PROCESS DEVELOPMENT FOR CO-DIGESTION OF TOXIC EFFLUENTS:

DEVELOPMENT OF SCREENING PROCEDURES.

Sithembile Dlamini
B-Tech (Chemical Engineering)

Submitted in fulfillment of academic requirements for a degree of

Masters of Technology

In the

Department of Chemical Engineering

Durban University of Technology

September, 2009

Abstract

The primary objective of this project was to establish a **screening protocol** which could be used to access high strength/toxic effluent for toxicity and degradability prior to being disposed in wastewater treatment works.

The serum bottle method (materials and method section) is simple, makes use of small glass vials (125 mℓ-volume were used in this research) which do not require any stirring nor feeding device or other engineered tool: a serum bottle is sealed immediately after all components are poured inside and thereafter conducted in a batch mode and occasionally shaken to ensure adequate homogenisation of the components. The only variables which are regularly measured are the volume of biogas produced and gas composition. The two assays, originally developed by Owen et al. (1979) to address the toxicity and the biodegradability have been combined in a single test called AAT, Anaerobic Activity Test, which enables one to assess simultaneously the inhibitory effect on the methanogenic biomass and the biodegradability of the test material as well as the ability of the biomass to adapt to the test material and therefore to overcome the initial inhibition.

The screening protocol is illustrated in **Annexure A**. The protocol consists of a sequence of assays which employ the serum bottle methodology. A first step of the procedure is aimed at rapidly estimating whether the effluent is potentially toxic to the methanogenic biomass and in what concentration. The second step is a more extensive screening, aimed at precisely characterising the toxicity of the effluent, the extent of biodegradation that can be achieved, as well as at establishing whether a potential for adaptation of the biomass exists upon exposure. If the sample passes the screening stage, the same serum bottle method will be used to conduct a series of batch co-digestion experiments aimed at evaluating a convenient volumetric ratio between the test material and the readily biodegradable substrate. Finally, a laboratory-scale co-digestion trial could simulate the full-scale process, thus enabling the selection of appropriate operating conditions for the start-up of the full-scale implementation.

This the protocol has been used to assess the amenability to be anaerobically (co)digested of four industrial effluents, i.e. size and distillery effluents which are classified as high strength and scour and synthetic dye effluents classified as toxic. From the biodegradability and toxicity assays the following conclusions were drawn. The size and distillery effluent were found to be

degradable at 32 g COD/ℓ and 16 g COD /ℓ concentrations respectively. Concentrations higher than these stipulated above were found inhibitory. Scour effluent was found to be recalcitrant at all concentration tested and synthetic dye was 100 % degradable at 0.12 g COD/ℓ and lower and highly inhibitory at concentration higher than 1.1 g COD/ℓ.

Co-digestion experiment using serum bottle AAT method were undertaken between effluents i.e. size + distillery, size + scour, distillery + synthetic dye in an attempt to verify whether the digestion performance benefits from simultaneous presence of the two substrates. The volumetric ratios between the effluents were 1:1, 1:2, 2:1. The presence of two mixtures in the case of size and distillery had better methane production compared to individual substrate i.e. size or distillery separate. The mixture with volumetric flow rate ratio of 2:1 (size: distillery) was preferable in terms of process performance as it had highest COD removal compared to the other mixtures /ratios and individual substrates. The mixture of size and scour (2:1) had highest degradation percentage compared to other ratios but not high enough to qualify as degradable (less than 50 %). The mixture of distillery and synthetic dye had the same pattern with ratio of 2:1 giving the best COD conversion. The pattern than can be drawn from the degradability of mixtures is: the degradability of mixtures increase with the increasing amount of the most biodegradable compound/effluent in the mixture.

