogas by chemoautotrophic fixed-film reactor. *Biotechnology and Bioengineering*, **34**: 410-414.

Gadre, R.V., Ranade, D.R. and Godbole, S.M. 1986. A note on survival of Salmonellas during anaerobic digestion of cattle dung. *Journal of Applied Bacteriology*, **60**: 93-96.

Gambrell, R.P. and Patrick, W.H. 1988. The influence of redox potential on the environmental chemistry of contaminants in soils and sediments. *In*: Hook, D.D., McKee, W.H., Smith, H.K., Gregory, J., Burrell, V.G., DeVoe, M.R., Sojka, R., Gilbert, E., Banks, R., Stolzy, L.H., Brooks, C., Mathews, T.D. and Shear, T.H (eds). *The Ecology and Management of Wetlands. Volume 1: Management, Use and Value of Wetlands.* pp 319-333. GoomHelm : London.

Gambrell, R.P., Taylor, B.A., Reddy, K.S. and Patrick, W.H. 1984. Fate of Selected Toxic Compounds Under Controlled Redox Potential and pH Conditions in Soil and Sediment-Water Systems. United States Environmental Protection Agency. EPA-600/S3-84-018.

Geeta, G.S., Gadees, K.S. and Reddy, T.K.R. 1990. Nickel as an accelerator of biogas production in water hyacinth (*Eichornia crassipes* Solms). *Biomass*, **21**: 157-161.

Gerhardt, G.G. 1988. The behaviour of facultative pathogenic bacteria in wastewater treatment process. *Water Science and Technology*, **20**: 533-535.

Gersberg, R.M., Gerhardt, R.A. and Ives, M. 1989. Pathogen removal in constructed wetlands. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural.* pp 431-445. Lewis Publishers : Chelsea, Michigan.

Ghaly, A.E. and Ben-Hassan, R.M. 1989. Continuous production of biogas from dairy manure using an innovative no-mix reactor. *Applied Biochemistry and Biotechnology*, **20** : 541-560.

Giacasso, P. 1996. The challenges of water supply. Proceedings of the Water Institute of Southern Africa Conference, Durban, South Africa. pp 1-4.

Gozo, L.R. 1993. The Bacteriophages Found in Sludge from a Rural Anaerobic Digester. BSc(Hons) Thesis, University of Natal, Pietermaritzburg.

Graetz, D.A. and Reddy, K.R. 1988. Anaerobic digester residues : Treatment and utilization. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach.* pp 445-454. Elsevier Applied Science : London.

Gray, S.F. and Griffiths, A. 1990. Observations on Aeromonas species isolated from human faeces. *Journal of Infection*, **20**: 267-268.

Gritzali, M., Shiralipour, A. and Brown, R.D. 1988. Cellulase enzymes for enhancement of methane production from biomass. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane From Biomass. A Systems Approach*. pp 367-383. Elsevier Applied Science : London.

Grundey, K. 1980. *Tackling Farm Waste*. p. 15. Farming Press Ltd : Ipswich.

Gunaseelan, V.N. and Lakshman, P. 1990. Biogas production potential of *Parthenium*. *Biological Wastes*, **33**: 311-314.

Guntenspergen, G.R., Stearns, F. and Kadlec, J.A. 1989. Wetland vegetation. *In*: Hammer, D.A. (eds) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 73-88. Lewis Publishers : Chelsea, Michgan.

Hach Water Analysis Handbook. 1983. Colorado, USA.

Hach Reactor Manual. 1994. Colorado, USA.

Hajarnis, S.R. and Ranade, D.R. 1994. Revival of *Methanobacterium formicicum* after its inhibition by high concentrations of ammonia. *Letters in Applied Microbiology*, **18**: 254-256.

Hamad, M.A., El-Gammal, M.A., Mitry, N.R. and El-Halwagi, N.R. 1989. Planning Egyptian villages in the new reclaimed areas with regard to accommodation of biogas technology. *Biological Wastes*, **28**: 53-60.

Hamburg, R.A. 1989. Household cooking fuel hydrogen sulphide and sulphur dioxide emissions from stalks, coal and biogas. *Biomass*, **19**: 233-245.

Hammer, D.A. 1992. *Creating Freshwater Wetlands*. pp 1-256. Lewis Publishers : Boca Raton.

Hammer, D.A. and Bastian, R.K. 1989. Wetlands ecosystems: Natural water purifiers. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 5-19. Lewis Publishers : Chelsea, Michigan.

Hashimoto, A.G., Chen, Y.R., Varel, V.H. and Prior, R.L. 1979. Anaerobic fermentation of animal manure. Paper presented at the Meeting of the American Society of Agricultural Engineers and Canadian Society of Agricultural Engineering, Winnipeg, Canada. pp 1-55.

Hashimoto, A.G., Chen, Y.R., Varel, V.H. and Prior, R.L. 1980. Anaerobic fermentation of agricultural residues. *In*: Shuter, M.L. (ed) *Utilization and Recycling of Agricultural Wastes and Residues*. pp 135-191. CRC Press : Boca Raton.

Hassan, A.E., Hassan, H.M. and Smith, N. 1975. Energy recovery and feed production from poultry wastes. *In*: Jewell, W.J. *Energy, Agriculture and Waste Management*. Proceedings of the 1975 Cornell Agricultural Waste Management Conference. pp 289-305. Ann Arbor Scientific Publications : Michigan.

Hassan Dar, G.H. and Tandon, S.M. 1987. Biogas production from pretreated wheat straw, lantana residues, apple and peach leaf litter with cattle dung. *Biological Wastes*, **21**: 75-83.

Haugen, V.J. and Lindley, J.A. 1988. Biogas production after solid liquid separation of dairy manure. *Transactions of the American Society of Agricultural Engineers*, **31**: 1782-1786.

Havelaar, A.H. 1991. Bacteriophages as model viruses in water quality control. *Water Research*, **25**: 529-545.

Havelaar, A.H. and Pot-Hogeboom, W.M. 1988. F-specific RNA-bacteriophages as model viruses in water hygiene: Ecological aspects. *Water Science and Technology*, **20**: 399-407.

Havelaar, A.H., Pot-Hogeboom, W.M., Furuse, K., Pot, R. and Hormann, M.P. 1990. F-specific RNA-bacteriophages and sensitive host strains in faeces and wastewater of human and animal origin. *Journal of Applied Bacteriology*, **69**: 30-37.

Hawkes, F.R., Guwy, A.J., Hawkes, D.L. and Rozzi, A.G. 1994. On line monitoring of anaerobic digestion: application of a device for continuous measurement of bicarbonate alkalinity. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 2-11.

Hayat, M.A. 1981. *Fixation For Electron Microscopy*. pp 77 and 150. Academic Press : New York. *Cited by*: Howgrave-Graham, A.R. and Wallis, F.M. 1991.

Heine-Dobbernack, E., Schoberth, S.M. and Sahm, H. 1988. Relationship of intracellular coenzyme F_{420} content to growth and metabolic activity of *Methanobacterium bryantii* and *Methanosarcina barkeri*. *Applied and Environmental Microbiology*, **54**: 454-459.

Hemond, H.F. and Benoit, J. 1988. Cumulative impacts on water quality functions of wetlands. *Environmental Management*, **12**: 639-653.

Henson, J.M., Bordeaux, F.M. and Smith, P.H. 1987. Effects of the addition of propionate and butyrate to thermophilic methane producing digester. *In*: Antonopoulos, A.A. (ed). *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 235-246. Noyes Data Corporation : New Jersey.

Hespell, R.B. and Bryant, M.P. 1981. The genera *Butyrivibrio, Succinovibrio, Succinomonas, Lachnospira* and *Selemonas*. *In*: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schlegel, H.G. (eds). *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria*. Volume 2. pp 1479-1494. Springer-Verlag : Berlin.

Heyes, R.H. and Hall, R.J. 1981. Anaerobic digestion, modelling the role of hydrogen. *Biotechnology Letter*s, **3**: 431-436.

Hiam, S.J., Hann, A.C., Furr, J.R. and Russel, A.D. 1995. X-ray microanalysis of chlorhexidine-treated cells of *Saccharomyces cerevisiae*. *Letters in Applied Microbiology*, **20**: 353-356.

Hobbs, G., Griffiths, A.J. and Hann, A.C. 1986. X-ray microanalysis of electron-dense bodies of *Cladosporium resinae*. *Microbiological Letters*, **33**: 285-288. *Cited by*: Hiam, S.J., Hann, A.C., Furr, J.R. and Russel, A.D. 1995.

