

*TOXICITY AND BIODEGRADABILITY ASSAYS FOR
HAZARDOUS LANDFILL LEACHATE AND TEXTILE
SIZE EFFLUENTS*

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PREFACE

I, Thabisile Rakgotho, declare that unless indicated, this dissertation is my own work and has not been submitted, in whole or part, for a degree at another institution.

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Thabisile Rakgotho

March 2005

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Glossary

Anaerobe

A microorganism capable of growing or metabolizing in the absence of free oxygen. These microorganisms may be facultative or obligate, the latter will perish in the presence of free oxygen.

Anaerobic digestion / biodegradation

The microbial degradation of an organic compound in the absence of oxygen.

Batch culture

A closed culture environment in which conditions are continuously changing according to the metabolic state of the microbial culture.

Biodegradable

A property which allows the microbial decomposition of an organic compound to inorganic molecules.

Biogas

The gas produced, mainly methane and carbon dioxide, by the action of anaerobic microorganisms on organic compounds.

Chemical Oxygen Demand

A measure of the total amount of organic material in a waste stream.

Co-disposal

The calculated and monitored treatment of industrial and commercial liquid and solid wastes by interaction with biodegradable wastes in a controlled landfill.

Co-metabolism

Metabolic transformation of a substance while a second substance serves as primary energy and carbon-source.

Effluent

A stream flowing from a sewage tank or industrial process

Headspace

The volume in a sealed vessel not occupied by the liquid phase.

Inhibition

An impairment of bacterial function.

Labile

Readily biodegradable.

Methanogens

Bacteria which utilize volatile organic acids as substrates and produce methane and carbon dioxide.

Pollution

An adverse alteration of the environment.

Recalcitrant

Resistant to microbial degradation.

Size

A coating applied to warp yarn to improve its weaving efficiency.

Sludge

The general term applied to the accumulated solids separated from wastewater.

Suspended solids

Undissolved non-settleable solids present in wastewater.

Total solids

The sum of dissolved and suspended solids in wastewaters and sludge.

Toxicity

An adverse effect (not necessarily lethal) on bacterial metabolism.

Volatile solids

Organic solids that are lost on ignition at 600°C

Warp

The longitudinal threads in a length of fabric.

List of Abbreviations

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AD - Anaerobic Digestion	10
ATA - Anaerobic Toxicity Assay	11
BMP - Biological Methane Potential	11
BOD - Biological Oxygen Demand	35
CMC - Carboxymethylcellulose	35
CMS - Water Soluble Starches	35
COD - Chemical Oxygen Demand	14
DO - Dissolved Oxygen	6
DWAF - Department of Water Affairs and Forestry	1
ECA - Environmental Conservation Act	2
GC - Gas Chromatography	44
H ₂ S - Hydrogen Sulphide	20
NEMA - National Environmental Management Act	2
NWA - National Water Act	1
NWP - National Water Policy	1
PAC - Polyacrylic	35
PAH - Polycyclic Aromatic hydrocarbon	29
PVA - Polyvinyl Alcohol	35
OFN - Oxygen Free Nitrogen	39
TSS - Total Suspended Solids	43
UV-VIS - Ultra Violet Spectrophotometer	49
VFA - Volatile Fatty Acids	20
VSS - Volatile Suspended Solids	43

Abstract

The cumulative effects of pollution have led to increased public concern, which is resulting in strict legislation on the discharge of wastes in whatever state they are present, i.e. solid, liquid or gas. Currently, in South Africa, effluents with a high organic load are sent to landfills or marine outfall because the cost of discharge to sewer is prohibitive. In regions where there is a net surplus of rainfall, landfill sites have the potential to pollute the groundwater due to saturated soil conditions. Therefore, many landfill sites should not receive liquid effluents. If liquid wastes are disposed onto landfills, then an alternate sink is required for the treatment of the high volumes of leachate that are generated. These concentrated effluents could then be treated by biological, chemical or physical methods to reduce the pollution load in the natural water resources. In this study, anaerobic digestion has been identified as one of the biological processes that can be applied to treat high-strength or toxic organic liquid effluents, since a survey conducted by Sacks (1997) indicated that many anaerobic digesters in the KwaZulu-Natal region have spare capacity. However before high strength industrial wastes can be treated in existing anaerobic digesters, their impact on the digestion process, i.e. their toxicity and biodegradability under anaerobic conditions, needs to be determined.

During this project, several high-strength or toxic industrial effluents were tested to assess their toxicity and biodegradability under anaerobic conditions. These include three synthetic textile size effluents from the textile industry (Textile effluent 1, 2, and 3) and three hazardous landfill leachates (Holfontein, Shongweni and Aloes). In addition, the components of a textile effluent, i.e., starch and wax, were tested to determine which

component may have contributed to the biodegradability and toxicity of size. Furthermore, a textile effluent was co-digested with a leachate sample at different ratios to determine the extent of biodegradability when two substrates are co-metabolized. These effluents were tested using the anaerobic toxicity assay and the biochemical methane production assay. Each effluent was tested at dilutions ranging from 0.04% to 40% (v/v) of the total reactor volume.

Biodegradability tests showed that textile effluents were highly biodegradable i.e. >60%. The leachate samples from Shongweni, Holfontein and Aloes landfill sites were found to be 52%, 19% and 38% biodegradable, respectively. Holfontein and Aloes leachate were found to be difficult to biodegrade and may be classed as a recalcitrant.

The co-digestion results showed that the addition of certain types of industrial wastewater does not inhibit anaerobic digestion. In this study, toxicity was concentration-dependent i.e. lower concentration of the effluents showed no toxic effects while the higher concentrations were found to be toxic. The wax component of the textile effluent was found to be toxic whereas starch was labile. This was evident from the initial gas production rates which were very low for wax component.

The results of this study indicate that the textile effluent and the hazardous landfill leachate from Shongweni site may be amenable to anaerobic treatment, and in Kwa-Zulu Natal region these effluents can be treated by using Anaerobic Digestion.

"Some , for all, forever" {Department of Water Affairs and Forestry (DWAF), 1999}

1.1 SOUTH AFRICAN LEGISLATION

Section 24 of the Constitution of the Republic of South Africa (*Act No. 108, 1996*) states that everyone has a right to an environment that is not harmful to his or her health and well-being. It further states that everyone has the right to have their environment protected, for the benefit of the present and future generations, through reasonable legislative and other measures that prevent pollution and ecological degradation. The Bill of Rights of the Constitution of South Africa gives effect to fundamental objectives outlined in the National Water Policy (NWP) and the National Water Act (NWA) (*Act No. 36 of 1998*) which are:

- a. To achieve sustainable use of water, and
- b. To achieve efficient and effective water use for optimum social and economic benefit.

The NWA contains comprehensive provisions for the protection, use, development, conservation, management and control of South African water resources. It further states that it is not practical or realistic to avoid all impacts to water quality, otherwise the country will be unable to grow economically and will not be able to alleviate poverty or to have social development. This Act requires a balance between using water and protecting water resources to be achieved as this is the principle of sustainability. The Department of Water Affairs and Forestry is mandated by this Act to ensure that South Africa's water resources are

protected and controlled in a sustainable and equitable manner, for the benefit of all persons (DWAF, 2000). This piece of legislation is the principal legal instrument relating to water resources management in South Africa, however, it is not the only instrument used to achieve the objectives of the NWP. There are many other policies and laws, administered by a number of departments within the South African government, which governs activities that are dependent on water, or which affect water resources.

The National Environmental Management Act (NEMA) (*Act No. 107, 1998*), for example, is one legislation used to manage the activities that might have detrimental impact on the water resources environment. NEMA legislates that the state must secure ecologically sustainable development and the use of natural resources while promoting justifiable economic and social development. Sustainable development requires the consideration of all relevant factors including that:

- a. the pollution and degradation of the environment are avoided, or where they cannot be altogether avoided, are minimized and remedied, and
- b. waste is avoided, or where it cannot be altogether avoided, minimized and reused or recycled where possible and otherwise disposed of in a responsible manner.

Other legislations include Environmental Conservation Act (ECA) (*Act No.73, 1989*) which under section 21(f) and (i) state that activities such as industrial processes and waste as well as sewage disposal are amongst those activities which will probably have a detrimental effect on environment and, hence, control of such activities needs to be implemented.

Due to tight national legislation, stringent disposal by law and the unavailability of sewer disposal options, there is a demand for on-site treatment of high strength or toxic effluents such as landfill leachate and industrial effluents. Various physical and chemical methods are being used to treat these effluents and are regarded as separation processes. The disadvantage with these methods is that they can be expensive, whereas, biological treatment methods such as anaerobic digestion can be cost-effective and have been proven to reduce and remove pollutants (Reinhard and Pohland, 1991).

1.2 SOUTH AFRICA'S WATER SITUATION

South Africa is located in a predominantly semi-arid part of the world. The climate varies from desert and semi-desert in the west to sub-humid along the eastern coastal area, with an average rainfall for the country of about 450 mm per year, well below the world's average of about 800 mm per year, while evaporation is comparatively high (DWAF, 1999). As a result, South Africa's water resources are, in global terms, scarce and extremely limited in extent. Due to poor spatial distribution of rainfall, the availability of water across the country is highly uneven and South Africa is regarded as a water scarce country that is poorly served with natural water systems. Since water is a universal solvent, it is utilized in many ways and then discarded. The discarded water carries suspended, floating, dissolved materials as well as toxic substances which lead to major environmental problems (Tothill and Turner, 1996). Around the world, pollution of water from industrial, agricultural and municipal operations continues to grow as a result of growing population and expanding industries, and these pollution loads and expanding demand will place additional pressure on the already limited

water resources of South Africa since the assimilation processes natural to the water resources cannot cope with the increasing contamination (Environmental Biotechnology 4001, 2003). Hence, in order to ensure that no potential risks of transmission of water-related pollutants to natural water resources, Horan (1996) stated that attention should be paid to the management of aquatic resources as well as the pollutants that enter them. In coastal areas, the biggest problem with industrial wastewater is that it is sent to marine outfall systems and the amount of fresh water lost via effluent pipelines to sea is phenomenal. In order to prevent the loss of fresh water and the deterioration of water quality in rivers, estuaries and oceans, DWAF has adopted a pollution prevention approach to control hazardous or toxic pollutants found in effluents (DWAF, 1999).

1.3 SOURCES OF WATER POLLUTION

Water that is used in industries usually becomes polluted and if allowed to escape into water resources, it pollutes the rivers with heavy metals which make the water unfit for use by others. For example, mining and industrial sectors have effluent dams on sites, and when rainfall seeps through these waste dumps, it becomes contaminated and can run off into water resources or into groundwater and can result in pollution of water resources. Some industries that treat and release wastewater directly into rivers through a pipe, sometimes have problems with their treatment works, and this results in poor quality effluent being released into rivers. This further worsens pollution in water resources.

Water courses such as streams and lakes are polluted in various ways, by physical, chemical and biological contaminants and the removal of priority pollutants is vital (Haghighi-Podeh and Bhattacharya, 1996). Biological contamination is caused by introduction of microorganisms such as bacteria, fungi, algae and viruses, whereas, physical contamination consists of substances that are suspended in wastewater such as solids and oils (Environmental Biotechnology 4001, 2003). Chemical contamination originates from chemicals such as acids dissolving in wastewaters and numerous test methods have been developed to assess the effect of chemical pollutants on the environment (Lopez-Fiuza *et al.*, 2002), including bioassays (Tothill and Turner, 1996) in which test organisms are exposed to various doses of pollutants for toxicity evaluations.

Water pollution is categorized as either point source or non-point source (ENBT, 2003):

- a. Point source pollutants are all dry weather pollutants that come from a concentrated originating point like a pipe or channel from a factory or a municipal wastewater treatment plant. Point source pollution is a registered source of pollution and is regulated by the state, government or local laws. Governments and industries are constantly on the lookout for technologies that will allow for more efficient and cost-effective measures to address pollution of point source origin.
- b. Non-point source pollution originates from many diffuse sources caused by rainfall moving through ground. As the runoff moves, it picks up and carries away natural and human-made pollutants, finally depositing them into lakes, rivers, and wetlands.

These pollutants include excess fertilizers, herbicides and, insecticides from agricultural lands and residential areas, oil, grease and toxic chemicals from urban runoff as well as illegal dump sites.

There are different major types of pollutants that can be found in wastewaters and these include:

- a. Oxygen demanding substances which comprise one of the most important types of pollutants because these materials decompose in the watercourse and can deplete the oxygen and create anaerobic conditions. Oxygen in watercourses is vital for aquatic life and to prevent odours. Dissolved oxygen (DO) is a variable that is widely used as an indicator of a water body's ability to support desirable aquatic life.
- b. Suspended solids also contribute to oxygen depletion since they are able to block light from reaching submerged aquatic vegetation. As the amount of light passing through water is reduced, photosynthesis slows down. The reduced rates of photosynthesis cause less dissolved oxygen to be released into the water by plants. If light is completely blocked, the bottom-dwelling vegetation will stop producing oxygen and eventually die. As they decompose, bacteria will use up even more oxygen from water and this decrease in dissolved oxygen could lead to fish kills. High suspended solids can also cause an increase in surface water temperature because the suspended solids absorb heat from sunlight and in addition, they create unsightly conditions and can cause odours.

- c. Nutrients, mainly nitrogen and phosphorus, can cause accelerated eutrophication, and some bio-concentrated metals can adversely affect aquatic ecosystems as well as making the water unsuitable for human contact or consumption.
- d. Heated discharges may drastically alter the ecology of a stream or lake. The primary effect is deleterious, lowering the solubility of oxygen in water, because gas solubility in water is inversely proportional to temperature, and thereby reducing the amount of DO available.
- e. Sediments from soil erosion may be classified as a pollutant. This is because contaminants from industries, land cultivation, construction, demolition, mining operations and even air make their way through water into sediments or bottom mud on our watercourses. These contaminants found in sediments can lead to human health since they make their way into the food web accumulating in fish, ducks and other wild life which eventually end up in humans.
- f. Petroleum compounds which can be extremely toxic and have the potential of polluting the environment when industries are drilling for them or during transportation or refinery can find their way to water courses.
- g. Acid mine drainage has polluted the surface waters since the beginning of ore mining. Wastewater leached from mines, including old and abandoned mines as well as active ones, contains sulphur compounds which are oxidized to sulphuric acids on contact

with air, resulting in acidity of the stream or lake, high enough to kill the aquatic ecosystems.

Organic and inorganic solids pollutants may be suspended or dissolved in water and some of these substances could be toxic to microorganisms at high concentrations. The majority of these organic and inorganic water pollutants come from discharges from manufacturing and industrial plants and leachates from solid waste disposal sites. Horan (1996) also added that the biological composition of effluents from these sites would vary dramatically and thorough care must be taken when conducting a selection of treatment options for such effluents. He further added that the main objective of effective wastewater treatment practices is to eliminate, if possible, or minimize, the hazardous volume and toxicity of all waste streams, thereby reducing the environmental impact of the waste prior to disposal at permitted and well-managed waste disposal facilities.

To ensure sustainable development, focus has moved to pollution prevention and the end-of-pipe approach has been replaced by a hierarchy approach (Figure 1.1). This new approach encompasses:

- a. The need to extend an acceptable level of waste management services to all communities and to ensure appropriate treatment of hazardous waste prior to disposal in an adequately designed waste disposal facility, and
- b. The pollution prevention and cleaner production or waste minimization measures that focus on the source of waste and moves away from the end-of-pipe solutions.
- c. Cleaner production is the continuous application of an integrated preventive environmental strategy, applied to processes, products and services to increase eco-efficiency and reduce risks to humans and the environment. Implementation of cleaner production and waste minimization practices, at the effluent source, will lead to the production of more concentrated effluents. These effluents will have to be handled in a responsible way.

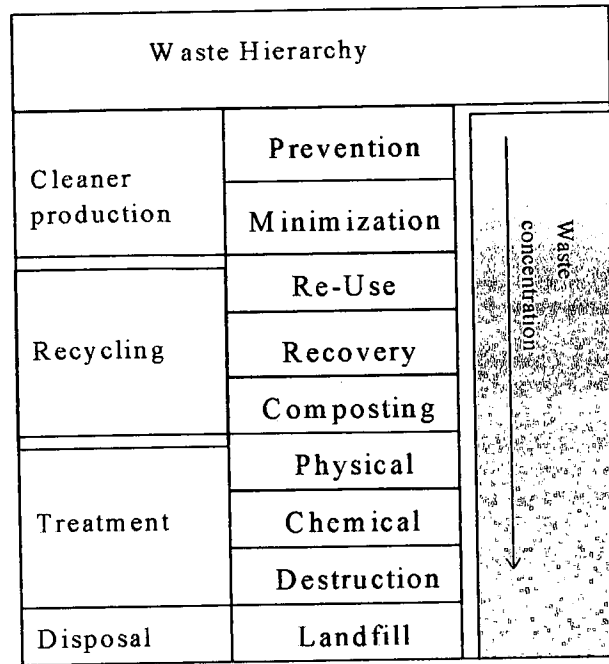


Figure 1.1: Waste hierarchy (National Waste Management Strategies, 1999)

Promotion of cleaner production techniques will result in the identification of numerous small streams of high strength liquid effluents. These concentrated effluents can then be treated by biological, chemical or physical methods to reduce the pollution load in the rivers. Anaerobic digestion (AD) has been identified as one of the biological processes that can be applied to treat industrial wastewater (Sacks, 1997).

This project aims to provide a screening protocol for high strength liquid industrial effluents before they are disposed of in anaerobic digesters. The main objective of this project is to evaluate whether high strength/toxic organic liquid industrial effluent can be disposed of in conventional anaerobic digesters. This objective is achieved by:

- a. Screening landfill leachate from different hazardous waste sites and textile size effluents for biodegradability in conventional anaerobic digesters. (Screening of these effluents is determined through a Biochemical Methane Potential (BMP) test. This is done by monitoring cumulative methane production from a sample, which is anaerobically incubated in a chemically defined medium).
- b. Determining the inherent toxicity of different hazardous landfill leachate and textile effluents by using the Anaerobic Toxicity Assay (ATA). (This measures the adverse effect of a compound on the rate of total gas produced from a labile methanogenic substrate relative to the control).

The above methods are effective techniques to determine optimum assay conditions for laboratory-scale batch assessments of organic effluent biodegradability and toxicity to anaerobic biomass. These bioassays are relatively simple; require minimal labour to set up, minimal monitoring and no sophisticated equipment is needed (Sacks1997).

This study was set up to evaluate whether high-strength organic liquid industrial effluents are amenable to AD. This included the screening of landfill leachates from different hazardous waste sites and textile size effluent for toxicity and biodegradability in conventional anaerobic digesters. The above were determined through BMP tests which measure substrate biodegradability. In addition, this study aimed to determine the inherent toxicity of the above substrates under defined conditions by using the ATA. This method measures the adverse effect of a compound on the rate of total gas produced from a labile methanogenic substrate relative to the control (Speece, 1996). At the start of the ATA test, an acetate/propionate solution was added in excess to determine the toxicity of the wastewater to the acetoclastic methanogens, which are the most sensitive group of bacteria in the anaerobic consortia.

Chapter 2 will give a brief literature review on Anaerobic Digestion as well as complexity of wastes regarding their treatment using anaerobic procedures. This review of the literature was undertaken to gain familiarity with the fundamentals of AD and also to give an indication of the parameters affecting the efficient functioning of the anaerobic system. Chapter 3 will focus on the materials and methods used during this research programme. Chapter 4 will cover aspects of toxicity, biodegradability and co-digestion. The thesis is concluded with Chapter 5 and recommendations for future research are dealt with in this section. Appendix

A to G provide a compilation of raw data of all experimental work conducted during this project.

The basic question is no longer whether an industrial waste can be anaerobically biodegraded to methane, since most organic molecules are amenable to anaerobic treatment, but rather; at what rate is it degradable and to what degree is it degradable (Speece, 1983).

2.1 ANAEROBIC DIGESTION

There is a growing interest in alternate energy sources as the result of increased demand for energy coupled with a rise in the cost of available fuels (Rajeshwari *et al.*, 1999). Rapid industrialization has resulted in the generation of a large quantity of effluents with high carbon contents, of which if treated suitably, can result in a perpetual source of energy. In spite of the fact that there is a negative environmental impact associated with industrialization, the effect can be minimized and energy can be tapped by means of AD (Rajeshwari *et al.*, 1999).

Anaerobic digestion is one technology that can successfully treat the organic fraction of waste as it does not only provide pollution prevention but also allows for sustainable energy and nutrient recovery. It is one of the oldest means of wastewater treatment and refers to biological engineering process whereby complex feedstock is converted into a range of simpler compounds and normally, there would be a significant reduction in the amount of organic matter, which is measured as a Chemical Oxygen Demand (COD). The main objectives for anaerobic treatment are to purify the wastewater before it is discharged into watercourses and also to transform sludge to innocuous and easily dewatered substances (Malina and Pohland, 1992).

Initially, anaerobic treatment technologies were developed for the treatment of readily biodegradable fractions of wastewater and sludge. As the technology matured, increased knowledge on toxicity and biodegradability enabled applications to include effluents containing toxic and recalcitrant compounds from the chemical, petrochemical and pulp and paper industries. And with the development of high rate-systems in the period from the 1950's to 1980's, the technology was applied to agro-industrial effluents (Speece, 1996).

Furthermore, Ali and Sreekrishnan (2001) mentioned that effluents from pulp and paper mills are highly toxic and are the major source of aquatic pollution; they further mentioned that more than 250 chemicals have been identified in effluents that are produced at different stages of papermaking. Their toxic nature is derived from the presence of several naturally-occurring and xenobiotic compounds which are formed and released during various stages of papermaking.

Other industrial wastes treated with this technology include wastewater from food processing, edible oil production, distillers, wineries, breweries, cheese processing and organic chemicals. These concentrated organic wastes generally create serious treatment or disposal problems for the industry or the local authority concerned because of their high organic load (Speece, 1983). Sacks (1997) reported that sanitary landfill leachates and leachates from hazardous wastes are also potential feedstock for AD.

Industrial wastewaters, which would be good candidates for anaerobic treatment, may be toxic to the anaerobic biomass. It would be rare to find an industrial wastewater devoid of a

toxic component (Carrieri *et al.*, 1993; Speece, 1996). It has become possible to anaerobically biodegrade many other organic toxicants when appropriate precautions are provided to protect the biomass, i.e. to carefully increase the toxicants concentration slowly, and to prevent loss of biomass from the system until acclimation commences (Speece, 1996). However, there has been a sceptism due to the lack of quantitative information on the capability of such process to handle potentially toxic or high-strength waste (Sacks, 1997).

Interest in the AD process, supported by advances in process engineering, has been translated into numerous treatability studies of various industrial wastewaters (Speece, 1983). The success of this activity has been manifested in the commissioning of a number of full-scale industrial treatment installations as reported by Sacks, (1997). Speece (1996) reported that anaerobic treatment of medium to high-strength wastewater is presently accepted as a proven technology. However, there is still a need for more quantitative information on the effects and fates of pollutants found in these effluents during anaerobic treatment.

2.1.1 Advantages and Disadvantages of Anaerobic Digestion

Speece (1996) reported that positive features of AD far out-weigh disadvantages in the majority of cases studied. Anaerobic digestion:

- a. Provides process stability, which is the capacity to achieve pollutant reduction under varying environments such as high-strength effluent and short hydraulic retention times.
- b. Reduces waste biomass disposal costs by significantly lessening the disposal costs involved with excess biomass synthesis.
- c. Reduces nitrogen and phosphorus supplementation costs.
- d. Reduces installation space requirements.
- e. Conserves energy and thus ensuring ecological and economical benefits.
- f. Eliminates off-gas air pollution since many organic contaminants are volatile and tend to be air stripped from the wastewater during aerobic treatment before they are biodegraded, thus contributing to air pollution. This significant drawback is eliminated when anaerobic treatment is utilized.
- g. Provides seasonal treatment of different wastewaters that are normally produced within two to four months each year.

The disadvantages are:

- a. Long start up requirements for the development of biomass consortium.
- b. Low kinetic rates at low temperatures, results in high retention times.
- c. Insufficient methane generation from dilute wastewater to provide for heating to the 37°C optimal temperature.
- d. It results in formation of methane gas which is a greenhouse gas. Methane emissions resulting from decomposition of organic waste, traps over 21 times heat per molecule than carbon dioxide and also absorbs 270 times heat per molecule than carbon dioxide. Hence, global warming potential associated with the production of methane gas during AD is a disadvantage.

Anaerobic digestion is becoming a key method for both waste reduction and recovery of renewable fuel as well as providing environmental benefits by allowing waste disposal facilities to meet increasingly stringent regulations (Carrieri *et al.*, 1993). With more and more substrate found to be biodegradable in the absence of molecular oxygen, the list of substrate not accessible by anaerobic degradation is shrinking (Schink, 1998).

There are numerous reasons accounting for the recalcitrance of organic compounds in anaerobic environments. Intrinsic limitations, can be due to the lack of cell uptake mechanisms, lack of proper enzymes initiating attack, lack of functionality, unfavourable thermodynamic reaction, or steric hindrance (Field, 2001). Bioavailability limitations can result from poor uptake of non-hydrolyzable polymers or from the slow dissolution of highly

apolar pollutants. Non-hydrolyzable polymers such as plastics, lignin and humic compounds are sources of pollutants that are resistant to biodegradation.

2.1.2 Anaerobic Digestion Process

This is one of the oldest means of wastewater treatment and refers to a biological engineering process by which a complex feedstock is converted into a range of simpler compounds. Anaerobic treatment of complex organic materials is a multi-phase process as outlined in **Figure 2.1** which involves hydrolysis, fermentation and methanogenesis stages. These phases are described in details below.

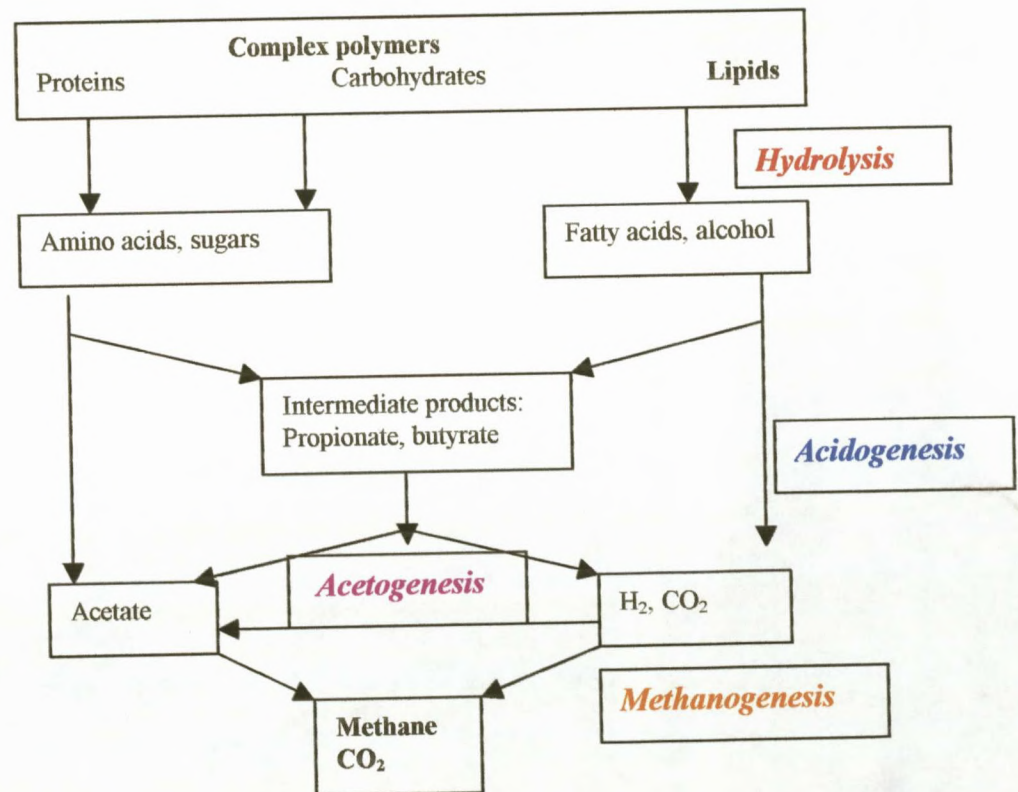


Figure 2.1: Anaerobic Digestion is a multi-phase process (Adapted from Brock and Madigan, 1991)

Anaerobic treatment does not require O_2 , the treatment rates are not limited by O_2 transfer and the non-requirements for O_2 also reduces the power requirements. In contrast, the methane gas produced is a good source of fuel energy (Brock and Madigan, 1991).

During hydrolysis long-chained complex macromolecules such as proteins, lipids and carbohydrates are hydrolyzed to short-chain compounds. These large molecules and suspended solids can not be directly metabolised by anaerobes, thus, proteins are degraded via peptides to amino acids, carbohydrates are transformed into soluble sugars (mono and disaccharides) and lipids are converted to long chain fatty acids and glycerine. Hydrolysis can be a slow process and can be a rate limiting step in the fermentation process if the influent contains large complex molecules in high quantities (Brock and Madigan, 1991).

