Assessment of the Anaerobic Baffled Reactor for Treatment of Vegetable Oil Effluent

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ASSESSMENT OF THE ANAEROBIC BAFFLED REACTOR FOR TREATMENT OF VEGETABLE OIL EFFLUENT

LEE-ANNE FROST

I hereby declare that the dissertation represents my own work. It has not been submitted before for any diploma/degree or examination at any other Technikon /University.

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I hereby approve the final submission of the following dissertation.

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This 23rd *day of* March, *2001, at Technikon Natal.*

DEDICATION

◊ To my parents, Albert and Arlene Frost, for your constant support and encouragement to see this project to the end,

◊ My brother Kurt, thank you for the endless trips to and from the laboratory,

◊ All my relatives and friends, for your time and effort to help me through this project,

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ABSTRACT

The vegetable oil industry produces effluent containing quantities of fat, oil, sodium, phosphates as well as other pollutants. Oils and greases tend to clog sewers and pumps, thus creating difficulties within the municipal wastewater treatment works. Physico-chemical treatment methods, such as (Dissolved Air Flotation) DAF, gravity separation and the use of coagulants have been attempted providing a considerable reduction in organic loading; however, discharge standards are still not met. Thus, biological treatment methods are being sought after. Aerobic treatment has been attempted however, shock loads cause problems while running such a process. The objective of this study was to assess the efficiency of anaerobic digestion to degrade Vegetable Oil Effluent (VOE) as well as the efficiency of the Anaerobic Baffled Reactor (ABR). Anaerobic digestion involves the breakdown of organic matter by the action of microorganisms in the absence of oxygen, producing methane-rich biogas. The VOE was characterized, providing significant information on its chemical composition. It was found that the effluent had high sulphate content as well as a high COD content. High sulpahte content of wastewaters have known to promote growth of Sulphate Reducing Bacteria (SRB), which utilize the same energy source as Methane Producing Bacteria (MPB) and therefore compete for the same energy source. Sulphate and lipid reduction pretreatment experiments were carried out, using barium chloride and gravitational separation respectively. The results obtained, showed that the use of barium chloride to reduce sulphate content in VOE was successful, with significant sulphate reduction. The lipid reduction experiments however, did not show any significant lipid reduction. Batch tests were conducted in serum bottles to assess the extent of biodegradation of the VOE in its raw state as well as with reduced sulpahte content. Methanogenic toxicity tests on the raw and pretreated VOE provided a range of toxicity results. These assays are relatively simple and inexpensive. Gas production was monitored to determine the rate and extent of biodegradation. The efficiency of digestion was assessed by COD reduction. Results indicated potential inhibition of the methanogenic bacteria responsible for methane production by the

presence of a toxic substance or substances (at elevated concentrations) in the VOE. Results showed raw VOE to be more susceptible to anaerobic degradation. A laboratory scale Anaerobic Baffled Reactor (ABR) was assessed to treat VOE anaerobically and compared to Fed-batch digestion. Both reactors were fed a combination of VOE (COD 2000mg/L) and artificial effluent. Results indicated that anaerobic fed-batch digestion is a promising method of treatment for VOE and that the ABR is not suitable for treatment of this type of effluent.

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ABR	-	Anaerobic Baffled Reactor
AFBR	-	Anaerobic Fluidised Bed Reactor
BOD	-	Biological Oxygen Demand
COD	-	Chemical Oxygen Demand
CSTR	-	Completely Stirred Tank Reactor
DAF	-	Dissolved Air Flotation
HRT	-	Hydraulic Retention Time
MLVSS	-	Mixed Liquor Volatile Suspended Solids
MPB	-	Methane Producing Bacteria
OFN	-	Oxygen Free Nitrogen
POME	-	Palm Oil Mill Effluent
SRB	-	Sulphate Reducing Bacteria
SRT	-	Solids Retention Time
SS	-	Suspended Solids
TDS	-	Total Dissolved Solids
TSS	-	Total Suspended Solids
VOE	-	Vegetable Oil Effluent
VS	-	Volatile Solids

Alkalinity	Represents the ability of digester contents to neutralize volatile fatty acids formed during anaerobic digestion.
Biological Oxygen Demand	Measures the biodegradable organic matter by monitoring oxygen utilised during a specified incubation period (usually 5 days) as well as oxygen to oxidize inorganic materials.
Biodegradation	Microbial decomposition of organic compounds into organic molecules.
Biogas	Product of the breakdown of organic matter caused by anaerobic microorganisms.
Biota	The living organisms of a region or system
Chemical Oxygen Demand	Measures the oxygen equivalent of the organic matter susceptible to oxidation by a strong chemical oxidant
Dissolved Air Flotation	A method used to remove fatty materials dispersed in liquid.

Decortication	The removal of husks and hulls (external coating from the oil seed to expose the oil-bearing portion of the seed.
Domestic Wastewater	Water that has been utilised and discharged from domestic dwellings, institutions and commercial establishments.
Effluent	A stream originating from a sewage tank or industrial process.
Headspace	The volume in a sealed vessel occupied by the gas phase.
Hydraulic Retention Time	The length of time liquid remains in the digester.
Hydraulic Overloading	Occurs when the residence time is reduced to the point at which organisms are washed out of the system before multiplying.
Hydrogenation	The addition of hydrogen gas in the presence of a catalyst (nickel) to the unsaturated constituents of the oil to produce more completely saturated triglycerides.
Industrial Wastewater	Water utilised and discharged from manufacturing industries.

Lipid	A generic term for fatty material, which is subdivided into simple lipids (including fats, oil and waxes) and complex lipids (including phospholipids).
Organic overloading	Occurs when an excessive mass rate of organic solids enters the digester, resulting in accumulation of volatile acids.
Soapstaock	The aqueous soap phase produced on the neutralization of free fatty acids in crude oil.
Toxic overloading	Caused by the introduction of materials such as heavy metals, detergents, organic chemicals, ammonia and various cations, which have an inhibitory effect on microorganisms at specific concentrations.
Volatile Fatty Acids	Short-chained, organic acids.

CHAPTER ONE

LITERATURE REVIEW

1.1. EFFECT OF WATER POLLUTION

As a result of industrial development, the occurrence of chemical pollutants in water has grown dramatically. These pollutants may have toxic, carcinogenic, mutagenic and teratogenic effects. They may bioaccumulate or persist in the environment and can cause health problems. They have also been implicated in genetic effects such as infertility and congenital deformities (Rodda and Kfir, 1991). Chemical and biochemical transformations of chemical pollutants in the aquatic environment may affect their biological availability, toxicity or may yield products more toxic than the original pollutants (Rodda and Kfir, 1991).

Resources for the supply of clean water are essential for maintaining a healthy community. They are a source of potable water and through supporting the growth of aquatic life as well as its use for irrigation in agriculture, provides food (Horan, 1990). High temperatures and seasonal rainfall in most of South Africa indicates that fresh water is a scarce resource (Dallas and Day, 1993). One of the prerequisites of water management policies in South Africa is that effluent must be re-used so as to help balance water supply with the demand for water, since water is a scarce resource (Dept. Water Affairs and Forestry, 1995). The supply of water is not the problem; earth has virtually the same amount today as it did billions of years ago. Ninety-seven percent of that is in the form of salt water. Only 3% is fresh and two-thirds of that is ice. Large reserves of fresh water underlies the earth's surface, however, much of it is too deep to remove economically (Parfit, 1993). The problem is increasing populations and flagrant abuse of our most precious and limited resources (Parfit, 1993).

Increasing human populations is causing pressure on South Africa's rivers, as a resource and in terms of pollution. The extent of pollution is dependent on the ability of riverine biota to deal with

pollutants entering rivers (Dallas and Day, 1993). The ability of the biota to purify polluted rivers depends on the extent and type of pollution entering them. The effect of pollution is also influenced by the degree to which a pollutant is diluted, as concentrated waste may inhibit or destroy this self-purifying ability (Dallas and Day, 1993). Domestic wastewater together with industrial wastewater contains a large number of potentially harmful compounds. Potentially serious damage could result from discharge of these wastewaters directly into watercourses (Horan, 1990). Most wastewaters contain quantities of organic matter which result in anaerobic conditions in receiving waters as well as growth of undesirable microorganisms and many other detrimental effects (Torien and Maree, 1987). Discharge of industrial effluents into aquatic ecosystems may affect Total Dissolved Solids (TDS), Total Suspended Solids (TSS), pH, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), toxicity (trace metals, toxic organics), colour, nutrients and temperature of receiving waters. The variables affected depend on the nature of the effluent (Dallas and Day, 1993).

1.2. WASTEWATER DISPOSAL

Effluent disposal options are governed by various factors. The Water Act (No.54 of 1956) administered by the Department of Water Affairs and Forestry require that all public water abstracted for industrial or municipal use must be returned to the stream of origin and all effluents must be purified to prescribed standards (WRC Report, 1992). Industries supplied with fresh water wishing to discharge effluent into the sea, require a permit issued in terms of the Act (WRC Report, 1992). The type of effluent to be disposed of is also a contributing factor in choosing a disposal option, since certain effluents cannot be considered due to their toxic nature (WRC Report, 1992).

Incineration is one of the disposal options, which involves complete destruction of all the combustible elements. Incineration can significantly contribute to air pollution as a result of incomplete combustion and the formation of intermediate combustion products. Therefore, this disposal option is only considered when alternative disposal is not possible. Many plants in

South Africa as well as other parts of the world have been closed down due to fuel requirements and air pollution problems (WRC Report, 1992).

The ocean can be viewed as a disposal option, provided that the quality of the seawater does not deteriorate beyond acceptable levels as a result of waste disposal (WRC Report, 1992). Therefore, strict control and responsible management of waste disposal to the ocean is required, whether by dumping at sea, surf zone discharges or discharge through ocean outfalls (WRC Report, 1992). Inefficient marine disposal of effluent can have adverse effects on the sea within discharge areas. In the case of persistent substances, areas beyond the discharge area are also affected. As a result, potential risks are associated with public health, the marine biota as well as economic risks (WRC Report, 1992).

Increasing demand for limited fresh water resources in South Africa as a result of growing urban and industrial complexes will necessitate, direct and indirect reuse of effluent (WRC Report, 1992). Efficient wastewater treatment is therefore required.

Most industrial wastewaters can be safely added to municipal sewage for treatment and disposal, however, there are toxic wastes that may damage collection systems and interfere with or overload treatment facilities (Fair *et al.*, 1966). Thus, pretreatment or separation from the collection system becomes mandatory. The degree of pretreatment or treatment with municipal sewage depends on the concentration, composition and condition of the wastewater. Treatment also depends on the nature and capacities of the treatment works as well as of the receiving waters (Fair *et al.*, 1966). There are some guiding principles to consider in solving industrial wastewater problems. The recovery of useful materials and improvement of manufacturing processes where waste is reduced to a minimum (Fair *et al.*, 1966). Recycling process waters and development of treatment methods are also ways to solve industrial wastewater problems (Fair *et al.*, 1966).

Vegetable oil effluent discharged into sewers contains quantities of fat, oil, sodium, sulphates, phosphates as well as other pollutants (WRC Report, 1989). Thus it is imperative to have some knowledge of the vegetable oil production process so as to develop a treatment method for vegetable oil effluent.

1.3. VEGETABLE OIL PRODUCTION

Liquid oil is the principle product of an edible oil refinery, which may either be sold as cooking or salad oil or may be further processed to margarine, cooking and baker's fat, peanut butter and mayonnaise (WRC Report, 1989). Vegetable oil may be obtained from a wide variety of seeds, including cottonseed, soya bean, peanuts, sunflower seed, maize, olives and coconut. In South Africa the most commonly grown oil bearing crops are sunflower, groundnut and maize, although other seeds such as cotton and soya are also processed (WRC Report, 1989).

There are two stages in the production of refined vegetable oil, namely, crude oil production conducted in an oil mill and oil processing conducted in a refinery (WRC Report, 1989).

1.3.1. Crude Oil Production

The seeds are first decorticated (removal of external coat) after which the meats (oil bearing portion of the seed) are separated from the hulls using vibratory screens and airlifts (WRC Report, 1989). The separated meats are made up of small tough walled cells containing oil. They are rolled into thin flakes and subjected to live steam where they are brought up to a predetermined optimum temperature and moisture content (WRC Report, 1989). The conditioned seed is then passed to expellers where approximately 75% of the oil is squeezed from the flakes (WRC Report, 1989).

1.3.2. Oil Processing

Crude oil is composed largely of glycerides of various saturated and unsaturated fatty acids, notably oleic and linoleic acids. There are a number of impurities which must be removed from

the oil before it can be considered fit for human consumption (WRC Report, 1989). These impurities include gums, free fatty acids, pigment (usually of chlorophyllic or carotenic base) and taste or odour (as s result of volatile aldehydes and ketones) (WRC Report, 1989). Constituents and properties of oils depend upon their source, necessitating the use of slightly different processing techniques (WRC Report, 1989).

There are two principle methods of refining, namely chemical refining and physical refining as shown in Figure 1-1.

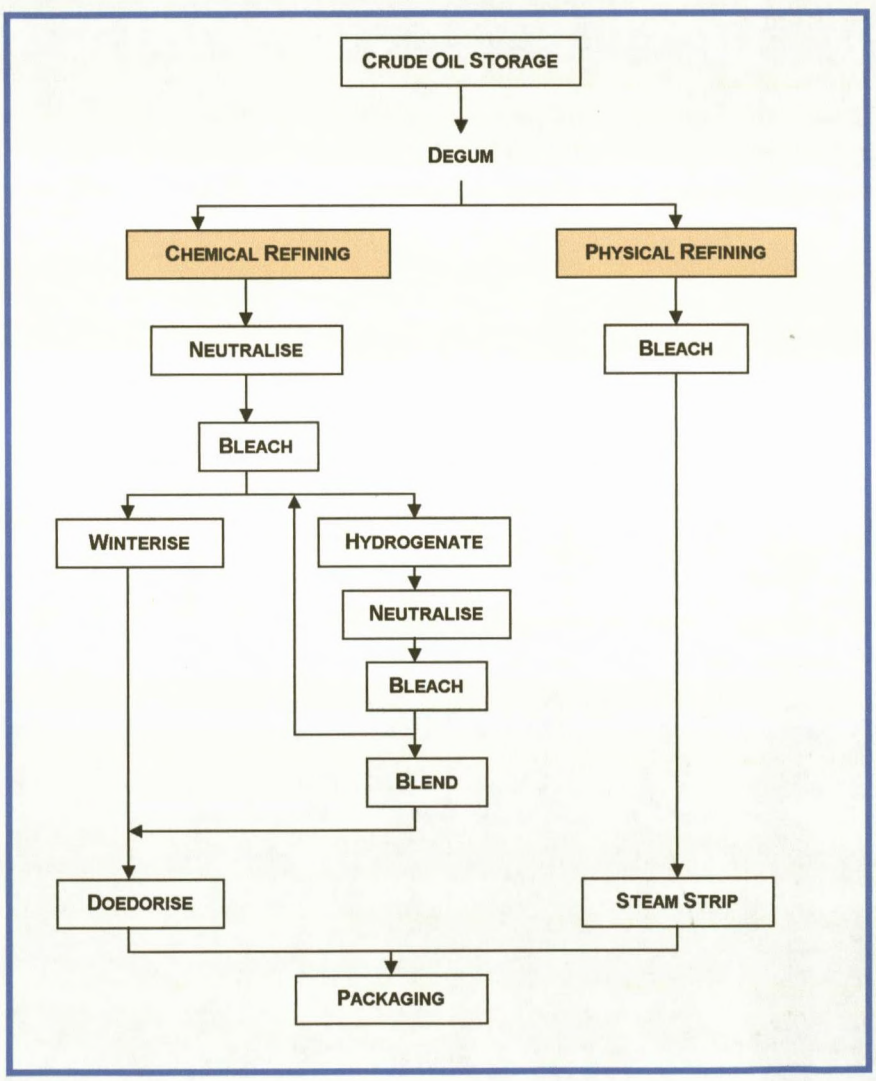

Figure 1-1: Diagram illustrating Vegetable Oil Effluent (WRC Report, 1989).

During the neutralization process, fatty acids are neutralized (Rohbrecht-Buck and Sekoulov, 1990; WRC Report, 1989) by the addition of caustic soda, which results in saponification (WRC Report, 1989). The soapstock produced is separated from the neutralized oil using centrifuges or by gravity settling, depending on whether batch or continuous operating equipment is used (WRC Report, 1989). Soapstock is further treated on-site, producing acid oil (WRC Report, 1989). The bleaching process, in addition to colour removal, acts as a purification step (WRC Report, 1989). During hydrogenation, hydrogen gas is dispersed in the oil in the presence of a catalyst (nickel). Naturally occurring oils contain fatty acids, which have up to six double bonds in the chain. Hydrogenation attempts to saturate these selectively, reducing the six double bonds to five, the five to four and so on (WRC Report, 1989). This produces fats with superior keeping qualities as well as higher melting points. Hydrogentaion also imparts a characteristic flavouring to the product; therefore, the oil must be deodorized (WRC Report, 1989).

Certain vegetable oils contain waxes, which cause an undesirable cloudy appearance at lower temperatures, when they begin to solidify. Winterising reduces the amount of waxes by cooling the oil to 5^0C followed by filtration to remove the solidified wax (WRC Report, 1989).

Deodorising involves steam distillation under vacuum, to remove residual free fatty acids, aldehydes and ketones (which are responsible for unacceptable odours and flavours) as well as to decolourise the final oil by thermal decomposition of the pigments (WRC Report, 1989; Rohbrecht-Buck and Sekoulov, 1990).

The physical refining technique (or steam stripping) involves the use of deodorizers for the team distillation of all free fatty acids as well as odouriferous volatiles without a prior neutralizing stage (WRC Report, 1989).

1.4. VEGETABLE OIL EFFLUENT

The vegetable oil industry consumes approximately 1.75 million.m^3 of water each year. An oil plant typically discharges about 35% incoming water to the sewers. The remaining 65% is either vapourised in cooling circuits, or leaves the site in secondary products or by-products (WRC Report, 1989). The effluent discharged into sewers contains quantities of fat, oil, sodium, sulphates, phosphates and other pollutants (WRC Report, 1989). The effluent therefore has a high inorganic as well as organic loading. The quantity and contamination of wastewater from vegetable oil refineries depends on the temperature and hardness of cooling water used, processing equipment used (continuous or discontinuous processes), process temperature and pressure as well as raw materials used (Rohbrecht-Buck and Sekoulov, 1990). The largest portion of effluent discharged by a vegetable oil plant arises from the refining process, which comprises approximately 80% of the effluent volume (WRC Report, 1989). Once the crude oil is neutralized with subsequent soap splitting, water is used for washing, resulting in effluents known as soapy water and acid water. These wastewaters can contain quantities of fats and oils as well as high levels of sodium and sulphate (WRC Report, 1989). The main pollutants in vegetable oil effluents are fats and oils. Oils are often emulsified following the use of detergents in washdown operations, leading to high levels of oil in the effluent, which are difficult to remove (WRC Report, 1989). Approximately 35% of incoming water from the vegetable oil industry is discharged into sewage works (WRC Report, 1989). Oils and greases tend to clog sewers and pumps, thus creating difficulties within the municipal wastewater treatment works (Sengul, 1990).

1.4.1. Vegetable Oil Effluent Treatment

The role of industry has a major impact on the deterioration and preservation of natural resources (Ozturk *et al.*, 1990). Effective treatment of vegetable oil effluent is dependent on total plant housekeeping, conservation of water usage, integrated treatment system design, accurate process control as well as close supervision and operation (Seng, 1980). Vegetable oil industries have incorporated methods into the production process to avoid or reduce pollutants.