Hobson, P.N. 1983. The kinetics of anaerobic digestion of farm wastes. *Journal of Chemical Technology and Biotechnology*, **33**: 1-20.

Hobson, P.N. and Shaw, B.G. 1976. Inhibition of methane production by *Methanobacterium formicum*. *Water Research*, **10**: 849-852.

Hoffman, J.R.H. 1986. Prospecton Brewery Effluent Pretreatment Plant Process Control Tests Manual. South African Breweries Central Laboratory, Johannesburg, South Africa. pp 1-26.

Holland, K.T., Knapp, J.S. and Shoesmith, J.G. 1987. *Anaerobic Bacteria*. pp 1-188. Blackie : Glasgow.

Holmes, B. 1991. The genera *Flavobacterium, Sphingobacterium* and *Weeksella*. *In*: Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (eds). *The Prokaryotes. A Handbook on the Biology of Bacteria; Ecophysiology; Isolation; Identification and Appreciations*. Second Edition. pp 3620-3630. Springer-Verlag : Berlin.

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T. 1994. *Bergeys Manual of Determinative Bacteriology*. Ninth Edition. pp 97, 181, 226 and 580. Williams and Wilkins : Baltimore.

Howard, P. and Posnik, S.J. 1994. The issues of socio-economics and waste management in developing nations. Proceedings of the All-Africa Congress, Summerset West. pp 132-142.

Howard-Williams, C. 1985. Cycling and retention of N and P in wetlands: a theoretical and applied perspective. *Freshwater Biology*, **15**: 391-431.

Howgrave-Graham, A.R. 1995. Microbiological Investigations into Granular Sludge from Two Anaerobic Digesters Differing in Design and Industrial Effluent Purified. PhD Thesis, University of Natal.

Howgrave-Graham, A.R. and Wallis, F.M. 1991. Preparation techniques for the electron microscopy of granular sludge from an anaerobic digester. *Letters in Applied Microbiology*, **13**: 87-89.

Howgrave-Graham, A.R. and Wallis, F.M. 1994. Scanning electron microscopy for qualitative studies of, and population quantification within anaerobic digester sludge granules. *Proceedings of the XIII International Conference on Electron Microscopy (ICEM) Paris*, **13**: 165-166.

Howgrave-Graham, A.R., Wallis, F.M. and Steyn, P.L. 1991. A bacterial population analysis of granular sludge from an anaerobic digester treating a maize-processing waste. *Bioresource Technology*, **37**: 149-156.

Huang, J.J.H. and Shih, J.C.H. 1981. The potential of biological methane generation from chicken manure. *Biotechnology and Bioengineering*, **26**: 2307-2314.

Hunt, D.T.E. and Wilson, A.L. 1986. *The Chemical Analysis of Water. General Principles and Techniques*. Second Edition. pp 560-563. Royal Society of Chemistry : Oxford.

Iannotti, E.L., Fischer, J.R. and Sievers, D.M. 1982. Characterisation of bacteria from swine manure digester. *Applied and Environmental Microbiology*, **43**: 136-143.

Iannotti, E.L., Mueller, R.E., Sievers, D.M., Georgacakis, D.G. and Gerhardt, K.O. 1986. Phenylacetic acid in an anaerobic swine manure digester. *Journal of Industrial Microbiology*, **1**: 57-61.

Ifeadi, C.N. and Brown, J.B. Jr. 1975. Technologies suitable for recovery of energy from livestock manure. *In*: Jewell, W.J. (ed) *Energy, Agriculture and Waste Management. Proceedings of the 1975 Cornell Agricultural Waste Management Conference.* pp 373-396. Ann Arbor Scientific Publications : Michigan.

Imhoff, K. and Maskew Fair, G. 1956. *Sewage Treatment*. Second Edition. pp 204-213. John Wiley and Sons : New York.

Ishihara, M., Toyama, S. and Yonaha, K. 1988. Biogas production from methane fermentation of sugarcane bagasse. *Science Bulletin College of Agriculture. University of Ryukyus*, **35**: 45-51.

Ito, M., Ohno, T. and Tanoka, R. 1992. A specific DNA probe for identification of *Bifidobacterium brevi. Microbial Ecology in Health and Disease*, **5**: 185-192.

Jackson, B.M. 1996. National sanitation policy: final stages of the draft white paper. Proceedings of the Water Institute of Southern Africa Conference, Durban, South Africa. pp 1-6.

Jagadeesh, K.S., Geeta, G.S. and Reddy, T.K.R. 1990. Biogas production by anaerobic digestion of *Euptorium odoratum* L. *Biological Wastes*, **33**: 67-70.

Jain, M.K. and Zeikus, J.G. 1987. A defined starter culture for biomethanation of proteinaceous wastes. *In*: Antonopoulus, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals.* pp 125-139. Noyes Data Corporation : New Jersey.

Jans, A.J.M. 1981. Detoxification and anaerobic treatment of formaldehyde containing wastewaters. Abstracts of the Second International Symposium on Anaerobic Digestion, Travemünde, Germany. p 10.

Jarrell, K.F., Saulnier, M. and Tey, A. 1987. Inhibition of methanogenesis in pure cultures by ammonia, fatty acids, and heavy metals and protection against heavy metal toxicity in sewage sludge. *Canadian Journal of Microbiology*, **33**: 551-554.

Jash, T., and Ghosh, D.N. 1990. Studies on residence time distribution in cylindrical and rectangular biogas digesters. *Energy*, **15**: 987-991.

Jewell, W.J., Dellórto, S., Fanfoni, K.J., Hayes, T.D., Leuschner, A.P. and Sherman, D.F. 1981. Low cost methane generation on small farms. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 2. pp 111-131. CRC Press : Boca Raton.

Jewell, W.J. and Loehr, R.C. 1977. Energy recovery from animal wastes: anaerobic digestion, pyrolysis, hydrogenation. *In*: Taiganides, E.P. (ed) *Animal Wastes*. pp 273-294. Applied Science Publishers : London.

Jiang, C-Q., Liu, T-W. and Zhong, J-L. 1989. A study on compressed biogas and its application to the compression ignition dual-fuel engine. *Biomass*, **20**: 53-59.

Jiang, Z., Steinsberger, S.C. and Shih, J.C.H. 1987. *In situ* utilization of biogas on a poultry farm: heating, drying and animal brooding. *Biomass*, **14**: 269-281.

Jones, W.J., Nagle, D.P. and Whitman, W.B. 1987. Methanogens and the diversity of Archaebacteria. *Microbiological Reviews*, **51**: 135-177.

Jungersen, G. and Ahring, B.K. 1994. Anaerobic digestion of liquefied cow manure pretreated by catalytic liquefaction. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 215-225.

Kadlec, R.H. and Kadlec, J.A. 1979. Wetlands and water quality. *In*: Greeson, P.E., Clark, J.R. and Clark, J.E. (eds) *Wetland Functions and Values: The State of Our Understanding*. pp 436-456. American Water Resource Association : Minneapolis.

Kai, S., Wantanabe, S., Furuse, K. and Osawa, A. 1985. Bacteroides bacteriophages isolated from human faeces. *Microbiology and Immunology*, **29**: 895-899.

Kalbermatten, J.M., Julius, D.S., Cunnerson, C.G. and Mara, D.D. 1982. *Appropriate Sanitation Alternatives. A Planning and Designing Manual*. pp 3, 23-26, 50, 94-100 and 142-152. John Hopkins University Press : London.

Kalia, A.K. and Kanwar, S.S. 1990. Anaerobic fermentation of *Ageratum* for biogas production. *Biological Wastes*, **32**: 155-158.

Kalia, V.C., Kumar, A., Jain, S.R. and Joshi, A.P. 1992. Methanogenesis of dumping wheat grains and recycling of the effluent. *Resources Conservation and Recycling*, **6**: 161-166.

Kanwar, S.S. and Kalia, A.K. 1993. Anaerobic fermentation of sheep droppings for biogas production. *World Journal of Microbiology and Biotechnology*, **9**: 1474-175.

Kelkar, A.S., Gadre, R.V. and Ranade, D.R. 1990. Comparative studies on cellulose degradation by three mesophilic species isolated from cattle dung digestion. *Tropical Ecology*, **31**: 123-128.

Khalil, E.F., Whitmore, T.N., Gamel-El-Din, H., El-Bassel, A. and Lloyd, D. 1989. The effect of detergent on methanogenesis by *Methanosarcina barkeri*. *Microbiology Letters*, **57**: 313-316.

Khatib, R., Usmani, N.F. and Husain, S.S. 1989. Studies on bioconversion. Part 4. On the efficiency of "bitumen-drum" made portable unit for biogas generation. *Pakistan Journal of Scientific and Industrial Research*, **32**: 431-434.