Dissolved compounds generated during hydrolysis are called monomers and are taken up in the cells of fermentative bacteria. This process whereby monomers are broken down to carboxylic acids (propionic and butyric acids) is called acidogenesis. After acidogenesis, the carboxylic acids are excreted as simple compounds such as volatile fatty acids (VFA), lactic acids, carbon dioxide, hydrogen and hydrogen sulphide gas (H_2S). This step is carried out by a diverse group of bacteria, most of which are obligate anaerobes. Horan (1996) mentioned the importance of this step since dissolved oxygen might become toxic to obligate anaerobes such as the methanogens.

Propionate is a common intermediate in the metabolism of sugars, proteins and complex organics. Propionate is normally converted to acetate and hydrogen, and this conversion is

only thermodynamically possible if H_2 pressure is below 100 ppm. Low concentrations of propionate are indicative of a well functioning anaerobic process. Elevated concentrations of propionic acids can have an inhibitory effect on methane production (Heinrichs *et al.*, 1990). The inhibitory effect can be harmful to the organisms producing the VFA as well as to the organisms responsible for VFA decomposition. However, the bacteria may acclimatize to the inhibitory effect of un-ionized propionic acid so that normal metabolism can resume (Speece, 1996).

The products of acidogenesis are converted into acetate, hydrogen, carbon dioxide and this process is called acetogenesis. In a mixture of different organic pollutants, it is possible for both processes to occur simultaneously. Some industrial wastewaters such as heat-treated biomass effluents have acetate as a primary organic pollutant. With complex organic pollutants such as carbohydrates, a major fraction of the methane results from acetate as the direct precursor. Since acetate is a major precursor, a well functioning anaerobic system must process acetate efficiently and remove it to low concentrations in the effluent (Speece, 1996).

Hydrogen is an important intermediate because it affects the substrate conversion potential of many major anaerobic bacteria groups. It is also an important intermediate in the metabolism of some substrates such as alcohols, carbohydrates, propionate and butyrate in anaerobic systems.

The last stage, methanogenesis is the stage whereby methane gas is produced from acetate or from the reduction of carbon dioxide and hydrogen and this is accomplished by acetotrophic

and hydrotrophic bacteria, respectively. This stage is often the rate-limiting step in the overall digestion process. Methane formed in this last stage is poorly soluble in water hence moves to the gaseous phase. It is slightly explosive, and therefore, has to be handled appropriately, e.g. it can be collected and used for its energy value (Sacks, 1997). The end result of the process is a well-stabilized sludge in which 40 to 60% of the volatile solids have been destroyed (Sacks, 1997).

In aerobic respiration, molecular oxygen serves as an external electron acceptor from carriers such as NADH by way of a transport chain. In the absence of O_2 , a number of other electron acceptors can be used in which case the process is called anaerobic respiration. When organic materials are anaerobically metabolized by bacteria, the final steps involve re-oxidation of the reduced nucleotides $NADH^+$ and $NADPH^+$. Because exogenous electron acceptors are not available, ones such as pyruvic acid must be used. The end products of these oxidation-reduction reactions are the oxidized nucleotides and low-molecular weight alcohols and organic acids (e.g. acetic, butyric, propionic and formic). Facultative bacteria, those that can function under aerobic and anaerobic conditions, are the predominant species in both aerobic and anaerobic wastewater treatment processes and under anaerobic conditions are the volatile acids.

A group of obligate bacteria, the methane bacteria, are able to use the volatile acids and simple alcohols as either energy or carbon source and produce CH_4 as an end product (Schroeder, 1977). Although this group is widespread in nature, there is little known about the metabolism of the methane bacteria. The microbial interactions in the biodegradation of

organic compounds is vital as most microorganisms have evolved the metabolic capacities of biodegrading most, if not all, naturally occurring organic substances. Some compounds such as cellulose are particularly resistant to microbial attack because of their limited water solubilities and complex chemical structures, only a limited diversity of microorganisms typically produce enzymes that attack such substances. Other simpler and water-soluble compounds, such as glucose and proteins are readily metabolised by numerous microorganisms, including many species of bacteria and fungi.

Organic compounds that flow into waste treatment facilities and pollutants that enter the environment most commonly occur within mixtures. The initial steps in the metabolism of the various compounds in such mixtures are performed by diverse microorganisms, with the growth and metabolism of each type of microorganisms being supported by one another. Even most synthetic compounds are sufficiently similar to naturally occurring compounds to be subjected to microbial metabolism. Although most organic compounds can be degraded, many are degraded only slowly so that they pass through waste treatment facilities without being biodegraded (van Haandel and Lettinga, 1994).

There are many interactions in microbial populations that influence the fates of compounds in the environment and within the bioreactors of waste treatment facilities. Microorganisms rarely exist in isolation, rather, numerous microbial population of different types coexist. Often there is an interactive association between microorganisms, called consortium that results in combined metabolic activities. These microorganisms interact within a community. A variety of positive and negative population interactions lead to a stable functional

community. The microbial community is structured so that each population contributes to its maintenance. Some microbial populations within the community adversely influence others and can even lead to their exclusion from the community. Other populations' interactions are beneficial so that multiple microbial populations can live together at a particular location.

One common type of population interaction, called commensal relationship, occurs when one population benefits and the other remains unaffected. Co-metabolism, in which an organism - growing on a particular substrate gratuitously oxidises a second substrate that it is unable to utilize as nutrient and energy source, is the basis for various commensal relationships. According to the strict definition of co-metabolism, the second substrate is not assimilated by the primary organism but the oxidation products are available for use by other microbial populations. The activities of one microbial population can also make a compound available to another population without actually transforming the particular compound. For example, acids produced by one microbial population can release compounds that are bound or inaccessible to the second population. Another basis for commensalisms between populations is the production of growth factors. Some microbial populations produce and excrete growth factors, such as vitamins and amino acids, which can be utilised by other microbial populations. Yet another basis for commensal relationship is the removal of a toxic material.

Other types of population interactions include synergism and consortia etc. Many diverse populations of microorganisms are involved in the biodegradation of organic compounds in wastewater treatment facilities and environment. The most resistant compounds to biodegrade

are xenobiotic and those with unusual chemical substitutions and have limited water solubilities. Even many of these complex compounds are subject to biodegradation, at slower rates and by only a limited number of microorganisms.

2.1.3 Factors Affecting Anaerobic Processes

Anaerobic digestion process is affected significantly by the operating conditions (Rajeshwari *et al.*, 1999) which are discussed below:

(a) Temperature

Anaerobic process is more sensitive to temperature variation than its aerobic counterpart (Rajeshwari *et al.*, 1999). Grommen and Verstraete (2002) stated that AD performs poorly at low temperatures and therefore needs to be linked to low-value, heat-recovery processes. Changes in temperature are sometimes resisted by anaerobes as long as they do not exceed the upper limit as defined by the temperature at which the decay rate begins to exceed the growth rate. Any form of heating increases the activity of the anaerobic bacteria, thus reducing the required digestion time (Malina and Pohland, 1992). The effect of temperature on the first stage of the digestion process (hydrolysis and acidogenesis) is not very significant, since among the mixed population there are always some bacteria which have their optimum within the range concerned (Rajeshwari *et al.*, 1999). The second and third stages of decomposition can only be performed by certain specialized microorganisms (acetogenic and methanogenic) and thus, these are more sensitive towards temperature changes (Rajeshwari *et al.*, 1999).

(b) pH

Rajeshwari *et al.* (1999) reported the fact that anaerobic reactions are highly pH dependent and restoring an upset anaerobic process to stable operation is sometimes accomplished by stopping the feed and raising the pH (Anderson and Yang, 1992). Adjustment of the pH is accomplished by adding a base such as lime or ammonia, but pH correction may result in problems if considerable care is not taken (Wang and Banks, 2002). In general, sodium bicarbonate is used for supplementing the alkalinity since it is the only chemical which shifts the equilibrium to the desired value without disturbing the physical and chemical balance of the fragile microbial population (Rajeshwari *et al.*, 1999)

(c) Nutrients

Other environmental factors affecting the rates of methanogenesis include ions that affect the granulation process and the stability of reactors (Rajeshwari *et al.*, 1999). The bacteria responsible for waste conversion and stabilization in the AD process requires micronutrients and trace elements such as nitrogen, phosphorus, sulphur, potassium, calcium, magnesium, iron, nickel, cobalt, zinc, manganese and copper for optimum growth (Rajeshwari *et al.*, 1999). Although these elements are needed in extremely low concentrations, the lack of these nutrients has an adverse effect upon microbial growth and performance. Rajeshwari *et al.* (1999) further stated that methane-forming bacteria have a relatively high internal concentration of iron, nickel and cobalt.

In addition to the fundamental requirements for macronutrients, such as nitrogen, the inability of many anaerobes to synthesize some essential vitamins or amino acids often necessitates supplementation of the culture medium with specific nutrients for growth and metabolism (Malina and Pohland, 1992).

(d) Toxicity

Other factors that can inhibit methanogenic process can be due to a variety of circumstances. This includes heavy metals toxicity which have been often implicated as a cause for failure of anaerobic microbial conversion processes (Rajeshwari *et al.*, 1999).

(e) Organic loading rate

In the case of non-attached biomass reactors, where the hydraulic retention time is long and overloading results in biomass washout. This in turn leads to process failure. Fixed film bed reactors can withstand higher organic loading rate. Even if there is a shockload resulting in failure, the system is rapidly restored to normal (Rajeshwari *et al.*, 1999).

2.1.4 Limits of Anaerobic Biodegradation

Not all compounds are biodegradable, many xenobiotic compounds, which are chemicals synthesised by man and have no molecular structures and chemical bond sequences that are recognized by existing degradative enzymes. Xenobiotic organic compound might be recalcitrant (totally resistant) to biodegradation for a number of reasons (ENBT 4001, 2003):

- a. Molecular structure (e.g. substitution with chlorine and other halogens).
- b. Failure of the compound to enter a cell owing to the absence of suitable permeases.
- c. Unavailability of the compound as a result of insolubility or adsorption may make it inaccessible to microbial action.
- d. Unavailability of the proper electron acceptor.
- e. Unfavourable environmental factors such as temperature, light, pH (Anderson and Yang, 1992), O₂, moisture or Redox potential.
- f. Compound toxicity can affect the biodegradation potential by microorganisms, which have numerous ways to detoxify chemicals.
- g. Low substrate concentration can also affect biodegradation of a compound by microorganisms. Organisms growing at very low substrate concentrations have a high affinity for substrate. Some microorganisms in the environment may not be able to assimilate and grow on organic substrates below a threshold concentration.

The anaerobic degradation of recalcitrant compounds have been the subject of considerable investigation, driven not only by waste management, but also more recently by relevance in

contaminated natural ecosystems (Malina and Pohland, 1992). A large number of compounds, previously regarded as extremely recalcitrant in anaerobic environment, have now shown to be degraded without oxygen. Perhaps, the greatest developments have been with the anaerobic biodegradation of unsubstituted hydrocarbons. Aromatic hydrocarbons like toluene, benzene and xylene are now known to be mineralized under anaerobic conditions under a wide variety of electron accepting conditions ranging from methanogenic, sulphate reducing, iron reducing, manganese reducing to denitrification (Field, 2001; Parkin and Owen, 1986). Polycyclic aromatic hydrocarbons (PAH) mineralization has also been observed under denitrifying, sulphate reducing and manganese reducing conditions.

Alkanes were also regarded as the most recalcitrant family of compounds for anaerobic degradation. However, in the last 10 years, a body of evidence has accumulated indicating that even these saturated hydrocarbons are degraded under denitrifying, sulphate reducing and even methanogenic conditions (Field, 2001).

New evidence for the anaerobic degradation of lower chlorinated hydrocarbons has been found with vinyl chloride. It was found to be degraded by halospiring bacterium and oxidized by acetogenic bacteria in a methanogenic consortium.

While humus is extremely recalcitrant as a carbon and energy source in anaerobic environment; it is, however, not inert in anaerobic environment. Redox active substructures in humus such as quinines are able to function as a terminal electron acceptor compounds supporting the anoxic degradation of many substrates.

Seasonal wastes such as those from canneries are generally not suitable for anaerobic treatment because start up times are long, but where a food-processing industry operates throughout the year, anaerobic processes may be well suited. Wastewaters containing large quantities of soluble fats are difficult to treat by anaerobic processes. Fat breakdown by β oxidation produces large amounts of reduced pyrimidine nucleotides (NADH^+). Because of the difficulty in reoxidizing the nucleotides, fat breakdown is relatively slow under anaerobic conditions as evidenced by layers forming in unmixed digesters.

In determining whether or not an anaerobic treatment process is used in treating a particular waste, several factors related to conversion rates and the stoichiometry must be considered (Schroeder, 1977).

HIGH STRENGTH EFFLUENTS-SOUTH AFRICAN DISPOSAL OPTION

In general, high-strength industrial effluents are obnoxious and potentially more dangerous than domestic wastewater and are being discharged into sewers.. However, some industrial wastes are not really amenable and may be toxic to normal sewage treatment processes and thus should never be allowed into a sewer (Water Quality Management Series, 1997).

Marine disposal of wastewater relies on the powerful dispersing capability of the sea (Gunaseelan, 1997), its ability to degrade many organic wastes and the binding ability of the sea sediments. Marine disposal has proved to be an inexpensive disposal option and has been widely used in South Africa (DWAF, 1994). DWAF (1994) sets standards for the discharge of wastewater into marine environment.

Gunnerson (1996) stated that the toxicity of a substance depends on its concentration in the environment, its availability to an organism, and its susceptibility of the organism. Persistent substances are most likely to accumulate to toxic levels. No environmental protection entity wants to see marine organisms killed with toxicants; neither does it want to see them sick. The problem is that sub lethal or chronic effects are not revealed by the bioassay tests in which organisms are subjected to a range of concentrations of known or suspected pollutants.

Gunnerson (1996) further mentioned that waste discharged into the marine environment undergoes various biochemical transformations, such as decomposition of organic matter,

alteration of the physical or chemical form of the constituents, adsorption onto particles and sediments, and incorporation into living matter. Where mixing and circulation are limited and where added nutrients and organics increase the biological activity, the oxygen content of the water is decreased.

2.2.1 Anaerobic Digested Sludge

Anaerobic digestion of sludge is another technology with the objective of transformation of wastewater sludge to innocuous and easily dewatered substance. It is a process typically employed at many wastewater treatment plants to treat or stabilize, the various primary and secondary sludges produced. Collins *et al.* (1998) stated that a significant potential exists to lower energy consumption and to reduce sludge production by incorporating anaerobic processes into wastewater treatment plants.

The anaerobic sludge digestion is not a recent development; it has been in operation for more than 100 years in other countries such as France (Malina and Pohland, 1992). In the process, a portion of the organic solids is microbiologically converted to CH₄ and CO₂ gases (Collins *et al.*, 1998). Destruction of pathogenic organisms is also accomplished during AD (Malina and Pohland, 1992). The final product is stable, innocuous sludge that can be used as a soil conditioner or fertilizer.

2.2.2 Landfill Leachate

Landfill leachate is a complex organic liquid formed primarily by percolation of precipitation water through the open landfill or through the cap of the completed site when rain falls on a landfill, sinks into the wastes, and picks up chemicals as it seeps downwards. The resulting leachate is a highly variable mixture of soluble organic, inorganic, bacteriological constituents and suspended solids in an aqueous medium (Senior, 1995) depending on the composition of the solid waste disposed in landfill, refuse moisture and the content as well as the age of the landfill (Mavinic, 2000). Since all organic material undergo partial or total microbial decomposition, all leachate contains intermediate products together with high concentrations of toxic organics, heavy metals and other xenobiotic material. The exact composition is variable and site-specific depending on the type and age of the materials that is land filled and the amount of precipitation.

At first, leachates are extremely high in organic and inorganic constituents such as volatile fatty acids, carbohydrates and heavy metals. As time progresses, constituents within the leachate may decline due to biological activities. If these liquid emissions are not correctly managed, they may pose a threat to the natural environment surrounding the landfill. Bredenhann (2000) mentioned that the emissions should be monitored to minimize the impact they have on the environment, natural resources and public health. Furthermore, minimizing the infiltration into sub-surface soil should control leachate and pipelines should be installed for the removal of leachate to collection and holding facilities. At present, leachate is managed by disposal rather than actual treatment.

One of the most important aspects concerning water control on landfills is the proportion of rainfall falling on a soil-covered landfill surface that runs off and enters surface water regime and the corresponding proportion that infiltrates the cover layers. Of the water that infiltrates, some will in general be evaporated or evapotranspired and some will continue to move into the refuse body, possibly to emerge at the base of the landfill as leachate. Pollution by leachate is most costly and difficult to remediate once it has occurred. If nothing is done to ameliorate the situation, the pollution may persist in the groundwater for decades even though the source of pollution has been removed (Senior, 1995). This of course, dictates the necessity for leachate treatment and disposal alternatives. Thus, the management of landfill leachate will be a significant concern for the years to come. Because of the high strength and labile nature of the leachate, rapid deoxygenation of the receiving watercourse can occur. Ammonia in leachates from older landfills is also a problem and will often cause deoxygenation and fish kills in the receiving waters (Senior, 1995).

2.2.3 Textile Size Effluent

The textile industry is characterized by using a large quantity of chemicals and huge quantities of water for its processes. Among the large number of chemicals needed by the textile industry to produce woven fabrics, the sizing agent is one of the most important. Sizing is the most critical steps in the preparation of warps for weaving and is mainly added to improve weaving by protecting warp yarns against abrasion during the weaving operation. Size, a kind of glue, acts as an abrasion-resistant yarn coating. Other chemicals used include acids, alkalis, oxidizing agent, dyestuff, surfactants and thickening agents.

The sizing agent would disturb the chemical processes tremendously by, for example, preventing the dyestuff molecules from migrating uniformly into the fibres. It is therefore essential to remove the sizing agent through a desizing operation, before dyeing, i.e. washing the size out. The washing liquor contains size and when this liquor is discharged, it contributes highly to the COD in the textile effluent. This effluent is particularly harmful to the environment.

Worldwide, the textile industry uses three types of sizing agents namely (i) polysaccharides including starch and modified starches, water-soluble cellulose, (ii) synthetic vinyl, such as polyvinyl alcohol (PVA), and (iii) protein-based sizes, such as those derived from gelatine and casein. Starch-based products are mostly used as sizing agents due to their low prices and they are not water-soluble by nature. During sizing, the starch eliminates the possibility of its recovery and also contributes to high biochemical oxygen demand (BOD). Other water-soluble sizing agents include carboxymethylcellulose (CMC), polyacrylic (PAC) and water-soluble starches (CMS) (Sacks and Buckley, 1999).

The annual consumption of sizing agents in countries like Egypt where the textile industry is the bedrock of the country's economy, is about 10, 000 tonnes, of which about 8, 000 tonnes are starch and the remainder synthetic vinyl and water-soluble cellulose derivatives (Manu and Chaudhari, 2002). The use of starch size results in low efficiency weaving and increases pollution when desizing effluent is discharged into a watercourse. None of these are toxic but the sizing agent represents 50 to 70% of the COD in textile effluent.

Regulators have begun to target the textile industry to clean up the wastewater that is being discharged from the textile mills. Increasingly strict environmental legislation has led to textile finishing industries being labelled as a high priority industry with respect to pollution (Sacks and Buckley, 1999). They are looking closely at toxicity due to high salts, non-destructible COD, and heavy metals. Several studies have been recently conducted to monitor the effect of salts and dyes that are present in textile wastewater on methanogenesis (Manu and Chaudhari, 2002). These tests are based on maximum methane production and the methanogenic activity (Manu and Chaudhari, 2002).

In South Africa, the feasibility of using tests based on maximum methane production for synthetic textile effluent produced by a mill in New Germany were determined. The mill is situated about 10 km from the Umbilo Sewage Purification Works. The size effluent is segregated at the mill, all the wastewater is mixed and the total effluent is disposed in the submarine pipeline (Sacks, 1997). Submarine pipelines are for the discharge of effluent to coastal waters.

As mentioned earlier, starch has been the traditional sizing material used in the textile industry. It is a major glucose containing carbohydrate polymer used as a energy source by many microbes. Starch is a branched polymer comprised of an α -1, 4 linked backbone and α -1,6 linked branches and is more readily degraded by microbes. Starch degrading enzymes, are ubiquitous in nature and they cleave α -1, 4 and α -1, 6 linkages of starch and related polymers to form a mixture of smaller saccarides ranging from glucose to maltodextrins with an average degree of polymerization of 10 glucose units (Ramesh *et al.*, 1991). The qualities, which give

starch its usefulness as a sizing agent, are its ability to form a pliable film and its ability to adhere and provide a good coating without excess penetration into the yarn (Sacks, 1997). Starch is a labile substrate and its addition to anaerobic digesters should enhance methane production.

Polyvinyl alcohol is a synthetic polymer resin and is produced by acid or alkaline hydrolysis of polyvinyl acetate. This is the most widely used synthetic size and several forms are available commercially. Polyvinyl alcohol contains acetate and acrylonitrile constituent groups to decrease rotting and decaying. The viscosity and hydrolysis of PVA are controlled in the manufacturing process and are important in the determining the end-product sizing characteristics (Sacks, 1997). Polyvinyl alcohol is an excellent textile warp size because of its strength, adhesion, flexibility and film-forming properties.

Waxes involve lubricants of solid nature. The chemical composition varies widely but is generally based on the long-chain hydrocarbon molecules or a derivative such as fatty acids (Sacks, 1997).

3.1 INTRODUCTION

As outlined in section 1.6 the bioassays that are employed in this project are effective techniques to determine optimum assay conditions for laboratory-scale batch assessments of organic effluent biodegradability and toxicity. The experimental part of this project has been divided into four phases:

- a. *Phase I – Screening of Landfill leachate.*
- b. *Phase II – Screening of Textile effluents.*
- c. *Phase III- Screening of Textile effluent 1-components.*
- d. *Phase IV- Co-digestion of a Selected Leachate (Shongweni) with Textile effluent 1.*

Phases I- IV include the utilization of two methods, i.e. ATA and BMP to evaluate the industrial wastewater and these methods are employed to give a preliminary estimate of the potential amount of COD, which can be biotransformed to CH₄, and a preliminary estimate of any toxicity inherent in wastewater.

3.2

EXPERIMENTAL SET-UP

Experiments were performed in 125 mL glass serum bottles sealed with butyl rubber septa and aluminium crimp seals. A 30% (v/v) inoculum was used for each serum bottle and the total working volume was 100 mL. The biomass was mixed with 30 mL of a defined nutrient medium (after equilibration of both to the assay temperature). A 40 mL sample of different concentrations of substrate was added to the assay bottles. The headspace was gassed with oxygen free nitrogen (OFN) at a flow rate of 0.5 mL/min for 5 min. The bottles were sealed with butyl rubber septa and aluminium crimp seals prior to incubation in a thermostated room at a constant temperature of 37°C (optimal temperature for mesophilic digestion.). After equilibration for 1 h, the headspace volumes were zeroed to ambient pressure with a glass syringe. The assay bottles were shaken manually once a day to facilitate contact between the microorganisms and the substrate. Anaerobic toxicity assay indicated the inherent toxicity of a substrate, which is determined by a decrease in metabolic rate, relative to a control. The test ran for duration of one week and the results obtained were used to guide the set-up of the biodegradability assay.

3.2.1 Reagents

The protocol for these assays (Annual Book of ASTM Standards, 1992a) was designed to assure that the degradation of the compound is not limited by factors such as nutrients, inoculum, pH, etc. Stock solutions were prepared and blended to make up a defined media to meet these requirements (Table 3.1). A stock solution containing resazurin A3, a Redox

indicator turns the media pink when O₂ is present ensuring an O₂ free medium.. A stock solution of macronutrients ensures that nitrogen, phosphorus and potassium are not limiting. A1 & A2 stock solution of micronutrients ensures appropriate trace metals are available in the final media. Previously, Owen *et al.*, 1979 showed that the micronutrients solution lacked a source for nickel, but studies showing the importance of nickel in methanogenic metabolism resulted to this media. Owen *et al.* (1979) also included a stock solution containing vitamins and cofactors which was also included in the defined medium, A5. Finally, sodium bicarbonate A6, was added to the media to provide a pH buffer to assure acidification of the substrate does not cause an inhibitory pH drop.

The stock solution for preparation of the medium is presented in Table 3.1 and the final medium method for preparation is described in Table 3.2.

Table 3.1: Composition of stock solutions for defined anaerobic nutrient medium

Stock Solution	Component	Concentration in Stock mg/L
A1	K_2HPO_4	65300
A2	$NaH_2PO_4 \cdot 2H_2O$	19500
A3	Resazurin	500
A4	Trace element solution	
	EDTA	500
	$FeCl_2 \cdot 4H_2O$	2000
	$MnCl_2 \cdot 4H_2O$	100
	$CoCl_2 \cdot 6H_2O$	190
	$ZnCl_2$	70
	$CuCl_2$	2
	$AlCl_3 \cdot 6H_2O$	10
	H_3BO_3	6
	Na_2MoO_4	36
	$NiCl_2 \cdot 6H_2O$	24
	1 mL HCl	
A5	Vitamin solution	
	Biotin	2
	p-aminobenzoate	5
	Pantothenate	5
	Folic Acid	2
	pyridoxine	10
	nicotinamide	5
	thiamine HCl	5
	riboflavin	5
	cyanobalamin	0.1
A6	NH_4HCO_3	440
	$NaHCO_3$	3730
A7	$Na_2S \cdot 9H_2O$	240200
A8	$MgCl_2 \cdot 4H_2O$	101000
	$CaCl_2 \cdot 2H_2O$	110 000

Table 3.2: Preparation of 1 L anaerobic nutrient medium

Stock	Volume added (mL)
A1	10
A2	10
A3	1
A6	add 0.44 g of NH_4HCO_3 and 3.73 g of $NaHCO_3$
A7	1
A5	10
A7	1
A8	1
Distilled water	966 and boil the mediums in a bottle with glass watch to prevent water loss.

A range of six concentrations for each of the three landfill leachate samples and three textile size effluents were prepared by diluting 20, 10, 4, 0.4, and 0.04 mL of effluent with distilled water to make up 40 mL altogether. No distilled water was added to a 40 mL effluent sample. These diluted samples were added to serum bottles containing defined medium and biomass. The control contained inoculum and defined medium for the BMP test and an inoculum, defined medium and sodium acetate-propionate solution for the ATA. For a sodium acetate-propionate solution a volume of 2 mL was added to give an approximate concentration of 75 mg acetate and 25.6 mg propionate per 100 mL working volume and this solution acted as a methanogenic precursor. All experiments were in triplicate as it is indicated in Appendix A-G and the standard deviation was determined for the replicates. Over the course of the assay, the serum bottles were periodically equilibrated to atmospheric pressure and the gas volume was collected and analysed to determine the methane and CO₂ content. The inoculum was an anaerobically digested sewage sludge collected from Umbilo Wastewater Treatment Plant. This sludge was chosen as it represents the sludge discharged from industries in close proximity to this treatment plant

3.2.2 Biomass and Anaerobically Digested Sludge

Inoculum or seed biomass, for all assays during this project was sampled from the primary anaerobic digesters at Umbilo Wastewater Treatment Plant. It comprised of 70% organic and 30% inorganic solids. Table 3.3 gives the characteristics of the sludge that was utilised during this project.

Table 3.3: Characteristics of Umbilo biomass.

Characteristics of Umbilo Anaerobically Digested Sewage Sludge (Biomass)	Value
Temperature	36 ⁰ C
Sludge pH	7.5
Sludge VFA	22.7 mg/L
Sludge TSS	31 mg/L

The biomass was stored, for a short period, at 4⁰C until utilized. The sludge activity was assessed before a high-strength or toxic organic effluent was fed into an anaerobic digester. The rate of gas production was indicative of the activity of the biomass.

3.3 ANALYTICAL PROCEDURES

Several analyses were performed for the ATA and the BMP tests. These include gas volumes, gas compositions, COD, total suspended solids (TSS) and volatile suspended solids(VSS).

3.3.1 Gas Volumes

Gas volume was measured with a graduated glass syringe fitted with a 22 gauge disposable needle. The sample syringe was flushed with Oxygen Free Nitrogen gas and lubricated with distilled water. It was then inserted through a rubber septum into the headspace. Readings

were taken at the incubation temperature by holding the syringe vertically. Volume determinations were made by allowing the syringe plunger to equilibrate between the bottle and atmospheric pressure. Readings were verified by drawing the plunger past the equilibrium point and releasing to ensure that the plunger returned to the original volume. To continue the assay, gas was re-injected into the bottles without contamination or loss. Alternatively, gas was released to prevent inadvertent gas leakages and gas overpressure effects. Gas was released when the difference between the internal and the atmospheric pressure was 0.5 atm, which is equivalent to approximately 12 mL under these experimental conditions.