1.4.1.1. Physical-chemical Treatment

Physico-chemical treatment methods have been attempted, providing a considerable reduction in organic loading; however, discharge standards are still not met (Rohbrecht-Buck and Sekoulov, 1990; Eroglu *et al.*, 1990).

1.4.1.1.a. Gravity Separation

Gravity fat traps have been used in an attempt to remove fatty materials from vegetable oil effluent. In its simplest form, the system involves separating fats and oils from the effluent by flotation (WRC Report, 1989). The flotation system may consist of a tank containing a series of baffles beneath which the aqueous phase can flow freely. A manometric arm maintains the liquid level at baffle height to trap fatty material, which necessitates periodical manual removal of fats from the surface. The fat removed can often be processed (WRC Report, 1989). The effectiveness of gravity fat traps depends on water temperature, the density and size of the oil globules as well as the amount and characteristics of suspended matter (WRC Report, 1989). It was found that such gravity fat traps could not reduce emulsified fatty material wastes to conform to standards of the sewer system (Eroglu *et al.*, 1990; WRC Report, 1989). Other parameters undergo limited reduction, any emulsifier, spent caustic or detergent present tends to reduce efficiency of removal, suspended solids attached to oil may not settle and hydraulic overloading reduces efficiency (WRC Report, 1989).

1.4.1.1.b. Dissolved Air Flotation (DAF)

An alternative treatment to gravity fat traps is Dissolved Air Flotation (DAF). Air is dissolved in the effluent in a pressure vessel before being released into an open flotation tank. A sudden drop in pressure causes the air to leave solution as fine bubbles, which adhere to oil, fat and other suspended solids and rise to the surface. A skimming device (rotating arm) then removes the layer of solids, fats and oils (WRC Report, 1989). DAF units have displayed satisfactory removal efficiencies when compared to the gravity fat traps. However, it has been proven ineffective without additional treatment (Eroglu *et al.*, 1990). Vegetable oil effluents have been treated with

coagulant acids, ferric chloride being the most effective; however, still do not meet discharge standards (Ozturk *et al.*, 1990). Thus, biological treatment methods are being sought after. At least one vegetable oil plant in South Africa runs an activated sludge treatment facility as a polishing unit to complement a DAF system. However, shock loads pf for example soap stock spillages, cause problems while running such a treatment process (WRC Report, 1989).

1.4.1.2. Aerobic Treatment of Vegetable Oil Effluent

Aerobic treatment of VOE has been investigated using activated sludge. The effluent was pretreated with flocculant C_{40}, to reduce the lipid content of the VOE, since the high lipid content of the effluent initially caused blockages of the system. The effluent was supplemented with nitrogen and phosphorous, as it was found that these elements were lacking in the substrate. The system was fed pretreated effluent at an initial COD of 500mg/L, gradually increasing the COD concentration as the microorganisms became acclimatized to the substrate. The percentage COD removed was found to be ± 90%, with the VOE fed at a COD of 1800mg/L (Frost, 2001).

1.4.1.3. Anaerobic Digestion of Palm Oil and Olive Oil Mill Effluent

Vegetable oil can be processed from a variety of sources. In South Africa the most commonly processed seeds are sunflower, groundnut and maize, although cotton and soya are also processed (WRC Report, 1989). In other parts of the world palm oil and olive oil are more commonly produced, Malaysia as the worlds leading producer and exporter of palm oil 9Idris and Al-Mamun, 1998). Effluent arising from the production of olive and palm oil contain various pollutants and attempts have been made to treat these effluents anaerobically.

1.4.1.3.a. Anaerobic Treatability of Palm Oil Mill Effluent (POME)

Effluent arising from palm oil production is a complex waste containing residual oils and greases, polysaccharides and proteins (Cail and Barford, 1985 a;b). Characteristics of palm oil mill effluent are shown in Table 1-1.

Table 1-1: Characteristics of Palm Oil Mill Effluent (Cail and Barford, 1985 a;b).

COMPONENT	RANGE
COD	45 00 – 75 000 mg/L
BOD	20 000 – 40 000 mg/L
TS	40 000 – 60 000 mg/L
SS	20 000 – 30 000 mg/L
pH	4.5 – 5.0

Although many POME industries in Malaysia use conventional ponding systems to treat POME, the trend is changing towards anaerobic treatment systems (Idris and Al-Mamun, 1998). Anaerobic treatability of POME using Anaerobic Fluidized Bed Reactors (AFBR) has been investigated. It was reported that the AFBR can be operated at HRT of 12 hrs with a COD removal of 85% and at HRT of 4hrs up to 65% COD removal (Idris and Al-Mamun, 1998). High rate semi-continuous anaerobic digestion of POME was also investigated and it was found that at HRT of 5.6 days with a space loading of 12.6kgCOD/m^3.d, soluble COD removals were greater than 97% (Cail and Barford, 1985a).

1.4.1.3.b. Anaerobic Treatability of Olive Oil Mill Effluent

Olive oil effluent consists of 15% VS and 2% inorganic matter. The major component of the inorganic fraction is potassium. Sugars, polyphenols, polyalcohols, proteinaceous and lipid compounds, pectins etc. constitute the organic fraction (Borai *et al.*, 1984). Anaerobic treatability and methane potential of olive oil mill effluent has been investigated. It was found that olive oil wastewater yielded biogas with a methane content of $77_{\pm}6\%$ and 57.5L CH_4/L wastewater. COD removal of 85-93% was achieved (Demirer *et al.*, 2000). Laboratory and pilot scale UASB reactors were used to investigate anaerobic treatment of olive oil mill effluent yielding COD removal efficiencies of 70% (Demirer *et al.*, 2000). It was concluded that anaerobic digestion is a feasible treatment method for vegetable oil produced from palms and olives. Various sources of vegetable oil require different production methods and therefore produce wastewaters with distinct differences (WRC Report, 1989).

1.5. ANAEROBIC DIGESTION

Anaerobic digestion, a process occurring widely in nature, was harnessed just over a century ago (1881) for the removal of putrecible (sewage) suspended solids from municipal wastewaters (McCarthy, 1982). Anaerobic digestion is the use of biological processes in the absence of oxygen for the stabilization of organic materials by conversion to methane and inorganic end products including carbon dioxide and ammonia (McCarthy, 1982). Anaerobic digestion of complex organic materials can be considered to be a mutli-stage process.

Anaerobic digestion offers tremendous potentials for treating high strength as well as low strength industrial wastewaters. As compared with conventional aerobic wastewater treatment processes, anaerobic digestion offers a number of significant benefits. Production of energy from pollutants is one of the major benefits (Lettinga, 1985). As a result of its proven capacity to degrade toxic pollutants as well as common organic pollutants present in industrial wastewaters, anaerobic digestion has advanced to a high level of usefulness in treating many industrial effluents (Speece, 1996).

1.5.1. Anaerobic versus Aerobic Biotechnology

The common alternative to anaerobic biotechnology for treatment of industrial wastewaters is the aerobic biological process. Aerobic processes use oxygen as the electron acceptor and anaerobic processes use sulphate or carbon dioxide as an electron acceptor (Speece, 1996). Major factors for comparison include electrical power usage, methane gas production and excess microbial cell production with an associated disposal cost. Table 2-1 shows the comparison of anaerobic / aerobic biotechnology.

1.5.1.1. Excess Biomass Production

Aerobic treatment usually produces about 10 times more refractory biomass than its anaerobic counterpart. Although excess biomass from anaerobic treatment is no less refractory than from aerobic, only 5-20% as much biomass is generated as compared to the aerobic treatment

process (Speece, 1996). The reduced costs of excess cell disposal or reduced electrical consumption are contributing factors favouring usage of anaerobic digestion rather than methane production as sole justification (Speece, 1983). The cell walls of biomass are very complex and therefore refractory to further biotransformation (Speece, 1996). Under aerobic and anaerobic conditions, only 25-40% of the resultant biomass can be further biodegraded. The remaining biomass is so refractory that it cannot practically be destroyed by any natural means short of fire or chemical hydrolysis (Gossett and Besler, 1982). More free energy is available to the microorganisms when oxygen is the electron accpetor. The abundance of free energy in aerobic processes is reflected by the larger amounts of excess biomass produced.

1.5.1.2. Organic Loading Rate

It is possible to maintain 5-10 times more MLVSS concentration in anaerobic processes than in aerobic processes (Speece, 1996). As a result, the anaerobic volumetric loading rates are often >10 times higher than possible for aerobic treatment. Due to higher loading rates, anaerobic biotechnology applied to industrial wastewaters utilizes a much smaller reactor and produces a greatly reduced waste biomass than would be necessary if an aerobic process were applied to the same effluent. The associated significant financial savings and reduction of environmental impact of anaerobic digestion therefore more than compensates for long start-up time requirements (Speece, 1996).

Table 2-1: Comparison of anaerobic /aerobic biotechnology (Speece, 1996)

COMPARISON OF ANAEROBIC / AEROBIC BIOTECHNOLOGY
• Anaerobic volumetric loading rates 5-10 times higher than for aerobic treatment
• Excess biomass production 5-20% of that for aerobic treatment
• Nutrient requirements 5-20% of those for aerobic treatment
• Anaerobic biomass can be preserved for years without serious deterioration in activity
• No aeration energy required for anaerobic treatment whereas 500-2000kwhr/1000kg COD for aerobic treatment
• Methane production of 12 000 000 BTU/1000kg COD destroyed

1.5.2. Anaerobic Digestion Process

Anaerobic digestion involves the breakdown of organic matter by the action of microorganisms in the absence of oxygen producing biogas, which contains methane and carbon dioxide as well as quantities of ammonia, nitrogen, hydrogen and hydrogen sulphide (Callander and Barford, 1983).

Although some fungi and protozoa can be found in anaerobic digesters, bacteria are the dominanat microorganisms. The consortia of microorganisms involved, consists of several trophic groups that possess different carbon catabolizing functions (Stafford, Wheatley and Hughes, 1980). The four different groups include hydrolytic bacteria, hydrogen-producing acetogenic bacteria, homoacetogenic bacteria and methanogenic bacteria as shown in Figure 1-2 (Stafford, Wheatley and Hughes, 1980; Bulock and Kristiansen, 1987).

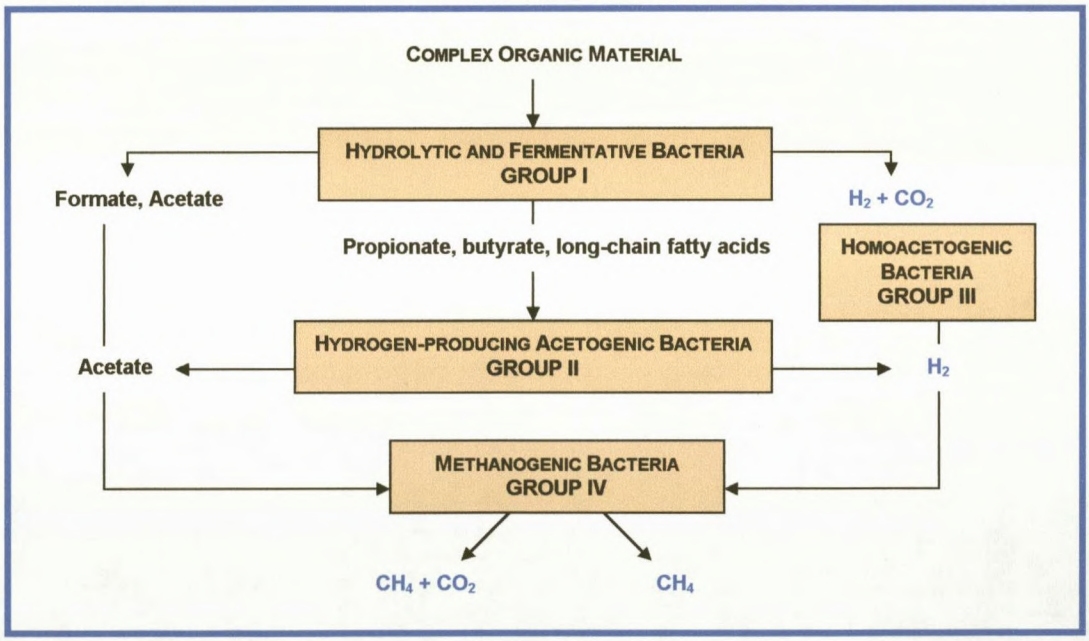

Figure 1-2: Diagram illustrating the four trophic groups involved in anaerobic digestion

1.5.2.1. Hydrolytic and Fermentative Bacteria

The group consists of both obligate and facultative anaerobes (e.g. *Bacteriodes, Bifidobacterium, Clostridium, Lactobacillus* and *Streptococcus*), which hydrolyse complex organic molecules (eg.

Proteins, cellulose, lignin and lipids) into monomers such as amino acids, glucose and fatty acids. These monomers are directly available to the next group of bacteria (Bitton, 1994). Ectracellular enzymes such as cellulase, proteases and lipases catalyze hydrolysis of the complex molecules (Bitton, 1994).

1.5.2.2. Acetogenic Bacteria

Acetogenic bacteria, such as *Syntrobacter wolinii* and *Syntrophomonas wolfei*, consist of hydrogen-producing and homoacetogenic bacteria (Bitton, 1994). Hydrogen-producing acetogenic bacteriacatabolize certain fatty acids (eg.propionic acid, butyric acid). Homoacetogenic bacteria catabolize unicarbon compounds or hydrolyse multicarbon compounds to acetic acid (Stafford, Wheately and Hughes, 1980), which becomes the substrate for a group of strictly anaerobic methanogenic bacteria.

1.5.2.3. Methanogenic Bacteria

The Methanogenic bacteria consist of gram-positive and gram-negative bacteria (Bitton, 1994). From a taxonomic viewpoint methanogenic bacteria, belong to a separate kingdom (Archaebacteria). Methanogens differ from other bacteria in that methanogens lack muramic acid in their cell walls; they have a specific coenzyme, F_{420}, which acts as an electron carrier in metabolism and they possess ribosomal RNA sequences that differ from those of other prokaryotes (Bitton, 1994). Methanogens are grouped into 3 orders, namely, Methanobacteriales (e.g. *Methanogenobacterium, Methanobrevibacter, Methanothermus*), Methanomicrobiales (e.g. *Methanomicrobium, Methanogenium, Methanospirillum, Methanosarcina* and *Methanococcoides*), and Methanococcales (e.g. *Methanococcus*) (Bitton, 1994).

Methanogenic bacteria are often considered the key class of microorganisms in anaerobic digestion as well as the most fastidious of all the microorganisms responsible for anaerobic conversion of organics to methane. They are also known for their low synthesis rate (Speece, 1983). Methanogenic bacteria can be subdivided into two categories, namely, hydrogenotrophic

and acetotrophic methanogens (Bitton, 1994). The hydrogenotrophic methanogens, hydrogen-utilizing chemolithotrophs, convert hydrogen and carbon dioxide to methane (Eq.1). The acetotrophic methanogens, also know as acetoclastic or acetate splitting bacteria, convert acetate to methane (Eq.2) and are comprised of two main genera, namely, *Merthanosarcina* and *Methanothrix* (Bitton, 1994).

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \qquad \textbf{Eq. 1}$$

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad \textbf{Eq.2}$$

An obligate, syntrophic relationship exists between the hydrogen-producing acetogenic bacteria and the hydrogenotrophic methanogens (Speece, 1983). The hydrogen partial pressure must be maintained at an extremely low level to ensure favourable thermodynamic conditions for the conversion of volatile acids and alcohols to acetate. Thus, it is necessary that the hydrogen-producing methanogens maintain these extremely low hydrogen partial pressures in the system (Stafford, Wheatley and Hughes, 1980; Bitton, 1994; Bulock and Kristiansen, 1987; Speece, 1983). If low hydrogen partial pressures are not maintained, higher volatile acids such as propionic and butyric acid will accumulate in the system (Speece, 1983).

1.5.2.4. Sulphate Reducing Bacteria (SRB)

Sulpahte is present in many wastewaters as a result of the use of sulphuric acid in chemical processing. Sulphate is utilised by SRB as an electron acceptor, with hydrogen sulphide as the main product of the reaction. SRB may also utilize sulphite, which is an intermediate step in sulphate reduction. Methane producing bacteria (MPB) and SRB utilize the same energy sources, namely, acetate and hydrogen. Therefore the SRB compete with the MPB for the same energy sources (Sarner *et al.*, 1988).

Methane Production

$4H_2 + HCO_3^- + H^+ \longrightarrow CH_4 + 3H_2O$ **Eq.3**

$CH_3COO^- + H_2O \longrightarrow CH_4 + HCO_3$ **Eq. 4**

Sulphate Reduction

$4H_2 + S + H^+ \longrightarrow HS^- + 4H_2O$ **Eq.5**

$CH_3COO^- + SO_4^- \longrightarrow HS^- + 2HCO_3^-$ **Eq.6**

These two groups of bacteria have many similarities, namely, most are strictly anaerobic, chemoheterotrophic and have similar temperature and pH requirements. Therefore they are found co-existing in many anaerobic ecosystems (Zhou and Fang, 1998) although, SRB are less sensitive to pH and temperature variations (Sarner *et al.*, 1988). Growth kinetics studies of SRB and MPB have shown that sulphate-reducing bacteria have a higher affinity for acetate (k_s = 9.5mg/L) than MPB (k_s = 32.8mg/L), therefore SRB will outcompete MPB under low acetate concentrations (Schonheit *et al.*, 1982). This competitive inhibition results in shunting of electrons from methane production to sulphate reduction (Schonheit *et al.*, 1982). This competitive inhibition results in shunting of electrons from methane production to sulphate reduction (Schonheit *et al.*, 1982; Speece, 1996).

1.5.3. Process Parameters

Anaerobic treatment must be monitored regularly to ensure successful operation (Switzenbaum et al., 1990). The principal causes of digester failure, apart from mechanical failure include, hydraulic and organic overloading of the microorganisms with a resultant pH and loss of biomass, as well as the presence of inorganic and organic toxic materials (Graef and Andrews, 1974; Ross and Louw, 1987; Switzenbaum *et al.*, 1990). As a result, whatever the cause of failure, a retardation or even complete cessation of gas production is observed. Revitalisation of the biomass (if possible) is often time consuming due to the slow growth rate of the methanogens (Ross and Louw, 1987).

There are various process control indicators, which measure digester progress and give warning of impending upset so that preventative action can be taken (Ross *et al.*, 1992). To date, no single parameter is sufficiently sensitive to permit reliable forecasting of incipient overloading or digester failure (Ross *et al.*, 1992; Ross and Louw, 1987). Important features of a good indicator are its ability to detect process imbalance at an early stage as well as its ability to reflect the metabolic state of the system directly (Ahring *et al.*, 1995). Some of the more commonly used indicators include VFA/Alkalinity ratio, gas production rates, gas composition, pH, volatile solids and COD reductions. Daily monitoring of several of these parameters with regards to general trends usually aids in applying corrective actions before the process fails (Switzenbaum *et al.*, 1992).

Mixing plays an important role in anaerobic digestion. Mixing promotes intimate contact between bacteria and substrate therefore allowing biological reactions to proceed more efficiently (Hughes, 1981; Ross *et al.*, 1992). Good mixing provides effective use of the entire reactor volume, aiding in maintaining uniform temperature and solids concentration throughout the digester (Hughes, 1981; Ross *et al.*, 1992). It reduces the effect of toxic substances through rapid dispersion and dilution and it also facilitates the release of biogas from lower levels of the digester (Ross *et al.*, 1992).