Kishore, V.V.N. 1989. A heat-transfer analysis of fixed-dome biogas plants. *Biological Wastes*, **30**: 199-215.

Korentajer, L. 1991. A review of the agricultural use of sewage sludge: benefits and potential hazards. *Water SA*, **17**: 189-196.

Koster, I.W. and Cramer, A. 1987. Inhibition of methanogenesis from acetate in granular sludge by long chain fatty acids. *Applied and Environmental Microbiology*, **53**: 403-409.

Kottner, M. 1994. Presentation of the activities and policies of the German Biogas Association ("Fachverband Biogas"). Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 177-178.

Kozuchowski, J. and Johnson, D.L. 1978. Gaseous emissions of mercury from an aquatic vascular plant. *Nature (London)*, **274**: 468-469.

Krieg, N.R. and Holt, J.G. 1984. *Bergeys Manual of Systematic Bacteriology*. Volume 1. pp 316-408. Williams and Wilkins : Baltimore.

Kroeker, E.J., Schulte, D.D., Sparling, A.B. and Lapp, H.M. 1979. Anaerobic treatment process stability. *Journal of Water Pollution Control Federation*, **51**: 718-727.

Krzycki, J.A. and Zeikus, J.G. 1987. Acetate metabolism by *Methanosarcina*. *In*: Antonopoulus, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 15-34. Noyes Data Corporation : New Jersey.

Kulkarni, S.V. and Godbole, S.H. 1990. Studies on interrelationships between different anaerobic bacteria in biogas digesters. *Current Science*, **59**: 603-605.

Kumar, S., Jain, M.C. and Chhonkar, P.K. 1987. A note on stimulation of biogas production from cattle dung by addition of charcoal. *Biological Wastes*, **20**: 209-215.

Laak, R. 1986. *Wastewater Engineering Design for Unsewered Areas.* pp 1, 11 and 19. Technomic Publishing Co : Lancaster, Pennsylvania.

Laak, R. and Crates, F.J. 1978. Sewage treatment by a septic tank. *In*: American Society of Agricultural Engineers. *Home Sewage Treatment. Proceedings of the Second National Home Sewage Treatment Symposium, Chicago, Illinois.* pp 54-60. American Society of Agricultural Engineers : Michigan.

Langton, E.W. 1981. Digestion design concepts. *In*: Wise, D.L. (ed) *Fuel Gas Production from Biomass.* Volume 2. pp. 63-109. CRC Press : Boca Raton.

Lapp, H.M., Schulte, D.D., Kroeker, E.J., Sparling, A.B. and Topnik, B.H. 1975. Start up of pilot scale swine manure digester for methane production. *In*: American Society of Agricultural Engineers. *Managing Livestock Wastes. Proceedings of the Third International Symposium on Livestock Wastes.* pp. 234-243. American Society of Agricultural Engineers : Michigan.

Large, P.J. 1983. Methylotrophy and methanogenesis. *In*: *Aspects of Microbiology No. 8.* pp 271-297. Van Nostrand Co Ltd : Wokingham, Berkshire. *Cited by*: Daneel, R.A., Percival, L.J. and Senior, E. 1997.

Laskin, A.I. 1967. Tetracyclines. *In*: Gottlieb, D. and Shaw, P.D. (eds) *Antibiotics. Volume I: Mechanism of Action.* pp 331-359. Springer Verlag : Berlin.

Letcher, T.M. 1991. Report on 17 day visit to India to investigate their methane from organic waste projects. pp 1-19. Report prepared for SA National Energy Council.

Letcher, T.M. and Kolbé, F. 1994. Collection, transportation and storage of biogas. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 173-176.

Lettinga, G., Roersma, R., Grin, P., de Zeeuw, W., Hulshof Pol, L., van Velsen, L., Hobma, S. and Zeeman, G. 1982. Anaerobic treatment of sewage and low strength wastewaters. *In*: Hughes, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L. (eds). *Anaerobic Digestion 1981, Proceedings of the Second International Symposium on Anaerobic Digestion*, Travemünde. pp 271-291. Elsevier Biomedical Press : Amsterdam.

Ling, B., Den, T.X., Lu, Z.P., Min, L.W., Wang, Z.X. and Yuan, A.X. 1993. Use of night soil in agriculture and fish farming. *World Health Forum*, **14**: 67-70.

Loehr, R.C. 1977. *Pollution Control for Agriculture*. pp 71-89 and 224-258. Academic Press : New York.

Lombard, R. 1994. Appropriate waste management technology in developing countries. Proceedings of the All-Africa Congress, Summerset West. pp 68-79.

Lotter, L.H. and Pitman, A.R. 1996. New approaches to the provision of water and sanitation in a metropolitan area. Proceedings of the Water Institute of Southern Africa Conference, Cape Town, South Africa. pp 1-10.

Louw, G. 1992. *Rural Poverty Alert. Challenging Science and Technology. FRD Report Series No. 2*. pp 1-28. Foundation for Research and Development : Pretoria, South Africa.

Lue-Hing, C., Zenz, D.R. and Kuchenrither, R. 1992. *Municipal Sewage and Sludge Treatment: Processing, Utilization, and Disposal*. pp 139-157. Technomic Publishers : Lancaster.

Macario, A.J.L., Earle, J.F.K., Chynoweth, D.P. and de Macario, E.C. 1989. Distinctive patterns of methanogenic flora determined with antibody probes in anaerobic digesters at different characteristics operated under controlled conditions. *Systems of Applied Microbiology*, **12**: 216-222.

Mackie, R.I. and Bryant, M.P. 1990. Efficiency of bacterial protein synthesis during anaerobic degradation of cattle waste. *Applied and Environmental Microbiology*, **56**: 87-92.

MacLeod, F.A., Guiot, S.R. and Costerton, J.W. 1990. Layered structures of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor. *Applied and Environmental Microbiology*, **56**: 1598-1607.

Macy, J.M. 1981. Non-pathogenic members of the genus *Bacteroides*. *In*: Starr, M.P., Stolp, H., Truper, H.G., Balows, A. and Schlegel, H.G. (eds). *The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria*. Volume 2. pp 1450-1463. Springer-Verlag : Berlin.

Madhukara, K., Nand, K., Raju, N.R. and Srilatha, H.R. 1993. Ensilage of mango peel for methane generation. *Process Biochemistry*, **28(2)**: 119-123.

Maeda, A., Tshii, K., Tamaka, M., Mikami, Y. and Arai, T. 1986. KM^{-1}, a bacteriophage of *Clostridium butyricum. Journal of General Microbiology,* **132**: 2271-2276.

Mah, R.A. and Smith, M.R. 1981. The methanogenic bacteria. *In*: Starr, M.P., Stolp, H., Trüper, H.G., Balows, A. and Schlegel, H.G. (eds). *The Prokaryotes, a Handbook on Habitats, Isolation, and Identification of Bacteria.* Volume 1. pp 948-977. Springer-Verlag : Berlin.

Mah, R.A., Ward, D.M., Baresi, L. and Glass, T.L. 1977. Biogenesis of methane. *Annual Review of Microbiology,* **31**: 309-341.

Mahadevaswamy, M. and Venkataraman, L.V. 1988. Integrated utilization of rabbit droppings for biogas and fish production. *Biological Wastes,* **25**: 249-256.

Mahadevaswamy, M. and Venkataraman, L.V. 1990. Integrated utilization of fruit-processing wastes for biogas and fish production. *Biological Wastes,* **32**: 243-251.

Makhetha, S.N. 1990. Training for sustainability of rural water supply and sanitation projects. *Water Sewage and Effluent,* **10**: 37-39.

Malik, R.K., Tauro, P. and Dahiya, D.S. 1989. Effect of delignification pretreatment and selective enrichment on methane production from cattle waste. *Biotechnology and Bioengineering,* **33**: 924-926.

Mallik, M.K., Singh, U.K. and Ahmad, N. 1990. Batch digester studies on biogas production from *Cannabis sativa,* water hyacinth and crop wastes mixed with dung and poultry litter. *Biological Wastes,* **31**: 315-319.

Mann, H.T. and Williamson, D. 1982. *Water Treatment and Sanitation, Simple Methods for Rural Areas.* pp 38 and 74-79. Intermediate Technology Publishers : London.

Marawanyika, G. 1986. Biogas technology for rural development in Zimbabwe. Renewable energy development in Africa. *In*: Wereko-Brobby, C.Y. and Breeze, E.M. (eds). *Proceedings of the International Conference on Research and Development Technologies in Africa.* Volume 2. pp 325-333. Commonwealth Science Council, Commonwealth Secretaria : London.