3.3.2 Gas Chromatograph (GC) Calibration

Calibration curves were prepared by injecting increasing volumes of high purity methane or nitrogen with known volumes of carbon dioxide (Fedgas). Instead of measuring the volume with a syringe, which can be quite inaccurate, a new device was used for the calibration. The apparatus was designed in the Department of Chemical Engineering, Durban.

The accuracy of gas chromatograph in practice is limited by the accuracy of the calibration procedure. A static volumetric device was used for the GC calibration of gas mixture. The device was based on a novel principle in which a piston with embedded valve operated in a uniform cylinder. A shaft compensating mechanism ensured no change in the total interior volume during piston movement. The latter was measured by counting the steps of the stepper motor, which drove the piston, with a precision of 0.0075 mm. The top and bottom compartments were magnetically stirred. A druck pressure transducer and 2 platinum

resistance thermometers allowed for measurement of the required pressure, volume and temperature data to determine single invariant equipment constant. Computer programs developed allowed for the preparation and analysis of binary, ternary and multi-component gas mixtures. Because of its accuracy, reproducibility of results and ease to use, it is expected that the device will be widely adopted.

Carbon dioxide was used as the reference gas. Mixtures of nitrogen and carbon dioxide, and methane and carbon dioxide were injected into the GC, with an oven temperature of 40°C.

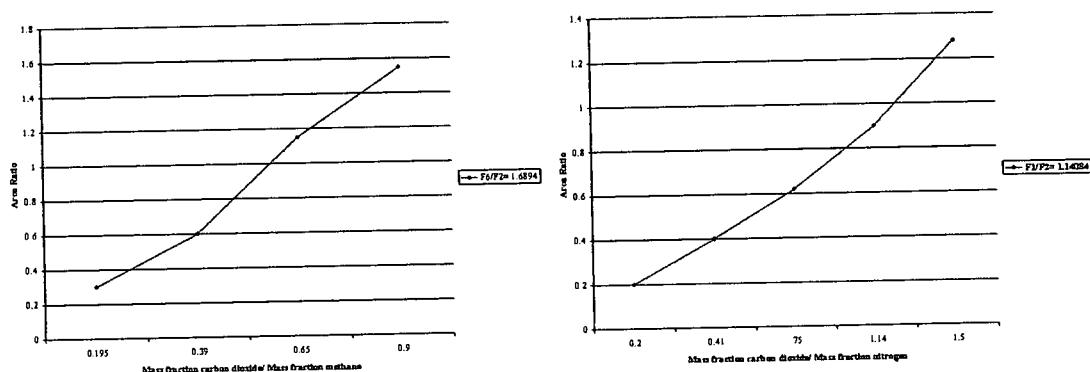


Figure 3.1: Gas chromatograph calibration curves

The calibration curves were used to calculate the mass of each gas in the biogas sample. The mass was converted to percentage (m/m).

3.3.2 Gas Composition

The composition of the gas was analyzed with GC, GowMac series 350 equipped with a thermal detector which can detect methane, carbon dioxide and nitrogen gases. The stainless steel column (Poropak N, 2m by 3 mm, 80-100 mesh) was used for the separation with the following conditions:

Conditions for Chrompak CP9000 and Conductivity Detector

Column Oven	:	40 °C
Detector	:	200 °C
Filaments	:	250 °C
Injection port	:	100 °C

The carrier gas was helium at a flow rate of 10 ml/min. The residence times of N₂, CH₄ and CO₂ were approximately 0.96, 1.54, 5.05 min, respectively.

Samples of biogas were drawn from each serum bottle (directly after the bottles had been equilibrated to atmospheric pressure) by inserting the needle of a gas-tight syringe (100 µL) precision syringe through the butyl rubber septum and withdrawing 100 µL of headspace gas.

The biogas composition was determined from the gas chromatograms. The method for calculation of percentage composition is detailed below. These calculations were formulated in a spread sheet.

1. The volumes of biogas produced were corrected to STP
2. biogas samples were analysed, by GC, for N₂, CH₄ and CO₂
3. The peak area for each biogas component was recorded.
4. CO₂ was used as the reference gas in the GC calibration. Area ratios were calculated with CO₂ as a common denominator i.e. N₂/CO₂ and CH₄/CO₂ (see Appendix A-G).
5. The area ratio was multiplied by the inverse of the fraction ratio for the Calibration curve i.e. area N₂/ area CO₂ x 1.14084 and area CH₄/area CO₂ X 1.16894
6. The resultant M value was used to calculate the mole fraction for each biogas component.
7. The proportion of each component were calculated on a mass basis since mass is conserved.
8. Fixed constants were set as follows:

Temperature	: 37 ⁰ C
pH ₂ O	: 47 mmHg (0.0618421 atm)
R (gas constant)	: 82.057 mL.atm/K
Head Space	: 25mL
Molecular masses	
Water vapour	: 18 g/mol
N ₂	: 28 g/mol
CH ₄	: 16 g/mol
CO ₂	: 44 g/mol

9. The total gas production was calculated by adding the headspace volume and the volume produced by the methanogens.
10. The atmospheric pressure, at the time of gas wastage, was recorded (atm)
11. The partial pressure of each gas component was calculated.
12. The number of moles of each component, in the biogas sample, was calculated from the equation $PV = nRT$
13. The mass of each gas was calculated (mg) from the equation
Mass = moles x molar mass.
14. The fraction of each component in the biogas was calculated as a percentage (m/m)

3.3.3 Chemical Oxygen Demand Measurements

Chemical Oxygen Demand measurement gives an accurate indication of the fraction of the waste effluent amenable to biodegradation (van Haandel and Lettinga, 1994). Filtered samples were placed in reflux tubes with 0.4 g $HgSO_4$, 2 mL standard potassium dichromate solution and 3 mL sulphuric acid reagent. The mixtures were refluxed in a COD reflux reactor at 150°C for 2 h (ASTM, 1992b). After cooling, samples were analysed at 610 nm wavelength on an Ultra Violet (UV-VIS) scanning spectrophotometer.

3.3.4 Solid Measurements

A method outlined in Standard Methods for Examination of Water and Wastewater (1995) was applied for determination of total and volatile suspended solids in anaerobically digested sewage sludge.

3.3.4.1 Total Suspended Solids Measurements

An evaporation dish is placed in a muffle furnace 550 ± 50 °C for 1 h. The dish is cooled in a desiccator, weighed and stored until used. A well mixed sample of 100 mL was transferred to the weighed dish and evaporated to dryness in a drying oven at 103 to 105°C. The dish was then cooled in a desiccator and re-weighed. The increase in weight represents the total residue. The following equation was used to calculate the total solids residue:

$$\text{Total suspended solids mg/L} = \frac{(A-B) \times 1000}{\text{Sample volume (mL)}} \quad \text{Equation 1.}$$

Sample volume (mL)

A = Weight of a sample (mg)

B = Weight of a dish (mg)

3.3.4.2 Volatile Suspended Solids Measurements

The dried residue from above was then ignited in a muffle furnace pre-heated to $550 \pm 50^{\circ}\text{C}$ for 1 h. The dish was partially cooled in air and then transferred to a desiccator for final cooling. The loss of weight on ignition represent the total volatile solids.

$$\text{Volatile suspended solids mg/L} = \frac{(A-B) \times 1000}{\text{Sample volume (mL)}} \quad \text{Equation 2.}$$

Sample volume (mL)

A = Weight of a dish + residues before ignition (mg)

B = Weight of dish + residue after ignition (mg)

3.4 ANAEROBIC TOXICITY ASSAY

The biomass to be evaluated was placed in a serum bottle into which the effluent sample was injected in increasing volumes into successive bottles. This procedure results in a range of dilutions of the wastewater with the initial inocula / biomass. Excess substrate was also added to the serum bottles to avoid substrate limitations. If there was toxicity in the wastewater sample, it would be reflected in a reduced initial rate of gas production in proportion to the volume of the wastewater added (Lu and Hegemann, 1998). The initial rate is the critical parameter in the ATA. Because, the acetoclastic methanogens are commonly the most sensitive to toxicity in the consortium, this characteristic can be assayed by adding a surplus of acetate. Some organic pollutants such as acrylic acids, chlorinated organics and lipids are

biodegradable but will manifest inhibition of the biomass above certain concentrations.

Figure 3.1 shows the ATA procedure at the start of the test.

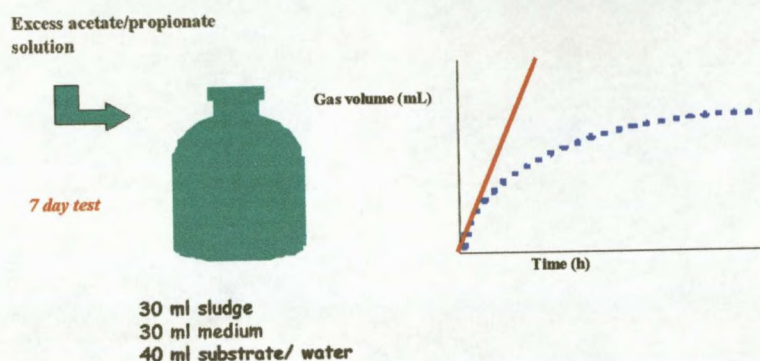


Figure 3.2: Anaerobic toxicity assay procedure (Naidoo, 2001)

Controls were included to show gas production rates when a readily biodegradable and non-toxic methanogenic substrate is fed to the biomass.

3.5 BIODEGRADABILITY TESTS

Biochemical methane potential tests were done for the three textile effluents and three leachate samples at volumes that were found to be non-toxic to the biomass. Gas volumes were produced from the consumption of the various effluent and leachate samples and the biogas composition was determined for all the test samples using procedures outlined in section 3.3.2.

The BMP is a procedure developed to determine the methane yield of an organic material during its anaerobic decomposition by a mixed microbial flora in a defined medium (ASTM,

1992a). This assay provides a simple means to monitor relative biodegradability of substrates. The procedures developed for the BMP are outlined in serum bottle techniques by Owen *et al.* (1979). The procedure involves placing an aliquot of the effluent sample, in a serum bottle with an anaerobic inoculum. The headspace is purged with OFN gas at a flow rate of 0.5mL/min for 5 min (Speece, 1996). The protocol for this assay (ASTM, 1992b) was designed to assure that the degradation of the compound is not limited by nutrients, inoculum, pH, substrate toxicity, oxygen toxicity or substrate overloading.

Biochemical methane potential has to be run for 30 days and this is regarded as a first-cut evaluation of the amount of organic pollutant in a wastewater, which is potentially converted to methane. An assay of short duration i.e. less than 30 days, can provide a valuable piece of data on the suitability of anaerobic treatment for a given wastewater (Speece, 1996).

The focus of this chapter was the assessment of the anaerobic treatability of high strength organic liquid effluents by the application of the strategies outlined in Sections 3.3, 3.4 and 3.5. For the purpose of this report, substrate volumes will be indicated as follows:-

A: 40 mL; B: 20 mL; C: 10 mL; D:4 mL; E: 0.4 mL and F: 0.04 mL.

The anaerobically digested sludge that was utilised during this project was obtained from the Umbilo Wastewater Works. A well mixed sample of 100mL was analysed according to section 3.3.4.1 and 3.3.4.2 and the results that were obtained are illustrated below in

Table 4.1.

Table 4.1: Total and volatile suspended solids (mg/L) for an anaerobically digested sewage sludge.

Replicate	Weight (g) of an empty dish	Weight of Dish plus a sample (g) after drying at 103-105°C	Weight of Dish plus a sample (g) after ignition at 550 ±50°C	TSS (mg/L)	VSS (mg/L)
1	53.81	54.11	53.89	30	22
2	55.22	55.54	55.31	32	23
3	57.17	57.48	57.25	31	23

The average TSS and VSS for the anaerobically digested sludge were found to be 31 mg/L and 22.7 mg/L respectively. This anaerobically digested sludge was then utilized for all the experimental tests conducted during this project.

The pH of the samples was determined at the beginning of the assays as well as at the end of the test to provide an indication of the efficiency of the process. pH analyses for all samples were found to be between 6-7 which is a suitable pH range for AD process.

4.1 HOLFONTEIN "HH" LANDFILL LEACHATE

Holfontein landfill site is in the eastern part of Gauteng on the border of Mpumalanga. It is presently the only legal toxic waste site (class HH) in Gauteng and deals with all the hazardous waste generated by industries around Gauteng. Enviroserve manages this site. Holfontein 'HH' landfill site became the first engineered high hazardous commercial waste disposal site designed and permitted according to DWAF's Minimum Requirements. Holfontein landfill site uses biodegradation to process the hazardous waste instead of chemicals. To do this Enviroserv co-dispose the hazardous waste with domestic waste and it is very interesting to note that some organisms can actually live in this toxic concoction. The domestic waste forms a substrate for anaerobic organisms to live on, and anaerobes slowly digest the organic matter in the domestic waste and nutrients from the toxic substances.

The leachate is carried away by herringbone drains laid on the sloping floor of the cell to leachate dams down slope of the cell. The leachate is then pumped back into the cells for further digestion.

This site contains a series of above ground cells, which are lined with layers of clay, and impervious sheeting and the latest cells on site are lined with polypropylene to prevent leachate from migrating into groundwater and contaminating the water resources.

Gas volumes obtained from ATA of Holfontein Leachate are given in Table 4.2. The amount of gas produced by experimental samples A, B and C, were lower than that of a control. This was also a definite concentration effect, which is evident as the volume of gas produced decreased with an increased in the amount of Holfontein leachate added. This is probably due to the high inherent inhibitory effect of Holfontein leachate on the anaerobic biomass.

Table 4.2: Average cumulative gas production (mL) for Holfontein "HH" landfill leachate

Time (days)	A	B	C	D	E	F	Control
0	0	0	0	0	0	0	0
1	0	0	0	9	14	15	13
2	0	0	6	39	38	34	37
3	0	0	18	67	55	59	49
4	0	4	29	81	63	68	55
5	0	6	40	91	68	75	59
6	0	8	50	97	73	81	63
7	1	10	66	105	79	87	66

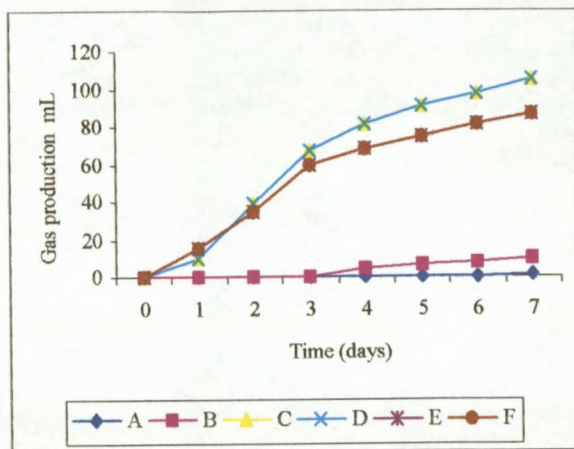
This inhibitory effect of the leachate also resulted in very low initial rates at the same volumes (Table 4.3). However, at lower volumes (D, E and F), the metabolic rates were higher than the control and this suggests that the substrate at these volumes were not inhibitory to

anaerobic microorganisms. In addition, it may also suggest that some of the substrate might have been degraded by the anaerobic biomass.

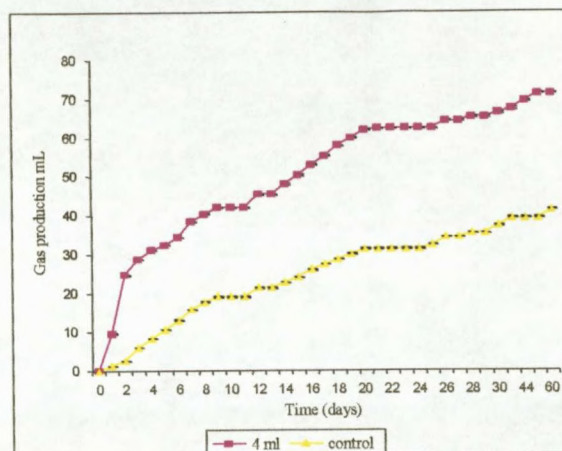
Table 4.3: Initial rates (mL/h) obtained for different volumes of Holfontein "HH" landfill leachate

Sample	Rate (mL/h)
0, Control	0.81
A	0.00
B	0.09
C	0.48
D	1.37
E	0.87
F	0.9

Figure 4.1b illustrates that when a $4 \text{ mL} \pm 0.27$ sample was subjected to BMP over a 60d period, an overall cumulative gas production of 32 mL was obtained. In Appendix A.2, 63% CH_4 was obtained from gas the composition analysis. The COD removal was calculated using Equation 3, to be 19%. Based on these results, it can be concluded that Holfontein Leachate is a highly complex recalcitrant effluent which cannot be easily biodegraded even at low concentrations. Figure 4.2 shows Holfontein effluent before it was subjected to BMP assay and afterwards. In this regard, AD had cleared the colour of the leachate.



(a) ATA



(b) BMP

Figure 4.1: Gas volumes (mL) produced from different volumes of Holfontein "HH" landfill leachate

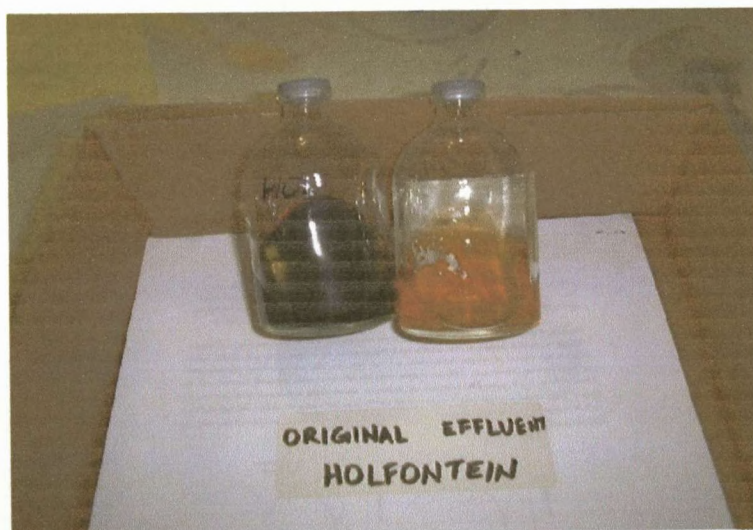


Figure 4.2: Holfontein 4 mL \pm 0.27 volume sample before and after Anaerobic Digestion

4.2

SHONGWENI "Hh" LANDFILL LEACHATE

Shongweni landfill is situated in KwaZulu-Natal and is one of the two hazardous sites that are currently accepting low hazardous (class "Hh") waste streams in the region. This site is unique in that it is currently in a very isolated position in terms of contact with industry and communities. Its large buffer zone as a waste disposal site is unique in a South African context. This site has a proven history in the waste management of low hazardous domestic and commercial waste. Co-disposal of liquid and sludge waste is occurring in a controlled manner into trenches excavated.

The quality of leachate has been reported to be typical of that produced by landfills with both methanogenic and acidophilic catabolism active microorganisms. Gas volumes obtained from ATA of Shongweni Leachate are given in Table 4.4.

Table 4.4: Average cumulative gas production (mL) for Shongweni "Hh" landfill leachate

Time (Days)	A	B	C	D	E	F	Control
0	0	0	0	0	0	0	0
1	2	8	10	13	13	13	13
2	15	32	36	44	45	35	37
3	29	60	65	72	69	46	50
4	51	81	84	88	87	54	56
5	67	91	91	95	95	59	60
6	84	96	95	99	96	63	64
7	98	100	100	104	104	68	67

From the results outlined in Table 4.4, all experimental samples had higher gas production than the control, which implies that Shongweni leachate is not toxic and can be biodegraded by anaerobic biomass. A 4 mL \pm 0.9 volume sample of Shongweni Leachate had the highest gas production of 104 mL in total and the highest initial rate of 1.25 mL/h. Also, a 10mL \pm 0.75 had a total of 100 mL gas being produced and an initial rate of 1.18 mL/h. The latter sample was selected for further testing using BMP assay.

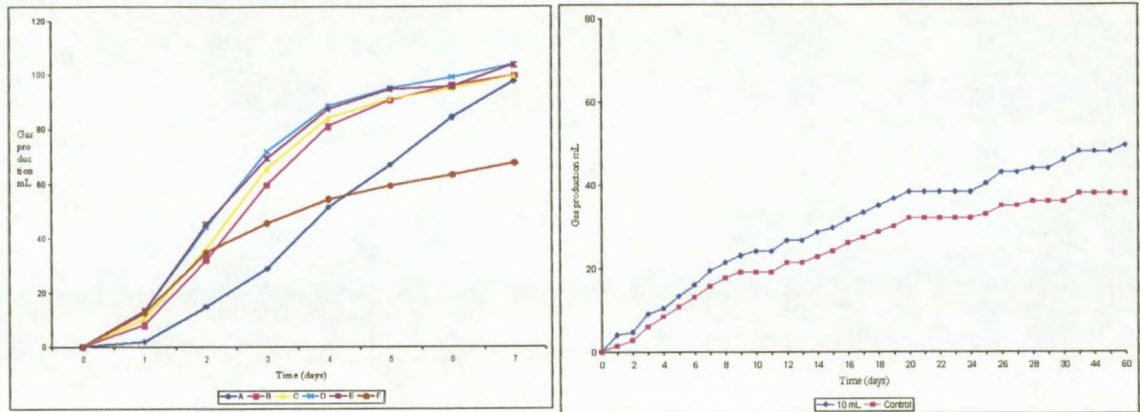
Table 4.5: Initial rates (mL/h) obtained for different volumes of Shongweni "Hh" landfill leachate

Sample	Rate (mL/h)
0, Control	0.81
A	0.57
B	1.1
C	1.18
D	1.25
E	1.21
F	0.42

In Figure 4.3a, C, D and E had the highest gas production and any of them could be used for the BMP assays, however, C was selected based on its high gas production as well as its high initial rate.

Figure 4.3(b) shows the biodegradability curve for Shongweni effluent. The total gas that was produced from the consumption of the leachate or effluent samples was calculated by the difference in total gas production between the control and the test samples. Shongweni BMP

test was conducted with effluent volume of $10 \text{ mL} \pm 0.32 \text{ (v/v)}$ and the gas that was produced from the consumption of this sample was found to be 9 mL in total.



(a) ATA

(b) BMP

Figure 4.3: Gas volumes (mL) produced from different samples of Shongweni “Hh” landfill leachate

Biogas composition was also analyzed as shown in **Appendix B.2** and about 50.2% CH_4 was detected. Since the percentage of methane composition and gas volumes of the experimental sample were known, the COD consumption was calculated based on Equation 3 and was found to be 59%. It was then concluded that Shongweni leachate is labile and not toxic and can be treated in anaerobic digesters.

4.3

ALOES "HH" LANDFILL LEACHATE

The Aloes tip site, located north of Port Elizabeth near municipal boundary, handles highly "HH" waste from the Eastern Cape province. This site is now operating under Enviroserv. This site, Aloes 1 is an unlined hazardous waste tip and is now closed and rehabilitated. The second tip Aloes 11 is currently operating. There have been several complaints from the residents around the Aloes site regarding strong smells from the leachate pond and some people even believe that leachate from the site is polluting the watercourses. This is of serious concern since the leachate from this site is very toxic and recalcitrant and if it finds its way to the nearby watercourses, it could have a detrimental impact on the water resource and the downstream users. This site produces high strength liquid effluent and was selected to try and see if anaerobic digestion will not treat the inherent toxicity of the effluent. Table 4.6 illustrates the average cumulative gas production (mL) for Aloes "HH" Landfill Leachate.

Table 4.6: Average cumulative gas production (mL) for Aloes "HH" landfill leachate

Time (Days)	A	B	C	D	E	F	Control
0	0	0	0	0	0	0	0
1	0	3	11	14	12	13	13
2	1	13	30	34	32	32	31
3	2	32	47	48	45	45	44
4	2	41	51	52	49	49	47
5	2	48	55	56	52	52	51
6	3	53	58	59	55	55	54
7	3	58	60	62	57	57	56

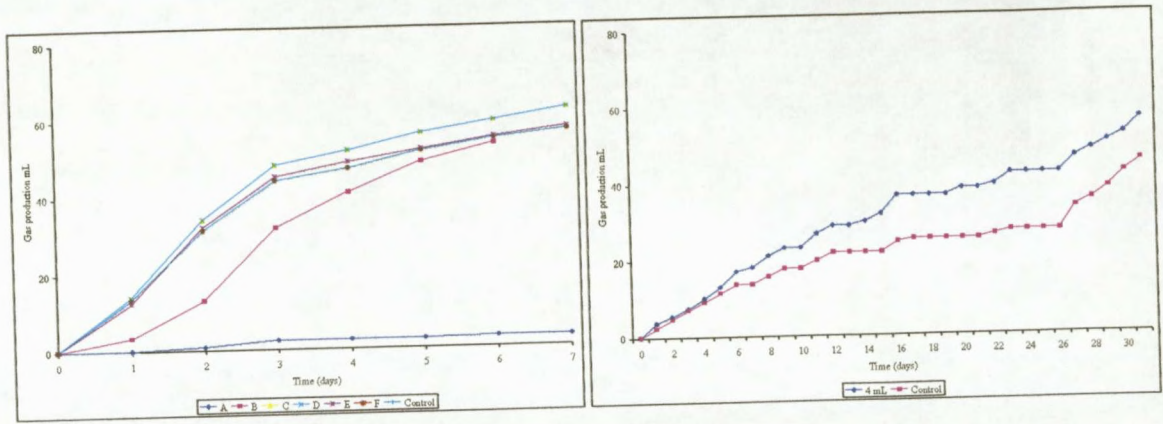
From the results outlined in Table 4.6, similar trends to those obtained for Holfontein leachate are seen. At A, approximately 3 mL cumulative gas was produced after seven days of incubation and this amount of gas production is below that of the control. For samples A-D, as the substrate concentration increased, the gas production decreased. This could be as a result of a high inherent toxicity effect of this type of leachate on anaerobic biomass and this effect was evident in the initial rates as shown in Table 4.7

Table 4.7: Initial rates (mL/h) obtained for different volumes of Aloes "HH" landfill leachate

Leachate added (mL)	Rate (mL/h)
0, Control	0.46
A	0.47
B	0.47
C	0.5
D	0.49
E	0.38
F	0.03

A 4 mL \pm 0.16 sample had the highest initial rate and also had the highest gas production and thus was used to further analyze for BMP. The results from BMP assay are shown below in Figure 4.4 (b).

The figure below further illustrates that from BMP assay, a total cumulative gas volume of 15 mL was produced and from Appendix C.2, the biogas had 55.8% CH₄ composition. Hence, a COD removal of 38% was calculated from Equation 3. It can then be concluded that Aloes leachate is not amenable to anaerobic digestion and can be classified as recalcitrant.



(a) ATA

(b) BMP

Figure 4.4: Gas volumes (mL) obtained from different volumes of Aloes "HH" landfill leachate

Figure 4.5 shows Aloes effluent before and after anaerobic biodegradation. An observation was made regarding clearance of the original dark colour during this process.

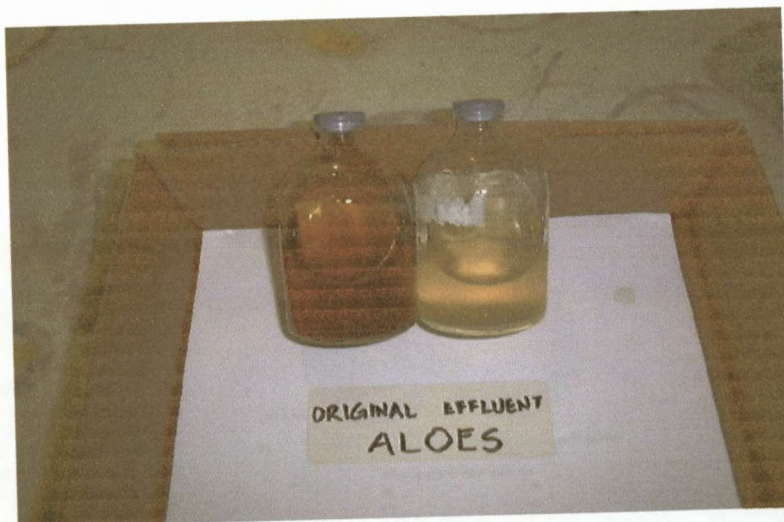


Figure 4.5: Aloes 4mL ± 0.16 volume sample before and after AD

All three textile effluents tested during this study, were synthesised in the laboratory according to three recipes submitted by Frametex Company, Durban. This company is one of the largest textile and textile related product manufacturers in Southern Africa with plants situated in KwaZulu-Natal and Western Cape. Frametex produces yarn, woven, and non-woven and knitted fabric. Frame fibres located in New Germany in Durban is the largest specialist producer of regenerated waste in Southern Africa. This industry was selected because it is situated in New Germany and can be easily accessible. For a size formula to approach perfection, it should have several basic characteristics, which will serve to eliminate warp breaks during weaving. The film forming part is an essential ingredient and the basic material for textile sizing (Cited in Naidoo, 2001). Starches and synthetic polymers such as PVA are very popular film formers. Historically, starches have been the film-formers of choice for textile sizing. From this study, it is clear that starch is labile and is easily biodegraded.