Anaerobic digesters are heated to increase the activity of the MPB as well as to liquefy fats and greases to hasten their degradation (Ross *et al.*,1992). The MPB are most sensitive to temperature changes and their activity is severely affected by sudden changes in excess of 2-3^{0}C, whereas the acid-forming bacteria are less sensitive to environmental changes (Ross *et al.*, 1992). Unbalanced conditions may encourage volatile acids to be produced faster than they can be utilised by MPB, thus increasing their concentration in solution (McCarthy and McKinney, 1961; Ross *et al.*, 1992; Speece and Kem, 1990). Accumulation of VFA to concentrations exceeding the buffering capacity of the digester leads to a drop in pH (McCarthy and McKinney,

1961; Switzenbaum *et al.*, 1990). In a healthy digester volatile acids are utilised by the MPB at about the same rate as they are produced and under these conditions, the volatile acid concentration of the digester contents is usually in the range 50-300mg/L (expressed as acetic acid) (Ross *et al.*, 1992).

The maintenance of proper pH is therefore one of the most important requirements for effective anaerobic digestion. The pH of the digester contents is controlled by volatile acids production and alkalinity. The best pH range for methanogenic bacteria is between 6.8 and 7.2 (Ross *et al.*, 1992; Switzenbaum *et al.*, 1990) and are inhibited when the pH falls below 6.8 (Ross *et al.*, 1992). Acid forming bacteria are not severely inhibited by pH changes (Ross *et al.*, 1992). An overload of feed will cause a drop in pH, as more volatile acids will be produced than alkalinity. Alkalinity of a digester is important as it represents the ability of the digester to neutralize volatile acids produced during the anaerobic digestion process (Ross *et al.*, 1992).

Effective anaerobic digestion cannot occur without methane production (Ross *et al.*, 1992); Speece, 1996), as no methane production indicates no energy yield, no growth of the crucial methanogenic bacteria as well as no COD/BOD reduction of the organic pollutant (Speece, 1996). The conversion of organic matter to biogas is thus a useful indicator, which provides warning of digester upset (Ross *et al.*, 1992). The CO_2 content of biogas usually increases with a corresponding decrease in CH_4 content, during digester overload conditions. This is caused by the inhibition of MPB at the reduced pH value as a result of increased concentration of volatile fatty acids (Ross *et al.*, 1992).

The main drawbacks of anaerobic digestion include long retention time as a result of the slow growth rate of anaerobic bacteria. As well as process instability due to hydraulic and organic shockloads. These drawbacks have been overcome by the development of high-rate digesters, which achieve separation between the Hydraulic Retention Time (HRT) and Solid Retention Time (SRT). Therefore, slow-growing anaerobic microorganisms are allowed to remain within the

reactor independent of the wastewater flow (Nachaiyasit and Stuckey, 1997a). Successful operation of the anaerobic digestion process necessitates detailed consideration in selecting an appropriate process configuration (Speece, 1983). Various configurations have implications for the ratio SRT/HRT. Maintenance of process stability requires maximal SRT (Switzenbaum *et al.*, 1990) and minimal HRT (Speece, 1996), since the lower the HRT the smaller the reactor volume, which reduces capital costs (Speece, 1983; Callander and Barford, 1983). There are two mechanisms for achieving this: a) The natural tendency of biomass to form flocs (aggregates), large enough to settle can be enhanced by providing optimal conditions in digesters, and b) Provision of surfaces within digesters to which bacteria can attach in films (Callander and Barford, 1983).

1.5.4. Kinetic Models

It is important to understand the kinetics of methane fermentation to operate and design optimum systems. Several kinetic models have been used to describe the anaerobic fermentation process. The Monod (1950) kinetic model is able to predict the conditions for maximum biological activity and when activity will cease, also the kinetic parameters (i.e. the microorganisms maximum specific growth rate and half velocity constant) have deterministic connotations, which describe the microbial processes (Hashimoto *et al.*, 1979). Disadvantages of the Monod model are that one set of kinetic parameters cannot describe the biological process at short and long retention times and that the kinetic parameters cannot be obtained for certain complex substances (Hashimoto *et al.*, 1979).

The Contois (1959) kinetic model has the advantages and avoids the disadvantages associated with the Monod model. The Contois model was adapted to describe the kinetics of methane fermentation (Chen and Hashimoto, 1979), which is as follows.

$$Yv = \frac{BoSo}{\theta} - [1 - \frac{k}{\theta\mu - 1 + k}] \qquad \textbf{Eq.7}$$

Where :

Yv	=	*Volumetric methane production rate, LCH_4/L fermenter.d*
Bo	=	*Ultimate methane yield, LCH_4/gVS added as $\theta \rightarrow \infty$*
θ	=	*Retention time, d.*
μm	=	*maximum specific growth rate of microorganisms, d^{-1}*
k	=	*Saturation constant, dimensionless*

The above equation states that for a given loading rate (So/θ), the daily volume of methane per volume of fermenter depends on the biodegradability of the substrate (Bo) and the kinetic constants μm and k (Hashimoto *et al.*, 1979).

1.5.4.1. Ultimate Methane Yield

Equation 7 shows that the amount of the methane produced is directly proportional to the ultimate methane yield (Bo). Ultimate methane yield can be obtained by two methods. One method involves plotting the steady-state methane yield (LCH_4/gVS added) versus the reciprocal of the retention time and extrapolating to an infinite retention time (i.e. 1/θ = 0) (Chen and Hashimoto, 1979). Another method involves incubating known amount of substrate until a negligible amount of methane is produced. Theorectically, mineralization of 1g of COD is equivalent to the production of 0.35liters of methane at STP (McCarthy, 1964). The ultimate methane yield is therefore a measure of the biodegradability of the waste.

1.6. SUMMARY

As a result of the development of high-rate systems together with advances in the fundamental microbiology and biochemistry of the anaerobic methane fermentation process, anaerobic digestion is being used more frequently for the treatment of various wastewaters (Switzenbaum *et al.*, 1990). Dwindling reserves of fossil fuels has also increased interest with regards to the

potential usage of anaerobic digestion processes for converting organic residues to methane gas (Owen *et al.*, 1979). This is undoubtedly one of the main benefits of anaerobic digestion since energy is produced from organic pollutants, whereas the energy requirements of the process are nil (Lettinga, 1985). Anaerobic digestion produces excess biomass 5-20% of that produced by aerobic processes, thus reducing financial and waste disposal site requirements (Speece, 1996). Substantially reduced nutrient requirements and no energy requirement for aeration also provide savings (Speece, 1996; Lettinga, 1985). This research project investigates the potential usage of anaerobic digestion for treatment of vegetable oil effluent.

2.1. INTRODUCTION

2.1.1. Vegetable Oil Effluent

The VOE used for this investigation has 3 main sources, namely refinery effluent, acid water and lye, as shown in Figure 2-1.

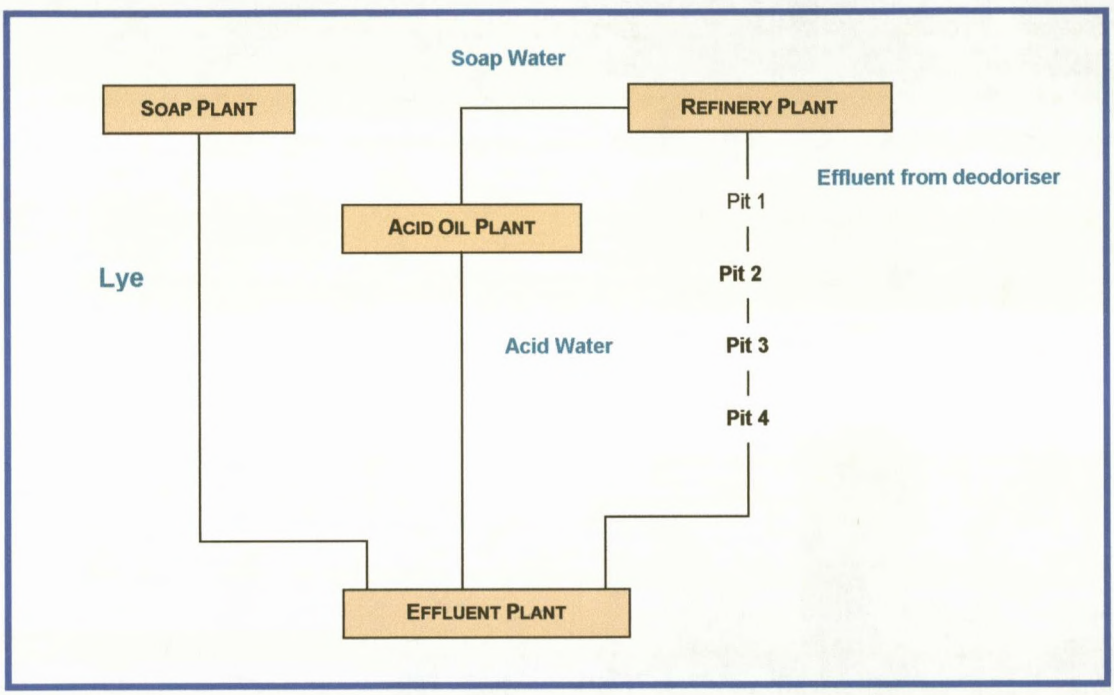

Figure 2-1: Schematic representation of the components of final vegetable oil effluent

The deodorizing process, in the refinery plant, produces refinery effluent. This effluent then proceeds to gravity separators or pits 1to 4 and is then pumped to the effluent plant. Soap water, generated from the degumming and neutralization stages in the refining process, proceeds to the acid oil plant where it is mixed with sulphuric acid to form acid oil. This is known as acid water, which proceeds to the effluent plant and mixed with lye. Lye effluent is generated from the

manufacture of soap. When no lye is produced, the acid water is mixed with caustic soda to raise the pH to about 4.

Effective treatment of refinery effluent may be achieved through a combination of screening, acid splitting of oil emulsions, skimming of fats and final neutralization. On-site treatment methods used on the effluent collected is illustrated in Figure 2-2.

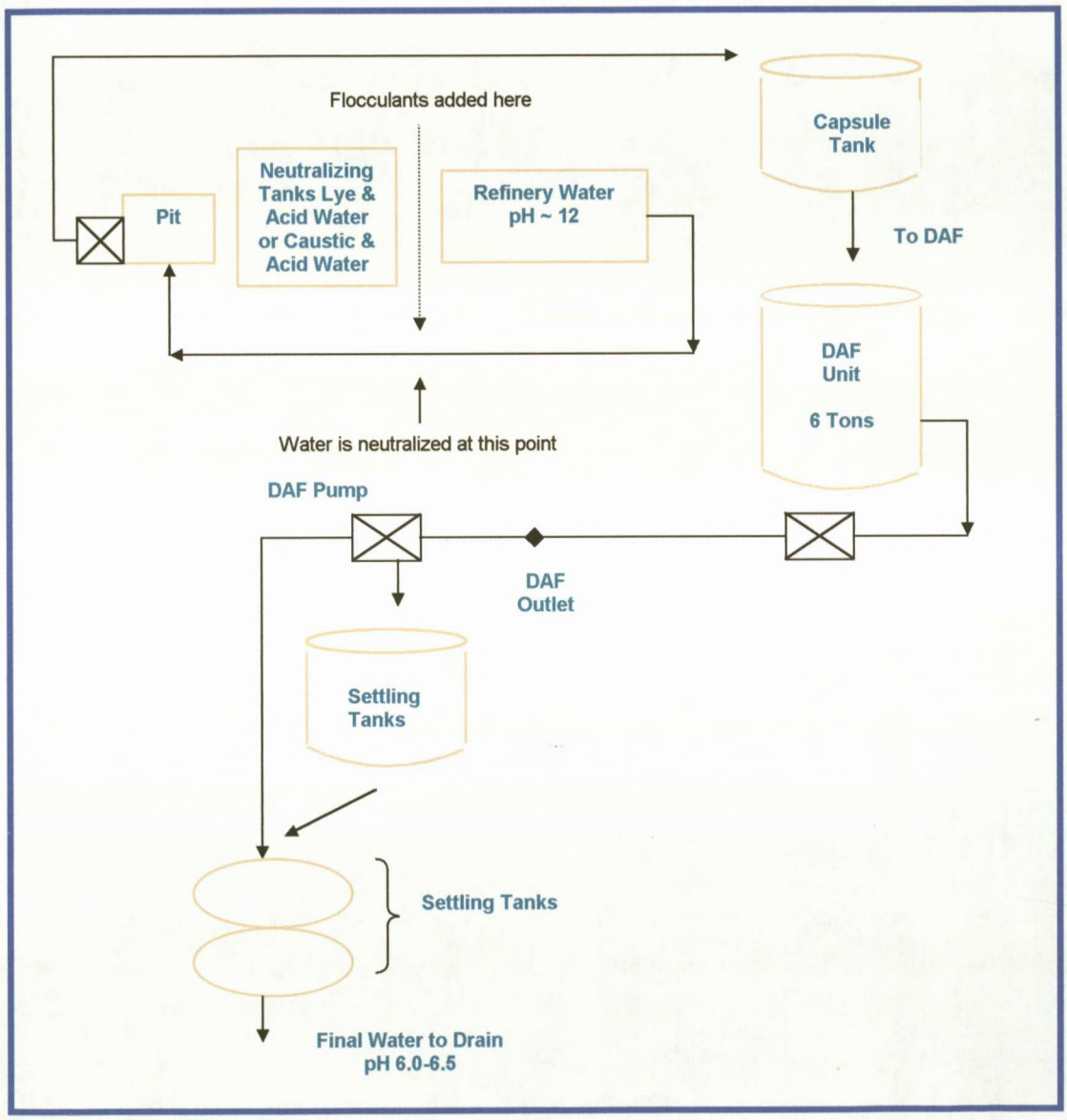

Figure 2-2: Schematic representation of on-site effluent treatment

The bulk of fatty material found in soapy water, deodorizer effluent and final effluent is an emulsified form and cannot be removed by direct application of gravity separation techniques. Destabilisation of these emulsions can be achieved by the use of sulphuric acid and as a result, the separated fatty material may be recovered. With prior soap splitting, the acid water generated may be used for this purpose and fresh used on a stanby basis (Eroglu, 1989).

Surujlal, (1999) assessed the VOE over a few months and found the following characteristics of final effluent. High sulphate concentrations (30 000mg/L) were caused by the use of sulphuric acid in the acid water and the absence of lye in the final effluent (which would have diluted the final sulphate concentration). The presence of lye also affected the COD content of the effluent. When the soap factory was in operation, lye was produced, increasing the COD content from approximately 8 500mg/L to 14 775mg/L. The fluctuation of the phosphorous concentration was due to the method of refining (i.e. chemical or physical refining). Thus it can be seen that the characteristics of the final effluent vary according to the refining methods used as well as the type of end products produced.

One of the objectives of this study is to investigate the anaerobic biodegradability of VOE. Two of the main pollutants in VOE are sulphates and lipids. Industrial effluents containing high sulphate concentrations discharged into surface waters contribute directly to mineralisation and corrosion potential of receiving waters (du Preez and Maree, 1991). Sulphate is utilised by SRB as an electron acceptor, with hydrogen sulphide as the main product (Sarner *et al.*, 1988). Since MPB and SRB compete for the same energy sources, it was decided to pretreat the wastewater so as to reduce the sulphate content of the substrate. Resulting in ultimate methane production rather than hydrogen sulphide. Lipids are known to be inhibitory to methanogenic bacteria, key microorganisms involved in anaerobic digestion (Hamdi, 1991). Pretreatment experiments to reduce the lipid content of the substrate were therefore also investigated.

2.1.2. BMP and Toxicity Assays

Doubts have been cast on the efficiency of anaerobic treatment, as many potential residues for bioconversion are relatively non-biodegradable and may also contain compounds, which are toxic to methanogenic bacteria. Bioassay techniques are essential for determining biodegradability, as no chemical procedure is available to distinguish between biodegradable and non-biodegradable organic compounds (Owen *et al.*, 1979).

Owen *et al.*, (1979) presented techniques for measuring biodegradability (Biochemical Methane Potential – BMP) as well as toxicity (Anaerobic Toxicity Assay – ATA) of a substrate by subjecting it to anaerobic treatment. The bioassays are relatively simple and inexpensive (Owen *et al.*, 1979). The BMP assay requires minimal labor for setting up and monitoring the process (Speece, 1996). BMP is a measure of the biodegradability of a substrate, which is determined by monitoring cumulative methane production from a sample, which is anaerobically incubated in a defined medium (Owen *et al.*, 1979). The toxicity assay measures the adverse effect of a compound on the rate of total biogas production from an easily utilised methanogenic substrate (Owen *et al.*, 1979). In other words, ATA indicates the inherent toxicity of an effluent determined by a decrease in metabolic rate, relative to a control. The acetate-propionate solution is added to the assay bottles as a direct methanogenic precusor. Substrate is then added and if the substrate or its constituents are inhibitory to the methanogenic bacteria, metabolism would decrease and gas production would be lower than the controls. Thus, anaerobic toxicity is determined as the adverse effect of a substrate on the predominant methanogenic bacteria (Owen *et al.*, 1979).

Both techniques (BMP and ATA) involve anaerobic serum bottles containing defined medium, and seed inoculum, which are incubated at a desired temperature. Gas volumes are monitored volumetrically using the syringe method (Owen *et al.*, 1979). In most cases the incubation period is 30 days, which allows virtually complete decomposition of biodegradable organics, however, some organics may require a longer period for the microorganisms to acclimatize (Owen *et al.*,

1979). An important factor for the BMP assay is that the biomass must be anaerobically converted to methane as well as to measure residual organic pollutants amenable for further anaerobic treatment (Speece, 1996). The assay may also be used to test for non-biodegradable matter remaining after treatment (Speece, 1996).

2.2. MATERIALS AND METHODS

2.2.1. Characterisation of Vegetable Oil Effluent

Final vegetable oil effluent was collected and stored at 4^0C until required. Figure 2-2 illustrates the various components of the final effluent collected.

2.2.2. Pretreatment of Vegetable Oil Effluent

Two methods of pretreatment was investigated, namely lipid reduction by gravitational separation and sulphate reduction using $BaCl_2$.

2.2.2.1. Lipid Reduction by Gravitational Separation

A well-mixed sample of VOE (100mL) was placed into a 100mL-separating funnel and allowed to stand for 20 minutes. Thereafter, 50mL was removed and referred to as the settled layer. The remaining 50mL was removed and referred to as the top layer. Both samples were then analysed. The experiment was repeated with the following settling time periods (1hr, 2hrs, 4hrs, 24hrs).

2.2.2.2. Sulphate Reduction using Barium Chloride

A BaCl2 solution was prepared 5 times in excess of the sulphate content present in the wastewater sample. 2.125g $BaCl_2$ was weighed and then dissolved in 20mL distilled water. The $BaCl_2$ solution was added slowly to the wastewater sample, with continuous mixing. The sample was allowed to stand for 1hr, producing a white precipitate. The supernatant was decanted and analysed.

2.2.3. Biochemical Methane Potential and Toxicity Assays

The BMP and anaerobic toxicity assays were carried out according to the method described by Owen *et al.*, 1979).

2.2.3.1. Anaerobic Sludge

The experiment was carried out with anaerobic sludge collected from a mesophilic digester at a watstewater treatment works. Properties of the anaerobic sludge used are outlined in Table 2-1.