Mata-Alvarez, J., Mitz-Viturtia, A. and Torres, R. 1986. A simple device to measure biogas production in laboratory scale digesters. *Biotechnology Letters,* **8**: 719-720.

Matsoga, V.R.T.C. 1994. Community participation in waste management. Proceedings of the All-Africa Congress, Summerset West. pp 80-87.

McCarty, P.L. 1964. Anaerobic waste treatment fundamentals. Part 2: Environmental requirements and control. *Public Works*, **96**: 123-126.

McCarty, P.L. and Smith, D. 1987. Effect of hydrogen concentration on population distribution and kinetics in methanogenesis of propionate. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 53-67. Noyes Data Corporation : New Jersey.

McGary, M.G. and Stainforth, J. 1978. *Compost, Fertilizer, and Biogas Production from Human and Farm Wastes in the Peoples Republic of China*. pp 1-94. International Development Research Centre (IDRC) : Canada.

McInery, M.J. and Bryant, M.P. 1981. Review of methane fermentation fundamentals. *In*: Wise, D.L. (ed) *Fuel Gas Production from Biomass*. Volume 1. pp 19-46. CRC Press : Boca Raton.

McKinney, R.E. 1962. *Microbiology for Sanitary Engineers*. pp 166-177 and 247-259. McGraw-Hill : New York.

Merkel, J.A. 1981. *Managing Livestock Wastes*. pp 339 and 344. Avi Publishing Co : Connecticut.

Meyer, V. and Oellermann, R.A. 1994. Special methanogenic activity test (SMA). Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 70-71.

Meynell, P.J. 1982. *Methane. Planning a Digester*. pp 1-163. Prism Press : Dorchester.

Mislevy, P., Gilreath, J.P., Prine, G.M. and Dunavin, L.S. 1988. Alternative production systems: non conventional herbaceous species. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach*. pp 261-276. Elsevier Applied Science : London.

Mitsch, W.J. and Gosselink, J.G. 1986. *Wetlands*. pp 1-537. Van Nostrand Reinhold : New York.

Molnar, L. and Bartha, I. 1988. High solids anaerobic fermentation for biogas and compost production. *Biomass*, **16**: 173-182.

Moody, B. 1969. *Comparative Inorganic Chemistry*. Second Edition. pp 400-402. Edward Arnold : London.

Moorhead, K.K. and Nordsted, R.A. 1993. Batch anaerobic digestion of water hyacinth: Effects of particle size, nitrogen content and inoculum volume. *Bioresource Technology*, **44**: 71-76.

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

Morris, G.R., Jewell, W.J. and Caster, G.L. 1975. Alternative animal waste anaerobic fermenter designs and their costs. *In*: Jewell, W.J. (ed) *Energy, Agriculture and Waste Management. Proceedings of the 1975 Cornell Agricultural Waste Management Conference*. pp 317-335. Ann Arbor Scientific Publications : Michigan.

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

Mozes, T. 1992. Range of Artificial Wetlands Now Avaliable. CSIR Water Technology.

Muller, H.W. and Trosch, W. 1986. Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. *Applied Microbiology and Biotechnology*, **24**: 180-185.

Murray, K.A. 1987. *Wastewater Treatment and Pollution Control*. pp 36-61 and 145-181. Heer Printing Co : Pretoria, South Africa.

Muyiboi, S.A. 1992. Planning water supply and sanitation projects in developing countries. *Journal of Water Resource Planning Management*, **118**: 351-355.

Nand, K., Devi, S.S., Viswanath, P., Deepak, S. and Sarada, R. 1991. Anaerobic digestion of canteen wastes for biogas production : process optimisation. *Process Biochemistry*, **26(1)**: 1-5.

Nandy, T., Kaul, S.N., Pathe, P.P., Deshpande, C.V. and Daryapurkar, R.A. 1992. Pilot plant studies on fixed bed reactor systems for biomethanation of distillery spent wash. *International Journal of Environmental Studies*, **41**: 87-107.

Nazir, M. 1991. Biogas plants construction technology for rural areas. *Bioresource Technology*, **35**: 283-289.

Nhlapo, M.P. 1996. Empowerment of water and sanitation committees. Proceedings of the Water Institute of Southern Africa Conference, Durban, South Africa. pp 1-8.

Nichols, D.S. 1983. Capacity of natural wetlands to remove nutrients from wastewater. *Journal of Water Pollution Control*, **55**: 495-505.

Nilsson, B.K. and Karlsson, H.T. 1990. Online failure analysis of a biogas plant by a tracer test. *Chemie Ingenieur Technik*, **62**: 124-125.

Novaes, R.F.V., Rech, C.M., Fiqueirido, M.G. and Giaj-Cevra, L.A. 1988. Bacterial identification of granular sludge from a domestic sewage UASB-Reactor. Poster Papers of the Fifth International Symposium on Anaerobic Digestion, Bologna, Italy. pp 61-64.

Nozhevnikova, A.N. and Kotsyurbenko, O.R. 1994. Anaerobic digestion under psychrophilic conditions. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 90-91.

Nyirenda, S.H.C. 1994. Appropriate waste management technology in developing world situations. Proceedings of the All-Africa Congress, Summerset West. pp 101-108.

Nyns, E.J. 1990. Biogas - the real fuel for developing countries. *Enzyme and Microbial Technology*, **12**: 151-152.

Nyns, E.J. and Naveau, H.P. 1981. Status of european work in methane fermentation from biomass. *In*: Wise, D.L. (ed) *Fuel Gas Production from Biomass*. Volume 1. pp 135-145. CRC Press : Boca Raton.

Obias, E.D. 1988. Practical applications of biogas technology. Proceedings of the International Symposium on Application of Biotechnology for Small Industries Development in Developing Countries. Bangkok. pp 1-11.

ÓHair, S.K., Snyder, G.H., White, J.M., Olson, S.M. and Dunavin, L.S. 1988. Alternative production systems: Root and other herbaceous crops. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach*. pp 235-247. Elsevier Applied Science : London.

Õmura, S. 1981. Screening of specific inhibitors of cell wall peptidoglycan synthesis: an approach to early identification of new antibiotics. *In*: Ninet, L., Bost, P.E., Bouanchaud, D.H. and Florent, J. (eds) *The Future of Antibiotherapy and Antibiotic Research*. pp 389-405. Academic Press : London.

Oremland, R.S. 1988. Biochemistry of methanogenic bacteria. *In*: Zehnder, A.J.B. (ed) *Biology of Anaerobic Microorganisms*. pp 641-705. John Wiley and Sons : New York.

Ortega, M.E., Rojas, O. and Perez-Gil, F. 1984. Nutritional evaluation of ensiled ground sorghum-pig faeces mixtures. *In*: Ketelaars, E.H. and Boer I Wema, S. (eds) *Animals as Waste Converters. Proceedings of an International Symposium.* Department of Animal Science, Wageningen. pp 31-32. Pudoc Wageningen : Wagenin, The Netherlands.

Pain, B. 1991. *UK Experience of Anaerobic Digestion on Farms. Proceedings of the UK Interdepartmental Anaerobic Digestion Seminar.* pp 77-90. Harwell Laboratories : United Kingdom.

Pandey, G.N., Misra, U., Singh, S. and Singh, A. 1992. A new temperature controlled digester for anaerobic digestion for biogas production. *Energy Conservation Management*, **33**: 983-986.

Pandya, J.D., Ghosh, K.K. and Rastogi, S.K. 1988. A phosphoric acid fuel cell coupled with biogas. *Energy*, **13**: 383-388.

Pantskhava, E.S., Varosyan, S.O., Tapaltsyan, S.K.H., Goldfarb, L.L. and Gyunter, L.I. 1989. Methane generation from viscous heterogeneous organic-mineral solutions. *Biological Bulletin of the Academy of Sciences of the USSR*, **16**: 85-89.

Parkes, R.J. 1982. Methods for enriching, isolating, and analysing microbial communities in laboratory systems. *In*: Bull, A.T. and Slater, J.H. (eds) *Microbial Interactions and Communities*. Volume 1. pp 45-101. Academic Press : London.

Patel, V., Desai, M. and Madamwar, D. 1993. Thermochemical pretreatment of water hyacinth for improved biomethanation. *Applied Biochemistry and Biotechnology*, **42**: 67-74.

Pearce, D.W. and Turner, R.K. 1990. *Economics of Natural Resources and the Environment*. pp 241-324. Harvester : New York.

Pelczar, M.J. Jr., Chan, E.C.S. and Kreig, N.R. 1986. *Microbiology*. Fifth Edition. pp 469-617. McGraw-Hill : New York.