Textile effluent 1 was composed of starch (Kollotex) and wax, textile effluent 2 was composed of starch, PVA and wax and textile effluent 3 was made up of starch, wax and elvanol. Gas volumes obtained from ATA of Textile effluent 1 are given in Table 4.8.

Table 4.8: Average cumulative gas production (mL) for Textile Effluent 1

Time (Days)	A	B	C	D	E	Control
0	0	0	0	0	0	0
1	8	9	13	12	9	11
2	36	46	44	37	30	32
3	55	66	62	47	47	45
4	68	75	71	56	54	52
5	79	3	79	63	60	56
6	89	91	87	71	66	62
7	4	95	90	73	68	64

From the results outlined in Table 4.8, all concentrations of samples tested had higher gas production volumes than the control sample. This signifies that textile size effluent is very amenable to anaerobic digestion. The initial rates for all the experimental samples were also higher than the initial rate for the control (see Table 4.9).

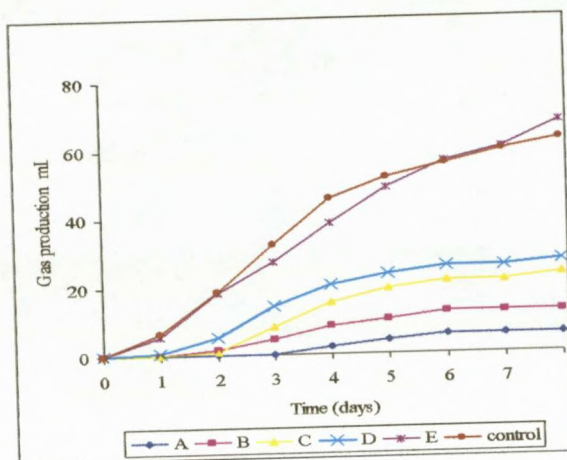
Table 4.9: Initial rates (mL/h) obtained for different volumes of Textile Effluent 1

Sample	Rate (mL/h)
0, Control	0.74
A	0.97
B	1.17
C	1.05
D	1.05
E	0.78

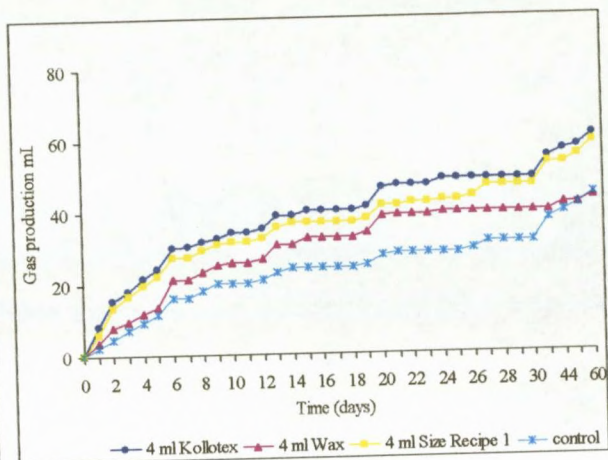
In addition to ATA, a BMP Assay was conducted for a $4 \text{ mL} \pm 0.26$ volume to determine the biodegradability of this effluent. A total gas production of 13 mL was obtained

(see Figure 4.6b) and 56% CH₄ was detected from the biogas composition (Appendix D.2).

Based on these results, COD removal of 68% was calculated.



(a) ATA



(b) BMP

Figure 4.6: Gas volumes (mL) obtained from different volumes of Textile Effluent 1

Textile effluent 1 is made up of a starch derivative, which is Kollotex. Starch is generally known to be highly biodegradable, however, the wax component of textile effluent 1 was very recalcitrant and hence when these two components were mixed together, a 68% COD removal was achievable. This brings us to the concept of co-digestion, which will be discussed later in this Chapter.

Table 4.10: Average cumulative gas production (mL) for Textile Effluent 2

Time (days)	A	B	C	D	E	Control
0	0	0	0	0	0	0
1	44	49	40	28	12	7
2	67	77	65	70	27	14
3	82	97	90	114	40	25
4	91	111	108	134	43	28
5	101	112	120	142	46	31
6	109	123	140	152	50	35
7	114	131	160	157	52	38

From the results outlined in Table 4.10, control sample had the lowest gas volume than any of the experimental samples. Furthermore, a definite concentration effect, was evident. As the volume of textile size increased, so did the gas production except for the 20 mL \pm 1.3 and 40 mL \pm 1.1. The initial rates for all the experimental samples were higher than the initial rate for the control as shown in Table 4.11.

Table 4.11: Initial rates (mL/h) obtained for different volumes of Textile Effluent 2

Sample	Rate (mL/h)
0, Control	0.36
A	1.2
B	1.72
C	1.46
D	1.90
E	0.6

What has been noticed as well is that the initial rate for the control decreased as compared to previous controls. This was also due to a different sample of anaerobic sludge being utilized for these sets of experiments. It is clear that the type of sludge used, does have a major impact on the experimental results. A $4 \text{ mL} \pm 0.18$ volume was selected to assess the biodegradability of the Textile effluent 2 based on both its production of gas and initial rate, which were high.

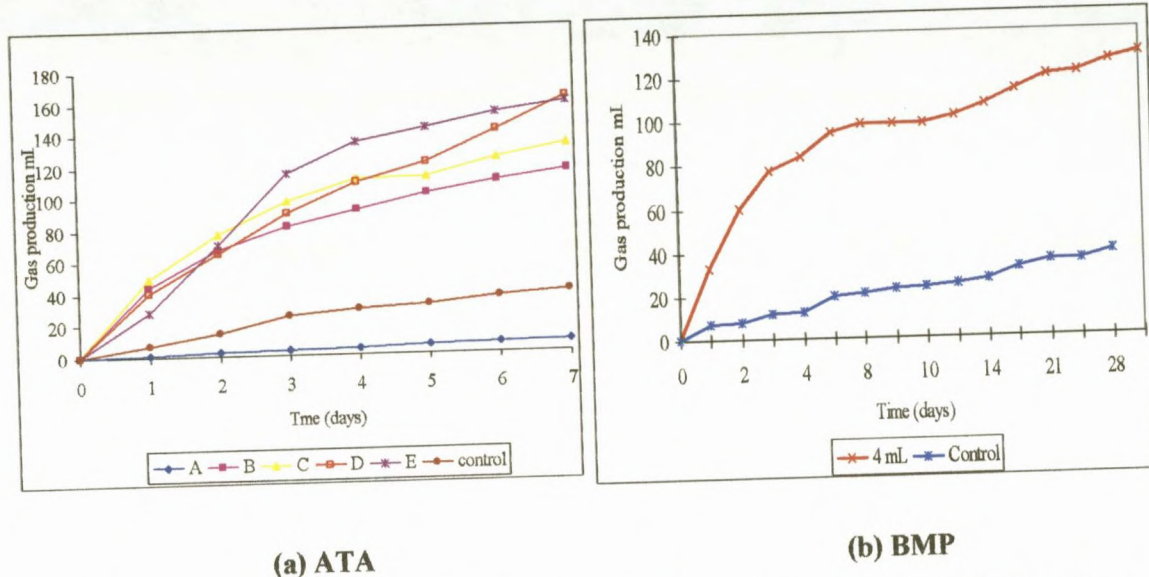


Figure 4.7: Gas volumes (mL) obtained from different volumes of Textile Effluent 2

The Biochemical Methane Potential Assay (**Figure 4.7 b**) was conducted for the $4 \text{ mL} \pm 0.32$ to determine the extent of biodegradability of this effluent. A total gas production of 100 mL was obtained and 70.4% CH_4 was calculated from biogas composition (**Appendix.E.2**). Textile effluent 2 is made up of starch, PVA and wax components. Polyvinly Alcohol and starch were highly biodegradable whereas wax was difficult to biodegrade. However, the combination of these three compounds made Textile effluent 2 fairly biodegradable.

Table 4.12 Average cumulative gas production (mL) for Textile Effluent 3

Time (days)	A	B	C	D	E	Control
0	0	0	0	0	0	0
1	43	24	26	27	14	14
2	89	66	68	69	37	31
3	143	110	118	122	57	49
4	165	121	136	136	64	53
5	178	128	144	144	66	56
7	187	133	150	144	68	58

From the results outlined in Table 4.12, the control had the lowest gas volume than any of the experimental samples. Furthermore, a definite concentration effect, which was evident. As the volume of textile size increased, so did the gas produced (Figure 4.8a). The initial rates for all the experimental samples were higher than the initial rate for the control as shown in Table 4.13.

The initial rate for the control has remained low as detected in the previous control sample in section 4.5. This was the same sample of anaerobic sludge being utilised for the previous set of experiments. It is once again clear that the type of sludge used does have a major impact on the experimental results.

Table 4.13: Initial rates (mL/h) obtained for different volumes of Textile Effluent 3

Sample	Rate (mL/h)
0, Control	0.4
A	1.17
B	0.83
C	0.89
D	0.92
E	0.48

From the initial rates data, a $4\text{mL} \pm 0.36$ volume was selected to assess the biodegradability of Textile effluent 3, based on its high production of gas and its initial rate. A total gas production of 140 mL was observed in **Figure 4. 8b** and 70% COD removal was calculated from **Appendix F.2**. This renders Textile effluent 3 as once again a suitable substrate for AD.

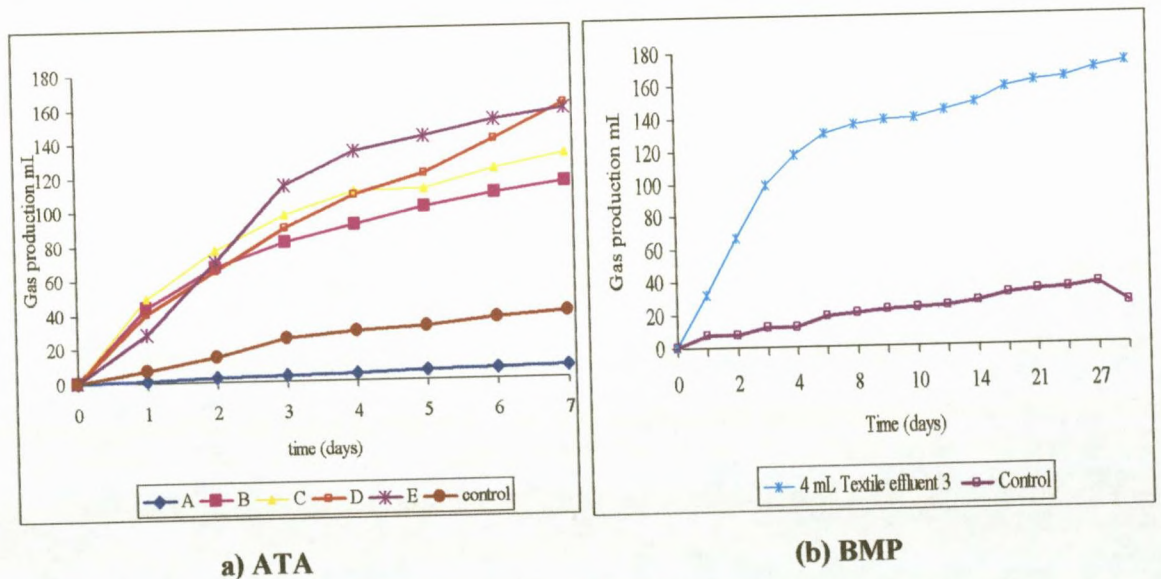


Figure 4. 8: Gas volumes (mL) obtained from different volumes of Textile Effluent 3

When looking at the constituents of Textile effluent 3, starch and elvanol are both highly biodegradable, whereas wax component is recalcitrant. It can be concluded that both starch

and ethanol made Textile effluent 3 very amenable. Once again, the concept of co-digestion had played a significant role.

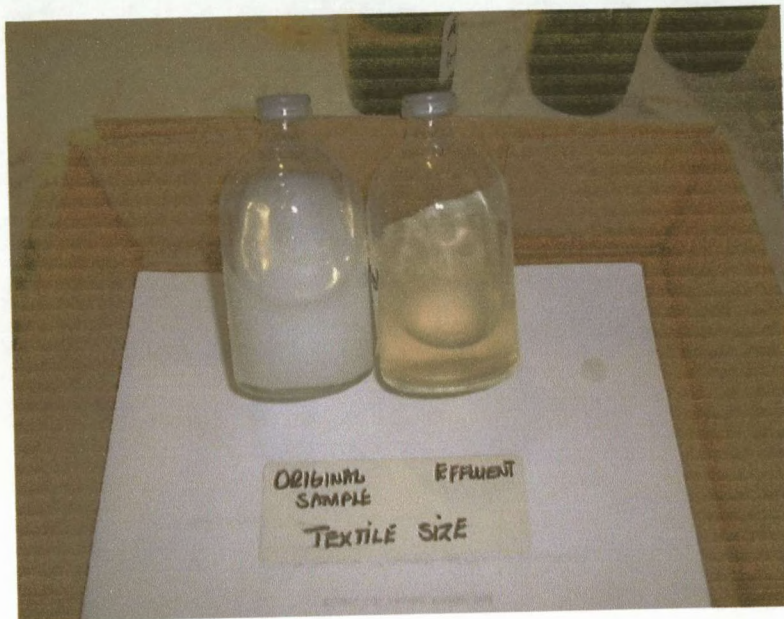


Figure 4.9: A sample of Textile Effluent before and after AD
Starch is common in all three types of textile effluent. In general, this is a labile substrate and its addition enhances methane production. It can be concluded that high volumes of biogas produced were due to the presence of starch derivatives.

Co-digestion is defined as transformation of a recalcitrant substrate in the obligate presence of a readily biodegradable labile substrate. In a dual substrate system, co-metabolism is defined as transformation of a non-growth substrate by growing organisms in the presence of a primary substrate or by resting microorganisms in the absence of a growth substrate (Criddle, 1993).

The addition of a labile substrate can at times increase the capacity of an anaerobic digester as the labile substrate overcomes certain inhibited pathways (Speece, 1983). Thus co-metabolism can be a vital mechanism since no organism could utilize potentially recalcitrant molecules as a sole source of carbon and energy and all require a co-substrate for growth.

In this study, Textile effluent 1 and Shongweni leachate were chosen as co-digestion substrates. Preliminary toxicity and biodegradability assays of the co-digestion mixture were conducted with a mixture of Textile effluent 1 and Shongweni leachate at different volumes [4, 10, 20 and 40 mL (v/v)] and with varying ratios of Textile effluent 1 to Shongweni leachate (0.5:1, 1:1, 2:1 and 3:1). A control was also set up for each assay. Results obtained are illustrated below in Figure 4.10. For the purpose of this report, substrate volumes will be indicated as follows:-

A: 4 mL; B: 10 mL; C: 20 mL and D:40 mL.

(a)

ATA

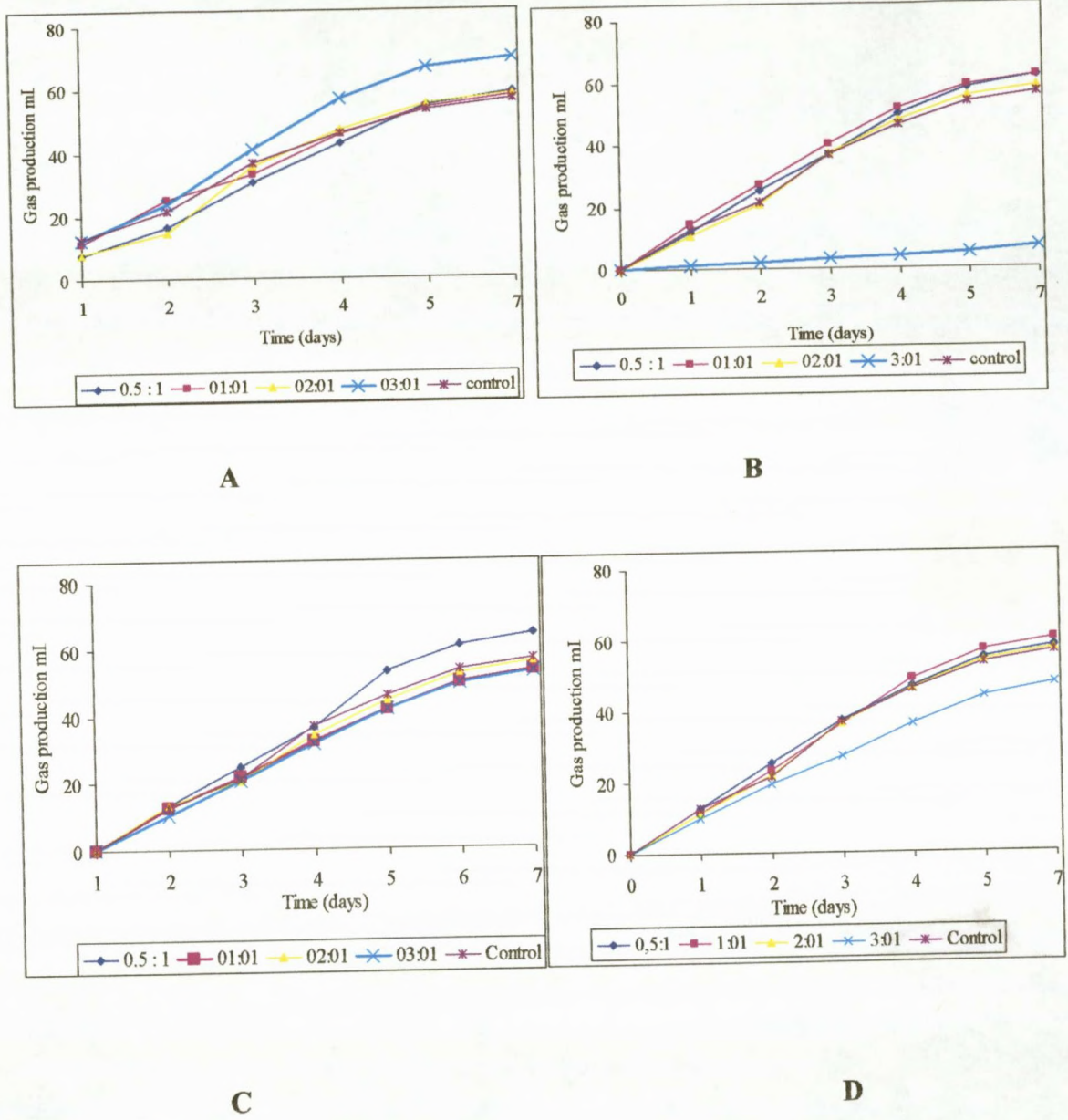


Figure 4.10 Gas volumes (mL) produced from different volumes of co-digested effluent

Table 4.14 compare initial gas production rates for the various ratios of mixed sample at different dilutions.

Table 4.14 Initial rates (mL/h) obtained from different volumes of a co-digestion effluent

Ratio	Sample			
	D	C	B	A
0.5:1	0.53	0.51	0.53	0.36
1:1	0.51	0.47	0.53	0.54
2:1	0.47	0.46	0.52	0.31
3:1	0.38	0.43	0.51	0.57
Control	0.46	0.46	0.46	0.46

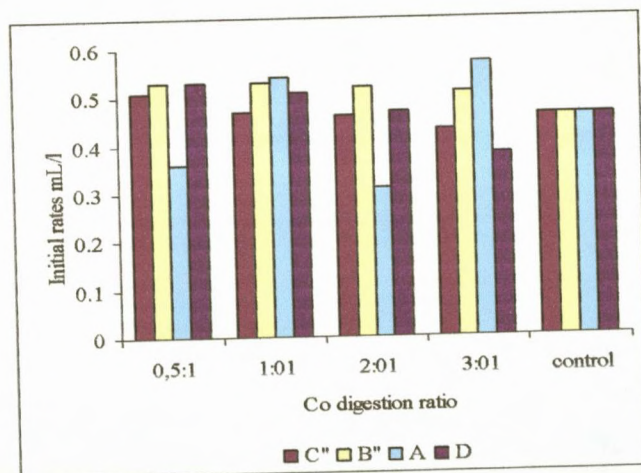


Figure 4.11 Initial rates (mL/h) obtained from different volumes of a co-digestion effluent

A comparison was made between results reported in **Figure 4.11** and those tabulated in **Table 4.5** and **Table 4.8**. A and B for Shongweni leachate (**Table 4.5**) and a 4mL sample for Textile Effluent 1 (**Table 4.9**) were found to be toxic to anaerobic bacteria whereas no toxic effect was evident at different dilutions and different ratios of the co-digestion mixture used.

From the results above, co-digestion mixture had lower rates and this could be attributed to the fact that more time was required for the biomass to acclimatize to the complex substrate. Thus, co-digestion of industrial effluents may not result in higher gas production rates but may reduce the effect of toxic loads within anaerobic bioreactors.

This screening method estimated the potential for anaerobic degradation and also facilitated identification of a concentration effect by comparison of the rates of metabolic activity with different dilutions. The above ATA results further prove the amenability of the co-digested effluent to anaerobic digestion. It further shows that co-digestion was possible at all the sample dilutions tested, i.e. A, B, C, and D.

(b) BMP

Since ATA illustrated that no toxicity effect was evident on all the various ratios tested, further screening for biodegradability was then conducted. It is believed that biodegradability of an effluent may be enhanced if a labile waste, e.g. Textile Effluent 1 is co-digested with a recalcitrant waste, e.g. hazardous landfill leachate. Although, Shongweni leachate was found to not be recalcitrant (Section 4. 2), it was chosen because Shongweni landfill site is situated in KwaZulu-Natal and samples were easily accessible for laboratory and pilot case studies. Below are the results obtained from BMP assay.

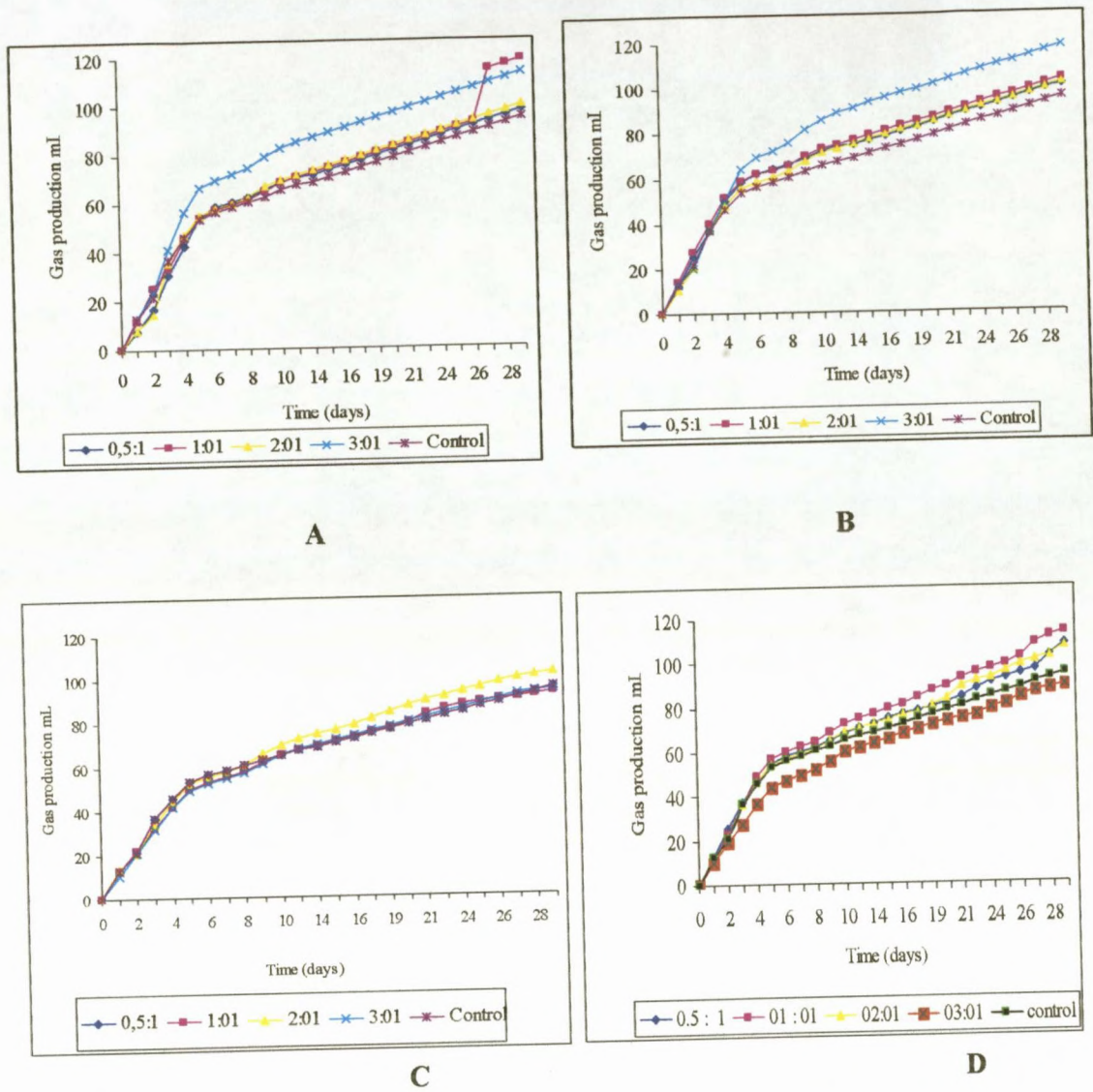


Figure 4.12 Gas volumes (mL) produced from different volumes tested for Textile Effluent 1: Shongweni leachate at different ratios- (a) 4mL, (b) 10mL, (c) 20mL and (d) 40 mL

Similar trends of gas production in all ratios and various dilutions were observed. High volumes of biogas were obtained (**Appendix G.3**) and the standard deviation was calculated

to be 0.36 mL, which suggested similarity between the duplicates. The biogas composition (Table 4.15) showed that samples contained about 59 to 70 % of CH₄.

Table 4.15 Methane percentage for co-digestion effluent at different ratios

Ratio	Sample			
	D	C	B	A
0.5:1	66	68	68	65
1:1	67	66	70	69
2:1	67	69	69	66
3:1	59	68	64	67
Control	74	74	74	74

The co-digested effluent was found to be biodegradable and it is highly likely that the biodegradability of this mixture may increase once the biomass becomes acclimatized to the effluent. Once again, the ATA and BMP showed that the co-digestion of the two effluents used was not toxic to sludge biomass. Further investigations involving co-metabolism of labile and recalcitrant industrial effluents in laboratory testing, is recommended for future studies.

4.8 DISCUSSION

Most of the BMP tests were conducted with effluent volumes that were less than 10 mL of the total working volume. Table 4.16 shows the gas that was produced from the consumption of the various effluent and leachate samples. The biogas composition was determined for all the tests and the result of CH₄ percentages are shown in Table 4.16 below. Most of the biogas samples contained about 50% to 75% CH₄. Since the methane

composition and gas volumes of the tests were known, the COD consumption was calculated based on Equation 3.

Table 4.16: COD consumption obtained from BMP assay

Sample	Sample Volume (mL)	Gas Volume (mL)*	CH ₄ (%)	COD Consumed (g/l)	Total COD (g/l)
Holfontein leachate	4	71.33	55	12.8	68
Control		41.33			
Shongweni leachate	10	49.50	50.2	1.5	2.8
Control		38			
Aloes leachate	4	52	56	5.8	18.3
Control		42			
Textile effluent 1	4	60.33	63	5.2	7.7
Control		43.33			
Textile effluent 2	4	129.33	70.4	19.6	80.5
Control		41.33			
Textile effluent 3	4	171.33	72.8	62.25	193
Control		45.67			

* gas volume is determined by the difference between total gas production of the control and tests containing the samples

$$\text{COD (g/L)} = [(V_{gc} - V_{gc}) \times \% \text{CH}_4 / (V_s \times 395)] \quad \text{Equation 3}$$

Where, V_{ge} is the volume of gas produced for tests containing effluent samples (mL)

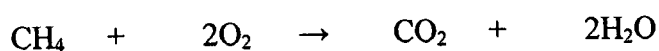
V_{gc} is the volume of gas produced in the control (mL)

V_s is the volume of sample added to the test bottles (L)

CH₄ is the methane composition (%)

395 is the ml CH₄ per g COD at 35°C.

From the above equation, COD equivalence of methane is determined as follows:



From the above equation it may be determined that for each mole of methane consumed (22.4L at 0 °C), two moles of oxygen equivalent are destroyed (64g). Thus, 0.35 L(22.4L/64g) of CH₄ at 0 °C and 760 mmHg pressure (STP) is equivalent to 1 g COD destruction. To compensate for a higher temperature, the COD equivalence is 0.395L at 35 °C and one atmosphere.