Table 2-1: Properties of Anaerobic Sludge

PARAMETER	mg/L
pH	7.16
COD	14 520
Alkalinity	12 845
Lipids	16 400
Sulphates	60
VSS	8 690
TSS	4 385
TDS	800

The sludge was stored at 4^0C until required. Sludge storage has been found to have no significant effect on the extent of degradation (Shelton and Tiedjie, 1984).

2.2.3.2. Preparation of assay bottles

The experiment was carried out in 125mL bottles with butyl rubber septa and metal caps as well as 225mL bottles with butyl rubber septa and screw caps. The bottles were gassed with OFN (oxygen free nitrogen), and then closed prior to the introduction of samples, defined medium and inoculum.

2.2.3.3. Defined Medium

The defined medium used for these experiments was adapted from the method outlined by Owen et al., 1979) (Appendix H). The defined medium was revised to avoid precipitation. Sodium

sulphide is used to provide a reducing environment (Owen *et al.*, 1979). However, when added to the medium caused a thick black precipitate. Thus, soudium sulphide was substituted with cysteine, which also provides a reducing environment. Ferric chloride was mixed with EDTA to facilitate dissolving. Sodium carbonate ($NaHCO_3$) was added to provide buffering which was important for pH control.

2.2.3.4. Procedure

Table 2-2: Sample Compositions for BMP and Toxicity assays

WASTEWATER% (v/v)	SLUDGE (mL)	DEFINED MEDIUM (mL)	WASTEWATER (mL)	DISTILLED WATER (mL)
Blank	300	0	0	700
Control	300	300	0	400
10	300	300	40	360
25	300	300	100	300
50	300	300	200	200
75	300	300	300	100
100	300	300	400	0

The components outlined in Table 2-2 were combined and mixed for each sample, and 100mL was decanted into each 125mL bottle. A working volume of 200mL was used for the 225L bottles. The bottles were overgassed with OFN, then sealed with butyl rubber septa and capped with aluminium crimp seals. Gas volumes of the bottles were zeroed (ambient pressure) with a syringe and the bottles were ready for incubation at 35^0C. The bottles were incubated in a shaking incubator to facilitate contact between the microorganisms and the substrate.

2.2.3.4.a. BMP assay

Controls (duplicate) were prepared containing sludge and defined medium, without the addition of organic substrate. Blanks (duplicate) were prepared containing sludge and distilled water, without the addition of defined medium and organic substrate. The sludge and medium in the case of the controls were mixed together and the sludge and distilled water for the blanks were mixed and a working volume of 100mL for each sample was decanted into each bottle, leaving a

headspace of 25mL. Duplicates of each sample were prepared incorporating a range of wastewater concentrations (Table 2-2). For the second BMP run as well as the run involving pretreated samples, 10%, 50% and 100% wastewater concentrations were used.

2.2.3.4.b. Anaerobic Toxicity Assay

Assay bottles were prepared as in the BMP assay, with defined medium, inoculum and wastewater samples. Two runs were carried out including the use of raw VOE as well as pretreated VOE. The wastewater concentrations investigated included 10%, 50% and 100% to provide a range from non-inhibitory to severely toxic. An additional 2mL spike, containing acetate and propionate, was added to each bottle via syringe injection to give a final concentration of 75mg acetate and 26.5mg propionate in each sample. Two sets of controls were prepared (in duplicate). Control A contained only sludge, medium and 100% v/v wastewater. Control B contained sludge, medium as well as the acetate-propionate spike. A blank was also prepared containing only sludge and the spike.

2.2.3.5. Gas Measurement

Gas volume sampling and removal during incubation was performed with a graduated syringe (20mL), fitted with a 22-gauge needle. Readings were taken at incubation temperature and the syringe was held vertical for measurement. During the second run using raw effluent as well as during pretreatment experiments, the syringe was filled to the 2mL mark with concentrated KOH (500g/L), which absorbs CO_2 present in the biogas. The gas as allowed to bubble through the KOH, thus gas volumes taken represented methane content of the biogas.

Gas production was measured daily for the first five days and periodically thereafter each measurement. Incubation was continued until gas production was complete.

2.2.4. Analyses

pH Measurement

pH was one of the parameters measured to characterize the VOE as well as for the BMP, ATA and pretreatment experiments. pH was measured using a calibrated pH meter (Beckman). Values obtained were accurate to within ± 0.2.

COD Measurement

The COD measurement for the first run of the BMP assay was carried out using a spectrophotometer as described in Appendix D. The measurement of COD for the second run of the BMP assay as well as for the ATA, VOE characterization and pretreatment experiments was based on the open reflux, colorimetric method described in Appendix C.

Sulphate Measurement

Sulphates were measured colorimetrically using Merck SQ118 Spectrophotometer as described in Appendix E to characterize the VOE as well as in the BMP, ATA and pretreatment experiments.

Lipid Measurement

The measurement of lipids was carried out gravimetrically according to Standard Methods (Clesceri *et al.*, 1989) as described in Appendix B for the BMP, ATA, pretreatment experiments and characterization of VOE.

Total solids and Volatile solids Measurement

Total solids and volatile solids were measured gravimetrically according to Standard Methods (Clesceri *et al.*, 1989) as described in Appendix F for the second run of the BMP assay, ATA, pretreatment experiments and characterization of VOE.

Total suspended solids and Total dissolved solids Measurement

TSS and TDS were measured gravimetrically according to Standard Methods (Clesceri *et al.*, 1989) as described in Appendix G for the first run of the BMP assay.

2.3. RESULTS

2.3.1. Chemical Characteristics of VOE

Two batches of VOE were collected and analysed as shown in Table 2-3. The batch referred to as sample A was used for the first run of the BMP assay. The batch referred to as sample B was used for the second run of the BMP assay, ATA and pretreatment studies.

Table 2-3: Chemical Characteristics of VOE

PARAMETER	SAMPLE A	SAMPLE B
pH	6.65	6.03
COD	6848 mg/L	7254 mg/L
Lipids	2492 mg/L	456 mg/L
Sulphates	2590 mg/L	2140 mg/L
TSS	1830 mg/L	-
TDS	24820 mg/L	-
TS	-	11840 mg/L
VS	-	10670 mg/L

2.3.2. Lipid Reduction by Gravitational Separation

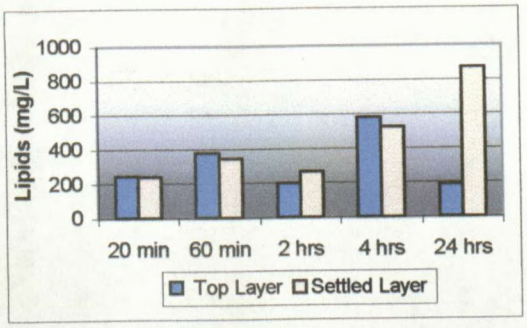

Figure 2-3: Lipids (mg/L) content of vegetable oil effluent after lipid reduction pre-treatment

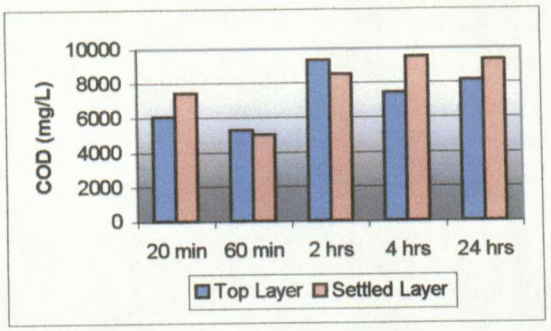

Figure 2-4: COD (mg/L) content of vegetable oil effluent after lipid reduction pretreatment

Table 2-4: Results of lipid reduction by gravitational separation

SETTLING TIME	PARAMETER	TOP LAYER	SETTLED LAYER
20 minutes	pH	5.07	5.06
	TS	6030 mg/L	8320 mg/L
	VS	5552.5 mg/L	8041.5 mg/L
60 minutes	pH	5.08	5.07
	TS	11700 mg/L	11090 mg/L
	VS	10200 mg/L	9550 mg/L
2 hours	pH	5.07	5.06
	TS	13030 mg/L	13110 mg/L
	VS	2260 mg/L	2710 mg/L
4 hours	pH	5.09	5.07
	TS	11160 mg/L	13070 mg/L
	VS	880 mg/L	2610 mg/L
24 hours	pH	5.06	5.07
	TS	14420 mg/L	14290 mg/L
	VS	3490 mg/L	3970 mg/L

2.3.3. Sulphate Reduction using Barium Chloride

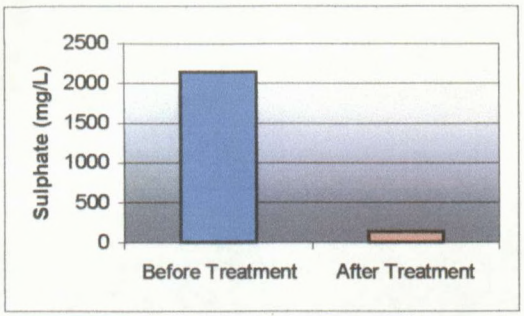

Figure 2-5: Sulphate (mg/L) content of vegetable oil effluent before and after sulphate reduction pretreatment

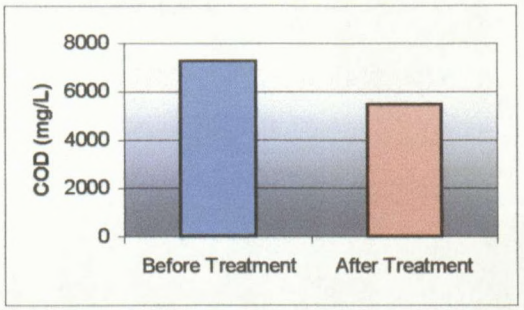

Figure 2-6: COD (mg/L) content of vegetable oil effluent before and after sulphate reduction pretreatment

Table 2-5: Results of Sulphate Reduction

PARAMETER	BEFORE PRE-TREATMENT	AFTER PRE-TREATMENT
PH	6.03	5.56
Lipids	456.8 mg/L	715 mg/L
TS	10670 mg/L	9345 mg/L
VS	11840 mg/L	10120 mg/L

2.3.4. Results of the Biodegradability Assay using Raw VOE

2.3.4.1. Results of the BMP Assay (First Run)

Table 2-6: Results of the Controls in the BMP Assay using raw VOE (First Run)

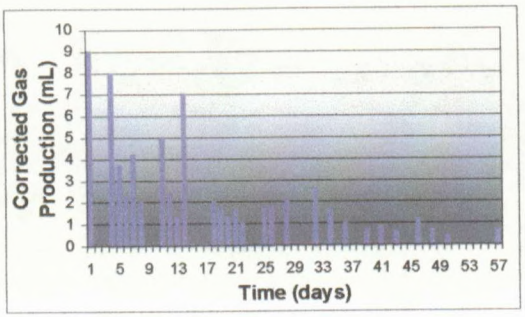

Figure 2-7: Average gas production of the controls in the BMP assay using raw VOE (First Run)

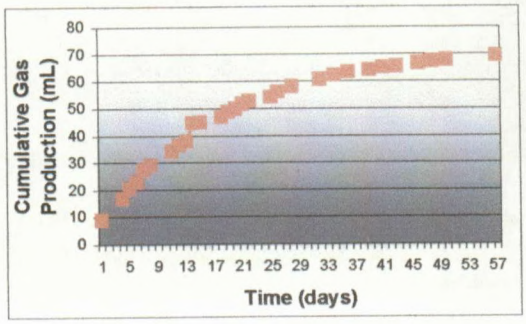

Figure 2-8: Cumulative gas production of the controls in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	68.33mL	
% COD Reduction:	33.32%	
Mineralised COD:	-	
% Lipid Reduction:	92.8%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.27	7.42
Alkalinity:	1844 mg/L	2128 mg/L
TSS:	8890 mg/L	5830 mg/L
TDS:	1790 mg/L	1315 mg/L

Table 2-7: Results of the BMP assay with 10% wastewater concentration using raw VOE (First Run)

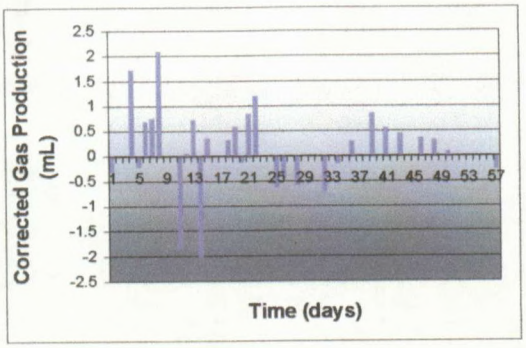

Figure 2-9: Corrected average gas production of 10% wastewater in the BMP assay using raw VOE (First Run)

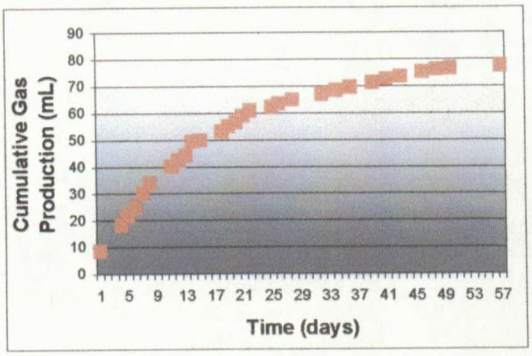

Figure 2-10: Cumulative gas production of 10% wastewater in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	77.46mL	
% COD Reduction:	16.77%	
Mineralised COD:	4.14mg	
Lipid Reduction:	25.34%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.34	7.53
Alkalinity:	1711 mg/L	2226 mg/L
TSS:	9360 mg/L	6070 mg/L
TDS:	2510 mg/L	1320 mg/L

Table 2-8: Results of the BMP assay with 25% wastewater concentration using raw VOE (First Run)

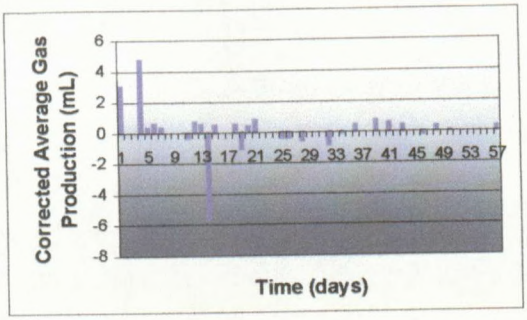

Figure 2-11: Corrected average gas production of 25% wastewater in the BMP assay using raw VOE (First Run)

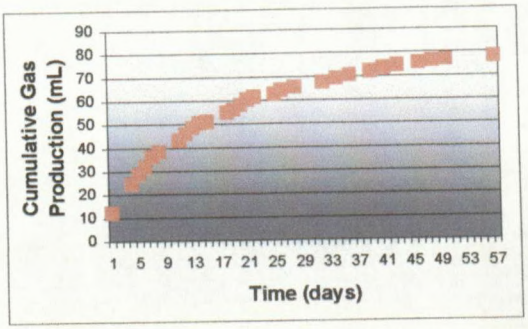

Figure 2-12: Cumulative gas production of 25% wastewater in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	77.58mL
% COD Reduction:	15.19%
Mineralised COD:	9.50mg
Lipid Reduction:	24.37%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.35	7.43
Alkalinity:	1716 mg/L	2472 mg/L
TSS:	9480 mg/L	6890 mg/L
TDS:	1790 mg/L	1650 mg/L

Table 2-9: Results of the BMP Assay with 50% wastewater concentration using raw VOE (First Run)

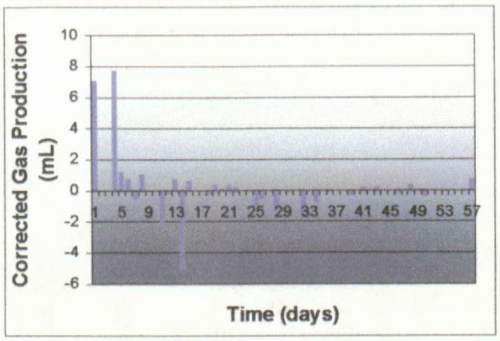

Figure 2-13: Corrected average gas production of 50% wastewater in the BMP assay using raw VOE (First Run)

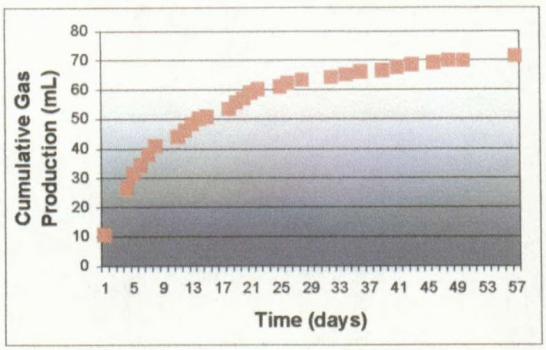

Figure 2-14: Cumulative gas production of 50% wastewater in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	71.22mL	
% COD Reduction:	10.73%	
Mineralised COD:	14.65mg	
Lipid Reduction:	35.49%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.34	7.53
Alkalinity:	1844 mg/L	2638 mg/L
TSS:	10630 mg/L	6960 mg/L
TDS:	2530 mg/L	2035 mg/L

Table 2-10: Results of the BMP Assay with 75% wastewater concentration using raw VOE (First Run)

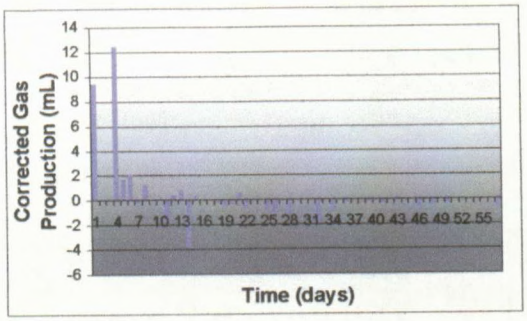

Figure 2-15: Corrected average gas production of 75% wastewater in the BMP assay using raw VOE (First Run)

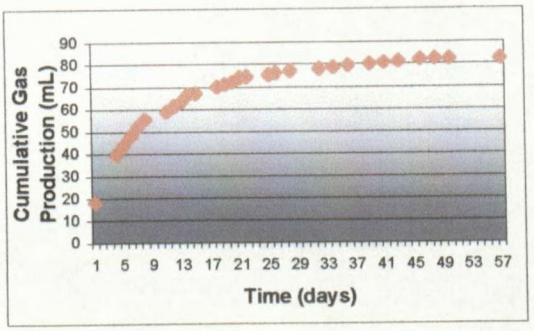

Figure 2-16: Cumulative gas production of 75% wastewater in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	82.32mL	
% COD Reduction:	8.01%	
Mineralised COD:	15.16mg	
Lipid Reduction:	45.79%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.32	7.59
Alkalinity:	1863 mg/L	2766 mg/L
TSS:	991 mg/L	6365 mg/L
TDS:	3060 mg/L	2625 mg/L

Table 2-11: Results of the BMP Assay with 100% wastewater concentration using raw VOE (First Run)

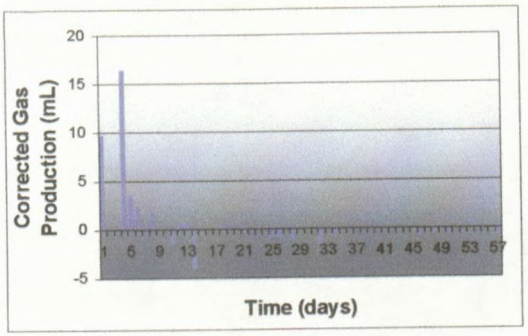

Figure 2-17: Corrected average gas production of 100% wastewater in the BMP assay using raw VOE (First Run)