Pelczar, M.J. Jr., and Reid, R.D. 1965. *Microbiology*. Second Edition. pp 317-353 and 511-522. McGraw-Hill : New York.

Perkins, R.J. 1989. *Onsite Wastewater Disposal*. pp 1-64. Lewis Publishers : Chelsea, Michigan.

Pfeffer, J.T. 1980. Anaerobic digestion processes. *In*: Stafford, D. (ed) *Anaerobic Digestion*. pp 15-35. Applied Science Publishers : London.

Pitt, A.J. 1996. The activated sludge process. *Water, Sewage and Effluent*, **16(2)**: 49-54.

Plaster, E.J. 1985. *Soil Science and Management*. pp 164-165. Delmar Publishers : New York.

Pohland, F.G. and Ghosh, S. 1971. Developments in anaerobic treatment processes. *Biotechnology and Bioengineering*, **2**: 85-106.

Poindexter, J.S. 1971. *Microbiology, an Introduction to Protists*. pp 458-460. Macmillan : New Jersey.

Ponnamperuna, F.A. 1972. The chemistry of submerged soils. *Advances in Agronomy*, **24**: 29-96.

Portier, R.J. and Palmer, S.J. 1989. Wetlands microbiology: Form, function, processes. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 89-105. Lewis Publishers : Chelsea, Michigan.

Pos, J., Eszes, R. and Pavlicik, V. 1985. Evaluation of two full size mixed mesophilic digesters processing swine manure. *In*: American Society of Agricultural Engineers. *Agricultural Waste Utilization and Management. Proceedings of the Fifth International Symposium on Organic Wastes, Chicago, Illinois*. pp 81-90. American Society of Agricultural Engineers : St Joseph, Michigan.

Postgate, J.R. 1984. *The Sulphate-Reducing Bacteria*. Second Edition. pp 30-50 and 163-168. Cambridge University Press : Cambridge.

Prasad, D.Y. 1991. Biogas generation from bagasse-based paper mills by anaerobic digestion. *Journal of Chemical Technology and Biotechnology*, **51**: 515-524.

Prasad, D.Y. 1992. The anaerobic biodegradation of a bagasse-based paper-mill waste in fixed-film reactor. *Journal of Chemical Technology and Biotechnology*, **53**: 67-72.

Prine, G.M., Dunavin, L.S., Brecke, B.J., Stanley, R.L., Mislevy, P., Karmbacher, R.S. and Hensel, D.R. 1988. Model crop systems: sorghum, Napiergrass. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach*. pp 83-102. Elsevier Applied Science : London.

Prior, R.L. and Hashimoto, A.G. 1981. Potential for fermented cattle residue as a feed ingredient for livestock. *In*: Wise, D.L. (ed) *Fuel Gas Production from Biomass*. Volume 2. pp 215-237. CRC Press : Boca Raton.

Rabezandrina, R. 1990. Biogas: Evolution of actions and prospects for the rural environment in Africa. *AMBIO*, **19**: 424-426.

Rajasekaran, K., Vasil, I.K. and Schank, S.C. 1988. Tissue culture of gramineous biomass species. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach*. pp 155-168. Elsevier Applied Science : London.

Raju, N.R., Devi, S.S. and Nand, K. 1991. Influence of trace elements on biogas production from mango processing waste in 1.5 m³ KVIC digesters. *Biotechnology Letters*, **13**: 461-464.

Ramamoorthy, S. and Sulochana, N. 1989. Enhancement of biogas production using algae. *Current Science*, **58**: 646-647.

Raman, P., Ranga Rao, V.V. and Kishore, V.V.N. 1989. A static scum-breaking net for fixed dome biogas plants. *Biological Wastes*, **30**: 261-273.

Ranade, D.R., Yeole, T.Y. and Godbole, S.H. 1987. Production of biogas from market waste. *Biomass*, **13**: 147-153.

Reddy, K.R. 1988. Water hyacinth (*Eichornia crassipes* (mart) solms) biomass cropping systems: I Production. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A Systems Approach*. pp 103-140. Elsevier Applied Science : London.

Richardson, C.J. and Marshall, P.E. 1986. Processes controlling movement, storage and export of phosphorus in a fen peatland. *Ecological Monographs*, **56**: 279-302.

Richardson, C.J. 1985. Mechanisms controlling phosphorus retention capacity in freshwater wetlands. *Science*, **228**: 1424-1427.

Ripley, L.E., Boyle, W.C. and Converse, J.C. 1986. Improved alkalimetric monitoring for anaerobic digestion of high strength wastes. *Journal of Water Pollution Control Federation*, **58**: 406-411.

Rivard, C., Himmel, M. and Grohmann, K. 1987. Anaerobic digestion of hemicellulosic feedstocks. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 261-289. Noyes Data Corporation : New Jersey.

Rivett-Carnac, J.L. 1982. *Biogas - A Literature Review*. pp 1-140. University of Natal Institute of Natural Resources : South Africa.

Rivett-Carnac, J.L. 1984. *Sanitation for Peri-Urban and Rural Communities in Natal/Kwazulu.* pp 1-21. University of Natal Institute of Natural Resources : South Africa.

Rockwood, D.L. and Prine, G.M. 1988. Alternative production systems: Woody crops. *In*: Smith, W.H. and Frank, J.R. (eds) *Methane from Biomass. A systems approach.* pp 277-289. Elsevier Applied Science : London.

Rodgers, F.E.J. 1983. Wetlands as accreting systems: Wastewater treatment. *Journal of Limnological Society of Southern Africa*, **9**: 110-116.

Rodgers, F.E.J., Rogers, K.H. and Buzer, J.S. 1985. *Wetlands for Wastewater Treatment With Special Reference to Municipal Wastewaters.* pp 1-122. Witwatersrand University Press : Johannesburg, South Africa.

Röhr, M. and Wimmerstedt, R. 1990. A comparison of two commercial membranes used for biogas upgrading. *Desalination*, **77**: 331-345.

Rorick, M.B., Spahr, S.L. and Bryant, M.P. 1980. Methane production from cattle waste in laboratory reactors at 40°C and 60°C after solid-liquid separation. *Journal of Dairy Science*, **63**: 1953-1956.

Ross, D.J., Tate, K.R., Speir, T.W., Stewart, D.J. and Hewitt, A.E. 1989. Influence of biogas-digester effluent on crop growth and soil biochemical properties under rotational cropping. *New Zealand Journal of Crop and Horticultural Science*, **17**: 77-87.

Ross, W.R. and Louw, J.M. 1987. Monitoring and control of anaerobic digestion. *Water SA*, **13**: 193-196.

Ross, W.R., Novella, P.H., Pitt, A.J., Lund, P., Thomson, B.A., King, P.B. and Fawcett, K.S. 1992. Anaerobic Digestion of Wastewater Sludge: Operating Guide. Water Research Commission Technology Transfer Workshop on Anaerobic Digestion of Wastewater Sludge, A Presentation, Durban. pp 1-182.

Russell, A.D. and Quesnel, L.B. 1983. *Antibiotics: Assessment of Antimicrobial Activity and Resistance.* pp 1-18. Academic Press : London.

Rybczynski, W., Polprasert, C. and McGarry, M. 1978. *Low Cost Technology Options for Sanitation.* pp 30-32. Unipub : NewYork.

S.A. Water Research Commission. 1992. Sanitation Evaluation. Draft Summary Report. pp 1-60.

S.A. Water Research Commission. 1993a. Urban Sanitation Evaluation. WRC Report Number 385/1/93. pp 1-84.

S.A. Water Research Commission. 1993b. Guidelines on the Cost Effectiveness of Rural Water Supply and Sanitation Projects. WRC Report Number 231/1/93. pp 1-191.

Safley, L.M. Jr and Westerman, P.W. 1988. Biogas production from anaerobic lagoons. *Biological Wastes*, **23**: 181-193.

Safley, L.M. Jr and Westerman, P.W. 1992. Performance of a low temperature lagoon digester. *Bioresource Technology*, **41**: 167-175.

Salkinoja-Salonen, M.S. and Hughes, D.E. Analytical Techniques. *In*: Hughes, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L. 1982. *Anaerobic Digestion 1981, Proceedings of the Second International Symposium on Anaerobic Digestion, Travemunde*. pp 383-393. Elsevier Biomedical Press : Amsterdam.

Samain, E., Moletta, R., Dubourguier, H.C. and Albagnac, G. 1987. Propionate conversion to butyrate in an anaerobic digester, *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 223-233. Noyes Data Corporation : New Jersey.