Textile effluents 1, 2 and 3 were found to be the most biodegradable and it is likely that the biodegradability of these high strength organic effluents may increase once the biomass becomes acclimatized to the effluent. Shongweni effluent was also fairly biodegradable. The results show that 54 % of Shongweni effluent was amenable to anaerobic treatment in the 60 d BMP test. While Aloes and Holfontein leachate were the least biodegradable with only 32 % and 19 % respectively of the total COD being consumed during the 60d test.

Once again, the anaerobic toxicity assays showed that textile effluent was the least toxic to sludge biomass, while the Holfontein effluent was the most toxic. The toxicity of textile effluent and leachate samples was concentration dependent. The anaerobic biodegradability BMP tests showed that textile effluents are labile and may be used as the primary substrate in future investigations into co-digestion of high strength organic industrial effluents. The Aloes and Holfontein leachate are recalcitrant (less than 50 % biodegradable) and may be used as the secondary substrate in the co-metabolism process. Laboratory investigations which involved co-metabolism of labile and recalcitrant industrial effluents in laboratory testing,

revealed that co-digestion effluent was not toxic and biodegradable and it is highly likely that this mixture may increase in biodegradability once biomass becomes acclimatised to the effluent.

It is envisaged that these screening tests will assist the municipalities in making informed decisions on whether or not to receive certain high strength organic liquid industrial effluents. Furthermore, the success of this project could provide an alternative sink for liquid wastes generated inland which have no other outlet for disposal other than landfills and it may provide an additional option for the treatment of landfill leachate.

From this study of the screening tests for high strength liquid industrial effluent in anaerobic digesters, the following can be concluded:

- High strength/organic effluent should be separated from bulk effluent and be treated anaerobically before being disposed of in sewers or marine pipeline. In KwaZulu-Natal, a study has already been conducted about the availability of anaerobic digesters, which can be utilised to treat these effluent (Sacks, 1997).
- ATA and BMP helped to determine whether loading a substrate into anaerobic digesters would impact anaerobic digestion positively or negatively. It also provided information on volumes and concentrations that could be effectively treated under AD. From ATA analyses for Holfontein and Aloes leachate, volumes of 4mL and below were proven not to be toxic to the anaerobic biomass. Whereas, Shongweni leachate was found to be less toxic to the biomass and a concentration of 10 mL (v/v) could effectively be treated anaerobically. For textile effluents, all effluent samples were

fairly biodegradable at all volumes tested, i.e. 0.4-40 Ml (v/v) and they were effectively treated with anaerobic digestion at 4 mL volumes.

- These tests also show that biodegradability protocol should run for a period of at least 30 days due to long retention times required for AD.
- For Textile Effluents, both ATA & BMP showed that it can be anaerobically treated and co-metabolism resulted in biodegradation of the substrate even though some of its component was found to be inhibitory to the anaerobic biomass.
- Shongweni Landfill leachate, though comes from a highly toxic hazardous landfill site, but the sample tested during this study showed that anaerobic treatment of this type of effluent is possible. However, Aloes and Holfontein leachate samples were not amenable to AD. Maybe they needed more time to biodegrade and also, the use of acclimatized biomass might help to biodegrade them. It should be noted that all these types of leachate are produced from highly hazardous landfill site and that renders them very toxic and complex in composition. This should explain their recalcitrant nature.

Based on the above conclusions, the following future work is recommended:

- Industries producing high strength effluent should be identified and be educated about treating their effluent before discharging it into a sewer or marine pipeline.

- Treatment of these types of effluent is promoting cleaner technology amongst industries thus reducing volumes of unwanted waste streams.

- Assessment of biomass acclimation and the influence it may have on increasing degradation rates.

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APPENDIX A
HOLFONTEIN LANDFILL LEACHATE DATA

A. SERUM BOTTLES FOR HOLFONTEIN "HH" LANDFILL LEACHATE

A.1 Biodegradability Data

Holfontein 4mL±0.27 and control samples

4 ml Holfontein sample							
Date	Time days	R 1	R 2	R 3	Average	Cumulative	Std Dev
23-Apr	0	0	0	0	0.00	0.00	0.00
24-Apr	1	10	10	9	9.67	9.67	0.58
25-Apr	2	14	16	15	15.00	24.67	1.00
26-Apr	3	3	4	5	4.00	28.67	1.00
27-Apr	4	4	2	1	2.33	31.00	1.53
28-Apr	5	2	0	2	1.33	32.33	1.15
29-Apr	6	2	2	2	2.00	34.33	0.00
30-Apr	7	4	4	4	4.00	38.33	0.00
1-May	8	2	2	2	2.00	40.33	0.00
2-May	9	1	2	2	1.67	42.00	0.58
3-May	10	0	0	0	0.00	42.00	0.00
4-May	11	0	0	0	0.00	42.00	0.00
5-May	12	3	4	3	3.33	45.33	0.58
6-May	13	0	0	0	0.00	45.33	0.00
7-May	14	3	3	2	2.67	48.00	0.58
8-May	15	2	3	2	2.33	50.33	0.58
9-May	16	2	3	3	2.67	53.00	0.58
10-May	17	2	2	3	2.33	55.33	0.58
11-May	18	2	3	3	2.67	58.00	0.58
12-May	19	2	2	2	2.00	60.00	0.00
13-May	20	2	2	2	2.00	62.00	0.00
14-May	21	1	0	0	0.33	62.33	0.58
15-May	22	0	0	0	0.00	62.33	0.00
16-May	23	0	0	0	0.00	62.33	0.00
17-May	24	0	0	0	0.00	62.33	0.00
18-May	25	0	0	0	0.00	62.33	0.00
19-May	26	2	2	2	2.00	64.33	0.00
20-May	27	0	0	0	0.00	64.33	0.00
21-May	28	1	1	1	1.00	65.33	0.00
22-May	29	0	0	0	0.00	65.33	0.00
23-May	30	1	1	1	1.00	66.33	0.00
28-May	35	1	1	1	1.00	67.33	0.00
6-Jun	44	2	2	2	2.00	69.33	0.00
13-Jun	51	2	2	2	2.00	71.33	0.00
23-Jun	60	0	0	0	0.00	71.33	0.00
						SD (TOT)	0.27

Cont. Biodegradability Data

Control							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
23-Apr	0	0	0	0	0.00	0.00	0.00
24-Apr	24	2	1	1	1.33	1.33	0.58
25-Apr	48	1	1	2	1.33	2.67	0.58
26-Apr	72	3	3	4	3.33	6.00	0.58
27-Apr	96	3	2	2	2.33	8.33	0.58
28-Apr	120	3	2	2	2.33	10.67	0.58
29-Apr	144	2	3	2	2.33	13.00	0.58
30-Apr	168	3	2	3	2.67	15.67	0.58
1-May	192	2	2	2	2.00	17.67	0.00
2-May	216	2	1	1	1.33	19.00	0.58
3-May	240	0	0	0	0.00	19.00	0.00
4-May	264	0	0	0	0.00	19.00	0.00
5-May	288	3	2	2	2.33	21.33	0.58
6-May	312	0	0	0	0.00	21.33	0.00
7-May	336	1	2	1	1.33	22.67	0.58
8-May	360	2	1	1	1.33	24.00	0.58
9-May	384	2	2	2	2.00	26.00	0.00
10-May	408	2	1	1	1.33	27.33	0.58
11-May	432	2	1	1	1.33	28.67	0.58
12-May	456	2	1	1	1.33	30.00	0.58
13-May	480	2	1	1	1.33	31.33	0.58
14-May	504	0	0	0	0.00	31.33	0.00
15-May	528	0	0	0	0.00	31.33	0.00
16-May	552	0	0	0	0.00	31.33	0.00
17-May	576	0	0	0	0.00	31.33	0.00
18-May	600	1	1	1	1.00	32.33	0.00
19-May	624	2	2	2	2.00	34.33	0.00
20-May	648	0	0	0	0.00	34.33	0.00
21-May	672	1	1	1	1.00	35.33	0.00
22-May	696	0	0	0	0.00	35.33	0.00
23-May	720	2	2		2.00	37.33	0.00
28-May	840	2	2		2.00	39.33	0.00
6-Jun	1056	0	0		0.00	39.33	0.00
13-Jun	1214	0	0		0.00	39.33	0.00
23-Jun	1440	2	2		2.00	41.33	0.00
						SD TOT	0.26

A.2

Biogas Composition Data

Holfontein 4mL±0.27 and control sample

DATE		AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AREA	VOLUME	%N2	TOT VOL
SAMPLE		CH4			CO2			N2			
25-Apr-01											
	4 ml	60371.0	125.0	43.8	9681.0	12.7	4.4	88178.0	148.1	51.8	285.8
4-May-01											
	4 ml	77799.0	161.1	61.2	21526.0	28.2	10.7	43975.0	73.8	28.1	283.1
	control	62125.0	128.7	47.3	18143.0	23.7	8.7	71289.0	119.7	44.0	272.1
9-May-01											
	4 ml	69277.0	143.5	54.6	20586.0	26.9	10.2	55098.0	92.5	35.2	262.9
	control	63538.0	131.6	45.6	18995.0	24.9	8.6	78717.0	132.2	45.8	288.6
14-May-01											
	4 ml	48924.0	101.3	56.4	13441.0	17.6	9.8	36259.0	60.9	33.9	179.8
	control	43519.0	90.1	51.4	11712.0	15.3	8.7	41630.0	69.9	39.9	175.4
18-May-01											
	4 ml	68784.0	142.4	55.0	23522.0	30.8	11.9	51074.0	85.8	33.1	259.0
	control	59649.0	123.5	50.5	16949.0	22.2	9.1	58911.0	98.9	40.4	244.6
21-May-01											
	4 ml	82317.0	170.5	56.6	21858.0	28.6	9.5	60893.0	102.3	33.9	301.3
	control	77383.0	160.3	60.5	18214.0	23.8	9.0	48098.0	80.8	30.5	264.9
28-May-01											
	4 ml	74989.0	155.3	54.0	27073.0	35.4	12.3	57577.0	96.7	33.6	287.4
	Control	85834.0	177.8	57.6	24373.0	31.9	10.3	58992.0	99.1	32.1	308.7
6-Jun-01											
	4 ml	82274.0	170.4	54.1	23232.0	30.4	9.7	67972.0	114.1	36.2	314.9
	control	85834.0	177.8	57.6	24373.0	31.9	10.3	58992.0	99.1	32.1	308.7
13-Jun-01											
	4 ml	73668.0	152.6	56.2	25389.0	33.2	12.2	50932.0	85.5	31.5	271.3
	control	75494.0	156.3	60.0	18804.0	24.6	9.4	47389.0	79.6	30.5	260.5
20-Jun-01											
	4 ml	78914.0	163.4	58.2	25292.0	33.1	11.8	50303.0	84.5	30.1	281.0
	control	77549.0	160.6	58.3	28337.0	37.1	13.5	46409.0	77.9	28.3	275.6
Average 4mL				55.0%							
Average Control				54.3%							

APPENDIX B
SHONGWNI "HH" LANDFILL LEACHATE

B. SERUM BOTTLES FOR SHONGWENI LANDFILL LEACHATE

B.1 Biodegradability Tests Data
Shongweni 10mL±0.32 and control samples

10 ml Shongweni sample							
Date	time (h)	R1	R2	R3	Average	Cumulative vol.	Std Dev
23-Apr	0	0	0	0	0.00	0.00	0.00
24-Apr	24	5	3	4	4.00	4.00	
25-Apr	48	0	0	2	0.67	4.67	1.15
26-Apr	72	5	2	6	4.33	9.00	2.08
27-Apr	96	2	1	1	1.33	10.33	0.58
28-Apr	120	2	4	3	3.00	13.33	1.00
29-Apr	144	2	4	2	2.67	16.00	1.15
30-Apr	168	4	3	3	3.33	19.33	0.58
1-May	192	2	2	2	2.00	21.33	0.00
2-May	216	1	2	2	1.67	23.00	0.58
3-May	240	1	1	1	1.00	24.00	0.00
4-May	264	0	0	0	0.00	24.00	0.00
5-May	288	3	3	2	2.67	26.67	0.58
6-May	312	0	0	0	0.00	26.67	0.00
7-May	336	2	2	2	2.00	28.67	0.00
8-May	360	1	1	1	1.00	29.67	0.00
9-May	384	2	2	2	2.00	31.67	0.00
10-May	408	2	2	1	1.67	33.33	0.58
11-May	432	1	2	2	1.67	35.00	0.58
12-May	456	2	2	1	1.67	36.67	0.58
13-May	480	2	2	1	1.67	38.33	0.58
14-May	504	0	0	0	0.00	38.33	0.00
15-May	528	0	0	0	0.00	38.33	0.00
16-May	552	0	0	0	0.00	38.33	0.00
17-May	576	0	0	0	0.00	38.33	0.00
18-May	600	2	2	2	2.00	40.33	0.00
19-May	624	3	3	2	2.67	43.00	0.58
20-May	648	0	0	0	0.00	43.00	0.00
21-May	672	1	1	1	1.00	44.00	0.00
22-May	696	0	0	0	0.00	44.00	0.00
23-May	720	2	2	2	2.00	46.00	0.00
28-May	840	2	2	2	2.00	48.00	0.00
6-Jun	1056	0	0	0	0.00	48.00	0.00
13-Jun	1214	0	0	0	0.00	48.00	0.00
23-Jun	1440	2	1	1.5	1.50	49.50	0.50
							0.32

Cont. Biodegradability Tests Data

Control							
Date	Time (h)	R1	R2	R3	Average	Cumulative vol.	Std dev
23-Apr	0	0	0	0	0.00	0.00	0.00
24-Apr	24	2	1	1	1.33	1.33	0.58
25-Apr	48	1	1	2	1.33	2.67	0.58
26-Apr	72	3	3	4	3.33	6.00	0.58
27-Apr	96	3	2	2	2.33	8.33	0.58
28-Apr	120	3	2	2	2.33	10.67	0.58
29-Apr	144	2	3	2	2.33	13.00	0.58
30-Apr	168	3	2	3	2.67	15.67	0.58
1-May	192	2	2	2	2.00	17.67	0.00
2-May	216	2	1	1	1.33	19.00	0.58
3-May	240	0	0	0	0.00	19.00	0.00
4-May	264	0	0	0	0.00	19.00	0.00
5-May	288	3	2	2	2.33	21.33	0.58
6-May	312	0	0	0	0.00	21.33	0.00
7-May	336	1	2	1	1.33	22.67	0.58
8-May	360	2	1	1	1.33	24.00	0.58
9-May	384	2	2	2	2.00	26.00	0.00
10-May	408	2	1	1	1.33	27.33	0.58
11-May	432	2	1	1	1.33	28.67	0.58
12-May	456	2	1	1	1.33	30.00	0.58
13-May	480	2	2	2	2.00	32.00	0.00
14-May	504	0	0	0	0.00	32.00	0.00
15-May	528	0	0	0	0.00	32.00	0.00
16-May	552	0	0	0	0.00	32.00	0.00
17-May	576	0	0	0	0.00	32.00	0.00
18-May	600	1	1	1	1.00	33.00	0.00
19-May	624	2	2	2	2.00	35.00	0.00
20-May	648	0	0	0	0.00	35.00	0.00
21-May	672	1	1	1	1.00	36.00	0.00
22-May	696	0	0	0	0.00	36.00	0.00
23-May	720	0	0	0	0.00	36.00	0.00
28-May	840	2	2	2	2.00	38.00	2.00
6-Jun	1056	0	0	0	0.00	38.00	0.00
13-Jun	1214	0	0	0	0.00	38.00	0.00
23-Jun	1440	0	0	0	0.00	38.00	0.00
							0.24

B.2 Biogas Composition Data

Shongweni 10mL±0.32 and control samples.

Sample Date	AREA CH4	VOLUME	% CH4	AREA CO2	VOLUME	% CO2	AREA N2	VOLUME	% N2	TOT VOL
25-Apr-01 10 ml	47430.0	98.3	35.1	14311.0	18.7	6.7	97051.0	163.0	58.2	280.0
control	54004.0	111.8	37.8	11348.0	14.9	5.0	100524.0	168.8	57.1	295.5
4-May-01 10 ml	67218.0	139.2	49.2	17339.0	22.7	8.0	71974.0	120.9	42.7	282.8
control	62125.0	128.7	47.3	18143.0	23.7	8.7	71289.0	119.7	44.0	272.1
9-May-01 10 ml	3338.0	6.9	38.6	1085.0	1.4	7.9	5698.0	9.6	53.5	17.9
control	59900.0	124.0	47.8	17799.0	23.3	9.0	66888.0	112.3	43.3	259.7
14-May-01 10 ml	40959.0	84.8	40.6	14640.0	19.2	9.2	62446.0	104.9	50.2	208.8
control	43519.0	90.1	51.4	11712.0	15.3	8.7	41630.0	69.9	39.9	175.4
18-May-01 10 ml	67609.0	140.0	55.6	19587.0	25.6	10.2	51358.0	86.2	34.2	251.9
control	59649.0	123.5	50.5	16949.0	22.2	9.1	58911.0	98.9	40.4	244.6
21-May-01 10 ml	69204.0	143.3	55.1	21298.0	27.9	10.7	52966.0	88.9	34.2	260.1
control	77383.0	160.3	60.5	18214.0	23.8	9.0	48098.0	80.8	30.5	264.9
28-May-01 10 ml	69204.0	143.3	55.1	21298.0	27.9	10.7	52966.0	88.9	34.2	260.1
control	77383.0	160.3	60.5	18214.0	23.8	9.0	48098.0	80.8	30.5	264.9
6-Jun-01 10 ml	82274.0	170.4	54.1	23232.0	30.4	9.7	67972.0	114.1	36.2	314.9
control	85834.0	177.8	57.6	24373.0	31.9	10.3	58992.0	99.1	32.1	308.7
13-Jun-01 10 ml	78911.0	163.4	56.7	21841.0	28.6	9.9	57156.0	96.0	33.3	288.0
control	75494.0	156.3	60.0	18804.0	24.6	9.4	47389.0	79.6	30.5	260.5
20-Jun-01 10 ml	2314.0	4.8	62.1	684.0	0.9	11.6	1211.0	2.0	26.3	7.7
control	77549.0	160.6	58.3	28337.0	37.1	13.5	46409.0	77.9	28.3	275.6
Average 10mL			50.2%							
Average Control			53.0%							

APPENDIX C
ALOES "HH" LANDFILL LEACHATE

C. SERUM BOTTLES FOR ALOES LANDFILL LEACHATE

C.1 Biodegradability Tests Data

Aloes 4mL±0.16 and control samples.

Date	Time (h)	R1	R2	R3	Average	Cumulative	SD
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	4	4	3	3.67	3.67	0.58
14-Jun	48	2	1	2	1.67	5.33	0.58
15-Jun	72	2	2	2	2.00	7.33	0.00
16-Jun	96	3	2	3	2.67	10.00	0.58
17-Jun	120	3	3	3	3.00	13.00	0.00
18-Jun	144	7	7	7	7.00	17.00	0.00
19-Jun	168	1	1	1	1.00	18.00	0.00
20-Jun	192	2	4	3	3.00	21.00	1.00
21-Jun	216	2	2	2	2.00	23.00	0.00
22-Jun	240	0	0	0	0.00	23.00	0.00
25-Jun	312	4	3	4	3.67	26.67	0.58
26-Jun	336	2	2	2	2.00	28.67	0.00
27-Jun	360	0	0	0	0.00	28.67	0.00
28-Jun	384	1	1	1	1.00	29.67	0.00
29-Jun	408	2	2	2	2.00	31.67	0.00
2-Jul	480	6	4	4	4.67	36.33	1.15
3-Jul	504	0	0	0	0.00	36.33	0.00
4-Jul	528	0	0	0	0.00	36.33	0.00
5-Jul	552	0	0	0	0.00	36.33	0.00
6-Jul	576	2	2	1	1.67	38.00	0.58
7-Jul	600	0	0	0	0.00	38.00	0.00
8-Jul	624	1	1	1	1.00	39.00	0.00
9-Jul	648	3	3	3	3.00	42.00	0.00
10-Jul	672	0	0	0	0.00	42.00	0.00
11-Jul	696	0	0	0	0.00	42.00	0.00
12-Jul	720	0	0	0	0.00	42.00	0.00
17-Jul	840	4	4	4	4.00	46.00	0.00
24-Jul	1056	2	2	2	2.00	48.00	0.00
2-Aug	1214	2	2	2	2.00	50.00	0.00
12-Aug	1440	2	2	2	2.00	52.00	0.00
	Average						0.16

Cont. Biodegradability Tests Data

Control

Date	Time (h)	R1	R2	R3	Average	Cumulative	SD
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	2	2	3	2.33	2.33	0.58
14-Jun	48	2	2	3	2.33	4.67	0.58
15-Jun	72	2	2	3	2.33	7.00	0.58
16-Jun	96	2	2	2	2.00	9.00	0.00
17-Jun	120	1	3	3	2.33	11.33	1.15
18-Jun	144	5	5	4	4.67	13.67	0.58
19-Jun	168	0	0	0	0.00	13.67	0.00
20-Jun	192	2	2	2	2.00	15.67	0.00
21-Jun	216	2	2	2	2.00	17.67	0.00
22-Jun	240	0	0	0	0.00	17.67	0.00
25-Jun	312	2	2	2	2.00	19.67	0.00
26-Jun	336	2	2	2	2.00	21.67	0.00
27-Jun	360	0	0	0	0.00	21.67	0.00
28-Jun	384	0	0	0	0.00	21.67	0.00
29-Jun	408	0	0	0	0.00	21.67	0.00
2-Jul	480	2	4	2	2.67	24.33	1.15
3-Jul	504	0	2	0	0.67	25.00	1.15
4-Jul	528	0	0	0	0.00	25.00	0.00
5-Jul	552	0	0	0	0.00	25.00	0.00
6-Jul	576	0	0	0	0.00	25.00	0.00
7-Jul	600	0	0	0	0.00	25.00	0.00
8-Jul	624	1	1	1	1.00	26.00	0.00
9-Jul	648	1	1	1	1.00	27.00	0.00
10-Jul	672	0	0	0	0.00	27.00	0.00
11-Jul	696	0	0	0	0.00	27.00	0.00
12-Jul	720	0	0	0	0.00	27.00	0.00
17-Jul	840	6	6	6	6.00	33.00	0.00
24-Jul	1056	2	2	2	2.00	35.00	0.00
2-Aug	1214	3	3	3	3.00	38.00	0.00
12-Aug	1440	4	4	4	4.00	42.00	0.00
						SD	0.19

C.2 Biogas Composition Data

Aloes, 4mL±0.16 and control samples

DATE	19-Jun	AREA	VOLUM	%CH4	AREA	VOLUM	%CO2	AREA	VOLUM	%N2	TOT VO.
SAMPLE		CH4			CO2			N2			
	4 ml	48493	100.42	41.083	8429	11.032	4.5132	79196	132.99	54.404	244.44
		54935	113.77	39.135	11220	14.685	5.0517	96623	162.25	55.813	290.7
		46864	97.051	39.716	9845	12.886	5.2732	80052	134.42	55.01	244.36
	Average	50097	103.75	39.978	9831.3	12.868	4.946	85290	143.22	55.076	259.83
	control	55479	114.89	42.678	10105	13.226	4.9129	84022	141.09	52.409	269.21
		52032	107.75	40.914	9554	12.505	4.748	85225	143.11	54.338	263.37
		49768	103.06	40.251	15053	19.702	7.6945	79375	133.29	52.054	256.05
	Average	52426	108.57	41.281	11571	15.144	5.7851	82874	139.16	52.934	262.88
DATE	26-Jun	AREA	VOLUM	%CH4	AREA	VOLUM	%CO2	AREA	VOLUM	%N2	TOT VO.
SAMPLE		CH4			CO2			N2			
	4 ml	48493	100.42	41.083	8429	11.032	4.5132	79196	132.99	54.404	244.44
		54935	113.77	39.135	11220	14.685	5.0517	96623	162.25	55.813	290.7
		46864	97.051	39.716	9845	12.886	5.2732	80052	134.42	55.01	244.36
	Average	50097	103.75	39.978	9831.3	12.868	4.946	85290	143.22	55.076	259.83
	control	61289	126.92	48.844	14149	18.519	7.1266	68135	114.41	44.029	259.86
		51272	106.18	47.144	12411	16.244	7.2124	61221	102.8	45.644	225.23
		61133	126.6	48.235	13555	17.741	6.7595	70346	118.13	45.006	262.47
	Average	57898	119.9	48.074	13372	17.501	7.0328	66567	111.78	44.893	249.18
DATE	3-Jul	AREA	VOLUM	%CH4	AREA	VOLUM	%CO2	AREA	VOLUM	%N2	TOT VO.
SAMPLE		CH4			CO2			N2			
	4 ml	105289	218.04	61.886	26166	34.247	9.7202	59576	100.04	28.394	352.33
		133650	276.78	65.385	23807	31.16	7.3611	68703	115.37	27.254	423.3
		98139	203.24	56.267	26678	34.917	9.6671	73275	123.04	34.065	361.2
	Average	112359	232.69	61.179	25550	33.442	8.9161	67185	112.82	29.904	378.94
	control	108033	223.73	62.454	24760	32.407	9.0465	60799	102.09	28.5	358.23
		92376	191.3	59.478	26490	34.671	10.78	56967	95.659	29.742	321.63
		137844	285.46	57.916	32903	43.065	8.7373	97879	164.36	33.346	492.89
	Average	112751	233.5	59.95	28051	36.715	9.5212	71882	120.7	30.529	390.92

Cont. Biogas Composition Data

DATE SAMPLE	19-Jun	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	117702	243.75	65.507	33080	43.297	11.636	50648	85.048	22.857	372.1
		117007	242.31	64.435	25976	33.999	9.0409	59400	99.745	26.524	376.05
		97790	202.51	62.794	26961	35.288	10.942	50444	84.706	26.265	322.51
	Average	110833	229.52	64.245	28672	37.528	10.54	53497	89.833	25.215	356.89
	control	112730	233.45	65.341	30875	40.411	11.31	49679	83.421	23.349	357.29
		109436	226.63	63.54	26712	34.962	9.8021	56624	95.083	26.658	356.68
		117192	242.69	63.737	29625	38.775	10.183	59138	99.305	26.08	380.77
	Average	113119	234.26	64.206	29071	38.049	10.432	55147	92.603	25.362	364.91
	17-Jul	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	127207	263.43	57.089	37599	49.211	10.665	88613	148.8	32.246	461.44
	control	126282	261.52	59.81	36341	47.565	10.878	76323	128.16	29.311	437.25
	24-Jul	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	84894	175.81	55.381	23967	31.369	9.8817	65669	110.27	34.737	317.45
	control	89661	185.68	60.153	42176	55.202	17.883	40375	67.798	21.964	308.68
	31-Jul	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	88115	182.48	61.431	22557	29.524	9.9392	50644	85.042	28.629	297.04
	control	127754	264.57	74.73	28496	37.297	10.535	31067	52.168	14.735	354.03
	6-Aug	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	97555	202.03	62.158	29634	38.786	11.934	50147	84.207	25.908	325.02
	control	88092	182.43	64.148	31599	41.358	14.543	36090	60.602	21.31	284.39
	13-Aug	AREA CH4	VOLUM	%CH4	AREA CO2	VOLUM	%CO2	AREA N2	VOLUM	%N2	TOT VO.
	4 ml	93470	193.57	61.136	29097	38.084	12.028	50598	84.964	26.835	316.62
	control	90074	186.53	64.588	26867	35.165	12.176	39965	67.109	23.237	288.81

APPENDIX D
TEXTILE EFFLUENT 1

D. SERUM BOTTLES FOR TEXTILE EFFLUENT 1

D.1 Biodegradability Tests Data

Textile effluent 1 4mL±0.26, its components and control samples.

KOLLOTEX 4 ml							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	8	8	8	8.00	8.00	
14-Jun	48	6	8	8	7.33	15.33	1.15
15-Jun	72	4	2	2	2.67	18.00	1.15
16-Jun	96	4	3	3	3.33	21.33	0.58
17-Jun	120	2	3	3	2.67	24.00	0.58
18-Jun	144	6	6	6	6.00	30.00	0.00
19-Jun	168	0	1	0	0.33	30.33	0.58
20-Jun	192	1	2	1	1.33	31.67	0.58
21-Jun	216	1	1	1	1.00	32.67	0.00
22-Jun	240	0	2	2	1.33	34.00	1.15
23-Jun	264	0	0	0	0.00	34.00	0.00
24-Jun	288	1	1	1	1.00	35.00	0.00
25-Jun	312	3	4	4	3.67	38.67	0.58
26-Jun	336	0	0	0	0.00	38.67	0.00
27-Jun	360	1	1	2	1.33	40.00	0.58
28-Jun	384	0	0	0	0.00	40.00	0.00
29-Jun	408	0	0	0	0.00	40.00	0.00
30-Jun	432	0	0	0	0.00	40.00	0.00
1-Jul	456	1	1	1	1.00	41.00	0.00
2-Jul	480	4	6	6	5.33	46.33	1.15
3-Jul	504	0	1	0	0.33	46.67	0.58
4-Jul	528	0	0	0	0.00	46.67	0.00
5-Jul	552	0	0	0	0.00	46.67	0.00
6-Jul	576	2	2	1	1.67	48.33	0.58
7-Jul	600	0	0	0	0.00	48.33	0.00
8-Jul	624	0	0	0	0.00	48.33	0.00
9-Jul	648	0	0	0	0.00	48.33	0.00
10-Jul	672	0	0	0	0.00	48.33	0.00
11-Jul	696	0	0	0	0.00	48.33	0.00
12-Jul	720	0	0	0	0.00	48.33	0.00
19-Jul	840	6	6	6	6.00	54.33	0.00
27-Jul	1056	2	2	2	2.00	56.33	0.00
3-Aug	1214	1	1	1	1.00	57.33	0.00
12-Aug	1440	3	3	3	3.00	60.33	0.00
						SD	0.26

Cont.