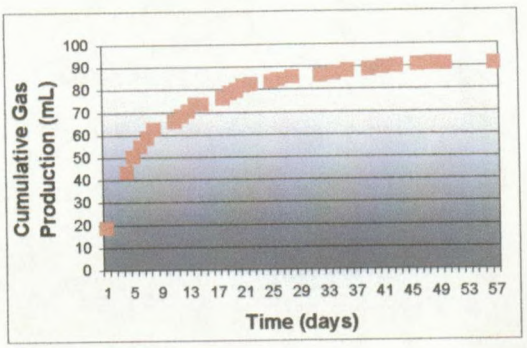

Figure 2-18: Cumulative gas production of 100% wastewater in the BMP assay using raw VOE (First Run)

Total gas production (35^{0}C):	90.95mL
% COD Reduction:	4.93%
Mineralised COD:	14.55mg
Lipid Reduction:	26.25%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.30	7.35
Alkalinity:	1746 mg/L	2962 mg/L
TSS:	9780 mg/L	7585 mg/L
TDS:	3630 mg/L	3180 mg/L

Table 2-12: Results of the Biodegradability Assay using Raw VOE (First Run)

WASTEWATER CONCENTRATION	WASTEWATER COD (MG/BOTTLE)	MINERALISED COD (mg)	GAS PRODUCED (mL/bottle)	CORRECTED GAS (mL/bottle)	ESTIMATED CH_4 (mL)	% CH_4	% COD REDUCTION
Control	-	-	44.8	-	-	-	-
10	24.68	4.14	49.3	4.5	1.64	36.4	16.77
25	62.58	9.50	50.43	5.63	3.75	66.6	15.19
50	136.50	14.65	50.13	5.33	5.79	108.6	10.73
75	185.24	15.16	66.75	21.95	5.99	27.29	8.01
100	295.20	14.55	73.37	28.52	5.75	20.16	4.93

2.3.4.2. Results of the BMP Assay (Second Run)

Table 2-13: Results of the Controls in the BMP Assay using raw VOE (Second Run)

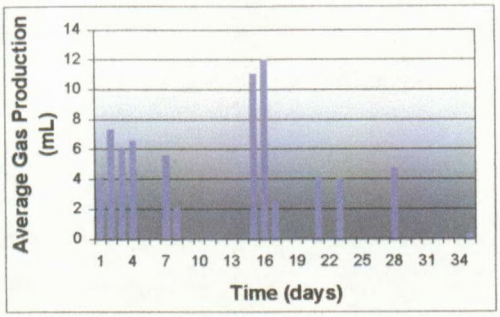

Figure 2-19: Average gas production for controls in the BMP assay using raw VOE (Second Run)

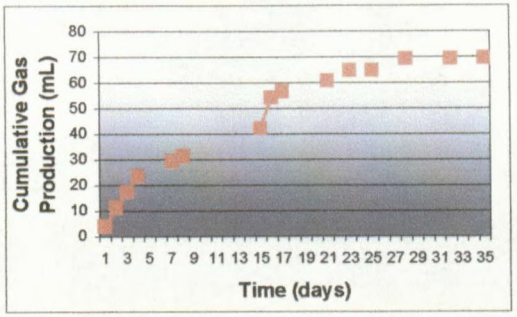

Figure 2-20: Cumulative gas production for controls in the BMP assay using raw VOE (Second Run)

Total gas production (35^{0}C):	69.45mL	
% COD Reduction:	-	
Mineralised COD:	-	
Lipid Reduction:	11.03%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.71	7.15
TS:	11940 mg/L	12260 mg/L
VS:	7670 mg/L	6380 mg/L

Table 2-14: Results of the BMP Assay with 10% wastewater concentration using raw VOE (Second Run)

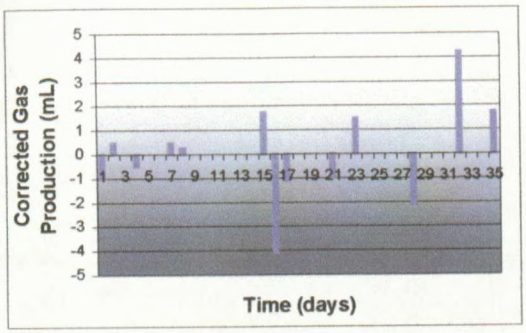

Figure 2-21: Corrected average gas production of 10% wastewater in the BMP assay using raw VOE (Second Run)

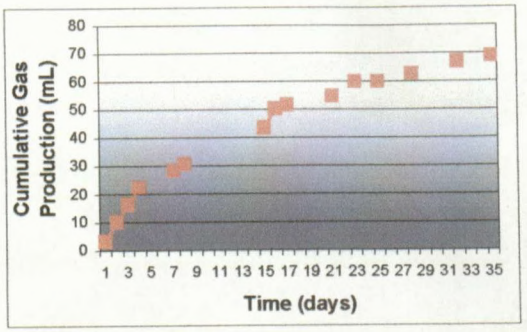

Figure 2-22: Cumulative gas production for 10% wastewater in the BMP assay using raw VOE (Second Run)

Total gas production (35^{0}C):	68.7mL	
% COD Reduction:	13.46%	
Mineralised COD:	11.1mg	
Lipid Reduction:	3.87%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.83	7.11
TS:	12910 mg/L	10920 mg/L
VS:	7370 mg/L	6350 mg/L

Table 2-15: Results of the BMP assay with 50% wastewater concentration using raw VOE (Second Run)

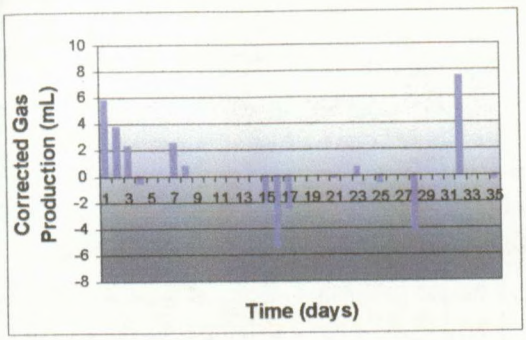

Figure 2-23: Corrected average gas production for 50% wastewater in the BMP assay using raw VOE (Second Run)

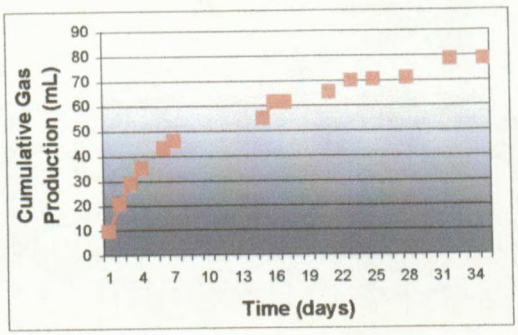

Figure 2-24: Cumulative gas production for 50% wastewater in the BMP assay using raw VOE (Second Run)

Total gas production (35^{0}C):	78.15mL	
% COD Reduction:	17.67%	
Mineralised COD:	72.1mg	
Lipid Reduction:	11.59%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.76	7.14
TS:	13020 mg/L	9200 mg/L
VS:	8090 mg/L	4970 mg/L

Table 2-16: Results of the BMP assay with 100% wastewater concentration using raw VOE (Second Run)

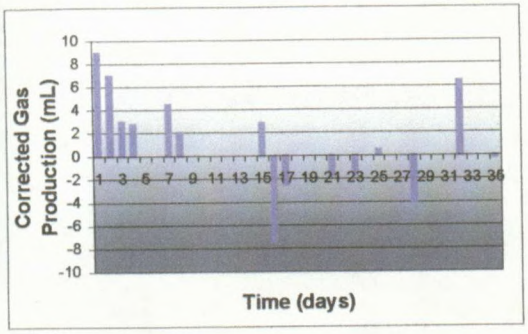

Figure 2-25: Corrected average gas production of 100% wastewater in the BMP assay using raw VOE (Second Run)

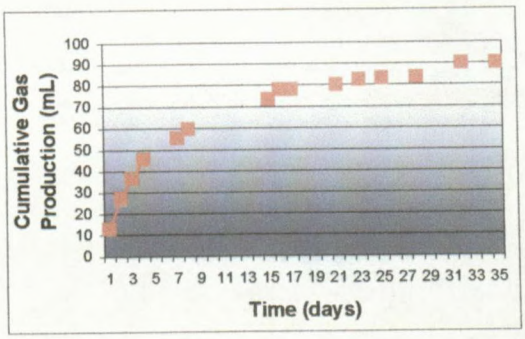

Figure 2-26: Cumulative gas production of 100% wastewater in the BMP assay using raw VOE (Second Run)

Total gas production (35^{0}C):	89.9mL	
% COD Reduction:	24.14%	
Mineralised COD:	224mg	
%Lipid Reduction:	11.0%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.67	7.13
TS:	13650 mg/L	11860 mg/L
VS:	8290 mg/L	5380 mg/L

Table 2-17: Results of the Biodegradability Assay using Raw VOE (Second Run)

%WASTEWATER CONCENTRATION	WASTEWATER COD (MG/BOTTLE)	MINERALISED COD (mg)	GAS PRODUCED (mL/bottle)	CORRECTED GAS (mL/bottle)	ESTIMATED CH_4 (mL)	% CH_4	% COD REDUCTION
Control	-	-	69.45mL	-	-	-	-
10	83.2 mg/L	11.1 mg	68.7mL	-0.75mL	4.38	-584	13.46%
50	408 mg/L	72.1 mg	78.15mL	8.7mL	28.48	327	17.67%
100	928 mg/L	224 mg	89.9mL	20.45mL	88.48	432	24.14%

2.3.5. Results of the Biodegradability Assay using Pre-treated VOE

Table2-18: Results of Controls in the BMP assay using pretreated VOE

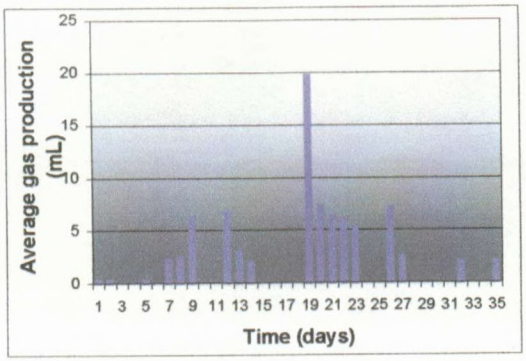

Figure 2-27: Average gas production of the controls in the BMP assay using pretreated VOE

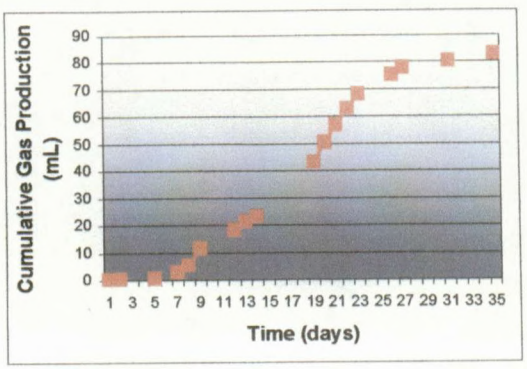

Figure 2-28: Cumulative gas production of the controls in the BMP assay using pretreated VOE

Total gas production (35^{0}C):	82.8mL	
% COD Reduction:	-	
Mineralised COD:	-	
% Lipid Reduction:	10.2%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.61	7.69
Sulpahtes:	510mg/L	417mg/L
TS:	13 280mg/L	11 350mg/L
VS:	8 150mg/L	6 580mg/L

Table 2-19 : Results of the BMP assay with 10% wastewater concentration using pretreated VOE

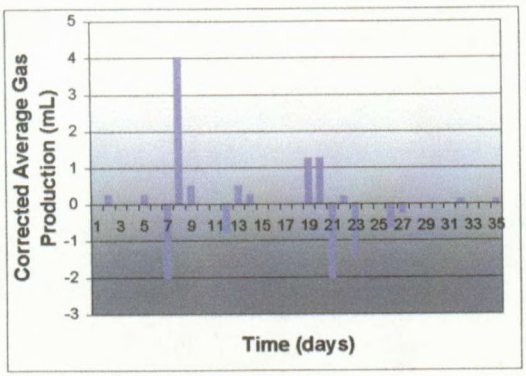

Figure 2-29: Corrected average gas production of 10% wastewater concentration in the BMP assay using pretreated VOE

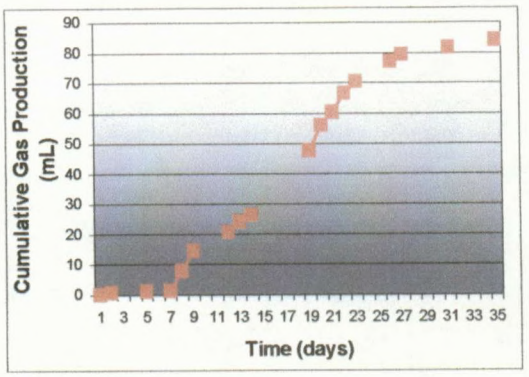

Figure 2-30: Cumulative gas production of 10% wastewater concentration in the BMP assay using pretreated VOE

Total gas production (35^0):	84mL
% COD Reduction:	12.5%
Mineralised COD:	10.2 mg
% Lipid Reduction:	26.5%

PARAMETER	BEFORE INCUBATION	AFTER INCUBATION
pH:	7.66	7.56
Sulphates:	430mg/L	170mg/L
TS:	13 650mg/L	10 750mg/L
VS:	8 650mg/L	5 980mg/L

Table 2-20: Results of the BMP assay with 50% wastewater concentration using pretreated VOE

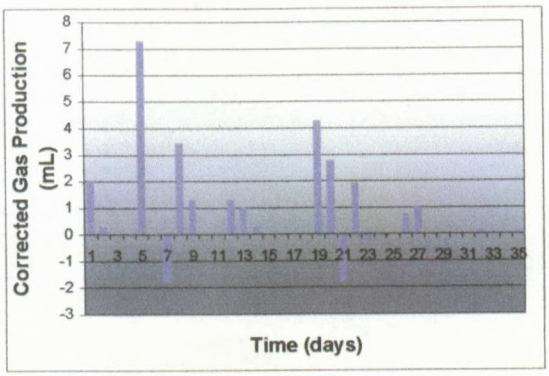

Figure 2-31: Corrected average gas production of 50% wastewater concentration in the BMP assay using pretreated VOE

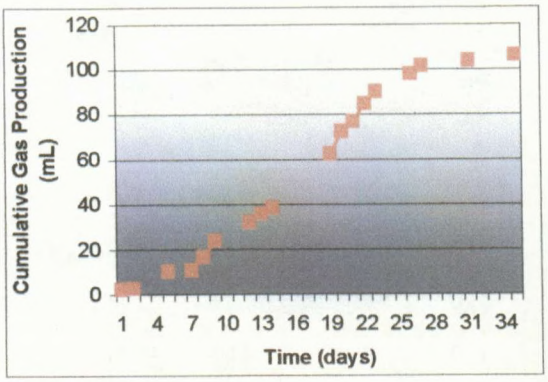

Figure 2-32: Cumulative gas production of 50% wastewater concentration in the BMP assay using pretreated VOE

Total gas production (35^{0}C):	106.1mL	
% COD Reduction:	14.9%	
Mineralised COD:	66.8 mg	
% Lipid Reduction:	37.4%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.73	7.59
Sulphates:	353mg/L	652mg/L
TS:	12 890mg/L	10 260mg/L
VS:	7 170mg/L	5 850mg/L

Table 2-21 : Results of the BMP assay with 100% wastewater concentration using pretreated VOE

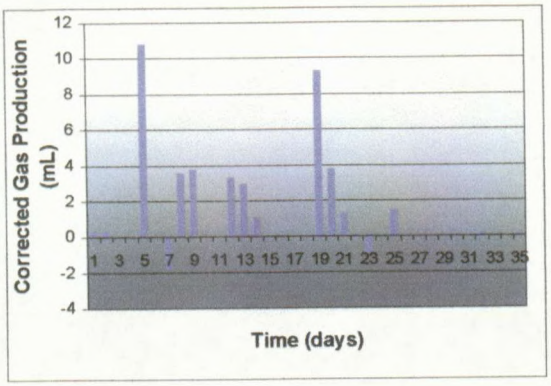

Figure 2-33: Corrected average gas production of 100% wastewater concentration in the BMP assay using pretreated VOE

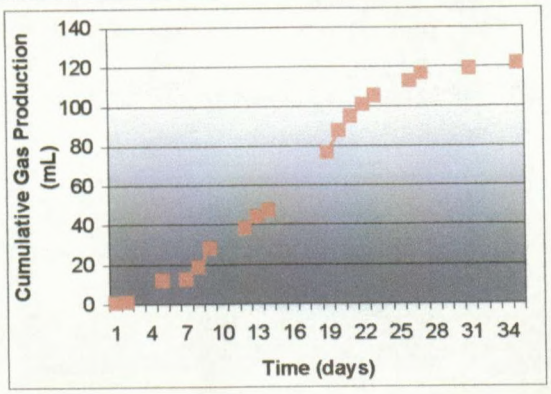

Figure 2-34: Cumulative gas production of 100% wastewater concentration in the BMP assay using pretreated VOE

Total gas production (35^{0}C):	121.25mL	
% COD Reduction:	5.2%	
Mineralised COD:	47 mg	
% Lipid Reduction:	49.4%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.71	8.28
Sulphates:	333mg/L	588mg/L
TS:	14 250mg/L	11 140mg/L
VS:	7 870mg/L	5 890mg/L

Table 2-22: Results of the Biodegradability Assay Pre-treated VOE

%WASTEWATER CONCENTRATION	WASTEWATER COD (mg/BOTTLE)	MINERALISED COD (mg)	GAS PRODUCED (mL/bottle)	CORRECTED GAS (mL/bottle)	ESTIMATED CH_4 (mL)	% CH_4	% COD REDUCTION
Control	-	-	82.8	-	-	-	
10	81.6	10.2	84	1.2	4.03	335	12.5
50	448	66.8	106.1	23.3	26.4	113	14.9
100	904	47	121.25	38.45	18.6	48.4	5.2

2.3.6. Results of the Toxicity Assay using Raw VOE

Table 2-23 : Results of Control A and Control B in the ATA using raw VOE

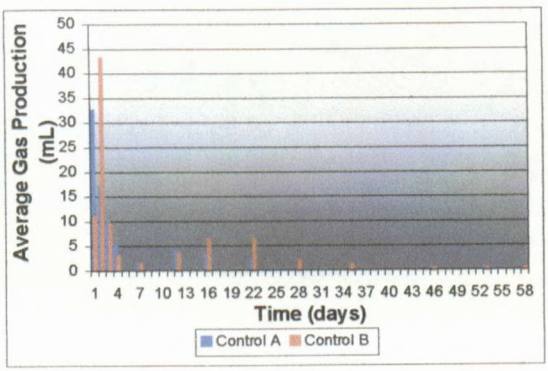

Figure 2-35: Average gas production of Controls A and B in the ATA using raw VOE

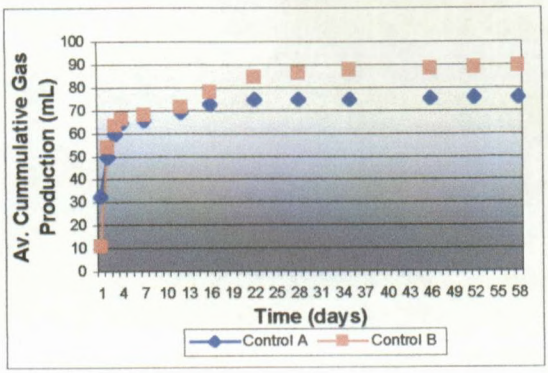

Figure 2-36: Average cumulative gas production of Controls A and B in the ATA using raw VOE