Sanchez, E.P., Weiland, P. and Traviesa, L. 1992. Effect of the hydraulic retention time on the anaerobic biofilm reactor efficiency applied to screened cattle waste treatment. *Biotechnology Letters*, **14**: 635-638.

Sandberg, M. and Ahring, B.K. 1992. Anaerobic treatment of fish meal process wastewater in a UASB reactor at high pH. *Applied Microbiology and Biotechnology*, **36**: 800-804.

Sasahara, T., Kitamura, Y. and Ogawa, T. 1982. Treatment of brewery effluent. Part 3: Decrease in pH of effluent in balancing tanks. *Brauwissenschaft*, **35**: 249-254.

Sather, J.H. and Smith, R.D. 1984. An Overview of Major Wetland Functions and Values. Fish and Wildlife Service, US Department of Interior Washington DC. 1-68.

Sato, K. and Ochi, S. 1994. Control of carbon dioxide gas concentration to increase methane gas production in anaerobic sewage sludge digestion. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. 610-613.

Saxena, K.K., Nath, K. and Srivastava, S.K. 1989. The effect of using dung from cattle fed high-low-or no-concentrate rations, on the quality and nutritive value of slurry from a biogas plant. *Biological Wastes*, **28**: 73-79.

Schellenbach, S. 1981. Designing a high quality system to produce biogas from animal manures. *In*: Wise, D.L. (ed) *Fuel Gas Production from Biomass*. Volume 1. pp 245-256. CRC Press : Boca Raton.

Schink, B. 1988. Principles and limits of anaerobic degradation : environmental and technological aspects. *In*: Zehnder, A.J.B. (ed) *Biology of Anaerobic Microorganisms*. pp 771-846. John Wiley and Sons : New York.

Scholten-Koerselman, I., Houwaard, F., Janssen, P. and Zehnder, A.J.B. 1986. *Bacteroides xylanolyticus* sp.nov, a xylanolytic bacterium from methane producing cattle manure. *Antonie van Leeuwenhoek*, **52**: 543-554.

Sehgal, H.S., Kaur, K. and Sehgal, G.K. 1991. Phytoplankton response to biogas slurry in carp ponds. *Bioresource Technology*, **37**: 229-234.

Seidel, S.F., Federico, A.C., Milleson, J.F. and Roosen, M. 1978. Sampling programs for using upland marsh to improve quality. American Society of Agricultural Engineers (ASAE) Technical Paper 78-2050, presented at ASAE, Utah.

Sell, T.L., Scharberg, D.R. and Fekety, F.R. 1983. Bacteriophage and bacteriocin typing scheme for *Clostridium difficile*. *Journal of Clinical Microbiology*, **17**: 1148-1152.

Senior, E. and Balba, M.T.M. 1984. The use of single-stage and multi-stage fermenters to study the metabolism of xenobiotic and naturally occurring molecules by interacting microbial associations. *In*: Grainger, J.M. and Lynch, J.M. (eds) *Microbial Methods for Environmental Biotechnology*. pp 275-293. Academic Press : London.

Sharma, S.K., Mishra, I.M., Sharma, M.P. and Saini, J.S. 1988. Effect of particle size on biogas generation from biomass residues. *Biomass*, **17**: 251-263.

Sharma, S.K., Saini, J.S., Mishra, I.M. and Sharma, M.P. 1987. *Mirabilis* leaves - a potential source of methane. *Biomass*, **13**: 13-24.

Sharma, S.K., Saini, J.S., Mishra, I.M. and Sharma, M.P. 1989. Biogasification of woody biomass : *Ipomoea fistulosa* plant stem. *Biological Wastes*, **28**: 25-32.

Sharma, V.K. and Hobson, P.N. 1986. Properties of a cellulolytic *Sporolactobacillus* and some non sporing cellulolytic rods, presumptive Clostridia, from an anaerobic digester. *Journal of Applied Bacteriology*, **61**: 257-262.

Sharma, V.K. and Hobson, P.N. 1990. Isolation and characterisation of a new Sarcina-like cellulolytic bacterium from an anaerobic, mesophilic, cattle-waste digester. *Biological Wastes*, **33**: 81-88.

Shelef, G., Kimchie, S. and Grynberg, H.G. 1981. Organic loadings and methane production of agricultural wastes. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 2. pp 259-271. CRC Press : Boca Raton.

Siegrist, H., Renggli, D. and Gujer, W. 1993. Mathematical modelling of anaerobic mesophilic sewage sludge treatment. *Water Science and Technology*, **27**: 25-36.

Simankova, M.N. and Nozhevnikova, A.N. 1991. Hydrolytic bacteria from biogas digesters. *Prikladnaya Biokhimiya Mikrobiologiya*, **27**: 228-234.

Sims, R.E.H. and Richards, K. M. 1990. Anaerobic digestion of crops and farm wastes in the United Kingdom. *Agriculture Ecosystems and Environment*, **30**: 89-95.

Singh, N. and Gupta, R.K. 1990. Community biogas plants in India. *Biological Wastes*, **32**: 149-153.

Sinha, C.S. and Kandpal, T.C. 1990. A framework for the financial evaluation of household biogas plants in India. *Biomass*, **23**: 39-53.

Skrinde, R.T., Fritz, J.J. and Seabloom, R.W. 1981. Engineering feasibility of fuels from biomass utilizing dairy farm residue. *In*: Wise, D.L. (ed) *Fuel Gas Production From Biomass*. Volume 2. pp 47-62. CRC Press : Boca Raton.

Slatter, N.P. and Alborough, H. 1992. Chemical oxygen demand using microwave digestion: a tentative new method. *Water SA*, **18**: 145-147.

Sleat, R. and Robinson, J.P. 1983. The bacteriology of anaerobic degradation of aromatic hydrocarbons. *Journal of Applied Bacteriology*, **57**: 381-394.

Slim, J.A. and Wakefield, R.W. 1991. The utilization of sewage sludge in the manufacture of clay bricks. *Water SA*, **17**: 197-202.

Smith, J.E. 1981. *Biotechnology. Studies in Biology No. 136.* pp 55-57. Edward Arnold : London.

Snowdon, J.A., Cliver, D.O. and Converse, J.C. 1988. Human and animal wastes mixed for disposal to land: inactivation of viruses and parasites in a laboratory model. *In*: American Society of Agricultural Engineers. *On Site Wastewater Treatment. Volume 5. Proceedings of the Fifth National Symposium on Individual and Small Community Sewage Systems*. Chicago, Illinois. pp 94-101. American Society of Agricultural Engineers : Illinois.

Somayaji, D. and Khanna, S. 1994. Biomethanation of rice and wheat straw. *World Journal of Microbiology and Biotechnology*, **10**: 521-523.

Souza, M.E., Fuzaro, G. and Polegato, A.R. 1991. Thermophilic anaerobic digestion of vinasse in pilot plant UASB reactor. Proceedings of the Sixth International Symposium on Anaerobic Digestion, São Paulo, Brazil. 191-200.

Speece, R.E. and Parkin, G.F. 1987. Nutrient requirements for anaerobic digestion. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp. 195-219. Noyes Data Corporation : New Jersey.

Sprott, G.D., Jarrell, K.F. and Knowles, R. 1981. Potent inhibitory action of acetylene on methanogenic bacteria. Proceedings of the Second International Symposium on Anaerobic Digestion, Travemünde. pp 23-24.

Sprott, G.D. and Patel, G.B. 1986. Ammonia toxicity in pure cultures of methanogenic bacteria. *Systematic and Applied Microbiology*, **7**: 358-363.

Srivastava, V.J., Biljetina, R., Isaacson, H.R. and Hayes, T.A. 1989. Biogasification of community-derived biomass and solids wastes in a pilot scale SOLCON super (+) reactor. *Applied Biochemistry and Biotechnology*, **20**: 587-602.

Staley, J.T., Bryant, M.P., Pfenning, N. and Holt, J.G. 1989. *Bergeys Manual of Systematic Bacteriology*. Volume 3. pp 2171-2216. Williams and Wilkins : Baltimore.

Stanier, R.Y., Ingraham, J.L., Wheelis, M.L. and Painter, P.R. 1987. *General Microbiology*. Fifth Edition. pp 330-338 and 567-674. Macmillan : London.

Stolp, H. 1988. *Microbial Ecology : Organisms, Habitats, Activities*. pp 232-241. Cambridge University Press : Cambridge.

Strauch, D. and Winterholder, K. 1985. Effect of disinfectants, additives and antimicrobial drugs on anaerobic digestion. *In*: American Society of Agricultural Engineers. *Agricultural Waste Utilization and Management. Proceedings of the Fifth International Symposium on Organic Wastes*, Chicago, Illinois. pp 516-522. American Society of Agricultural Engineers : Illinois.