Biodegradability Tests Data

WAX 4 ml							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
12-Jun	0	0	0	0	0	0	0
13-Jun	24	4	4	3	3.67	3.67	0.58
14-Jun	48	4	6	2	4.00	7.67	2.00
15-Jun	72	2	1	2	1.67	9.33	0.58
16-Jun	96	2	3	2	2.33	11.67	0.58
17-Jun	120	1	2	2	1.67	13.33	0.58
18-Jun	144	7	8	8	7.67	21.00	0.58
19-Jun	168	0	0	0	0.00	21.00	0.00
20-Jun	192	2	2	2	2.00	23.00	0.00
21-Jun	216	2	2	2	2.00	25.00	0.00
22-Jun	240	0	1	1	0.67	25.67	0.58
23-Jun	264	0	0	0	0.00	25.67	0.00
24-Jun	288	1	1	1	1.00	26.67	0.00
25-Jun	312	4	4	4	4.00	30.67	0.00
26-Jun	336	0	0	0	0.00	30.67	0.00
27-Jun	360	2	2	2	2.00	32.67	0.00
28-Jun	384	0	0	0	0.00	32.67	0.00
29-Jun	408	0	0	0	0.00	32.67	0.00
30-Jun	432	0	0	0	0.00	32.67	0.00
1-Jul	456	1	1	1	1.00	33.67	0.00
2-Jul	480	4	5	5	4.67	38.33	0.58
3-Jul	504	0	0	1	0.33	38.67	0.58
4-Jul	528	0	0	0	0.00	38.67	0.00
5-Jul	552	0	0	0	0.00	38.67	0.00
6-Jul	576	0	1	1	0.67	39.33	0.58
7-Jul	600	0	0	0	0.00	39.33	0.00
8-Jul	624	0	0	0	0.00	39.33	0.00
9-Jul	648	0	0	0	0.00	39.33	0.00
10-Jul	672	0	0	0	0.00	39.33	0.00
11-Jul	696	0	0	0	0.00	39.33	0.00
12-Jul	720	0	0	0	0.00	39.33	0.00
19-Jul	840	0	0	0	0.00	39.33	0.00
27-Jul	1056	2	2	2	2.00	41.33	
3-Aug	1214	0	0	0	0.00	41.33	
12-Aug	1440	2	2	2	2.00	43.33	
						SD	0.21

Cont. Biodegradability Tests Data

TEXTILE EFFLUENT 1" 4 ml±0.36							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	6	6	6	6.00	6.00	
14-Jun	48	6	8	8	7.33	13.33	1.15
15-Jun	72	4	2	4	3.33	16.67	1.15
16-Jun	96	3	3	3	3.00	19.67	0.00
17-Jun	120	2	2	3	2.33	22.00	0.58
18-Jun	144	5	5	6	5.33	27.33	0.58
19-Jun	168	0	0	0	0.00	27.33	0.00
20-Jun	192	2	2	2	2.00	29.33	0.00
21-Jun	216	2	2	1	1.67	31.00	0.58
22-Jun	240	0	0	2	0.67	31.67	1.15
23-Jun	264	0	0	0	0.00	31.67	0.00
24-Jun	288	1	1	1	1.00	32.67	0.00
25-Jun	312	4	2	2	2.67	35.33	1.15
26-Jun	336	0	2	2	1.33	36.67	1.15
27-Jun	360	0	0	0	0.00	36.67	0.00
28-Jun	384	0	0	0	0.00	36.67	0.00
29-Jun	408	0	0	0	0.00	36.67	0.00
30-Jun	432	0	0	0	0.00	36.67	0.00
1-Jul	456	1	1	1	1.00	37.67	0.00
2-Jul	480	3	4	4	3.67	41.33	0.58
3-Jul	504	0	0	0	0.00	41.33	0.00
4-Jul	528	0	1	1	0.67	42.00	0.58
5-Jul	552	0	0	0	0.00	42.00	0.00
6-Jul	576	0	1	1	0.67	42.67	0.58
7-Jul	600	0	0	0	0.00	42.67	0.00
8-Jul	624	1	1	1	1.00	43.67	0.00
9-Jul	648	3	3	3	3.00	46.67	0.00
10-Jul	672	0	0	0	0.00	46.67	0.00
11-Jul	696	0	0	0	0.00	46.67	0.00
12-Jul	720	0	0	0	0.00	46.67	0.00
19-Jul	840	6	6	6	6.00	52.67	0.00
27-Jul	1056	0	0	0	0.00	52.67	0.00
3-Aug	1214	2	2	2	2.00	54.67	0.00
12-Aug	1440	4	4	4	4.00	58.67	0.00
						SD	0.26

Cont. Biodegradability Tests Data

Size 10 ml							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	2	2	3	2.33	2.33	0.58
14-Jun	48	2	2	3	2.33	4.67	0.58
15-Jun	72	4	4	4	4.00	8.67	0.00
16-Jun	96	2	2	2	2.00	10.67	0.00
17-Jun	120	1	1	1	1.00	11.67	0.00
18-Jun	144	5	5	6	5.33	17.00	0.58
19-Jun	168	0	0	0	0.00	17.00	0.00
20-Jun	192	1	2	2	1.67	18.67	0.58
21-Jun	216	2	2	2	2.00	20.67	0.00
22-Jun	240	0	0	0	0.00	20.67	0.00
23-Jun	264	0	0	0	0.00	20.67	0.00
24-Jun	288	1	2	1	1.33	22.00	0.58
25-Jun	312	5	6	6	5.67	27.67	0.58
26-Jun	336	2	1	1	1.33	29.00	0.58
27-Jun	360	0	0	1	0.33	29.33	0.58
28-Jun	384	0	0	0	0.00	29.33	0.00
29-Jun	408	0	0	0	0.00	29.33	0.00
30-Jun	432	0	0	0	0.00	29.33	0.00
1-Jul	456	1	1	1	1.00	30.33	0.00
2-Jul	480	4	4	6	4.67	35.00	1.15
3-Jul	504	2	2	2	2.00	37.00	0.00
4-Jul	528	0	0	1	0.33	37.33	0.58
5-Jul	552	0	0	0	0.00	37.33	0.00
6-Jul	576	2	2	1	1.67	39.00	0.58
7-Jul	600	0	0	0	0.00	39.00	0.00
8-Jul	624	0	0	0	0.00	39.00	0.00
9-Jul	648	2	2	2	2.00	41.00	0.00
10-Jul	672	0	0	0	0.00	41.00	0.00
11-Jul	696	0	0	0	0.00	41.00	0.00
12-Jul	720	0	0	0	0.00	41.00	0.00
19-Jul	840	5	5	5	5.00	46.00	0.00
27-Jul	1056	2	2	2	2.00	48.00	0.00
3-Aug	1214	2	2	2	2.00	50.00	0.00
12-Aug	1440	6	6	6	6.00	56.00	0.00
						SD	0.20

Cont. Biodegradability Tests Data

CONTROL							
Date	Time (h)	R1	R2	R3	Average	Cumulative	Std Dev
12-Jun	0	0	0	0	0.00	0.00	0.00
13-Jun	24	2	2	3	2.33	2.33	0.58
14-Jun	48	2	2	3	2.33	4.67	0.58
15-Jun	72	2	2	3	2.33	7.00	0.58
16-Jun	96	2	2	2	2.00	9.00	0.00
17-Jun	120	1	3	3	2.33	11.33	1.15
18-Jun	144	5	5	4	4.67	16.00	0.58
19-Jun	168	0	0	0	0.00	16.00	0.00
20-Jun	192	2	2	2	2.00	18.00	0.00
21-Jun	216	2	2	2	2.00	20.00	0.00
22-Jun	240	0	0	0	0.00	20.00	0.00
23-Jun	264	0	0	0	0.00	20.00	0.00
24-Jun	288	0	1	1	0.67	20.67	0.58
25-Jun	312	2	2	2	2.00	22.67	0.00
26-Jun	336	2	2	0	1.33	24.00	1.15
27-Jun	360	0	0	0	0.00	24.00	0.00
28-Jun	384	0	0	0	0.00	24.00	0.00
29-Jun	408	0	0	0	0.00	24.00	0.00
30-Jun	432	0	0	0	0.00	24.00	0.00
1-Jul	456	0	1	1	0.67	24.67	0.58
2-Jul	480	2	4	2	2.67	27.33	1.15
3-Jul	504	0	2	0	0.67	28.00	1.15
4-Jul	528	0	0	0	0.00	28.00	0.00
5-Jul	552	0	0	0	0.00	28.00	0.00
6-Jul	576	0	0	0	0.00	28.00	0.00
7-Jul	600	0	0	0	0.00	28.00	0.00
8-Jul	624	1	1	1	1.00	29.00	0.00
9-Jul	648	2	2	2	2.00	31.00	0.00
10-Jul	672	0	0	0	0.00	31.00	0.00
11-Jul	696	0	0	0	0.00	31.00	0.00
12-Jul	720	0	0	0	0.00	31.00	0.00
19-Jul	840	6	6	6	6.00	37.00	0.00
27-Jul	1056	2	2	2	2.00	39.00	0.00
3-Aug	1214	2	2	2	2.00	41.00	0.00
12-Aug	1440	3	3	3	3.00	44.00	0.00
						SD	0.24

D.2

Biogas Composition Data

Textile effluent 1" 4mL±0.26, its components and control samples

DATE		AREA	VOLUM	%CH4	AREA	VOLUM	%CO2	AREA	VOLUM	%N2	TOT VO
SAMPLE		CH4			CO2			N2			
13-Jun	10 ml	242656	502.52	39.938	384026	502.63	39.947	150732	253.11	20.116	1258.3
		186667	386.57	39.181	290666	380.44	38.559	130788	219.62	22.26	986.63
		169352	350.71	39.064	277133	362.73	40.402	109786	184.35	20.534	897.79
		199558	413.27	39.394	317275	415.27	39.636	130435.33	219.03	20.97	1047.6
4 ml	46722	96.757	37.544	17334	22.688	8.8032	82345	138.27	53.653	257.72	
	48567	100.58	38.985	12355	16.171	6.268	84112	141.24	54.747	257.99	
	52177	108.05	42.976	14321	18.744	7.455	74221	124.63	49.569	251.43	
	49165.3	101.8	39.835	14670	19.201	7.6087	80226	134.72	62.656	265.71	
19-Jun	10 ml	63792	132.11	47.973	24537	32.115	11.662	66197	111.16	40.365	275.38
		67192	139.15	57.177		0	0	62062	104.21	42.823	243.36
		66405	137.52	54.144	47982	62.801	24.726	31961	53.669	21.13	253.99
		65796.3	136.26	63.098	24173	31.639	12.129	53406.667	89.681	34.773	257.59
4 ml	62011	128.42	47.849	21111	27.631	10.295	66897	112.33	41.856	268.38	
	67953	140.72	45.371	24219	31.699	10.22	82028	137.74	44.409	310.17	
	64643	133.87	46.879	21696	28.397	9.9441	73426	123.3	43.177	285.56	
	64869	134.34	46.7	22342	29.242	10.153	74117	124.46	43.147	288.04	
control	55479	114.89	42.678	10105	13.226	4.9129	84022	141.09	52.409	269.21	
	52032	107.75	40.914	9554	12.505	4.748	85225	143.11	54.338	263.37	
	49768	103.06	40.251	15053	19.702	7.6945	79375	133.29	52.054	256.05	
	52426.3	108.57	41.281	11570.6667	15.144	5.7851	82874	139.16	52.934	262.88	
26-Jun	10 ml	76067	157.53	54.917	35249	46.136	16.084	49537	83.183	28.999	286.85
		61332	127.01	53.594	31994	41.875	17.67	40556	68.102	28.736	236.99
		21344	44.201	55.386	10211	13.365	16.747	13244	22.239	27.867	79.806
		52914.3	109.58	64.633	26818	33.792	16.833	34445.667	67.841	28.534	201.21
4 ml	64894	134.39	55.508	21126	27.651	11.421	47681	80.066	33.071	242.11	
	57622	119.33	56.383	18912	24.753	11.696	40232	67.558	31.921	211.64	
	58933	122.04	58.658	16822	22.017	10.582	38112	63.998	30.759	208.06	
	60483	125.25	56.85	18953.3333	24.807	11.233	42008.333	70.541	31.917	220.6	
control	61289	126.92	48.844	14149	18.519	7.1266	68135	114.41	44.029	259.86	
	51272	106.18	47.144	12411	16.244	7.2124	61221	102.8	45.644	225.23	
	61133	126.6	48.235	13555	17.741	6.7595	70346	118.13	45.006	262.47	
	57898	119.9	48.074	13371.6667	17.501	7.0328	66567.333	111.78	44.893	249.18	
3-Jul	10 ml	94121	194.92	54.969	30857	40.387	11.39	71041	119.29	33.642	354.6
		81383	168.54	55.209	28598	37.43	12.261	59136	99.301	32.529	305.27
		104385	216.17	66.416	28204	36.915	11.342	43112	72.394	22.242	325.48
		93296.3	193.21	68.665	29219.6667	38.244	11.664	57763	96.996	29.471	328.45
4 ml	88387	183.04	52.457	41135	53.84	15.429	66733	112.06	32.114	348.94	
	90973	188.4	52.937	31564	41.313	11.608	75143	126.18	35.455	355.89	
	125154	259.18	62.011	33204	43.459	10.398	68677	115.32	27.592	417.96	
	101505	210.21	65.801	35301	46.204	12.479	70184.333	117.85	31.72	374.26	
control	108033	223.73	62.454	24760	32.407	9.0465	60799	102.09	28.5	358.23	
	92376	191.3	59.478	26490	34.671	10.78	56967	95.659	29.742	321.63	
	137844	285.46	57.916	32903	43.065	8.7373	97879	164.36	33.346	492.89	
	112751	233.5	59.95	28051	36.715	9.5212	71881.667	120.7	30.529	390.92	

Cont. Biogas Composition Data

DATE	AREA	VOLUM	%CH4	AREA	VOLUM	%CO2	AREA	VOLUM	%N2	TOT VO	
SAMPLE	CH4			CO2			N2				
10-Jul	10 ml	107959	223.57	64.194	43305	56.68	16.274	40510	68.025	19.532	348.28
		102766	212.82	68.76	42006	54.98	17.763	24840	41.711	13.477	309.51
		118732	245.88	65.065	38398	50.257	13.299	48693	81.766	21.636	377.91
		109819	227.43	66.006	41236.3333	53.972	15.779	38014.333	63.834	18.215	345.23
	4 ml	115091	238.34	57.361	41880	54.815	13.192	72867	122.36	29.447	415.52
		100684	208.51	60.124	37274	48.786	14.068	53300	89.502	25.808	346.79
		97211	201.32	67.4	34316	44.914	15.037	31240	52.458	17.563	298.69
		104329	216.06	61.628	37823.3333	49.505	14.099	52469	88.106	24.273	353.67
	control	112730	233.45	65.341	30875	40.411	11.31	49679	83.421	23.349	357.29
		109436	226.63	63.54	26712	34.962	9.8021	56624	95.083	26.658	356.68
		117192	242.69	63.737	29625	38.775	10.183	59138	99.305	26.08	380.77
		113119	234.26	64.206	29070.6667	38.049	10.432	55147	92.603	25.362	364.91
17-Jul	10 ml	134567	278.68	59.062	64595	84.545	17.918	64682	108.61	23.02	471.84
	4 ml	123459	255.67	57.344	46638	61.042	13.691	76906	129.14	28.965	445.86
	control	126282	261.52	59.81	36341	47.565	10.878	76323	128.16	29.311	437.25
24-Jul	10 ml	102710	212.7	59.801	45090	59.016	16.592	50003	83.965	23.607	355.68
	4 ml	100083	207.26	61.453	37142	48.613	14.414	48471	81.393	24.133	337.27
	control	91570	189.63	58.737	22281	29.162	9.0328	61966	104.05	32.23	322.85
31-Jul	10 ml	98560	204.11	69.952	49422	64.686	22.169	13691	22.99	7.8791	291.78
	4 ml	104475	216.36	65.076	39168	51.265	15.419	38617	64.846	19.504	332.47
	control	127754	264.57	74.73	28496	37.297	10.535	31067	52.168	14.735	354.03
6-Aug	10 ml	59286	122.78	59.303	34309	44.905	21.69	23434	39.35	19.007	207.03
	4 ml	91538	189.57	62.089	34779	45.52	14.909	41821	70.226	23.001	305.31
	control	99092	205.21	66.807	31599	41.358	13.464	36090	60.602	19.729	307.17

APPENDIX E
TEXTILE EFFLUENT 2

E. SERUM BOTTLES FOR TEXTILE EFFLUENT 2

E.1

Biodegradability Tests Data

Textile effluent 4mL±0.3, its components and control.

Date	Time (hrs)	Effluent 2		Control Acetate
		4ml	Control	
2-Jul	0	0	0	0
3-Jul	20	32.67	7	15
4-Jul	44	59.67	7.67	23.33
5-Jul	68	77	11.67	29
6-Jul	92.75	83.67	12.33	29
9-Jul	164	94.67	19	34.33
10-Jul	191.75	98.33	20.67	35
11-Jul	213.75	98.33	22.33	35.67
12-Jul	237.25	98.33	23.33	37
13-Jul	261.75	101.33	24.33	38.33
16-Jul	333.5	106.67	26.33	41.33
19-Jul	405.5	113	31.67	45.67
23-Jul	502.25	119.33	34.67	47.33
26-Jul	574.25	121	35	48.33
30-Jul	669.75	126	38.33	51.33
2-Aug	740.75	129.33	41.33	54.67
Std Dev		0.18	0.21	0.24

E.2

Biogas Composition Data

Textile effluent 2 4mL ± 0.3 , its components and control samples.

BMP GAS COMPOSITION										
6/7	AREA	VOLUME	%CH ₄	AREA	VOLUME	%CO ₂	AREA	VOLUME	%N ₂	TOT VOL
SAMPLE	CH ₄			CO ₂			N ₂			
Starch										
1	37358.00	77.36	39.98	100459.00	43.89	22.65	43059.00	72.30	37.37	193.50
2	43440.00	89.96	42.95	111240.00	48.53	23.17	42257.00	70.96	33.88	209.45
3	28557.00	59.14	30.32	103861.00	45.31	23.23	53944.00	90.58	37.37	195.04
AVERAGE	36451.67	75.49	37.75	105186.67	45.89	23.02	46420.00	77.95	36.20	199.33
PVA										
1	39968.00	82.77	30.96	12954.00	5.65	2.11	106568.00	178.95	66.93	267.37
2	35929.00	74.41	24.30	9952.00	4.34	1.42	135448.00	227.44	74.28	306.19
AVERAGE	37948.50	78.59	27.63	11453.00	5.00	1.77	121008.00	203.20	70.61	286.78
Wax										
1	34986.00	72.45	22.00	9822.00	4.29	1.30	150418.00	252.58	76.70	329.32
SIZE COMP 2										
1	86860.00	179.88	62.10	54824.00	23.92	8.26	51125.00	85.85	29.64	289.65
2	86414.00	178.96	64.35	56935.00	24.84	8.93	44241.00	74.29	26.71	278.09
3	77612.00	160.73	57.66	50992.00	22.25	7.98	57034.00	95.77	34.36	278.75
AVERAGE	83628.67	173.19	61.37	54250.33	23.67	8.39	50800.00	85.30	30.24	282.16
Control										
1	37799.00	78.28	25.84	10429.00	4.55	1.50	131068.00	220.09	72.66	302.92
2	42592.00	88.20	29.81	13426.00	5.86	1.98	120180.00	201.81	68.21	295.87
AVERAGE	40195.50	83.24	27.83	11927.50	5.20	1.74	125624.00	210.95	70.43	299.39
Na-acet-prop control										
1	111326.00	230.55	63.61	13277.00	5.79	1.60	75083.00	126.08	34.79	362.42
2	84269.00	174.51	49.23	11547.00	5.04	1.42	104198.00	174.97	49.35	354.52
AVERAGE	97797.50	202.53	56.42	12412.00	5.42	1.51	89640.50	150.52	42.07	358.47

Cont. Biogas Composition Data

7/9	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
Starch										
1	51378.00	106.40	61.21	97672.00	42.61	24.52	14778.00	24.82	14.28	173.83
2	57001.00	118.04	55.52	92041.00	40.16	18.89	32411.00	54.42	25.60	212.63
3	39844.00	82.51	40.14	98547.00	43.00	20.92	47663.00	80.04	14.28	205.55
AVERAGE	49407.67	102.32	52.29	96086.67	41.92	21.44	31617.33	53.09	18.05	197.33
PVA										
1	25805.00	53.44	23.64	18576.00	8.10	3.59	97954.00	164.48	72.77	226.03
2	54952.00	113.80	36.96	14129.00	6.16	2.00	111899.00	187.90	61.03	307.87
3	66003.00	136.69	45.95	15699.00	6.85	2.30	91652.00	153.90	51.74	297.44
AVERAGE	48920.00	101.31	35.52	16134.67	7.04	2.63	100501.67	168.76	61.85	277.11
Wax										
1	46880.00	97.08	33.20	12188.00	5.32	1.82	113182.00	190.06	64.99	292.46
2	52945.00	109.64	35.09	13515.00	5.90	1.89	117289.00	196.95	63.03	312.49
3	45779.00	94.80	32.99	12153.00	5.30	1.85	111523.00	187.27	65.17	287.38
AVERAGE	48534.67	100.51	33.76	12618.67	5.51	1.85	113998.00	191.43	64.39	297.44
Recipe 2										
1	86702.00	179.55	63.97	58458.00	25.51	9.09	45037.00	75.63	26.94	280.68
2	91102.00	188.66	67.37	60813.00	26.53	9.47	38623.00	64.86	23.16	280.05
3	77722.00	160.96	61.67	54331.00	23.70	9.08	45453.00	76.32	29.24	260.98
AVERAGE	85175.33	176.39	64.34	57867.33	25.25	9.21	43037.67	72.27	26.45	273.91
Control										
1	63377.00	131.25	41.27	14402.00	6.28	1.98	107483.00	180.49	56.75	318.02
2	69278.00	143.47	41.38	15367.00	6.70	1.93	117023.00	196.51	56.68	346.68
3	49849.00	103.23	31.09	14457.00	6.31	1.90	132531.00	222.55	67.01	332.09
AVERAGE	60834.67	125.98	37.91	14742.00	6.43	1.94	119012.33	199.85	60.15	332.26
Na-acet-prop control										
1	150517.00	311.71	78.39	27349.00	11.93	3.00	44079.00	74.02	18.61	397.66
2	140721.00	291.42	80.23	47618.00	20.78	5.72	30402.00	51.05	14.05	363.25
3	109393.00	226.54	64.22	58544.00	25.54	7.24	59960.00	100.69	28.54	352.77
AVERAGE	133543.67	276.56	74.28	44503.67	19.42	5.32	44813.67	75.25	20.40	371.22

Cont. Biogas Composition Data

7/16	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
Starch										
1	1815275.00	3759.27	96.93	188509.00	82.25	2.12	21928.00	36.82	0.95	3878.34
2	71555.00	148.18	55.99	26726.00	11.66	4.41	62412.00	104.80	39.60	264.65
3	80322.00	166.34	81.99	49618.00	21.65	10.67	8866.00	14.89	0.95	202.88
AVERAGE	655717.33	1357.93	78.30	88284.33	38.52	5.73	31068.67	52.17	13.83	1448.62
PVA										
1	112707.00	233.41	69.67	39752.00	17.34	5.18	50169.00	84.24	25.15	334.99
2	62150.00	128.71	59.10	99262.00	43.31	19.89	27257.00	45.77	21.02	217.79
3	98535.00	204.06	63.87	32120.00	14.01	4.39	60398.00	101.42	31.74	319.49
AVERAGE	91130.67	188.72	64.21	57044.67	24.89	9.82	45941.33	77.14	25.97	290.76
Wax										
1	85094.00	176.22	52.71	22108.00	9.65	2.89	88408.00	148.46	44.40	334.32
2	82257.00	170.35	52.39	22041.00	9.62	2.96	86449.00	145.17	44.65	325.13
3	79366.00	164.36	55.47	26132.00	11.40	3.85	71784.00	120.54	40.68	296.30
AVERAGE	82239.00	170.31	53.52	23427.00	10.22	3.23	82213.67	138.05	43.24	318.58
Recipe 2										
1	106846.00	221.27	79.56	65204.00	28.45	10.23	16918.00	28.41	10.21	278.13
2	104144.00	215.67	80.81	67535.00	29.47	11.04	12959.00	21.76	8.15	266.90
3	115602.00	239.40	83.96	74660.00	32.57	11.42	7835.00	13.16	4.61	285.13
AVERAGE	108864.00	225.45	81.44	69133.00	30.16	10.90	12570.67	21.11	7.66	276.72
Control										
1	97845.00	202.63	65.37	30198.00	13.18	4.25	56073.00	94.16	30.38	309.96
2	85922.00	177.94	51.46	20866.00	9.10	2.63	94530.00	158.74	45.91	345.78
3	90984.00	188.42	62.46	20467.00	8.93	2.96	62108.00	104.29	34.57	301.64
AVERAGE	91583.67	189.66	59.77	23843.67	10.40	3.28	70903.67	119.06	36.95	319.13
Na-acet-prop control										
1	127776.00	264.61	81.01	20832.00	9.09	2.78	31536.00	52.96	16.21	326.66
2	107648.00	222.93	71.47	17052.00	7.44	2.39	48566.00	81.55	26.15	311.92
3	133818.00	277.12	82.05	29781.00	12.99	3.85	28363.00	47.63	14.10	337.75
AVERAGE	123080.67	254.89	78.18	22555.00	9.84	3.00	36155.00	60.71	18.82	325.44

Cont. Biogas Composition Data

7/26	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
Starch										
1	82784.00	171.44	61.39	62913.00	27.45	9.83	47870.00	80.38	28.78	279.27
2	82494.00	170.84	67.03	64577.00	28.17	11.05	33270.00	55.87	21.92	254.88
3	107919.00	223.49	68.36	64492.00	28.14	8.61	44837.00	75.29	28.78	326.92
AVERAGE	91065.67	188.59	65.59	63994.00	27.92	9.83	41992.33	70.51	26.50	287.02
PVA										
1	93706.00	194.06	63.63	30931.00	13.50	4.43	58017.00	97.42	31.94	304.97
2	100338.00	207.79	63.93	28819.00	12.57	3.87	62323.00	104.65	32.20	325.02
3	85517.00	177.10	55.85	25189.00	10.99	3.47	76814.00	128.99	40.68	317.07
AVERAGE	93187.00	192.98	61.14	28313.00	12.35	3.92	65718.00	110.35	34.94	315.69
Wax										
1	80158.00	166.00	52.10	21925.00	9.57	3.00	85192.00	143.05	44.90	318.62
2	86773.00	179.70	57.20	23221.00	10.13	3.23	74031.00	124.31	39.57	314.14
3	82973.00	171.83	52.86	23205.00	10.12	3.11	85232.00	143.12	44.03	325.08
AVERAGE	83301.33	172.51	54.05	22783.67	9.94	3.11	81485.00	136.83	42.83	319.28
Recipe 2										
1	103817.00	215.00	69.12	74869.00	32.67	10.50	37736.00	63.37	20.37	311.03
2	104293.00	215.98	75.31	65080.00	28.39	9.90	25260.00	42.42	14.79	286.79
3	112695.00	233.38	71.71	69751.00	30.43	9.35	36707.00	61.64	18.94	325.45
AVERAGE	106935.00	221.45	72.05	69900.00	30.50	9.92	33234.33	55.81	18.03	307.76
Control										
1	95756.00	198.30	61.71	25117.00	10.96	3.41	66733.00	112.06	34.87	321.32
2	89598.00	185.55	55.86	17200.00	7.50	2.26	82842.00	139.11	41.88	332.16
AVERAGE	92677.00	191.93	58.79	21158.50	9.23	2.83	74787.50	125.58	38.38	326.74
Na-acet-prop control										
1	119721.00	247.93	76.10	17728.00	7.73	2.37	41771.00	70.14	21.53	325.81
2	114974.00	238.10	78.28	20288.00	8.85	2.91	34070.00	57.21	18.81	304.16
AVERAGE	117347.50	243.02	77.19	19008.00	8.29	2.64	37920.50	63.68	20.17	314.99

Cont. Biogas Composition Data

8/2 SAMPLE	AREA CH4	VOLUME	%CH4	AREA CO2	VOLUME	%CO2	AR EA N2	VOLUME	%N2	TOT VOL
Starch										
1	88687.00	183.66	72.03	68571.00	29.92	11.73	24645.00	41.38	16.23	254.96
2	98365.00	203.70	76.48	67861.00	29.61	11.12	19668.00	33.03	12.40	266.34
3	74263.00	153.79	68.95	62651.00	27.33	12.26	24958.00	41.91	16.23	223.04
AVERAGE	87105.00	180.39	72.49	66361.00	28.95	11.70	23090.33	38.77	14.95	248.11
PVA										
1	134009.00	277.52	74.62	32437.00	14.15	3.81	47791.00	80.25	21.58	371.92
2	100913.00	208.98	67.86	30952.00	13.50	4.38	50908.00	85.48	27.76	307.97
3	91346.00	189.17	60.00	28650.00	12.50	3.96	67649.00	113.60	36.03	315.27
AVERAGE	108756.00	225.22	67.49	30679.67	13.39	4.05	55449.33	93.11	28.46	331.72
Wax										
1	83085.00	172.06	55.65	25905.00	11.30	3.66	74931.00	125.82	40.70	309.19
2	80181.00	166.05	56.20	16001.00	6.98	2.36	72900.00	122.41	41.43	295.44
3	86743.00	179.64	59.64	25201.00	11.00	3.65	65849.00	110.57	36.71	301.21
AVERAGE	83336.33	172.58	57.16	22369.00	9.76	3.22	71226.67	119.60	39.61	301.95
Recipe 2										
1	89242.00	184.81	72.96	59953.00	26.16	10.33	25213.00	42.34	16.71	253.31
2	78480.00	162.52	70.25	52689.00	22.99	9.94	27300.00	45.84	19.81	231.36
3	92497.00	191.55	74.84	58781.00	25.65	10.02	23076.00	38.75	15.14	255.95
AVERAGE	86739.67	179.63	72.68	57141.00	24.93	10.09	25196.33	42.31	17.22	246.87
Control										
1	92632.00	191.83	64.57	30134.00	13.15	4.43	54852.00	92.11	31.00	297.09
2	84513.00	175.02	67.18	22776.00	9.94	3.81	45000.00	75.56	29.01	260.52
3	102942.00	213.18	66.52	27210.00	11.87	3.70	56829.00	95.43	29.78	320.48
AVERAGE	93362.33	193.34	66.09	26706.67	11.65	3.98	52227.00	87.70	29.93	292.70
Na-acet-prop control										
1	92561.00	191.69	67.34	21159.00	9.23	3.24	49859.00	83.72	29.41	284.64
2	113081.00	234.18	75.12	24870.00	10.85	3.48	39737.00	66.73	21.40	311.76
3	107949.00	223.55	79.84	21064.00	9.19	3.28	28146.00	47.26	16.88	280.01
AVERAGE	104530.33	216.47	74.10	22364.33	9.76	3.34	39247.33	65.90	22.57	292.13

APPENDIX F
TEXTILE EFFLUENT 3

F. SERUM BOTTLES FOR TEXTILE EFFLUENT 3

F.1 Biodegradability Tests Data.