PARAMETER	CONTROL A	CONTROL B
Total Gas Production (35^0):	75.8mL	89.3mL
% COD Reduction:	25.94%	14.7%
% Lipid Reduction:	2.7%	12.96%
pH (Before incubation):	7.56	7.82
pH (After incubation):	9.16	8.12
TS (Before incubation):	11370mg/L	9930mg/L
TS (After Incubation):	11230mg/L	9600mg/L
VS (Before incubation):	5220mg/L	4970mg/L
VS (After incubation):	4530mg/L	4760mg/L

Table 2-24 : Results of the ATA with 10% wastewater concentration using raw VOE

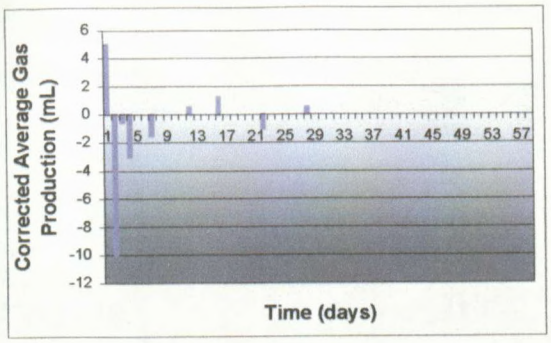

Figure 2-37: Corrected average gas production of 10% wastewater concentration in the ATA using raw VOE

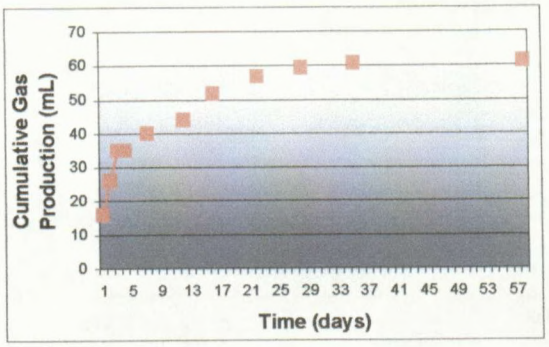

Figure 2-38: Cumulative gas production of 10% wastewater concentration in the ATA using raw VOE

Total gas production (35^{0}C):	61.1mL	
% COD Reduction:	31.8%	
% Lipid Reduction:	9.4%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.64	8.16
Sulphates:	380mg/L	178mg/L
TS:	10 150mg/L	10 490mg/L
VS:	5 170mg/L	6 420mg/L

Table 2-25 : Results of the ATA with 50% wastewater concentration using raw VOE

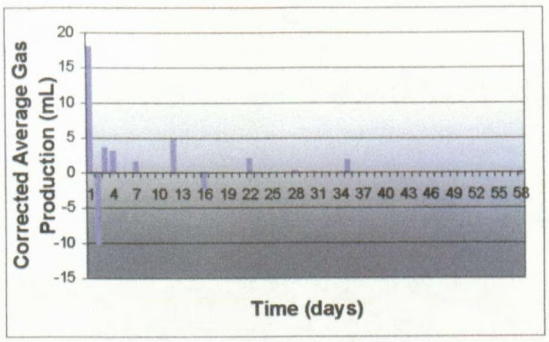

Figure 2-39: Corrected average gas production of 50% wastewater concentration in the ATA using raw VOE

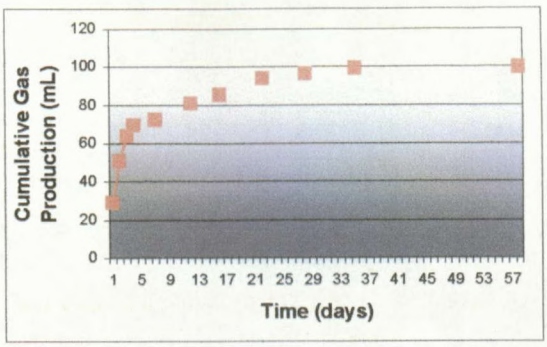

Figure 2-40: Cumulative gas production of 50% wastewater concentration in the ATA using raw VOE

Total gas production (35^{0}C):	99.4mL	
% COD Reduction:	28.3%	
% Lipid Reduction:	1.5%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.70	7.70
Sulphates:	600mg/L	343mg/L
TS:	11 390mg/L	11 900mg/L
VS:	5 620mg/L	5 830mg/L

Table 2-26 : Results of the ATA with 100% wastewater concentration using raw VOE

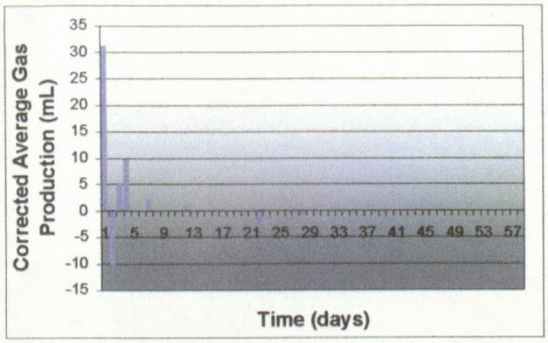

Figure 2-41: Corrected average gas production of 100% wastewater concentration in the ATA using raw VOE

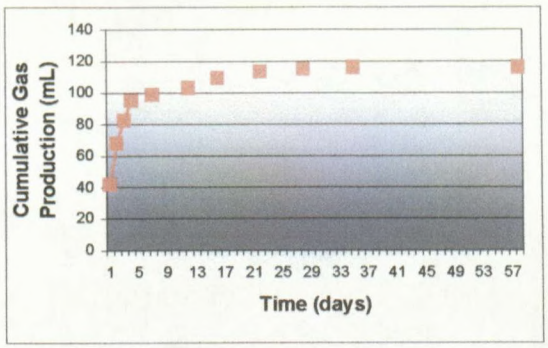

Figure 2-42: Cumulative gas production of 100% wastewater concentration in the ATA using raw VOE

Total gas production (35^0):	115.9mL	
% COD Reduction:	38.5%	
% Lipid Reduction:	3.8%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.58	9.18
Sulphates:	980mg/L	530mg/L
TS:	11 480mg/L	13 490mg/L
VS:	4 950mg/L	5 800mg/L

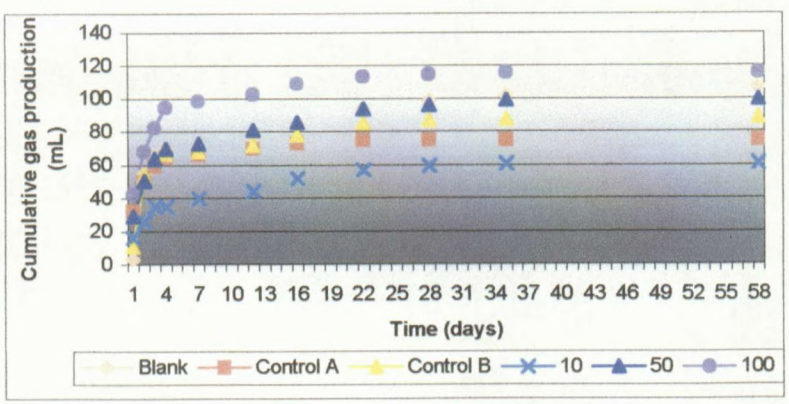

Figure 2-43: Comparison of the cumulative gas results for the ATA using raw VOE

2.3.7. Results of the Toxicity Assay using Pre-treated VOE

Table 2-27: Results of the Controls in the Toxicity assay using pretreated VOE

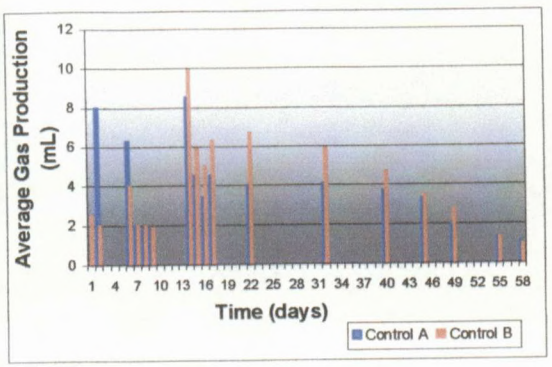

Figure 2-44: Average gas production of Control A and B in the ATA using pretreated VOE

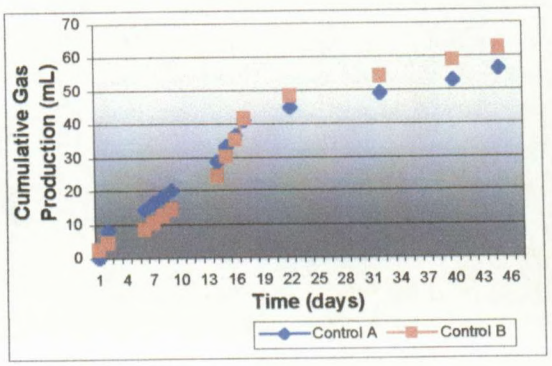

Figure 2-45: Cumulative gas production of Control A and B in the ATA using pretreated VOE

	CONTROL A	CONTROL B
Total gas production (35^{0}C):	60.5mL	67.4mL
% COD Reduction:	5.5%	7.2%
% Lipid Reduction:	11%	10%
pH (Before incubation):	7.76	7.66
pH (After incubation):	7.45	7.57
Sulphates (Before incubation):	353mg/L	588mg/L
Sulphates (After incubation):	25mg/L	502mg/L
TS (Before incubation):	12 070mg/L	12 350mg/L
TS (After incubation):	12 120mg/L	12 500mg/L
VS (Before incubation):	5 670mg/L	5 950mg/L
VS (After incubation):	5 570mg/L	6 180mg/L

Table 2-28 : Results of the Toxicity assay with 10% wastewater concentration using pretreated VOE

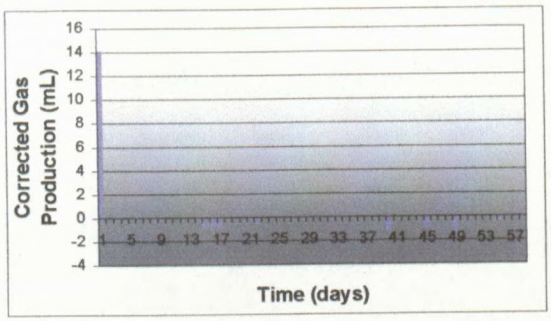

Figure 2-46: Corrected average gas production of 10% wastewater concentration in the ATA using pretreated VOE

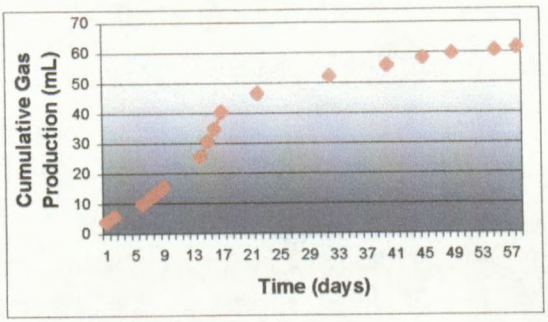

Figure 2-47: Cumulative gas production of 10% wastewater in the ATA using pretreated VOE

Total gas production (35^{0}C):	61.55mL	
% COD Reduction:	8.4%	
% Lipid Reduction:	27.5%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.65	7.65
Sulphates:	396mg/L	117mg/L
TS:	11 700mg/L	10 800mg/L
VS:	6 090mg/L	5 920mg/L

Table 2-29 : Results of the Toxicity assay with 50% wastewater concentration using pretreated VOE

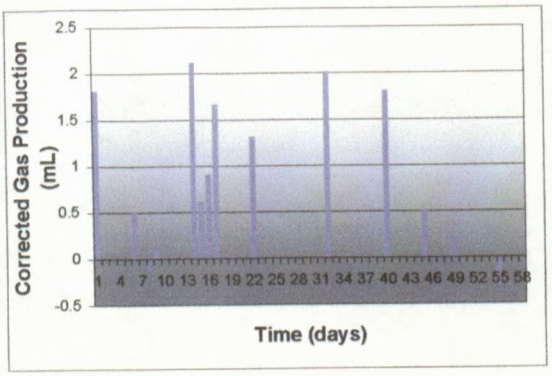

Figure 2-48: Corrected average gas production of 50% wastewater concentration in the ATA using pretreated VOE

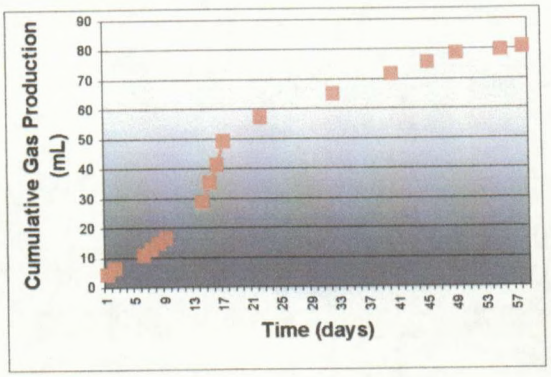

Figure 2-49: Cumulative gas production of 50% wastewater concentration in the ATA using pretreated VOE

Total gas production (35^{0}C):	80.45mL	
% COD Reduction:	11.6%	
% Lipid Reduction:	37.5%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.71	7.62
Sulphates:	400mg/L	113mg/L
TS:	13 400mg/L	12 500mg/L
VS:	5 790mg/L	5 580mg/L

Table 2-30 : Results of the Toxicity assay with 100% wastewater concentration using pretreated VOE

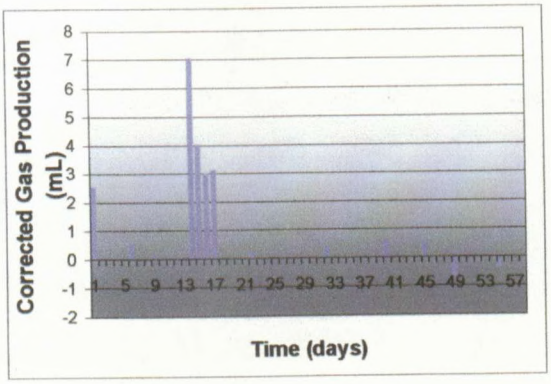

Figure 2-50: Corrected average gas production of 100% wastewater concentration in the ATA using pretreated VOE

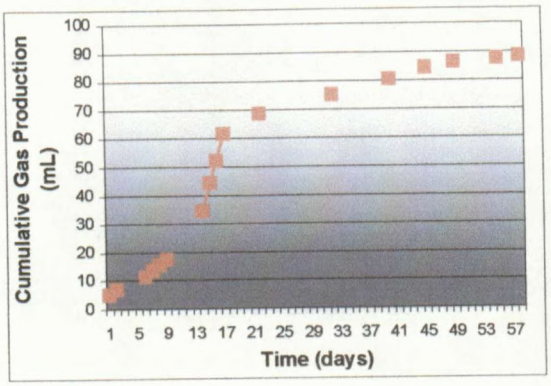

Figure 2-51: Cumulative gas production of 100% wastewater concentration in the ATA using pretreated VOE

Total gas production (35^{0}C):	88.2mL	
% COD Reduction:	1.2%	
% Lipid Reduction:	24.9%	
PARAMETER	**BEFORE INCUBATION**	**AFTER INCUBATION**
pH:	7.65	7.58
Sulphates:	544mg/L	409mg/L
TS:	12 550mg/L	11 900mg/L
VS:	5 660mg/L	5 450mg/L

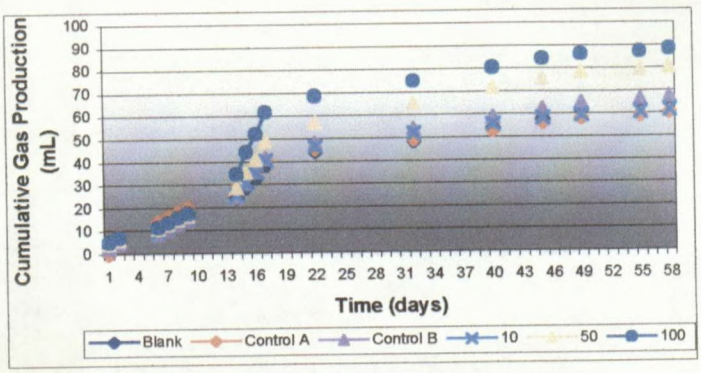

Figure 2-52: Comparison of the cumulative gas results for the ATA using pretreated VOE

2.4. DISCUSSION

2.4.1. Characterization of VOE

Two batches of effluent were collected and analysed (Table 2-3). Both batches were final VOE, with the treatment process differing for each. A filtration system was implemented following the DAF unit, after sample A was collected and analysed. A significant change in the lipid content of the effluent was seen, which suggests that the filtration system removed some of the lipids present in the effluent. No drastic changes were seen in the COD and sulphate content as well as the pH. The high sulphate concentration in both samples could be attributed to the use of sulphuric acid in the production process.

2.4.2. Lipid Reduction by Gravity Separation

The objective of this experiment was to observe the settling properties of lipids contained in VOE. It was expected that the lipids would float to the surface of the solution if allowed to stand, with a decrease in lipid concentration in the settled layer of VOE. Figure 2-1 illustrates the lipid concentration of the top and settled layers for various time intervals. For time intervals 20min, 60min, 2hrs and 4hrs there was no significant difference in lipid concentration for the top and settled layers. However, for the time interval of 24hrs a noticeable difference was seen, with the lipid concentration of the top layer at 184.5 mg/L and the settled layer at 870 mg/L. This suggests

that the lipids settled in solution. It is possible that the fatty compounds adhered to suspended solids and settled out of solution, thus the higher lipid concentration in the settled layer. No significant difference was observed for pH (Table 2-4). The COD concentrations (Figure 2-2) for the settled layers were slightly higher than the top layers since the TS and VS (Table 2-4) were higher in the settled layers.

2.4.3. Sulphate Reduction using Barium Chloride

The results in Table 2-5 illustrate a decrease in TS and VS. Sedimentation occurs as a result of the pretreatment, which removes TS and VS from the sample. Figure 2-4 illustrates the COD content of the VOE sample before and after treatment. It is clear that a decrease in COD content occurred. This could be explained by the fact that solids settled out of solution, decreasing the organic content of the sample. Figure 2-3 illustrates the sulphate results obtained before and after treatment. A sharp decrease in sulphate content was observed as a result of the pretreatment process. No changes were observed for pH.

2.4.4. Biodegradability Assay

BMP is a measure of sample biodegradability. The objective of this experiment was to evaluate whether anaerobic microbial populations were able to utilize the VOE with resultant methane production. The BMP assay was run using raw as well as pretreated VOE. Measurement of gas production provided an indication of the metabolic activity and the degradability of the substrate. Experimental controls were run for each sample as well as a range of wastewater concentrations. The results of the two runs using raw VOE are illustrated in Table 2-5 to 2-13. Tables 2-15 to 2-18 illustrate the results of the BMP assay using pretreated VOE.

Table 2-5 and 2-10 show results of the experimental controls for the first and second runs respectively. Tables 2-6 to 2-9 and Tables 2-11 to 2-13 illustrate the results of the bioassay for the different substrate concentrations of the first and second runs respectively. Results of the experimental controls of the BMP assay using pretreated VOE are shown in Tables 2-15 and the

results of the various wastewater concentrations are shown in Tables 2-16 to 2-18. Average gas production samples (mean value of each sample) was corrected by subtracting the gas production from that of the control in order to show the gas production resulting from degradation of the substrate. The graphs of cumulative gas production are useful as they illustrate gas production rate (gradient of the slope) and time at which gas production stabilized.