Suflita, J.M., Robinson, J.A. and Tiedge, J.M. 1983. Kinetics of microbial dehalogenation of haloaromatic substances in methanogenic environments. *Applied and Environmental Microbiology*, **45**: 1466-1473.

Suthaker, S., Polprasert, C. and Droste, R.L. 1991. Sequencing batch anaerobic reactors for treatment of a high-strength organic wastewater. *Water Science and Technology*, **23**: 1249-1257.

Taber, W.A. 1976. Wastewater microbiology. *Annual Review of Microbiology*, **30**: 263-277.

Tafdrup, S. 1994. Centralized biogas plants combine agricultural and environmental benefits with energy production. Proceedings of the Seventh International Symposium on Anaerobic Digestion, Cape Town, South Africa. pp 460-468.

Taheruzzaman, Q. and Kushari, D.P. 1989. Evaluation of some common aquatic macrophytes cultivated in enrichment water as possible source of protein and biogas. *Hydrological Bulletin*, **23**: 207-212.

Tamada, H., Harasawa, R. and Shingo, T. 1985. Isolation of a bacteriophage in *Fusobacterium necrophum. Japanese Journal of Veterinary Science*, **47**: 483-486.

Tang, W. and Wang, M. 1989. Experiment and research on a mesophilic anaerobic digester with dairy cattle manure in northern China. *Biomass*, **20**: 41-52.

Tartera, C. and Jofre, J. 1987. Bacteriophages active against *Bacteroides fragilis* in sewage polluted water. *Applied and Environmental Microbiology*, **53**: 1632-1637.

Taşdemiroğlu, E. 1988. Review of the biogas technology in Turkey. *Biomass*, **17**: 137-148.

Taşdemiroğlu, E. 1991. Economics of biogas space heating systems in rural Turkey. *Bioresource Technology*, **36**: 147-155.

Taylor, D.E. and Gruha, A. 1974. Asymmetric transcription during development of F^{-1}, a bacteriophage specific for *Clostridium sporogenes. Virology*, **59**: 190-200.

Taylor, M.A., Bandu, V.H. and Howgrave-Graham, A.R. 1991. Microbial distribution within granules from an upflow anaerobic sludge blanket digester treating brewery waste. Proceedings of the Electron Microscopy Society of Southern Africa, 21: 159-160.

Taylor, M.A. and Howgrave-Graham, A.R. 1992. Assessment of anaerobic sludges for inoculation of rural biogas digesters. Proceedings of the Electron Microscopy Society of Southern Africa, 22: 93-94.

Taylor, M.A. and Howgrave-Graham, A.R. 1993. Microbial segregation within anaerobic digester granules treating brewery wastewater. *Letters in Applied Microbiology*, **16**: 21-23.

Taylor, M.A., Howgrave-Graham, A.R. and Kruger, E. 1992. The feasibility of biogas technology as applied to the rural situation in Natal (South Africa). *In*: Senior, E., Senior ter Laag, M. and Britz, T.J. (eds). Proceedings of the Third Southern African Anaerobic Digestion Symposium. Pietermaritzburg, South Africa. pp 276-284.

Taylor, M.A., Senior, E. and Howgrave-Graham, A.R. 1993. Biogas technology in the rural areas of Natal, South Africa. Abstracts of the First South African Biotechnology Conference (Biotech SA '93). Biotechnology and Development in Southern Africa, South Africa. p 103.

Tchobanoglous, G. and Bruton, F.L. 1991. *Wastewater Engineering. Treatment, Disposal and Reuse*. Third Edition. pp 1021-1027. McGraw-Hill : New York.

Thom, C. and Banks, D.I. 1992. Biogas as rural domestic energy source: a pilot study. *In*: Senior, E., Senior ter Laag, M. and Britz, T.J. (eds). Proceedings of the Third Southern African Anaerobic Digestion Symposium, Pietermaritzburg, South Africa. pp 285-293.

Toerien, D.F. and Hattingh, W.H.J. 1969. Anaerobic digestion. I The microbiology of anaerobic digestion. *Water Research*, **3**: 385-416.

Toerien, D.F. and Maree, J.P. 1987. Reflections on anaerobic process biotechnology and its impact on water utilization in South Africa. *Water SA*, **13**: 137-144.

Tomei, F.A., Rouse, D., Maki, J.S. and Mitchell, R. 1988. Presence of unusual methanogenic bacteria in coal gasification wastes. *Applied and Environmental Microbiology*, **54**: 2964-2970.

Tong, X. and McCarty, P.L. 1991. Microbial hydrolysis of lignocellulosic materials. *In*: Isaacson, R. (ed) *Methane From Community Wastes*. pp 61-100. Elsevier Applied Science : London.

Touzel, J.P. and Albagnac, G. 1987. Acetoclastic methanogens in anaerobic digesters. *In*: Antonopoulos, A.A. (ed) *Biotechnological Advances in Processing Municipal Wastes for Fuels and Chemicals*. pp 33-39. Noyes Data Corporation : New Jersey.

Touzel, J.P., Samain, E., Albagnac G. and Morfaux, J.N. 1981. Microbiology of anaerobic digesters. Proceedings of the Second International Symposium on Anaerobic Digestion, Travemünde. pp 1-6.

Trattner, R.B. and Woods, S.J.E. 1989. The use of wetland systems for municipal wastewater treatment. *In*: Cheremisinoff, P.N. (ed) *Encyclopedia of Environmental Control Technology. Volume 3. Wastewater Treatment Technology*. pp 591-622. Gulf Publishing : Houston, Texas.

Tyagi, R.D. and Couillard, D. 1989. Bacterial leaching of metals from sludge. *In*: Cheremisinoff, P.N. (ed) *Encyclopedia of Environmental Control Technology. Volume 3. Wastewater Treatment Technology*. pp 557-590. Gulf Publishing : Houston, Texas.

Udo Ndebbio, J.E., Ekpo, A.H. and Forje, J.W. 1989. Energy and Development. *In*: Forje, J.W. (ed) *Science and Technology in Africa*. pp 167-183. Longman : Halow, Essex.

Ukei, K., Ukei, A. and Simogoh, Y. 1988. Terminal steps in the anaerobic digestion of municipal sewage sludge: effects of inhibitors of methanogenesis and sulphate reduction. *Journal of General Microbiology*, **34**: 425-432.

United Kingdom Department of the Environment. 1994. Landfill Co-disposal. Waste Management Paper No. 26F. A Draft for Consultation. *Cited by*: Daneel, R.A., Percival, L.J. and Senior, E. 1997.

United Nations Environment Programme. 1981. *Biogas Fertilizer System. Technical Report on a Training Seminar in China. UNEP Reports and Proceedings 2*. pp 13-29. United Nations Environment Programme : Nairobi.

van Andel, J.G. and Breure, A.M. 1984. Anaerobic wastewater treatment. *Trends in Biotechnology*, **2**: 16-20.

van Andel, J.G., Breure, A.M. and Cohen, A. 1986. Role of anaerobic spore forming bacteria in the acidogenic fermentation of glucose. *Process Biochemistry*, **21(2)**: 45-49.

van der Valk, A.G., Davis, C.B., Baker, J.L. and Beer, C.E. 1979. Natural fresh water wetlands as N and P traps for land runoff. *In*: Greeson, P.E., Clarke, J.R. and Clark, J.E. (eds) *Wetlands Functions and Values: The State of Our Understanding.* pp 457-467. American Water Resources Association : Minneopolis.

van der Westhuizen, P.C. 1990. Selection of, investigation into and implementation of a small bore sewer system for Marselle black township. *Water Sewage and Effluent*, **10**: 25-34.

van Rooyen, C.R. and Slatter, P. T. 1996. Aerobic small bore sewage system. Proceedings of the Water Institute of Southern Africa Conference. Durban, South Africa. 1-12.

van Ryneveld, M.B. 1994. The current extent of coverage and the costs of water supply and sanitation provision in the urban areas of South Africa. *Water SA*, **20**: 99-106.

Varel, V.H., Hashimoto, A.G. and Chen, Y.R. 1980. Effect of temperature and retention time on methane production from beef cattle waste. *Applied and Environmental Microbiology*, **40**: 217-222.

Varel, V.H., Isaacson, H.R. and Bryant, M.P. 1977. Thermophilic methane production from cattle waste. *Applied and Environmental Microbiology*, **33**: 298-307.