Textile Effluent 3 its components and control.

		Starch (kollotex) 4ml				
Date	Time	R1	R2	R3	Average	Cumulative gas
2-Jul	0	0	0	0	0.00	0.00
3-Jul	19	20	27	15	20.67	20.67
4-Jul	48	50	50	42	47.33	56.00
5-Jul	72	46	43	42	43.67	87.67
6-Jul	96	32	28	36	32.00	107.67
9-Jul	168	55	48	45	49.33	145.00
10-Jul	192	42	44	40	42.00	175.00
11-Jul	216	31	32	29	30.67	193.67
12-Jul	240	22	22	28	24.00	205.67
13-Jul	264	19	20	18	19.00	212.67
16-Jul	336	21	22	21	21.33	222.00
19-Jul	408	22	24	24	23.33	233.34
23-Jul	504	19	18	19	18.67	240.00
26-Jul	600	15	15	14	14.67	242.67
30-Jul	696	17	18	16	17.00	247.67
2-Aug	768	18	18	17	17.67	253.34

		Wax 4ml				
Day		R1	R2	R3	Average	Cumulative production gas
2-Jul	1:45	0	0	0	0.00	0.00
3-Jul	9:15	6	6	6	6.00	6.00
4-Jul	9:15	9	10	10	9.67	9.67
5-Jul	9:15	14	14	5	11.00	11.00
6-Jul	10:00	14	15	8	12.33	12.33
9-Jul	9:15	21	20	10	17.00	17.33
10-Jul	12:30	14	15	10	13.00	18.33
11-Jul	10:30	13	11	12	12.00	18.33
12-Jul	10:00	12	12	14	12.67	19.00
13-Jul	10:30	14	10	15	13.00	20.00
16-Jul	9:15	14	13	14	13.67	21.67
19-Jul	9:15	17	19	16	17.33	27.00
23-Jul	10:00	15	13	13	13.67	28.67
26-Jul	10:00	12	13	13	12.67	29.33
30-Jul	9:30	15	16	16	15.67	33.00
2-Aug	8:30	15	16	15	15.33	36.33

Cont. Biodegradability Tests Data.

Date	Time	Elvanol			Average	Cumulative
		R1.0	R2.0	R3.0		
2-Jul	0	0.00	0.00	0.00	0.00	0.00
3-Jul	19	6.00	4.00	7.00	5.67	5.67
4-Jul	48	11.00	8.00	12.00	10.33	10.33
5-Jul	72	14.00	12.00	12.00	12.67	12.67
6-Jul	96	16.00	16.00	12.00	14.67	15.34
9-Jul	168	20.00	19.00	15.00	18.00	21.34
10-Jul	192	14.00	13.00	14.00	13.67	23.00
11-Jul	216	13.00	13.00	13.00	13.00	24.00
12-Jul	240	12.00	12.00	12.00	12.00	24.00
13-Jul	264	15.00	14.00	15.00	14.67	26.67
16-Jul	336	15.00	16.00	15.00	15.33	30.00
19-Jul	408	19.00	17.00	15.00	17.00	35.00
23-Jul	504	14.00	14.00	14.00	14.00	37.00
26-Jul	600	14.00	13.00	13.00	13.33	38.34
30-Jul	696	16.00	15.00	15.00	15.33	41.67
2-Aug	768	17.00	15.00	16.00	16.00	45.67

Day		Effluent 3 4ml			Average	Cumulative	Std Dev
		R1.00	R2.00	R3.00			
2-Jul	0	0.00	0.00	0.00	0.00	0.00	0.00
3-Jul	19	32.00	30.00	34.00	32.00	32.00	2.00
4-Jul	48	50.00	47.00	42.00	46.33	66.33	4.04
5-Jul	72	46.00	45.00	43.00	44.67	99.00	1.53
6-Jul	96	32.00	28.00	30.00	30.00	117.00	2.00
9-Jul	168	25.00	24.00	25.00	24.67	129.67	0.58
10-Jul	192	17.00	17.00	18.00	17.33	135.00	0.58
11-Jul	216	17.00	15.00	14.00	15.33	138.33	1.53
12-Jul	240	12.00	12.00	14.00	12.67	139.00	1.15
13-Jul	264	16.00	17.00	16.00	16.33	143.33	0.58
16-Jul	336	18.00	16.00	17.00	17.00	148.33	1.00
19-Jul	408	21.00	21.00	20.00	20.67	157.00	0.58
23-Jul	504	17.00	17.00	13.00	15.67	160.67	2.31
26-Jul	600	14.00	15.00	14.00	14.33	163.00	0.58
30-Jul	696	18.00	16.00	16.00	16.67	167.67	1.15
2-Aug	768	16.00	16.00	15.00	15.67	171.33	0.58
						SD	0.98

F.2 Biogas Composition Data
Textile effluent 3 its component and control samples.

5-Jul	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
kollotex										
1	61815	128.0132	49.462	96210	41.97644	16.21895	52895	88.82153	34.31905	258.8111
2	66386	137.4793	54.15407	96273	42.00393	16.54565	44297	74.38373	29.30028	253.8669
3	65197	135.017	54.24563	107519	46.91056	18.84721	39883	66.97172	34.31905	248.8993
AVERAGE	64466	133.5031	52.62057	100000.7	43.63031	17.20393	45691.67	76.72566	32.64613	253.8591
elvanol										
1	49532	102.5762	33.03259	10391	4.533695	1.459952	121141	203.4205	65.50746	310.5303
2	39622	82.05351	25.87462	10925	4.766579	1.503085	137148	230.2996	72.62229	317.1197
	44577	92.31486	29.4536	10658	4.650087	1.481519	129144.5	216.8601	69.06488	313.825
Wax										
1	45882	95.0174	30.0119	10489	4.576353	1.445473	129231	217.0053	68.54263	316.5991
2	43823	90.7534	26.95269	15777	6.883508	2.044321	142375	239.0768	71.00299	336.7137
AVERAGE	44852.5	92.8854	28.4823	13133	5.72993	1.744897	135803	228.041	69.77281	326.6564
Recipe 3										
1	91168	188.8005	70.37084	68422	29.85253	11.12681	29562	49.64065	18.50235	268.2937
2	94616	195.941	66.56099	74505	32.50654	11.04244	39263	65.93062	22.39657	294.3782
3	74737	154.7734	60.11613	58152	25.37173	9.854727	46041	77.31226	30.02914	257.4574
AVERAGE	86840.33	179.8383	65.68265	67026.33	29.2436	10.67466	38288.67	64.29451	23.64269	273.3764
Control										
1	37799	78.27825	25.84136	10429	4.550175	1.502112	131068	220.09	72.65653	302.9184
2	42592	88.20411	29.81191	13426	5.857766	1.979853	120180	201.8068	68.20824	295.8687
AVERAGE	40195.5	83.24118	27.82664	11927.5	5.20397	1.740983	125624	210.9484	70.43238	299.3936
Na-acet-prop control										
1	111326	230.5459	63.61319	13277	5.792757	1.598362	75083	126.0797	34.78845	362.4184
2	84269	174.5133	49.2251	11547	5.037958	1.42106	104198	174.9698	49.35384	354.5211
AVERAGE	97797.5	202.5296	56.41914	12412	5.415358	1.509711	89640.5	150.5248	42.07115	358.4697

Cont.

Biogas Composition Data

10-Jul	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
kollotex										
1	106908	221.3966	72.03853	83258	36.32548	11.81967	29543	49.60875	16.1418	307.3308
2	118086	244.5452	78.39622	100217	43.72469	14.01725	14093	23.66503	7.586528	311.935
3	101237	209.6525	81.15804	96196	41.97033	16.24703	3992	6.703385	16.1418	258.3262
AVERAGE		225.1981	77.1976	93223.67	40.6735	14.02798	15876	26.65905	13.29005	292.5307
elvanol										
1	121208	251.0106	43.966	25861	11.28316	1.976312	183793	308.6261	54.05769	570.9198
2	73061	151.3026	56.0329	15781	6.885253	2.549862	66601	111.8367	41.41724	270.0246
3	99000	205.0199	61.52386	20558	8.969459	2.691621	71014	119.247	35.78452	333.2364
AVERAGE	97756.33	202.4444	53.84092	20733.33	9.045957	2.405932	107136	179.9033	43.75315	391.3936
Wax										
1	87327	180.8462	54.31733	20118	8.777487	2.636327	85350	143.3201	43.04634	332.9438
2	90063	186.5122	47.17291	18408	8.031414	2.031316	119602	200.8362	50.79577	395.3798
3	65819	136.3051	35.26558	19048	8.310646	2.150175	144053	241.8945	62.58424	386.5102
AVERAGE	81069.67	167.8878	45.58528	19191.33	8.373182	2.272606	116335	195.3503	52.14212	371.6113
Recipe 3										
1	96247	199.3187	75.01234	75828	33.08377	12.45087	19838	33.31206	12.53679	265.7145
2	106908	221.3966	76.21133	87330	38.10209	13.11588	18464	31.00484	10.67279	290.5036
3	111410	230.7198	82.29465	93804	40.9267	14.598	5188	8.711714	3.107351	280.3583
AVERAGE	104855	217.145	77.83944	85654	37.37086	13.38825	14496.67	24.34287	8.77231	278.8588
Control										
1	63377	131.2479	41.27066	14402	6.283595	1.975865	107483	180.486	56.75347	318.0175
2	69278	143.4684	41.38368	15367	6.704625	1.93396	117023	196.5056	56.68236	346.6786
3	49849	103.2327	31.08604	14457	6.307592	1.899379	132531	222.5467	67.01458	332.087
AVERAGE	60834.67	125.983	37.91346	14742	6.431937	1.936401	119012.3	199.8461	60.15014	332.261
Na-acet-prop control										
1	150517	311.7068	78.38588	27349	11.93237	3.000671	44079	74.01767	18.61345	397.6569
2	140721	291.4202	80.22643	47618	20.77574	5.719451	30402	51.05118	14.05412	363.2472
3	109393	226.5428	64.21815	58544	25.54276	7.240612	59960	100.6851	28.54124	352.7707
AVERAGE	133543.7	276.5566	74.27682	44503.67	19.41696	5.320244	44813.67	75.25132	20.40293	371.2249

Cont. Biogas Composition Data

16-Jul	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
kollotex										
1	103396	214.1236	81.47184	83118	36.2644	13.79823	7403	12.43115	4.729927	262.8191
2	99432	205.9145	70.21669	85667	37.37653	12.74537	29755	49.96474	17.03794	293.2558
3	101237	209.6525	81.15804	96196	41.97033	16.24703	3992	6.703385	4.729927	258.3262
AVERAGE	101355	209.8969	77.61553	88327	38.53709	14.26354	13716.67	23.03309	8.832597	271.467
elvanol										
1	86705	179.5581	57.67383	32037	13.97775	4.489636	70151	117.7979	37.83654	311.3337
2	102787	212.8624	64.18212	24515	10.6959	3.22502	64373	108.0954	32.59286	331.6538
3	106194	219.918	67.97193	22405	9.775305	3.021337	55889	93.84907	29.00673	323.5424
AVERAGE	98562	204.1128	63.27596	26319	11.48298	3.578664	63471	106.5808	33.14538	322.1766
Wax										
1	90348	187.1024	64.9722	25084	10.94415	3.80041	53553	89.92645	31.22739	287.973
2	80228	166.1448	53.7684	20901	9.11911	2.951161	79643	133.7369	43.28044	309.0008
3	96944	200.7621	58.19197	20315	8.863438	2.569115	80618	135.3741	39.23892	344.9997
AVERAGE	89173.33	184.6698	58.97752	22100	9.642234	3.106895	71271.33	119.6792	37.91558	313.9912
Recipe 3										
1	97430	201.7686	77.06388	72109	31.46117	12.01634	17026	28.59014	10.91977	261.8199
2	91170	188.8047	69.82419	87854	38.33072	14.17556	25765	43.26471	16.00026	270.4001
3	110925	229.7155	82.33137	87435	38.14791	13.67243	6640	11.14992	3.996197	279.0133
AVERAGE	99841.67	206.7629	76.40648	82466	35.97993	13.28811	16477	27.66826	10.30541	270.4111
Control										
1	97845	202.628	65.372	30198	13.17539	4.250656	56073	94.15805	30.37734	309.9614
2	85922	177.9365	51.46012	20866	9.103839	2.632875	94530	158.7352	45.907	345.7756
3	90984	188.4195	62.46475	20467	8.929756	2.960389	62108	104.292	34.57486	301.6413
AVERAGE	91583.67	189.6613	59.76562	23843.67	10.403	3.281307	70903.67	119.0618	36.95307	319.1261
Na-acet-prop control										
1	127776	264.6123	81.00624	20832	9.089005	2.782433	31536	52.9554	16.21133	326.6567
2	107648	222.9291	71.4697	17052	7.439791	2.385151	48566	81.55226	26.14515	311.9211
3	133818	277.1248	82.05135	29781	12.99346	3.847114	28363	47.62728	14.10153	337.7455
AVERAGE	123080.7	254.8887	78.17576	22555	9.84075	3.0049	36155	60.71165	18.81934	325.4411

Cont.

Biogas Composition Data

26-Jul	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AR EA	VOLUME	%N2	TOT VOL
SAMPLE	CH4			CO2			N2			
kollotex										
1	84015	173.9873	69.04967	70146	30.60471	12.14597	28217	47.38212	18.80436	251.9742
2	76064	157.5215	71.83725	62568	27.29843	12.44937	20519	34.4556	15.71338	219.2756
3	99836	206.7512	73.79047	90851	39.63831	14.1471	20127	33.79735	18.80436	280.1868
AVERAGE	86638.33	179.42	71.55913	74521.67	32.51382	12.91415	22954.33	38.54503	17.77403	250.4788
elvanol										
1	91853	190.2191	56.85823	32806	14.31326	4.278365	77428	130.0175	38.86341	334.5498
2	89302	184.9362	62.95314	22979	10.02574	3.412809	58841	98.80609	33.63405	293.768
3	100463	208.0496	68.94391	23944	10.44677	3.461873	49589	83.27008	27.59421	301.7665
AVERAGE	93872.67	194.4016	62.91843	26576.33	11.59526	3.717682	61952.67	104.0312	33.36389	310.0281
Wax										
1	72331	149.7908	67.56525	17855	7.79014	3.513851	38183	64.11707	28.9209	221.6981
2	93243	193.0977	60.94198	21672	9.455497	2.984172	68069	114.3018	36.07385	316.8549
3	78326	162.2059	50.28622	20153	8.792757	2.725884	90261	151.5667	46.9879	322.5654
AVERAGE	81300	168.3648	59.59781	19893.33	8.679465	3.074636	65504.33	109.9952	37.32755	287.0395
Recipe 3										
1	99804	206.6849	66.78349	71373	31.14005	10.06189	42675	71.66006	23.15462	309.485
2	102013	211.2595	74.24425	78668	34.32286	12.0623	23204	38.96427	13.69345	284.5467
3	104557	216.5279	75.58624	76181	33.23778	11.60275	21855	36.69902	12.81101	286.4647
AVERAGE	102124.7	211.4908	72.20466	75407.33	32.90023	11.24231	29244.67	49.10778	16.55303	293.4988
Control										
1	95756	198.3019	61.71499	25117	10.95855	3.410492	66733	112.0584	34.87452	321.3188
2	89598	185.5492	55.86102	17200	7.504363	2.259246	82842	139.1087	41.87974	332.1622
AVERAGE	92677	191.9255	58.788	21158.5	9.231457	2.834869	74787.5	125.5835	38.37713	326.7405
Na-acet-prop control										
1	119721	247.9312	76.09733	17728	7.734729	2.374015	41771	70.14206	21.52865	325.808
2	114974	238.1006	78.28065	20288	8.851658	2.910172	34070	57.21051	18.80918	304.1627
AVERAGE	117347.5	243.0159	77.18899	19008	8.293194	2.642093	37920.5	63.67628	20.16891	314.9853

Cont. **Biogas Composition Data**

2-Aug SAMPLE	AREA CH4	VOLUME	%CH4	AREA CO2	VOLUME	%CO2	AR EA N2	VOLUME	%N2	TOT VOL
kollotex										
1	89600	185.5533	67.75787	74377	32.4507	11.84991	33256	55.84363	20.39222	273.8477
2	18920	39.18158	72.92927	8668	3.78185	7.039215	6409	10.76202	20.03152	53.72545
3	68797	142.4722	70.20541	59315	25.87914	12.75235	20596	34.5849	20.39222	202.9363
AVERAGE	59105.67	122.4024	70.29751	47453.33	20.7039	10.54716	20087	33.73019	20.27199	176.8365
elvanol										
1	83550	173.0244	59.80083	30093	13.12958	4.537858	61446	103.1804	35.66131	289.3343
2	103640	214.6289	67.20242	27016	11.78709	3.690652	55360	92.96077	29.10693	319.3768
3	87829	181.8858	66.40801	23220	10.13089	3.698872	48758	81.87466	29.89312	273.8913
AVERAGE	91673	189.8463	64.47042	26776.33	11.68252	3.975794	55188	92.67195	31.55379	294.2008
Wax										
1	88825	183.9484	65.453	22297	9.728185	3.461509	52026	87.36231	31.08549	281.0389
2	78214	161.974	58.45305	22513	9.822426	3.544709	62711	105.3046	38.00224	277.101
3	92697	191.9669	64.23706	23092	10.07504	3.371368	57646	96.79944	32.39157	298.8414
AVERAGE	86578.67	179.2964	62.71437	22634	9.875218	3.459195	57461	96.48878	33.82643	285.6604
Recipe 3										
1	83089	172.0697	68.86295	59179	25.81981	10.33319	30957	51.98314	20.80386	249.8726
2	74910	155.1317	71.42992	55638	24.27487	11.17729	22495	37.77371	17.39279	217.1803
3	93195	192.9983	75.30256	72740	31.73647	12.38269	18796	31.56233	12.31475	256.2971
AVERAGE	83731.33	173.3999	71.86515	62519	27.27705	11.29772	24082.67	40.43973	16.83713	241.1167
Control										
1	92632	191.8323	64.57098	30134	13.14747	4.425453	54852	92.10774	31.00357	297.0875
2	84513	175.0186	67.18049	22776	9.937173	3.81436	45000	75.56421	29.00515	260.52
3	102942	213.1834	66.51948	27210	11.87173	3.704328	56829	95.42753	29.77619	320.4827
AVERAGE	93362.33	193.3448	66.09032	26706.67	11.65212	3.98138	52227	87.69983	29.9283	292.6967
Na-acet-prop control										
1	92561	191.6853	67.34296	21159	9.231675	3.243276	49859	83.72347	29.41376	284.6404
2	113081	234.1803	75.11614	24870	10.85079	3.480519	39737	66.72656	21.40334	311.7577
3	107949	223.5524	79.83857	21064	9.190227	3.282159	28146	47.2629	16.87927	280.0056
AVERAGE	104530.3	216.4727	74.09922	22364.33	9.757563	3.335318	39247.33	65.90431	22.56546	292.1346

APPENDIX G
CO-DIGESTION OF TEXTILE EFFLUENT 1: SHONGWENI LANDFILL
LEACHATE

G. SERUM BOTTLES FOR CO-DIGESTION

G.1 Toxicity Assay Data

4 ml	Average	Cumulative	Average	Cumulative	Average	Cumulative	Average	Cumulative	Cumulative
Time (h)	0,5 :1		1,0 :1		2,0 :1		3,0 :1		control
0	0	0	0	0	0	0	0	0	0
22	12.67	12.67	11.33	11.33	11.33	11.33	10	10	12.67
46	12.67	25.34	11.67	23	10.5	21.83	9.3	19.3	21.34
70	12	37.34	13	36	14.67	36.5	7.67	26.97	36.67
96	9.33	46.67	12.67	48.67	9.67	46.17	9.33	36.3	46
144	8	54.67	8	56.67	8	54.17	7.67	43.97	53.33
169	3.3	57.97	3.3	59.97	3	57.17	3.3	47.27	56.30
Rates		0.53		0.51		0.47		0.38	0.460

10 ml	Average	Cumulative	Average	Cumulative	Average	Cumulative	Average	Cumulative	Cumulative
Time (h)	0,5 :1		1,0 :1		2,0 :1		3,0 :1		Control
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22.00	13.30	13.30	12.67	12.67	13.30	13.30	10.33	10.33	12.67
46.00	11.33	24.63	9.00	21.67	7.00	20.30	10.00	20.33	21.34
70.00	11.67	36.30	10.50	32.17	13.67	33.97	11.00	31.33	36.67
96.00	16.67	52.97	9.67	41.84	10.33	44.30	10.33	41.66	46.00
144.00	7.67	60.64	7.67	49.51	8.00	52.30	7.67	49.33	53.33
169.00	3.30	63.94	3.67	53.18	3.30	55.60	3.30	52.63	56.33
Rates		0.51		0.47		0.46		0.43	0.4600

20 ml	Average	Cumulative	Average	Cumulative	Average	Cumulative	Average	Cumulative	Cumulative
Time (h)	0,5 :1		1,0 :1		2,0 :1		3,0 :1		Control
0	0	0	0	0	0	0	0	0	0
22	12	12	14.33	14.33	10.67	10.67	13	13	12.67
46	13	25	12.67	27	10	20.67	6.67	19.67	21.34
70	11.67	36.67	13	40	16.33	37	17.67	37.34	36.67
96	13	49.67	11.33	51.33	10.67	47.67	12.67	50.01	46
144	8.33	58	7.33	58.66	7.67	55.34	13.67	63.68	53.33
169	3.67	61.67	3	61.66	3	58.34	5.3	68.98	56.33
Rate mL/h		0.53		0.53		0.52		0.51	0.46006

Cont Toxicity Assay Data

40 ml	Average	Cumulative	Average	Cumulative	Average	Cumulative	Average	Cumulative	cumulati
Time (h)	0,5 :1		1,0 :1		2,0 :1		3,0 :1	Control	
0	0	0	0	0	0	0	0	0	0
22	7.67	7.67	11.33	11.33	8	8	12.33	12.33	12.67
46	8.67	16.34	13.5	24.83	6.5	14.5	11.33	23.66	21.34
70	14	30.34	8	32.83	21	35.5	17.33	40.99	36.67
96	12.33	42.67	12.67	45.5	11.67	47.17	15.67	56.66	46
144	12	54.67	8.33	53.83	8	55.17	10	66.66	53.33
169	4	58.67	3.67	57.5	3	58.17	3	69.66	56.33
Rate mL/h		0.36		0.54		0.31		0.46007	0.46

G.2 Biodegradability Tests Data.

14-Aug 11:00					15-Aug 9:00						
0 hrs	RATIOS	0.5:1	1:01	2:01	3:01	22 hrs	RATIOS	0.5:1	1:01	2:01	3:01
	1	0	0	0	0		1	12	11	14	12
	2	0	0	0	0		2	13	11	12	11
	3	0	0	0	0		3	13	12	7	7
		0	0	0	0			12.67	11.33	11.00	10.00
	10 ml						10 ml				
	1	0	0	0	0		1	13	15	14	12
	2	0	0	0	0		2	14	10	12	10
	3	0	0	0	0		3	13	13	14	9
		0	0	0	0			13.33	12.67	13.33	10.33
	20 ml						20 ml				
	1	0	0	0	0		1	12	15	12	11
	2	0	0	0	0		2	12	14	8	11
	3	0	0	0	0		3	12	14	12	17
		0	0	0	0			12.00	14.33	10.67	13.00
	40 ml						40 ml				
	1	0	0	0	0		1	7	12	10	14
	2	0	0	0	0		2	7	12	9	13
	3	0	0	0	0		3	9	10	5	10
		0	0	0	0			7.67	11.33	8.00	12.33
	control						control				
	1	0	0	0	0		1	10	0	0	0
	2	0	0	0	0		2	14	0	0	0
	3	0	0	0	0		3	14	0	0	0
		0.00	0.00	0.00	0.00			12.67	0.00	0.00	0.00
16-Aug 8:45					17-Aug 9:00						
46 hrs	RATIOS	0.5:1	1:01	2:01	3:01	70 hrs	RATIOS	0.5:1	1:01	2:01	3:01
	1	13	13	6	8		1	12	13	12	8
	2	13	12	10.5	11		2	12	14	24	12
	3	12	10	15	9		3	12	12	8	3
		12.67	11.67	10.50	9.33			12.00	13.00	14.67	7.67
	10 ml						10 ml				
	1	12	9	7	10		1	10	10.5	14	11
	2	10	9	4	10		2	12	11	13	14
	3	12	9	10	10		3	13	10	14	8
		11.33	9.00	7.00	10.00			11.67	10.50	13.67	11.00
	20 ml						20 ml				
	1	13	14	8	1		1	9	14	18	18
	2	14	12	12	6		2	14	12	17	24
	3	12	12	10	13		3	12	13	14	11
		13.00	12.67	10.00	6.67			11.67	13.00	16.33	17.67
	40 ml						40 ml				
	1	13	14	6.5	13		1	15	6	24	18
	2	3	13.5	6	10		2	14	10	21	16
	3	10	13	7	11		3	13	8	18	18
		8.67	13.50	6.50	11.33			14.00	8.00	21.00	17.33
	control						control				
	1	10	0	0	0		1	12	0	0	0
	2	8	0	0	0		2	14	0	0	0
	3	8	0	0	0		3	20	0	0	0
		8.67	0.00	0.00	0.00			15.33	0.00	0.00	0.00

Cont. Biodegradability Tests Data.