Looking at the graphs illustrating corrected gas production for the first and second runs using raw VOE, it can be seen that the highest gas production was recorded for samples containing 100% substrate solution. This suggests that the organics in the raw VOE were readily degraded by the microorganisms, with greater volumes of the effluent resulting in greater volumes of gas produced. Corrected gas production graphs (Figures 2-7, 2-9, 2-11, 2-13 and 2-15) for the first run using raw VOE illustrates gas volumes of between 0.5mL and 12.5mL for the first 9 days for the various wastewater samples. Thereafter gas production began decreasing, which suggested that the degradable organic materials were also decreasing. Figures 2-27, 2-29 and 2-31 illustrate corrected gas production for pretreated VOE samples. The graphs indicate the highest gas production for samples containing 100% wastewater concentration, which shows (as in the case with raw VOE) that the organics present in the pretreated VOE were degraded by the microorganisms.

The cumulative gas production graphs illustrate the total gas produced (i.e. not corrected using the control). Figures 2-8, 2-10, 2-12, 2-14 and 2-16 represent the cumulative gas produced for the various wastewater samples of the bioassay using raw VOE. After 30 days, the graphs indicate almost zero gas production suggesting that all degradable compounds present in the substrate had been utilised. Figures 2-26, 2-28, 2-30 and 2-32 illustrate cumulative gas production for the BMP assay using pretreated VOE. It ca be seen that for the first 7 days gas production was low in comparison to the runs using raw VOE. Thereafter a sharp increase was seen between days 9 and 27, after which gas production seemed to stabilize. The low gas production in the first few days suggests an acclimatization period for the microorganisms

thereafter the sharp increase in gas production suggests that the degradable organic material present in the VOE was utilised by the microorganisms.

Table 2-5 to 2-9 illustrating the results of the BMP assay using raw VOE for the first run, includes results for pH, alkalinity, TSS and TDS before and after incubation as well as COD reduction and lipid reduction. Alkalinity indicates the buffering capacity of a digester or the ability of the digester to neutralize the effect of volatile acid formation. The results show an increase in alkalinity for all samples. The pH readings before and after incubation of both runs of the bioassay using raw VOE as well as the run using pretreated VOE remained within optimal working range. The optimal pH range for methanogenic bacteria is between 6.5 and 8.2 (Ross *et al.*, 1992). This suggests that the methanogenic bacteria were able to utilize the volatile acids produced by acetogenic bacteria at an optimum rate. An accumulation of volatile acids exceeding the buffering capacity of the digester will lead to a drop in pH (McCarthy and McKinney, 1969; Switzenbaum *et al.*, 1990).

The results of both runs of the BMP assay using raw as well as pretreated VOE indicate lipid reduction, which suggests that the microorganisms were capable of degrading lipids. The first run using raw VOE showed a general decrease in lipid reduction with increasing substrate concentration, whereas an increase in lipid reduction was seen in the second run with increasing wastewater concentration. Also, the percentages of lipids reduced in the first run are higher than the second run. The VOE sample used for the first run had a lipid content of 2492 mg/L, whereas the sample used for the second run had a lipid content of 456mg/L. The higher lipid content of the VOE used in the first run could explain the decrease in reduction with increasing wastewater concentration, as methanogenic bacteria are inhibited by lipids (Hamdi, 1991). The results of the bioassay using pretreated VOE indicate an increase in lipid reduction with an increase in wastewater concentration. Which suggests that the microorganisms were capable of utilizing lipids with increasing lipid concentration in the pretreated VOE.

The COD of a sample is an indication of the amount of the organic matter present. Degraded organic matter is converted to methane in the biogas, thus recalcitrant organic matter contributes to the final solution. From the results obtained, it was found that the %COD reduction for the various substrate concentrations of the first BMP run decreased with increasing wastewater concentration although all samples showed some degree of COD reduction. The average COD reduction for the first run of the 10% wastewater sample was 16.7% and 4.9% for the 100% wastewater sample. This suggests that the methanogenic bacteria responsible for methane production were inhibited by the presence of a toxic substance or substances (at elevated concentrations) present in the VOE.

Biochemical methane potential is referred either to sample volume (m^3CH_4/m^3 sample), sample mass (m^3CH_4/kg sample), or sample organic content (m^3CH_4/kg COD). The latter permits direct transfer of results into percent organic matter converted to methane by the theoretical $0.350m^3$ CH_4 produced per kilogram COD catabolized (at STP) (McCarthy, 1964). Tables 2-12, 2-17 and 2-22 show results of the BMP assay for the first and second runs using raw VOE as well as pretreated VOE. Looking at the estimated CH_4 and mineralized COD columns, the amount of methane gas produced as a result of the mineralisation of 1mg COD can be determined. It was found that the amount of gas produced per mg COD mineralised, did not correlate with the findings of McCarthy, (1964) for the first run using raw VOE, with 4.05mL/mg and 0.34mL/mg for 10% and 100% wastewater concentrations respectively. This may have been due to experimental error, since the results obtained for the second run using raw VOE as well as the run using pretreated VOE, showed that for every milligram COD reduced, 0.395mL methane was produced.

2.4.5. Anaerobic Toxicity

Toxicity testing involves exposing living biological material to a test substrate and the toxicity of that substance is determined by measuring the response of the biological system. Anaerobic toxicity is determined as the adverse effect of a substance on the methanogenic bacteria, which

convert acetate and propionate to methane (Owen *et al.*, 1979). The rate at which the acetate-propionate spike was metabolized, was monitored by the total gas production of controls. The results of the controls are illustrated in Tables 2-23 and 2-27 for toxicity assays using raw and pretreated VOE respectively. Control A contained sludge, defined medium and 100% (v/v) wastewater concentration with no spike, whereas, Control B contained sludge, defined medium and spike with no wastewater. It can be seen from Figures 2-35 and 2-36 in Table 2-21as well as Figures 2-44 and 2-45 in Table 2-27, that Control B produced more gas than Control A. This is due to the presence of readily degradable substrate (i.e. acetate-propionate spike) for the methanogenic bacteria responsible for methane production. Tables 2-22 to 2-24 (using raw VOE) and Tables 2-28 to 2-30 (using pretreated VOE) illustrate the results of the various wastewater concentrations. Figure 2-52 is a comparison of the controls, blank as well as the various sample concentrations. The largest volumes of gas were produced by samples containing 100% (v/v) substrate concentration. Total gas volumes were higher for samples containing raw VOE as compared to samples containing pretreated VOE, suggesting that more degradable substrate was available in samples containing raw VOE. Lower COD reduction was also observed foe samples containing pretreated VOE. Therefore it can be said that the raw VOE more susceptible to anaerobic degradation than pretreated VOE.

Lipid reduction for all samples containing raw VOE was fairly low as compared to samples containing pretreated VOE. This suggests that the lipids were more readily available for degradation by the microorganisms after pretreatment of the effluent. The pH results of samples containing raw VOE were fairly high, with Control A and 100% wastewater samples above optimal working range, whereas pH results of samples containing pretreated effluent remained within normal working range. The high pH in samples containing raw VOE could indicate that the methaogenic bacteria were not capable of utilizing volatile acids produced by acetogenic bacteria at an optimal rate. It is possible that one of the acid forming populations were inhibited by the VOE although, it is generally known that methanogenic bacteria are more fastidious.

CHAPTER THREE

ANAEROBIC DIGESTION OF VOE USING THE ANAEROBIC BAFFLED REACTOR AND FED-BATCH DIGESTION

3.1. INTRODUCTION

This chapter deals with the investigation of the ABR as a means to treat VOE in comparison to fed-batch digestion.

3.1.1. The Anaerobic Baffled Reactor

The Anaerobic Baffled Reactor (ABR) was devised by Bachmann, Beard and McCarthy (1983), who initially named it the Modified Sludge Blanket Reactor. The ABR has been described as a series of Upflow Anaerobic Sludge Blanket (UASB) reactors, which do not require granulation (Bachmann *et al.*, 1985). The ABR, as illustrated in Figure 3-1, contains alternately hanging and standing baffles, dividing it into a number of compartments, which segregate both biomass and gas phases (Nachaiyasit and Stuckey, 1985).

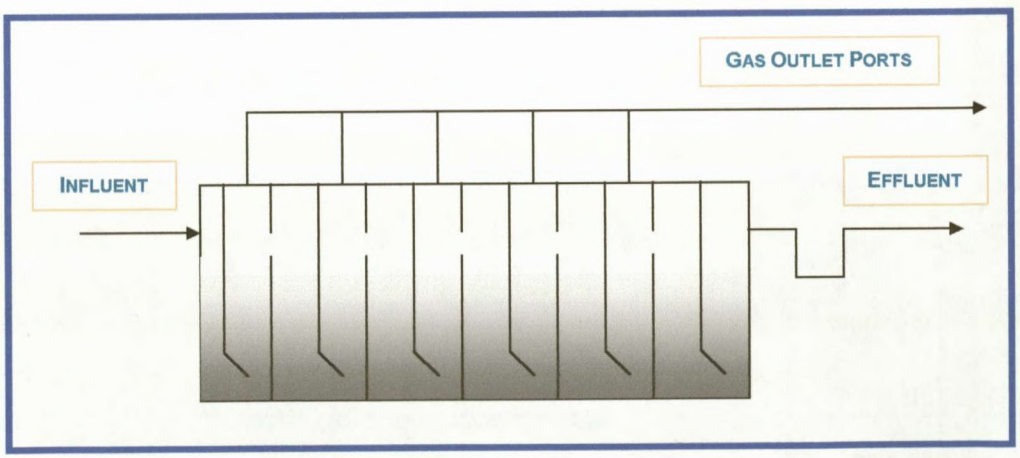

Figure 3-1: Schematic representation of the anaerobic baffled reactor

The slanted lower edges of the hanging baffles route flow of the liquid through the middle of the sludge bed, therefore reducing channeling. The liquid flow is alternately upward and downward between partitions. The biomass within the reactor tends to rise and settle with gas production and move horizontally down the reactor at a relatively slow rate (Nachaiyasit and Stuckey, 1995). The wastewater can therefore come into contact with a large active biomass as it passes through the ABR. The microorganisms in each compartment differ depending on the specific environmental conditions prevailing within each compartment. Also, the remaining compounds or intermediates to be degraded influence the sludge characteristics (Nachaiyasit and Stuckey, 1997b).

The ABR is well suited to intermittent high organic or hydraulic loads. With biomass, which has been acclimatized to all possible components in the effluent, the ABR will withstand toxic shocks (Sacks, Buckley and Stuckey, 1998). These attributes are largely due to the compartmentalization of different microbial associations, which have been acclimatized to the range of constituents in the wastewater (Sacks, Buckley and Stuckey, 1998). The various microbial associations allow specialized bacteria to degrade the effluent stepwise, producing degradation products, which may be toxic or inhibitory to a mixed culture (Sacks, Buckley and Stuckey, 1998). The compartmentalized structure of the ABR prevents much of the biomass being exposed to low pH during organic shock loads and maintains the biomass within the reactor for long SRT (Nachaiyasit and Stuckey, 1997b). One of the most significant advantages of the ABR is its ability to separate acidogenesis and methanogenesis longitudinally, allowing the reactor to behave as a two-phase system without the associated control problems and high costs. The reactor design is simple, with no moving parts or mechanical mixing, making it relatively inexpensive to construct. High solids retention time is achieved without the need for biomass to be fixed to media particles or a solid-settling chamber. Increased volumes of wastewater can be treated since the HRT and SRT are separate, relative to CSTR where HRT=SRT. The ABR has been found to be stable to hydraulic and shock loads and the reactor configuration provides protection of the biomass to toxic compounds in the effluent.

Digester start-up is one of the major problems associated with anaerobic treatment systems. Investigations have shown that with long initial HRT (80h), gradually reducing it, whilst keeping the substrate concentration constant, superior performance was observed in comparison to a reactor started up with a constant and low HRT coupled with a step wise decrease in substrate concentration (Barber and Stuckey, 1998). Investigations have also shown low levels of dead space (8-18% hydraulic dead space) in comparison with other anaerobic reactor designs (Grobicki and Stuckey, 1992). Investigations involving operation of the ABR at low temperatures showed that biochemical reactions double in relative activity for every 10^0C increase in temperature (Nachaiyasit and Stuckey, 1997a). However, no significant reduction in overall COD removal efficiency was observed when the temperature was decreased from 35^0C to 25^0C. A further reduction to 15^0C resulted in 20% decrease in COD removal (Nachaiyasit and Stuckey, 1997a). Changes in performance were gradual which is advantageous, as this would provide improved protection to shocks, in comparison to other reactors.

3.2. MATERIALS AND METHODS

3.2.1. Fed-Batch Digestion

3.2.1.1. Reactor

A reactor of 2.5L with a working volume of 2L was used. The reactor was equipped with a temperature control system and a mechanical mixer. The gas port was attached to a saline displacement system to aid in monitoring gas production. The saline solution consisted of 20%(v/v) $NaCl_2$, which was acidified to pH 3 to 4. A glass syringe (50mL) was used to feed substrate to the digester. A peristaltic pump was used to feed samples with volumes larger than 50mL

3.2.1.2. Artificial Effluent

The artificial effluent used in this experiment is illustrated in Table 3-1.

Table 3-1: Composition of Artificial Effluent

COMPONENT	CONCENTRATION (mg/L)
$MgCl_2.6H_2O$	468
$CaCl_2.2H_2O$	117
$FeSO_4.7H_2O$	5.25
$ZnSO_4.7H_2O$	1.5
$MnSO_4$	1.5
$CuSO_4.5H_2O$	0.3
$CoCl_2.6H_2O$	0.3
$Na_2MoO_4.2H_2O$	0.15
H_3BO_3	0.3
KI	0.075
Yeast Extract	10

3.2.1.3. Digester Start-up

Prior to inoculation, the reactor was filled with water. The reactor was examined for gas leaks and the temperature control system and mixer were switched on. This was carried out to ensure proper working order of the system. The digester was then inoculated with 2L anaerobic sludge, which had a COD content of 14 500mg/L and pH 7.39. Digestion was carried out at 35^0C, with continuous mixing.

3.2.1.4. Fed-Batch Digestion

Initially the digester was fed with 50mL artificial effluent, which had a COD content of approximately 400mg/L. With regular pH and COD monitoring the feed volume was then increased to 100mL/day except Saturdays and Sundays. After 20 days, the maximum working volume was reached and the reactor contents were drained, leaving 950mL of active sludge. Anaerobic conditions were maintained by pumping OFN into the reactor after drainage. Vegetable oil effluent (COD 2000mg/L) was then fed to the digester and gas production, as well as pH and COD monitored.

3.2.2. Anaerobic digestion using the ABR

3.2.2.1. Anaerobic Baffled Reactor

The working volume of the ABR used in this experiment was 6L. The reactor was operated at a temperature of 35^{0}C, using a temperature controlled water bath. The reactor was equipped with an inlet for feeding and an outlet for effluent overflow and sampling. Gas ports enabled monitoring of gas production.

3.2.2.2. Digester Start-up

The ABR was inoculated with anaerobic sludge and allowed to stand for 15 days for sludge settlement and acclimatization of the microorganisms. A decline in pH was observed with no gas production. The drop in pH indicated that the methanogenic bacteria were not capable of utilizing volatile acids produced by acetogenic bacteria, and as a result, did not produce any biogas.

The second start-up involved inoculation of the reactor with 2L anaerobic sludge, with a COD content of 11 060mg/L and pH 7.10. The remaining working volume of 4L was composed of artificial effluent. The artificial effluent used is illustrated in Table 3-1. The reactor was allowed to stand for 7 days for sludge settlement. Feeding was initiated with artificial effluent, monitoring gas production and pH. The reactor was fed a volume of 200mL, composed of 90% VOE and 10% artificial effluent. Initially, a glass syringe was used to feed the reactor, thereafter a peristaltic pump was used.

3.2.3. Analyses

pH Measurement

pH was measured using a calibrated pH meter (Beckman). Values obtained were accurate to within $\pm$ 0.2.

COD Measurement

The COD measurement was based on the open reflux, colorimetric method described in Appendix C.

Alkalinity Measurement

Alkalinity was measured potentiometrically as described in Appendix A.

3.3. RESULTS

3.3.1. Results of Fed-Batch Digestion

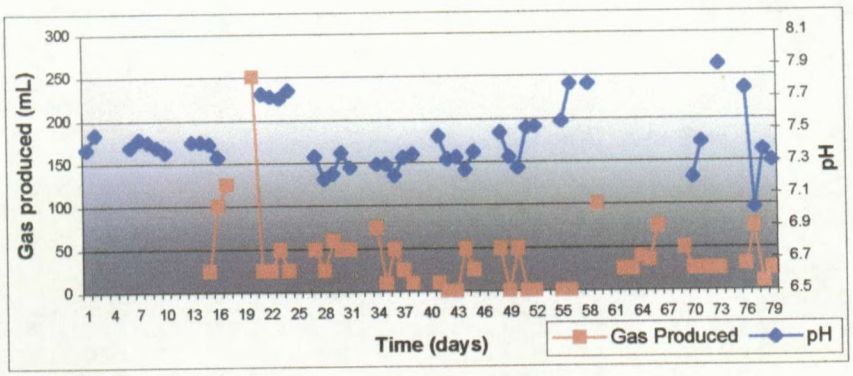

Figure 3-2: Comparison of gas production and pH during fed-batch digestion

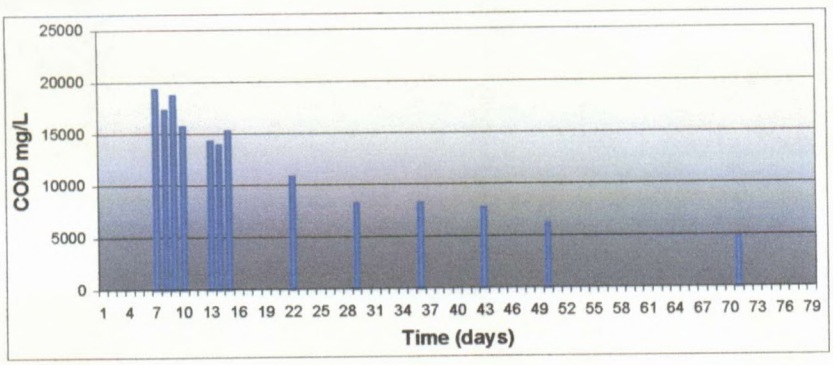

Figure 3-3: Graph illustrating COD (mg/L) during fed-batch digestion

3.3.2. Results of the Anaerobic Baffled Reactor

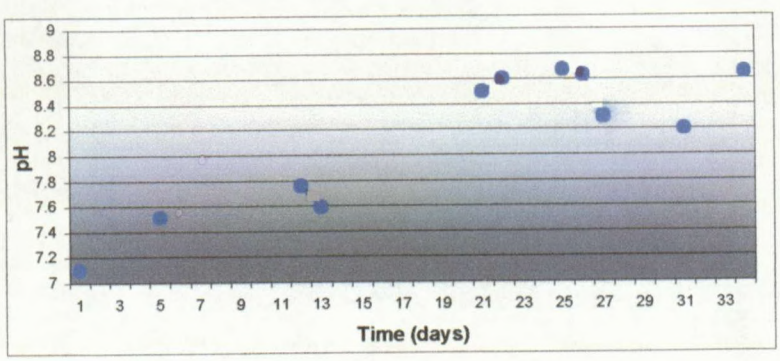

Figure 3-4: pH profile of the Anaerobic Baffled Reactor

3.4. DISCUSSION

3.4.1. Fed-Batch Digestion

The digester was initially fed artificial effluent to aid in acclimatization of the microorganisms to the environmental conditions. Gas production, pH and COD were monitored to ensure proper digester operation. Figure 3-1 illustrates the comparison of pH and gas production of the fed-batch digester. During the first 19 days, with HRT of 20 days, the graph indicates steady pH readings between 7.35 and 7.48. This suggests that the buffering capacity of the digester was sufficient to maintain the pH within normal working range. Thereafter, the working capacity of the digester was reached and the contents drained, leaving 950mL sludge. The substrate was then changed to 50mL VOE with a COD content of 2000mg/L. Looking at Figure 3-1, the graph indicates an increase in pH to 7.78 after day 20. This could be attributed to adaptation of the microorganisms to the new substrate. Thereafter, the pH stabilized between 7.2 and 7.35. Gas production was low (average 25mL) but stable. This suggests that the methanogenic bacteria were not severely inhibited by the increase in pH. The best pH range for the anaerobic bacteria is between 6.8 and 7.2 (Ross *et al.*, 1992; Switzenbaum *et al.*, 1990).