Vázquez, D. 1981. Screening of inhibitors of protein biosynthesis. *In*: Ninet, L., Bost, P.E., Bouanchaud, D.H. and Florent, J. (eds) *The Future of Antibiotherapy and Antibiotic Research.* pp 273-283. Academic Press : London.

Verstraete, W., Baere, L. and Rozzi, A. 1981. Phase separation in anaerobic digestion: motives and methods. *Trib. Bebedean Nas*, **34**: 367-453. *Cited by*: Howgrave-Graham, A.R. 1995.

Vestergaard, P. 1979. A study of indication of trace metal pollution of marine areas by analysis of salt marsh soils. *Marine Environmental Research*, **2**: 19-31.

Viitasalo, I. 1981. Laboratory method for biogas production assessment. Abstracts of the Second International Symposium on Anaerobic Digestion, Travemünde, Germany. p 70.

Vlissidis, A. and Zouboulis, A.I. 1993. Thermophilic anaerobic digestion of alcohol distillery wastewaters. *Bioresource Technology*, **43**: 131-140.

Vriens, L., van Soest, H. and Verachtert, H. 1990. Biological treatment of malting and brewing effluents. *Critical Reviews in Biotechnology*, **10**: 1-46.

Walsh, J.L., Ross, C.C., Smith, M.S. and Harper, S.R. 1989. Utilization of biogas. *Biomass*, **20**: 277-290.

Ward, R.F. 1982. Digesters for the third world. *In*: Hughes, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, J., Verstraete, W. and Wentworth, R.L. (eds). *Anaerobic Digestion 1981, Proceedings of the Second International Symposium on Anaerobic Digestion*, Travemünde. pp 315-341. Elsevier Biomedical Press : Amsterdam.

Water Pollution Control. 1993. "Wetlands down-under": wetlands in water pollution control. *Water Sewage and Effluent*, **13**: 57-61.

Watson-Craik, I.A. and Senior, E. 1989. Effects of phenol wastewater co-disposal on the attenuation of the refuse leachate molecule hexanoic acid. *Letters in Applied Microbiology*, **9**: 227-232.

Weigant, W.M. and de Man, A.W.A. 1986. Granulation of biomass in thermophilic upflow anaerobic sludge blanket reactors treating acidified wastewaters. *Biotechnology and Bioengineering*, **28**: 718-727.

Weiland, P. 1993. One-and two-step anaerobic digestion of solid agro industrial residues. *Anaerobic Digestion of Solid Waste*, **27**: 145-151.

Weland, A.F. and Cheremisinoff, P.N. 1975. Anaerobic digestion of sludge Part I. *Water and Sewage Works*, **121**: 80-83.

Weller, J.B. and Willetts, S.L. 1977. *Farm Waste Management*. pp 113-119. Crosby Lockwood Staples : London.

Wheelis, M.L. and Segel, W.P. 1979. *Introduction to the Microbial World*. pp 57-60. Prentice-Hall : New Jersey.

White, L.P. and Plaskett, L.G. 1981. *Biomass as Fuel*. pp 34-43. Macmillan : London.

Whitmore, T.N., Lloyd, P., Jones, G. and Williams, T.N. 1987. Hydrogen dependent control of the continuous anaerobic digestion process. *Applied and Microbial Biotechnology*, **26**: 383-388.

Wieder, R.K. and Lang, G.E. 1986. Fe, Al, Mn and S chemistry of sphagnum peat in four peatlands with different metal and sulphur input. *Water, Air and Soil Pollution*, **29**: 309-320.

Williams, A.T. and Eberhard, A.A. 1986. An overview of biogas applications in rural Southern Africa. Proceedings of the First South African Anaerobic Digestion Symposium, Bloemfontein. pp 283-291.

Williams, C.M. and Shih, J.C.H. 1989. Enumeration of some microbial groups in thermophilic poultry waste digesters and enrichment of a feather-degrading culture. *Journal of Applied Bacteriology*, **67**: 25-35.

Williams, J.D. and Drasar, F.A. 1979. Clinical relevance of antibiotic synergy. *In*: Williams, J.D. (ed) *Antibiotic Interactions*. pp 13-33. Academic Press : London.

Wohlt, J.E., Frobish, R.A., Davis, C.L., Bryant, M.P. and Mackie, R.I. 1990. Thermophilic methane production from dairy cattle waste. *Biological Wastes*, **32**: 193-207.

Wolverton, B.C. 1987. Artificial marshes for wastewater treatment. *In*: Reddy, K.R. and Smith, W.H. (eds). *Aquatic Plants for Wastewater Treatment and Resource Recovery*. pp. 141-152. Magnolia Publishing : Florida.

Wolverton, B.C. 1989. Aquatic plant/microbial filters for treating septic tank effluent. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 173-178. Lewis Publishers : Chelsea, Michigan.

Wong, M.H. and Cheung, Y.H. 1989. Anaerobic digestion of pig manure with different agro-industrial wastes. *Biological Wastes*, **28**: 143-144.

Wood, A. 1996. Investigation into the application and performance of constructed wetlands for wastewater treatment in South Africa. Proceedings of the Water Institute of Southern Africa Conference, Durban, South Africa. pp 1-6.

Wood, A. and Hensman, L.C. 1989. Research to develop engineering guidelines for implementation of constructed wetlands for wastewater treatment in southern Africa. *In*: Hammer, D.A. (ed) *Constructed Wetlands for Wastewater Treatment: Municipal, Industrial and Agricultural*. pp 581-589. Lewis Publishers : Chelsea, Michigan.

Wood, A. and Pybus, P. 1993. Artificial Wetland Use for Wastewater Treatment. Theory, Practice and Economic Review. Water Research Commission Report 232/1/93. pp. 1-66.

Wood, A. and Rowley, M. 1987. Artificial wetlands for wastewater treatment. Proceedings of the Symposium on Ecology and Conservation of Wetlands in South Africa, Pretoria. pp 1-7. National Institute for Water Research, Council for Scientific and Industrial Research : South Africa.

Wright, F. B. 1956. *Rural Water Supply and Sanitation*. pp 207-252. John Wiley and Sons : New York.

Yabuuchi, E., Kaneko, T., Yano, I., Moss, C.W. and Miyoshi, N. 1983. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov: Glucose-non fermenting Gram-negative rods in CDC Groups IIK-2 and IIb. *In*: Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K.H. (eds). *The Prokaryotes. A Handbook on the Biology of Bacteria; Ecophysiology; Isolation; Identification and Appreciations*. Second Edition. pp 3620-3630. Springer-Verlag : Berlin.

Youssef, F.M., Muller-Buethow, M. and Hanel, H. 1970. Isolation and characterization of *Lactobacillus bifidus* phages. *Zentralblatt fuer Bakteriologie und Hygiene. Abteilungl. Originale A. Medizin, Schemikrobiolgie, Infektienskrankheiten und Parasitologie*, **213**: 495-509.

Zafiriou, O.C., Joussof-Dubien, J., Zepp, R.G. and Zika, R.G. 1984. Photochemistry of natural wastes. *Environmental Science and Technology*, **18**: 358-371.

Zehnder, A.J.B., Ingvorsen, K. and Marti, T. 1982. Microbiology of methane bacteria. *In*: Huges, D.E., Stafford, D.A., Wheatley, B.I., Boader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L. (eds). *Anaerobic Digestion 1981. Proceedings of the Second International Symposium on Anaerobic Digestion*. Travemunde. pp 45-68. Elsevier Biomedical Press : Amsterdam.

Zehnder, A.J.B. and Stumm, W. 1988. Geochemistry and biogeochemistry of anaerobic habitats. *In*: Zehnder, A.J.B. (ed) *Biology of Anaerobic Microorganisms*. pp 1-38, 641-705 and 771-846. John Wiley and Sons : New York.

Zeikus, J.G. 1980. Microbial populations in anaerobic digesters. *In*: Stafford, D. (ed) *Anaerobic Digestion*. pp 61-87. Applied Science Publishers Ltd : London.

Zeikus, J.G. 1982. Microbial intermediary metabolism in anaerobic digestion. *In* Hughes, D.E., Stafford, D.A., Wheatley, B.I., Baader, W., Lettinga, G., Nyns, E.J., Verstraete, W. and Wentworth, R.L. (eds). *Anaerobic Digestion 1981. Proceedings of the Second International Symposium on Anaerobic Digestion*. Travemunde. pp 23-35. Elsevier Biomedical Press : Amsterdam.

Zoetemeyer, R.J., Van Den Heuvel, J.C. and Cohen, A. 1982. pH influence on acidogenic dissimilation of glucose in an anaerobic digester. *Water Research*, **16**: 303-311.