18-Aug sat					20-Aug mon						
96 hrs	RATIOS	0.5:1	1:01	2:01	3:01	144 hrs	RATIOS	0.5:1	1:01	2:01	3:01
	1	10	13	9	8		1	8	8	8	7
	2	9	12	10	11		2	8	8	8	8
	3	9	13	10	9		3	8	8	8	8
		9.33	12.67	9.67	9.33			8.00	8.00	8.00	7.67
	10 ml						10 ml				
	1	11	9	11	10		1	7	8	8	8
	2	11	10	10	10		2	8	7	8	8
	3	10	10	10	11		3	8	8	8	7
		10.67	9.67	10.33	10.33			7.67	7.67	8.00	7.67
	20 ml						20 ml				
	1	13	12	14	11		1	8	8	9	7
	2	14	11	10	11		2	9	7	7	14
	3	12	11	8	16		3	8	7	7	20
		13.00	11.33	10.67	12.67			8.33	7.33	7.67	13.67
	40 ml						40 ml				
	1	12	13	12	14		1	14	9	8	9
	2	13	12	11	19		2	12	8	8	12
	3	12	13	12	14		3	10	8	8	9
		12.33	12.67	11.67	15.67			12.00	8.33	8.00	10.00
	control						control				
	1	9	0	0	0		1	8	0	0	0
	2	9	0	0	0		2	7	0	0	0
	3	10	0	0	0		3	7	0	0	0
		9.33	0.00	0.00	0.00			7.33	0.00	0.00	0.00
21-Aug 10:00					22-Aug 10:30						
tue	RATIOS	0.5:1	1:01	2:01	3:01	wed	RATIOS	0.5:1	1:01	2:01	3:01
169 hrs	1	3	4	3	4	194 hrs	1	2	3	2	2
	2	4	3	3	3		2	2	2	2	2
	3	3	3	3	3		3	2	2	2	3
		3.33	3.33	3.00	3.33			2.00	2.33	2.00	2.33
	10 ml						10 ml				
	1	3	4	3	3		1	3	2	3	2
	2	4	4	3	4		2	2	3	2	2
	3	3	3	4	3		3	2	2	2	2
		3.33	3.67	3.33	3.33			2.33	2.33	2.33	2.00
	20 ml						20 ml				
	1	4	3	3	3		1	2	2	2	2
	2	3	3	3	5		2	3	2	2	3
	3	4	3	3	8		3	2	2	2	4
		3.67	3.00	3.00	5.33			2.33	2.00	2.00	3.00
	40 ml						40 ml				
	1	4	4	3	2		1	2	2	2	2
	2	4	4	3	4		2	2	2	2	3
	3	4	3	3	3		3	2	2	3	2
		4.00	3.67	3.00	3.00			2.00	2.00	2.33	2.33
	control						control				
	1	3	0	0	0		1	2	0	0	0
	2	3	0	0	0		2	2	0	0	0
	3	3	0	0	0		3	2	0	0	0
		3.00						2.00			

Cont. Biodegradability Tests Data.

29-Aug						30-Aug									
RATIOS						RATIOS									
	0.5:1	1:01	2:01	3:01		0.5:1	1:01	2:01	3:01		0.5:1	1:01	2:01	3:01	
350 hrs	1	2	2	2	2	374 hrs	1	2	2	2	2	2	2	2	
	2	2	2	2	2		2	2	2	2	2	2	2	2	
	3	2	2	2	2		3	2	2	2	2	2	2	2	
		2.00	2.00	2.00	2.00			2.00	2.00	2.00	2.00	2.00	2.00	2.00	
10 ml	1	2	2	2	2	10 ml	1	2	2	2	2	2	2	2	
	2	1	1	2	2		2	2	2	2	2	2	2	2	
	3	1	2	2	2		3	2	2	2	2	2	2	2	
		1.33	1.67	2.00	2.00			2.00	2.00	2.00	2.00	2.00	2.00	2.00	
20 ml	1	1	2	2	3	20 ml	1	1	2	2	2	2	2	2	
	2	1	2	2	2		2	3	2	2	2	2	2	2	
	3	1	2	2	2		3	1	2	2	2	2	4	2	
		1.00	2.00	2.00	2.33			1.67	2.00	2.00	2.00	2.67	2.00	2.00	
40 ml	1	1	2	2	2	40 ml	1	2	2	2	2	2	2	2	
	2	1	2	2	2		2	2	2	2	2	2	2	2	
	3	1	2	2	2		3	2	2	2	2	2	2	2	
		1.00	2.00	2.00	2.00			2.00	2.00	2.00	2.00	2.00	2.00	2.00	
control	1	2	0	0	0	control	1	2	0	0	0	0	0	0	
	2	1	0	0	0		2	2	0	0	0	0	0	0	
	3	1	0	0	0		3	2	0	0	0	0	0	0	
		1.33						2.00							
31-Aug						3-Sep									
RATIOS						RATIOS									
	0.5:1	1:01	2:01	3:01			0.5:1	1:01	2:01	3:01		0.5:1	1:01	2:01	3:01
400 hrs	1	2	2	2	2	mon	1	0	0	0	0	0	0	0	0
	2	2	2	2	2		2	0	0	0	0	0	0	0	0
	3	2	2	2	2		3	0	0	0	0	0	0	0	0
		2.00	2.00	2.00	2.00			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
10 ml	1	2	2	2	2	10 ml	1	2	2	2	2	2	2	2	
	2	2	2	2	2		2	2	2	2	2	2	2	2	
	3	2.00	2.00	2.00	2.00		3	2	2	2	2	2	2	2	
		2.00	2.00	2.00	2.00			2.00	2.00	2.00	2.00	2.00	2.00	2.00	
20 ml	1	2	2	2	2	20 ml	1	2	2	2	2	2	2	2	
	2	2	2	2	2		2	2	2	2	2	2	2	2	
	3	2	2	2	2		3	2	2	2	2	2	2	2	
		2.00	14.33	10.67	13.00			2.00	14.33	10.67	13.00	2.00	14.33	10.67	13.00
40 ml	1	2	2	2	2	40 ml	1	2	2	2	2	2	2	2	
	2	2	2	2	2		2	2	2	2	2	2	2	2	
	3	2	2	2	2		3	2.00	2	2	2	2	2	2	
		2.00	11.33	8.00	12.33			2.00	11.33	8.00	12.33	2.00	11.33	8.00	12.33
control	1	1	0	0	0	control	1	2	0	0	0	0	0	0	
	2	2	0	0	0		2	2	0	0	0	0	0	0	
	3	2.00	0	0	0		3	2	0	0	0	0	0	0	
		1.67						2.00							

Cont. Biodegradability Tests Data.

4-Sep					5-Sep						
RATIOS	0.5:1	1:01	2:01	3:01	RATIOS	0.5:1	1:01	2:01	3:01		
1	12	11	14	12	1	12	11	14	12		
2	13	11	12	11	2	13	11	12	11		
3	13	12	7	7	3	13	12	7	7		
	12.67	11.33	11.00	10.00		12.67	11.33	11.00	10.00		
10 ml					10 ml						
1	13	15	14	12	1	13	15	14	12		
2	14	10	12	10	2	14	10	12	10		
3	13	13	14	9	3	13	13	14	9		
	13.33	12.67	13.33	10.33		13.33	12.67	13.33	10.33		
20 ml					20 ml						
1	12	15	12	11	1	12	15	12	11		
2	12	14	8	11	2	12	14	8	11		
3	12	14	12	17	3	12	14	12	17		
	12.00	14.33	10.67	13.00	0.00	12.00	14.33	10.67	13.00		
40 ml					40 ml						
1	7	12	10	14	1	7	12	10	14		
2	7	12	9	13	2	7	12	9	13		
3	9	10	5	10	3	9	10	5	10		
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33		
control					control						
1	7	12	10	14	1	7	12	10	14		
2	7	12	9	13	2	7	12	9	13		
3	9	10	5	10	3	9	10	5	10		
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33		
6-Sep					7-Sep						
thursd	RATIOS	0.5:1	1:01	2:01	3:01	frid	RATIOS	0.5:1	1:01	2:01	3:01
	1	12	11	14	12		1	12	11	14	12
	2	13	11	12	11		2	13	11	12	11
	3	13	12	7	7		3	13	12	7	7
		12.67	11.33	11.00	10.00			12.67	11.33	11.00	10.00
	10 ml						10 ml				
	1	13	15	14	12		1	13	15	14	12
	2	14	10	12	10		2	14	10	12	10
	3	13	13	14	9		3	13	13	14	9
		13.33	12.67	13.33	10.33			13.33	12.67	13.33	10.33
	20 ml						20 ml				
	1	12	15	12	11		1	12	15	12	11
	2	12	14	8	11		2	12	14	8	11
	3	12	14	12	17		3	12	14	12	17
		12.00	14.33	10.67	13.00			12.00	14.33	10.67	13.00
	40 ml						40 ml				
	1	7	12	10	14		1	7	12	10	14
	2	7	12	9	13		2	7	12	9	13
	3	9	10	5	10		3	9	10	5	10
		7.67	11.33	8.00	12.33			7.67	11.33	8.00	12.33
	control						control				
	1	7	12	10	14		1	7	12	10	14
	2	7	12	9	13		2	7	12	9	13
	3	9	10	5	10		3	9	10	5	10
		7.67	11.33	8.00	12.33			7.67	11.33	8.00	12.33

Cont. Biodegradability Tests Data.

10-Sep					11-Sep				
RATIOS	0.5:1	1:01	2:01	3:01	RATIOS	0.5:1	1:01	2:01	3:01
1	12	11	14	12	1	12	11	14	12
2	13	11	12	11	2	13	11	12	11
3	13	12	7	7	3	13	12	7	7
	12.67	11.33	11.00	10.00		12.67	11.33	11.00	10.00
10 ml					10 ml				
1	13	15	14	12	1	13	15	14	12
2	14	10	12	10	2	14	10	12	10
3	13	13	14	9	3	13	13	14	9
	13.33	12.67	13.33	10.33		13.33	12.67	13.33	10.33
20 ml					20 ml				
1	12	15	12	11	1	12	15	12	11
2	12	14	8	11	2	12	14	8	11
3	12	14	12	17	3	12	14	12	17
	12.00	14.33	10.67	13.00		12.00	14.33	10.67	13.00
40 ml					40 ml				
1	7	12	10	14	1	7	12	10	14
2	7	12	9	13	2	7	12	9	13
3	9	10	5	10	3	9	10	5	10
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33
control					control				
1	7	12	10	14	1	7	12	10	14
2	7	12	9	13	2	7	12	9	13
3	9	10	5	10	3	9	10	5	10
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33
12-Sep					13-Sep				
wed	RATIOS				thurs	RATIOS			
	0.5:1	1:01	2:01	3:01		0.5:1	1:01	2:01	3:01
1	12	11	14	12	1	12	11	14	12
2	13	11	12	11	2	13	11	12	11
3	13	12	7	7	3	13	12	7	7
	12.67	11.33	11.00	10.00		12.67	11.33	11.00	10.00
10 ml					10 ml				
1	13	15	14	12	1	13	15	14	12
2	14	10	12	10	2	14	10	12	10
3	13	13	14	9	3	13	13	14	9
	13.33	12.67	13.33	10.33		13.33	12.67	13.33	10.33
20 ml					20 ml				
1	12	15	12	11	1	12	15	12	11
2	12	14	8	11	2	12	14	8	11
3	12	14	12	17	3	12	14	12	17
	12.00	14.33	10.67	13.00		12.00	14.33	10.67	13.00
40 ml					40 ml				
1	7	12	10	14	1	7	12	10	14
2	7	12	9	13	2	7	12	9	13
3	9	10	5	10	3	9	10	5	10
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33
control					control				
1	7	12	10	14	1	7	12	10	14
2	7	12	9	13	2	7	12	9	13
3	9	10	5	10	3	9	10	5	10
	7.67	11.33	8.00	12.33		7.67	11.33	8.00	12.33

Cont. Biodegradability Tests Data.

14-Sep					
	RATIOS	0.5:1	1:01	2:01	3:01
	1	12	11	14	12
	2	13	11	12	11
	3	13	12	7	7
		12.67	11.33	11.00	10.00
	10 ml				
	1	13	15	14	12
	2	14	10	12	10
	3	13	13	14	9
		13.33	12.67	13.33	10.33
	20 ml				
	1	12	15	12	11
	2	12	14	8	11
	3	12	14	12	17
		12.00	14.33	10.67	13.00
	40 ml				
	1	7	12	10	14
	2	7	12	9	13
	3	9	10	5	10
		7.67	11.33	8.00	12.33
	control				
	1	7	12	10	14
	2	7	12	9	13
	3	9	10	5	10
		7.67	11.33	8.00	12.33

G.3

Biogas Composition Data : Co-digestion

16-Aug	AREA CH4	VOLUME CH4	%CH4	AREA CO2	VOLUME CO2	%CO2	AREA N2	VOLUME N2	%N2	TOT VOL
0.5 :1										
4 ml	70662	146.334	52.95	6932	9.07294	3.28	72032	120.956	43.77	276.364
10 ml	55370	114.666	54.74	4787	6.26546	2.99	52729	88.5428	42.27	209.474
20 ml	65326	135.284	54.09	6363	8.32821	3.33	63419	106.493	42.58	250.106
40 ml	55779	115.513	45.78	7453	9.75485	3.87	75677	127.077	50.36	252.345
1:01										
4 ml	68073	140.973	48.05	6560	8.58605	2.93	85640	143.807	49.02	293.366
10 ml	59024	122.233	51.22	6112	7.99989	3.35	64550	108.393	45.42	238.628
20 ml	66196	137.086	51.66	6560	8.58605	3.24	71292	119.714	45.11	265.386
40 ml	62904	130.268	50.92	7585	9.92762	3.88	68851	115.615	45.20	255.811
2:01										
4 ml	59274	122.751	50.82	6010	7.86618	3.26	66060	110.928	45.92	241.545
10 ml	70409	145.811	53.03	6772	8.86353	3.22	71632	120.285	43.75	274.959
20 ml	63469	131.438	50.65	7662	10.0284	3.86	70294	118.038	45.49	259.505
40 ml	70991	147.016	55.71	8781	11.493	4.36	62761	105.389	39.94	263.897
3:01										
4 ml	59671	123.573	45.91	5884	7.70127	2.86	82130	137.913	51.23	269.187
10 ml	64994	134.597	50.43	8210	10.7457	4.03	72390	121.558	45.54	266.9
20 ml	72302	149.731	55.76	32279	42.2483	15.73	45576	76.5314	28.50	268.511
40 ml	62080	128.562	49.18	10897	14.2625	5.46	70635	118.611	45.37	261.435
Control										
	28144	58.2836	82.43	6367	8.33344	11.79	2434	4.08718	5.78	70.7043
Control Ace/Prop										
	62443	129.314	49.95	6440	8.42899	3.26	72148	121.151	46.80	258.894

Cont. Biogas Composition Data : Co-digestion

23-Aug	AREA	VOLUME%	CH4	AREA	VOLUME%	CO2	AREA	VOLUME%	N2	TOT VOL
	CH4			CO2			N2			
0.5 :1										
4 ml	87726	181.672	64.63	19298	25.2582	8.99	44161	74.1554	26.38	281.086
10 ml	98056	203.065	68.29	21358	27.9544	9.40	39499	66.3269	22.31	297.346
20 ml	106347	220.235	69.01	22471	29.4111	9.22	41387	69.4972	21.78	319.143
40 ml	107083	221.759	69.08	24407	31.9451	9.95	40073	67.2908	20.96	320.995
1:01										
4 ml	100298	207.708	75.09	2912	3.81137	1.38	38763	65.081	23.53	276.61
10 ml	100596	208.325	67.05	26490	34.8714	11.16	40322	67.7089	21.79	310.705
20 ml	119805	248.105	77.66	26028	34.0667	10.66	22208	37.2918	11.67	319.464
40 ml	103981	215.335	77.27	25400	33.2448	11.93	17926	30.1014	10.80	278.681
2:01										
4 ml	110425	228.68	79.47	24480	32.0406	11.13	16108	27.0486	9.40	287.769
10 ml	116253	240.749	79.20	31439	41.1489	13.54	13144	22.0715	7.26	303.97
20 ml	116687	241.648	80.51	25744	33.695	11.23	14779	24.817	8.27	300.16
40 ml	106418	220.382	63.70	26744	35.0039	10.12	53945	90.5847	26.18	345.97
3:01										
4 ml	94362	195.415	60.79	19467	25.4794	7.93	59898	100.581	31.29	321.475
10 ml	113120	234.261	73.29	26790	35.0641	10.97	29973	50.3308	15.75	319.656
20 ml	88019	182.279	66.60	48784	63.8508	23.33	16411	27.5574	10.07	273.688
40 ml	111739	231.401	76.07	28984	37.9357	12.47	20748	34.8401	11.45	304.177
Control										
	37246.33	77.13373	76.07	9661.333	12.64523	12.47	6916	11.61338	11.45	101.392
Control Ace/Prop										
	64607	133.795	56.52	13484	17.6485	7.46	50784	85.2767	36.02	236.72

Cont. Biogas Composition Data : Co-digestion

28-Aug	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AREA	VOLUME	%N2	TOT VOL
	CH4			CO2			N2			
0.5:1										
	72112	149.337	78.38	17964	23.5122	12.34	10530	17.682	9.28	190.632
4 ml	75291	155.9	80.79	18062	23.6404	12.25	7997	13.4286	6.96	192.989
AVE	73696.5	152.619	79.58	18013	23.6763	12.30	9263.5	15.5553	8.12	191.75
10 ml	74198	153.857	80.29	19903	26.05	13.61	8955	11.8789	6.10	181.388
	87822	140.039	79.40	14904	19.5071	11.06	10021	16.8273	9.54	176.373
AVE	70910	146.848	79.84	17403.5	22.7786	12.34	8488	14.2531	7.82	183.88
20 ml	73933	153.108	80.95	19227	25.1652	13.31	8470	10.8645	5.74	189.138
	87593	139.979	79.22	15283	19.977	11.31	9974	16.7484	9.48	176.704
AVE	70763	146.544	80.08	17245	22.5711	12.31	8222	13.8064	7.61	182.921
40 ml	76061	157.515	77.62	24041	31.466	15.51	8306	13.9475	6.87	202.929
	80687	187.054	80.73	20140	26.3602	12.74	8045	13.5092	6.53	208.923
AVE	78384	162.285	79.18	22090.5	28.9131	14.12	8175.5	13.7283	6.70	204.926
1:01										
4 ml	80212	186.112	78.42	21880	28.3758	13.40	10318	17.326	8.18	211.814
	88178	182.609	82.28	20264	26.5225	11.95	7628	12.809	5.77	221.94
AVE	84195	174.36	80.35	20972	27.4492	12.67	8973	15.0675	6.98	216.877
10 ml	88151	141.134	79.85	18227	21.2387	11.99	8821	14.8123	8.36	177.185
	80023	165.72	78.00	18563	24.2982	11.44	13363	22.4392	10.56	212.458
AVE	74087	153.427	78.83	17395	22.7674	11.71	11092	18.6257	9.46	194.821
20 ml	81978	169.769	81.62	19827	25.9606	12.48	7319	12.2901	5.91	208.01
	74029	153.307	78.96	18732	24.5174	12.63	9730	16.3387	8.41	194.183
AVE	78003.5	161.538	80.29	19279.5	25.234	12.55	8524.5	14.3144	7.16	201.088
40 ml	70938	146.908	81.46	17929	23.3355	12.94	8019	10.1071	5.60	180.349
	83519	172.96	78.03	20777	27.194	12.27	12801	21.4955	9.70	221.85
AVE	77228.5	159.933	79.74	19303	25.2647	12.60	9410	15.8013	7.65	200.999
2:01										
4 ml	87554	139.898	69.67	15345	20.0843	10.00	24318	40.8315	20.33	200.814
	98509	204.003	72.82	23031	30.1441	10.76	27399	46.0085	16.42	280.156
AVE	83031.5	171.951	71.24	19188	25.1142	10.38	25857.5	43.42	18.38	240.485
10 ml	105093	217.638	79.61	25314	33.1322	12.12	13471	22.6206	8.27	273.391
	84988	176.002	72.20	18901	24.7386	10.15	25624	49.0279	17.65	243.789
AVE	95040.5	196.82	75.90	22107.5	28.9354	11.13	19547.5	32.8243	12.96	258.58
20 ml	81420	188.613	76.46	20326	26.6037	12.06	15065	25.2972	11.47	220.514
	77631	160.767	77.47	17755	23.2386	11.20	14001	23.5105	11.33	207.518
AVE	79525.5	164.69	76.97	19040.5	24.9211	11.63	14533	24.4039	11.40	214.015
40 ml	90978	188.407	78.33	24939	32.8414	13.57	11806	19.4889	8.10	240.537
	96518	199.88	78.05	24319	31.8298	12.43	14528	24.3921	9.52	256.102
AVE	93748	194.143	78.19	24629	32.2356	13.00	13066	21.9405	8.81	248.32
3:01										
4 ml	73786	152.804	61.90	17148	22.4441	9.09	42849	71.6184	29.01	246.885
	99216	205.467	80.10	22331	29.2279	11.39	12985	21.8045	8.50	256.5
AVE	86501	179.136	71.00	19739.5	25.836	10.24	27817	46.7104	18.76	251.682
10 ml	71716	148.517	80.03	18214	23.8394	12.85	7878	13.2288	7.13	185.585
	87122	160.422	79.20	21848	28.5957	12.55	11180	18.7735	8.24	227.791
20 ml	75839	157.056	70.29	46089	60.3235	27.00	3804	6.05185	2.71	223.431
	71716	148.517	69.79	39004	51.0504	23.99	7878	13.2288	6.22	212.796
AVE	73777.5	152.786	70.04	42546.5	55.6869	25.49	5741	9.64031	4.46	218.114
40 ml	83501	172.923	75.46	34971	45.7718	19.97	8233	10.4665	4.57	229.161
	71716	148.517	74.75	28214	36.9279	18.59	7878	13.2288	6.66	198.674
AVE	77608.5	160.72	75.11	31592.5	41.3498	19.28	7055.5	11.8476	5.61	213.917
Control	74387	154.007	62.30	21373	27.974	11.32	38948	65.2337	26.39	247.215
	84333	133.228	64.67	28984	37.9357	16.42	20748	34.8401	16.91	206.004
AVE	69350	143.617	63.48	25178.5	32.9549	14.87	29798	50.0389	21.65	226.609
Control Ace/Prop										
	84145	132.838	68.06	20833	27.0055	13.84	21041	35.3321	18.10	195.178
	72588	150.278	76.05	15725	20.5817	10.42	15932	26.7531	13.54	197.612
AVE	68355.5	141.558	72.05	18179	23.7936	12.13	18486.5	31.0426	15.82	196.394

Cont. Biogas Composition Data : Co-digestion

GAS COMPOSITION FOR SIZE : SHONGWENI LEACHATE AT 2:1 RATIO										
22-Aug	AREA	VOLUME	%CH4	AREA	VOLUME	%CO2	AREA	VOLUME	%N2	TOT VOL
	CH4			CO2			N2			
Size : Shongweni										
10 ml	39990	82.8158	49.74	7579	9.91977	5.96	43933	73.7725	44.31	166.508
	56711	117.443	52.89	15446	20.2185	9.11	50247	84.375	38.00	222.035
	56722	117.466	53.92	14001	18.3252	8.41	48870	82.0627	37.67	217.854
	51141	105.9083	52.18	12342	16.15382	7.82	47683.33	80.07008	39.99	202.1322
20 ml	69849	144.651	54.07	17942	23.4834	8.78	59188	99.3888	37.15	267.523
	68116	141.062	48.42	25669	33.5968	11.53	69464	116.644	40.04	291.303
	56171	116.325	49.20	16122	21.1013	8.92	58963	99.0109	41.88	236.437
	64712	134.0126	50.56	19911	26.0605	9.75	62538.33	105.0147	39.69	265.0878
Na-Ace/Prop : Shongweni										
10 ml	119180	246.811	86.26	16545	21.6549	7.57	10521	17.6689	6.17	286.133
	115999	240.223	82.25	16310	21.3473	7.31	18153	30.4826	10.44	292.053
	118561	245.529	87.11	16817	22.0109	7.81	8533	14.3287	5.08	281.868
	117913.3	244.1877	85.21	16557.33	21.67105	7.56	12402.33	20.82606	7.23	286.6848
20 ml	120037	248.586	76.05	15783	20.6578	6.32	34316	57.6236	17.63	326.867
	112029	232.002	88.28	13796	18.0569	6.87	7585	12.7368	4.85	262.795
	104070	215.519	80.63	10885	14.2468	5.33	22343	37.5185	14.04	267.285
	112045.3	232.0356	81.66	13488	17.65376	6.17	21414.67	35.95961	12.17	285.6489
Control	44465	92.0829	35.37	12182	15.9444	6.13	90690	152.287	58.50	260.314
	67276	139.322	48.06	16660	21.8054	7.52	76675	128.753	44.42	289.881
	51922	107.526	40.22	12758	16.8983	6.25	85227	143.114	53.53	287.338
Ave	54554.33	112.977	41.22	13866.67	18.14937	6.63	84197.33	141.3846	52.15	272.5109

Cont. Biogas Composition Data : Co-digestion

31-Aug	AREA CH4	VOLUME%CH4	AREA CO2	VOLUME%CO2	AREA N2	VOLUME%N2	TOT VOL			
Size : Shongweni										
10 ml	60580	125.456	62.23	18200	23.8211	11.82	31152	52.3106	25.95	201.587
	63256	130.997	62.80	19174	25.0959	12.03	31263	52.497	25.17	208.59
	63256	130.9973	62.52	19174	25.09587	11.92	31263	52.49698	25.56	208.5902
20 ml	82126	170.075	63.64	19539	25.5736	9.57	42636	71.5948	26.79	267.244
	69024	142.942	65.20	24799	32.4581	14.81	26103	43.8323	19.99	219.233
	69024	142.9423	64.42	24799	32.45815	12.19	26103	43.83228	23.39	219.2328
Na-Ace/Prop : Shongweni										
10 ml	89137	184.595	81.72	22632	29.6219	13.11	6954	11.6772	5.17	225.894
	83740	173.418	80.27	21169	27.707	12.82	8882	14.9147	6.90	216.04
	83740	173.4178	80.99	21169	27.70703	12.97	8882	14.9147	6.04	216.0395
20 ml	91885	190.285	84.43	22536	29.4982	13.09	3327	5.58671	2.48	225.368
	102685	212.61	88.34	19712	25.8	10.72	1344	2.25685	0.94	240.667
	97275	201.4476	86.39	21124	27.64813	11.90	2335.5	3.921783	1.71	233.0175
Control	66552	137.823	65.94	19120	25.0252	11.97	27499	46.1785	22.09	209.025
	77846	161.212	65.20	25017	32.7435	13.24	31738	53.2912	21.55	247.247
Ave	72199	149.5175	65.57	22068.5	28.88434	12.61	29617.5	49.73385	21.82	228.1357

Cont. Biogas Composition Data : Co-digestion

27-Aug	AREA	VOLUME%	CH4	AREA	VOLUME%	CO2	AREA	VOLUME%	N2	TOT VOL
	CH4			CO2			N2			
Size : Shongweni										
10 ml	79968	185.806	59.59	40136	52.532	18.90	35587	59.7579	21.50	277.896
	80553	186.818	68.64	18119	23.715	9.76	31263	52.497	21.60	243.03
	62883	130.225	56.15	57505	75.2654	32.45	15749	26.4458	11.40	231.936
	74468	154.2164	61.46	38586.67	50.50413	20.37	27533	46.23354	18.17	250.954
20 ml	71216	147.482	61.71	22325	29.2201	12.23	37088	62.2783	26.06	238.98
	66601	137.925	62.83	18267	23.9087	10.89	34347	57.6756	26.27	219.509
	73562	152.34	60.92	21355	27.9505	11.18	41543	69.7592	27.90	250.05
	70459.67	145.9155	61.82	20649	27.02643	11.43	37659.33	63.23773	26.74	236.1796
Na-Ace/Prop : Shongweni										
10 ml	138435	286.886	71.84	33041	43.2457	10.84	41163	69.1211	17.32	399.053
	107481	222.583	71.28	22227	29.0918	9.32	38079	80.584	19.40	312.259
	88820	183.938	66.83	18386	24.0383	8.73	40048	67.2488	24.43	275.225
	111578.7	231.0691	69.99	24544.67	32.12527	9.63	39096.67	65.65131	20.39	328.8457
20 ml	119154	246.757	86.23	15135	19.8094	6.92	11865	19.5879	6.85	286.154
	118816	241.915	90.57	16973	22.2151	8.32	1774	2.97891	1.12	267.109
	104788	217.008	90.89	13024	17.0465	7.14	2808	4.71521	1.97	238.768
	113586	235.2261	89.23	15044	19.69033	7.46	5415.667	9.094013	3.31	264.0105
	64145	132.838	68.06	20633	27.0055	13.84	21041	35.3321	18.10	195.176
	72566	150.278	76.05	15725	20.5817	10.42	15932	26.7531	13.54	197.612
AVE	68355.5	141.558	72.05	18179	23.7936	12.13	18486.5	31.0426	15.82	196.394