After 55 days, the digester had reached its working capacity and the contents drained, leaving 1L of sludge. At this point, the pH of the digester contents was 7.78, alkalinity 1759.3 mg/L as $CaCO_3$ and COD content of 5700mg/L. Although the pH seemed high, alkalinity concentration suggested sufficient buffering capacity of the digester. The feed rate was then increased to 100mL/day, consisting of 90% VOE and 10% artificial effluent. It was thought that the VOE lacked essential nutrients for the microorganisms, as gas production was fairly low when fed 50mL VOE. Therefore the substrate was supplemented with artificial effluent. An increase in pH was observed, initially, but stabilized between 7.3 and 7.4. Gas production averaged at about 45mL per day. The increase in gas production with a feed rate of 100mL per day in comparison with a feed rate of 50mL per day suggests that the increased feed volume increased the readily degradable organics. Which resulted in an increase in gas production.

After day 88, the digester contents were drained, again leaving 1L of anaerobic sludge. The feed rate was continued at 100mL per day. The pH remained stable, between 7.25 and 7.30. Gas production increased to an average of 62mL per day (not shown on graph). This indicates that the microorganisms had acclimatized to the VOE at a feed rate of 100mL per day. After 98 days, the reactor was drained, leaving 1L of anaerobic sludge. The feed rate was increased to 150mL per day. The pH was stable, between 7.25 and 7.3 and gas production increased to an average of 95mL per day (not shown on graph).

It is clear that with an increase in feed volume, there is an increase in gas production. Therefore, the increasing feed volumes did not cause an organic overload. An organic overload causes increased acid production, with a decrease in pH and little or no gas production.

Figure 3-2 illustrates the COD profile of the digester, which indicates a gradual decrease in total COD of the digester contents. This suggests that the microorganisms were capable of degrading the available organic material present in the wastewater.

Results show that anaerobic fed-batch digestion is a promising method of treatment for VOE.

3.4.2. Anaerobic Baffled Reactor

The reactor was fed a combination of artificial effluent (10%) and VOE (90%), with pH at ± 7 and HRT of 30 days. The initial pH of the digester contents was within the normal working range of anaerobic digestion i.e. 6.5 and 8.2 (Ross *et al.*, 1992), with alkalinity at 2370mg/L as $CaCO_3$. Figure 3-3 illustrates the pH profile of the ABR during digestion. It can be seen that the pH gradually increases over time up to 8.7 on day 25. A gradual decrease in pH was seen after day 25 as the substrate fed to the digester was decreased to 6.5. However, the pH continued to increase thereafter. This suggests that the digester was unable to maintain adequate buffering capacity. It is possible that high sulphate content of the VOE, promoted growth of SRB producing

H_2S which can be toxic at elevated concentrations to sulphate reducers as well as other microbial activity (Speece, 1996). Inhibition caused by H_2S is dependant on the pH of the environment. At alkaline levels the inhibitory effect of H_2S is higher than at neutral or acidic pH levels (Hilton and Oleszkiewicz, 1989).

It was observed that the first few compartments (closest to the influent port) contained more settled biomass than the other compartments. This is not characteristic of an ABR, as biomass within the reactor tends to rise and settle with gas production and move horizontally down the reactor (Nachaiyasit and Stuckey, 1995). Also, different levels of liquid were observed in various compartments. It is possible that the fats and oils contained in the substrate formed a lipid barrier, disrupting normal circulation of the liquid.

After day 33, a decline was observed in pH of the digester (not shown on graph), with no gas production. The experiment was terminated, since no gas production indicated that the methanogenic bacteria were no longer active. It was therefore concluded that the ABR is not suitable for treatment of this type of effluent.

CHAPTER FOUR

CONCLUSIONS AND RECOMMENDATIONS

4.1 CONCLUSIONS

From this investigation of anaerobic digestion of vegetable oil effluent, the following can be concluded,

Gravitational separation of lipids is not a recommended method for reducing the lipid content of VOE due to the long settling time required. Settling times below 24hrs showed no significant difference in lipid content.

The use of barium chloride to reduce the sulphate content in VOE is an effective method.

VOE contains approximately 30% biodegradable fraction that under anaerobic conditions has the potential to produce 0.395mL CH_4 per mg COD mineralized.

Anaerobic toxicity of VOE indicates that the effluent is not harmful to the existing process if added at a COD of 2000mg/L.

Anaerobic digestion using fed-batch digestion configuration can achieve 30% biodegradation of COD present in VOE.

Our experiments show that the ABR configuration is unsuitable for anaerobic digestion of VOE.

4.2. RECOMMENDATIONS

Improvement of physical methods (i.e. DAF) of VOE pretreatment will reduce the oil content of VOE and make it more suitable for anaerobic digestion using the ABR.

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APPENDIX A: ALKALINITY POTENTIOMETRIC TITRATION (STD. METHODS, 1989).

Background

Alkalinity represents the ability to neutralize volatile fatty acids formed during anaerobic digestion. The ability to neutralize or absorb acid at a certain pH is called the buffering capacity of the liquid. The buffer capacity of a digester at its operating pH is estimated by measuring alkalinity. Total alkalinity is calculated by determining the mass of acid added per liter of solution to reduce the pH of digester effluent to pH 4.3. Different acids can be used to titrate down to pH 4.3, therefore the mass of acid added is specified in terms of a standard compound, calcium carbonate ($CaCO_3$).

Reagents

0.1N H_2SO_4

0.005M Na_2CO_3

Phenolphthalein indicator

50% Alcohol

xylene cyanol FF

methyl orange

Standardization of H_2SO_4

- A 0.1N H_2SO_4 solution was prepared by diluting 2.8mL concentrated H_2SO_4 in a 1L volumetric flask.
- 2.5g of the primary standard (Na_2CO_3) was weighed out and diluted in a 1L volumetric flask to produce a 0.05N solution. This solution was not kept for longer than two weeks.
- Screened methyl orange indicator was prepared by weighing out 1.4g xylene cyanol FF and 1g methyl orange indicator. These were then diluted in 500mL 50% alcohol solution.

◊ 20mL of 0.05N Na_2Ca_3 was placed in a 150mL Erlenmeyer flask together with 2 to 3 drops of screened methyl orange indicator and titrated against 0.1N H_2SO_4.

◊ The end point of the titration was taken as a sharp colour change from green to grey.

Potentiometric Titration for Alkalinity

◊ A well-mixed sample of 50mL was placed in a 250mL beaker.

◊ The burette was filled with 0.1N H_2SO_4 and the pH meter standardized.

◊ The solution was titrated against the acid until the pH reached 4.3.

◊ The volume of acid used was recorded and alkalinity calculated.

Alkalinity Calculation

Alkalinity mg $CaCO_3$/L = A x N x 50 000 / mL sample

Where: *A* = *mL standard acid used*

N = *normality of standard acid*

APPENDIX B: FATS, OILS AND GREASE EXTRACTION

Background

In determining oils and greases, groups of substances with similar physical characteristics are determined quantitatively on the basis of their common solubility in petroleum ether. Oil and grease is any material recovered as a soluble substance in petroleum ether. Oils and greases may influence wastewater treatment systems if present in excessive amounts. They may interfere with aerobic and anaerobic biological processes and lead to decreased efficiency of the wastewater treatment process. When discharged in wastewater treatment effluent, they may cause surface films and shoreline deposits leading to environmental degradation.

Reagents

AR Grade Petroleum Ether

Extraction

1. Effluent sample was well agitated the 20mL was transferred into a measuring cylinder
2. A 250mL separating funnel was rinsed with 15ml petroleum ether and discarded into residue storage vessel.
3. Effluent sample was transferred into the separating funnel and the measuring cylinder rinsed with 15mL petroleum ether and transferred into the funnel.
4. Petroleum ether (10mL) was added to the separating funnel.
5. The separating funnel was closed with a stopper and shaken vigorously for 2 minutes. The mixture was vented by opening the stopcock.
6. The mixture was allowed to stand and the ether layer to separate.
7. The aqueous layer was drained from the funnel into a beaker.
8. The ether extracts were drained into a dried and pre-weighed 250mL distillation flask (W1-grams).
9. The aqueous layer was returned from the beaker into the separating funnel.

10. The beaker was rinsed with 10mL petroleum ether and transferred into the separating funnel.
11. Steps 4 and 8 were repeated twice more.
12. The total volume of petroleum ether used was recorded (V2-mL).

Distillation

1. The 250mL distillation flask was connected to a condenser and a beaker placed at the discharge end of the condenser.
2. The distillation flask was placed into an isomantle and the water supply to the condenser turned on.
3. The ether mixture was distilled until approximately 5-10mL remained. The remainder of the ether was boiled off on the isomantle after removing the condenser.
4. The distillation flask and residue was then dried in a dessicator for 30 minutes.
5. The flask was re-weighed (W2-grams).
6. An aliquot of petroleum ether, 100mL (V1) was evaporated using a condenser and mass of residue determined (M1-grams).

Calculations

a) Pure Solvent

$$M3 = M1 \times V2 / V1 \text{ (mg)}$$

Where:

M1	=	*mass (mg) of petroleum ether extract residue (Blank)*
V1	=	*mL ether evaporated for blank*
V2	=	*Ml ether collected following extraction of effluent*
M3	=	*mass of background residue in V2mL of ether*

b) Oils and Grease

Ppm Oil/Grease = (M2-M3) x 1000 / V3

Where:

W1 = *mass of dried empty distillation container (g)*

W2 = *mass of distillation container and residue of effluent (g)*

M2 = *mass of residue (mg) following extraction of effluent*

= *(W2-W1) X 1000*

V3 = *sample volume (mL)*

APPENDIX C: CHEMICAL OXYGEN DEMAND: OPEN REFLUX METHOD (STD. METHODS, 1989)

Background

Chemical Oxygen Demand (COD) is a measure of the oxygen equivalent of the organic matter content that is susceptible to oxidation by a strong chemical oxidant. This test utilizes potassium dichromate in boiling concentrated sulphuric acid, in the presence of a silver catalyst. The catalyst acts as a strong oxidizing agent. Under these conditions most of the carbon in the sample is oxidized to carbon dioxide and the hydrogen present is oxidized to water. The dichromate is reduced to trivalent chromium. The more organic material present in the sample, the more dichromate will be reduced to chromium. The COD of the sample is determined by titrating the remaining dichromate with ferrous sulphate.

Reagents

Standard Potassium Dichromate Solution: 0.0417M

12.259g of $K_2Cr_2O_7$ was dissolved in distilled water and diluted to 1L.

Sulphuric Acid Reagent

Silver sulphate was added to concentrated sulphuric acid at the rate of 5.5g Ag_2SO_4 per liter of H_2SO_4 and allowed to stand for 2 days.

Ferroin Indicator

1.485g of 1,10 – phenanthroline monohydrate and 695mg $FeSO_4.7H_2O$ was dissolved in 100mL distilled water.

Standard Ferrous Ammonium Sulphate (FAS): 0.25M

98g of $Fe(NH_4)_2(SO_4)_2.6H_20$ was dissolved in distilled water. 20mL concentrated sulphuric acid was added to the mixture and diluted to 1L.

◊ Standardization of FAS

◊ 10ml of standard K_2CrO_7 was diluted to 100mL with distilled water.

◊ 30mL of concentrated sulphuric acid was added and cooled.

◊ This solution was titrated against FAS using 2/3 drops of ferroin indicator.

Molarity FAS = Vol.$K_2Cr_2O_7$ titrated (mL) x 0.25 / Vol.FAS used (mL)

Mecuric Sulphate

$HgSO_4$ crystals or powder

Potassium Hydrogen Phthalate (KHP)

425mg of KHP was dissolved in distilled water and diluted to 1L. KHP has a theoretical COD of 1.176mg O_2/mL and this solution has a theoretical COD of 500µg O_2/mL.

Procedure

◊ Samples with COD>50mg O_2/L 50ml sample was used and those with COD>900mg O_2/L smaller sample volumes were used and diluted to 50mL.

◊ Samples were placed in 500mL refluxing flasks and 1g of $HgSO_4$, glass beads and 5mL sulphuric acid reagent added.

◊ Samples were cooled to prevent loss of volatile materials.

◊ 25mL of $K_2Cr_2O_7$ solution was added. The flask was attached to a condenser and cooling water turned on.

◊ The remaining sulphuric acid reagent (70mL) was added through the open end of the condenser.

◊ The open end of the condenser was covered and the sample refluxed for 2hrs.

◊ Thereafter, the condenser was disconnected and the sample cooled to room temperature. Excess $K_2Cr_2O_7$ was titrated against FAS using Ferroin indicator.

◊ The endpoint of the titration was taken as the first sharp colour change from blue-green to reddish brown.

◊ A blank was titrated in the same manner containing reagents and a volume of distilled water equal to the sample volume used.

Calculation

$$\text{COD as mg } O_2/L = (A-B) \times M \times 8000 / \text{mL sample}$$

Where:

A = *mL FAS used for blank*

B = *ml FAS used for sample*

M = *molarity of FAS*

APPENDIX D: CHEMICAL OXYGEN DEMAND (COD) USING MERCK SQ118 SPECTROPHOTOMETER

Procedure

- ◊ 2.20mL of solution A (mercury (II) sulphate and sulphuric acid) and 1.8mL of solution B (sulphuric acid and potassium dichromate) was placed in a reaction cell and mixed.
- ◊ 3mL of the sample was placed in the reaction and mixed.
- ◊ A blank was prepared in the same manner containing distilled water of the same sample volume.
- ◊ The reaction cells were placed into the thermoreactor and heated at 148^{o}C for 2 hrs.
- ◊ Thereafter, the reaction cells were removed from the thermoreactor and placed into the cooling rack to cool.
- ◊ After 10 minutes, the reaction cells were shaken and replaced in the cooling rack to cool to 20-40^{o}C.
- ◊ The blank cell was placed into the cell compartment and the blank value read.
- ◊ The blank cell was removed and the sample cell inserted into the cell compartment. The COD content of the sample was then read.

Appendix E: Sulpahtes (Merck SQ118 Spectrophotometer)

Procedure

- ◊ A well-mixed sample was filtered through whatman no.1 filter paper and the filtrate filtered through a 0.45μm pore size filter.
- ◊ 2.5mL of the filtered sample was pipetted into a test tube (sample solution)
- ◊ 2.5mL of distilled water was pipetted into a second test tube (blank solution)
- ◊ Two drops of SO_4 – 1A was added into each test tube and mixed
- ◊ One green microspoonful of SO_4 – 2A was added to each test tube and mixed.
- ◊ Both sample and blank solutions were placed into a water bath at 40^0C for 5 minutes and the test tubes shaken several times during this period.
- ◊ 2.5mL of SO_4 – 3A was added to each test tube and mixed.
- ◊ Both sample and blank solutions were filtered into an empty round cell each through Ederol no.2 filter paper.
- ◊ Four drops of SO_4 – 4A was added to each test tube and mixed.
- ◊ Both test tubes were placed into a water bath at 40^0C for 7 minutes.
- ◊ Thereafter, each solution was transferred to a separate cell.
- ◊ The blank cell was placed into the cell compartment and the blank value read.
- ◊ The blank cell was removed and the sample cell inserted into the cell compartment. The sulphate content of the sample was then read.

APPENDIX F: TOTAL SOLIDS AND VOLATILE SOLIDS (STD. METHODS, 1989)

◊ A clean evaporating dish was ignited at 550^{0}C for 1hr in a muffle furnace and stored in a desiccator to cool.

◊ The dried dish was weighed immediately before use.

◊ A measured volume of a well-mixed sample was placed into the evaporating dish and evaporated to dryness at 180^{0}C before re-weighing.

Calculation: Total Solids

Total Solids mg/L = (A-B) x 1000 / sample volume (mL)

Where:

A = *weight dish and residue (mg)*

B = *weight dish (mg)*

◊ The residue was ignited in a muffle furnace at 550^{0}C for 15 to 20 minutes and allowed to cool in a desiccator before re-weighing.

Calculation: Volatile Solids

Volatile Solids mg/L = (A-B) x 1000 / sample volume (mL)

Where:

A = *weight residue and dish before ignition (mg)*

B = *weight residue and dish after ignition (mg)*

APPENDIX G: TOTAL SUSPENDED SOLIDS AND TOTAL DISSOLVED SOLIDS (STD. METHODS, 1989)

◊ A well-mixed sample of known volume was filtered through a weighed standard glass fiber filter paper.

◊ The residue retained on the filter paper was dried to a constant weight at 103 – 105^{0}C.

◊ The increase in weight of the filter paper represents total suspended solids.

Calculation: Total Suspended Solids

Total Suspended Solids (TSS) mg/L = (A-B) x 1000 / sample volume (mL)

Where:

A = *weight of filter paper and dried residue (mg)*

B = *weight of filter paper (mg)*

◊ The filtrate was evaporated to dryness in a weighed evaporating dish and dried to constant weight at 180^{0}C.

◊ The increase in weight of the dish represents total dissolved solids.

Calculation: Total Dissolved Solids

Total Dissolved Solids (TDS) = (A-B) x 1000 / sample volume (mL)

Where:

A = *weight of dried residue and dish (mg)*

B = *weight of dish (mg)*

APPENDIX H: PREPARATION OF THE NUTRIENT MEDIUM ACCORDING TO OWEN *et al.*, (1979)

The defined nutrient medium containing trace elements, minerals and vitamins was prepared according to Owen *et al.*, (1979). The stock solutions for preparation of the nutrient medium are presented in Table H.1 and the method for preparation is presented in Table H.2.

Table H.1: Stock solutions for preparation of mineral salts medium

STOCK SOLUTION	COMPOSITION	CONCENTRATION (g/L)
S2	Rezazurin	1
S3	$(NH_4)_2HPO_4$	26.7
S4	$CaCl_2.2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MoO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2.4H_2O$	20
	EDTA	20
S6	Cysteine	50
S7	Biotin	0.002
	Folic acid	0.002
	Pyridoxine HCl	0.01
	Roboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Pantothenic Acid	0.005
	Vitamin B12	0.0001
	p-aminobenzoic acid	0.005
	Thiotic acid	0.005

Table H.2: Preparation of the defined mineral salts solution

Step	Method	Volume (mL)	Mass (g)
1	1L of deionised water was added to a 2L vessel		
2	The following were added:		
	Stock solution S2	1.8	
	Stock solution S3	5.4	
	Stock solution S4	27	
3	Deionised water was added up to 1.8L		
4	Boiled for 15 min		
5	Cooled to room temperature		
6	The following were added:		
	Stock solution S7	1.8	
	Stock solution S5	1.8	
	Stock solution S6	1.8	
7	NaHCO3 was added as powder		8.4