Serum bottle results provided the detailed information regarding the safe operating parameters which should be used during the starting point for the larger scale investigation i.e. lab-scale investigations. The lab scale investigations were conducted primarily to validate screening and monitor how the digestion progresses and also to provide data for future project i.e. pilot plant investigation. Other effluents i.e. scour and synthetic dye and their co-digestion mixture were excluded from the lab-scale investigations since they were found to be non- biodegradable i.e. their COD conversion was less the 50 % in the screening protocol. Due to time constrains and other technical difficulties in the laboratory, the co-digestion of size and distillery mixture trials we not conducted on the laboratory scale.

Laboratory-scale digestion trials showed that the best organic loading rate for distillery effluent in terms of reactor performance and stability was 1.0g COD/ℓ with efficiency of about 45 %, and for size was 2.0g COD/ℓ with an efficiency of 40 %. The efficiencies obtained in both effluents trials could be greatly improved by acclimation; however these results showed that the digestion of these effluents on the bigger scale is possible.

Preface

I, Sithembile Dlamini, declare that unless indicated, this dissertation is my own work and that it has not been submitted, in whole or in part, for a degree at another Technikon or Institution

……

Sithembile Dlamini

September, 2009

Acknowledgement

Firstly I would like to thank God for giving me the strength to finish this work, and acknowledge the following people and organizations for their help and contribution:

Prof Chris Buckley for giving the opportunity to undertake this study and trusting me to finish it

Dr E. Remigi, for his dedication, patience and leadership: Thank you for always being there Enrinco, I am extremely grateful for your influence on my work

Prof. V.L. Pillay for enabling me to finish my studies through Durban University of Technology

My fellow postgraduate students for their friendship and support

The Water Research Commission for funding the project

The Workshop staff of the Department of Chemical Engineering for helping with equipment maintenance

The Umbilo Wastewater Works for providing the samples

My two girls, Andisa and Anelisa for being my inspiration, and my husband Musa for never loosing faith in me.

Glossary

Acclimation : The adaptation of a microbial community to degrade an inherently *recalcitrant* compound through prior exposure to that compound.

Adaptation : A change in the microbial community that increases the rate of transformation of a test compound as a result of prior exposure to that test compound.

Anaerobic digestion : The microbial degradation of an organic compound in the absence of oxygen. It is affected by anaerobic micro-organisms which degrade the compound in a step-wise process, yielding organic acids, carbon dioxide and hydrogen and, ultimately, methane and carbon dioxide.

Anaerobic Toxicity Assay (ATA): It allows for the determination of the *toxic* or *inhibitory* potential of a test compound, which is reflected in a reduced initial rate of gas production (compared to a reference system, not exposed to the compound), in proportion to the relative volume of compound added.

Batch culture : A close culture environment in which conditions are continuously changing, according to the metabolic state of the microbial culture.

Biodegradability : The property of a substance, primarily dependent on the molecular structure, that refers to its susceptibility to undergo a biologically mediated degradation.

Biogas : The gas produced, principally methane and carbon dioxide, by the action of anaerobic micro-organisms on organic compounds.

Biological Methane Potential (BMP): A measure of the portion of organic material in a waste stream which can be anaerobically converted to methane. Units: g COD/ℓ (or %).

Chemical Oxygen Demand (COD): A measure of the total amount of organic material in a waste stream.

Co-digestion : Term used to describe the treatment of high-strength or toxic organic effluents with municipal sewage sludge whereby the *organic load* to an anaerobic digester can be increased by the addition of a concentrated wastewater, without compromising the overall performance of the system.

Cometabolism : Phenomenon whereby complex organic compounds which would normally persist undegraded in the environment due to their inherent toxicity and/or very low (trace) concentrations, undergo a biodegradation process in presence of an external source of energy, which acts as the *primary* substrate. This type of process generally does not result in biomass growth.

Effluent : A stream flowing from a sewage tank or industrial process.

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

Hydraulic Retention Time (HRT): Ratio between the reactor volume and the incoming flow rate. It defines the window of opportunity afforded the microbes to accomplish their task (Speece, 1996). Units: hours or days.

Inhibition : Impairment (generally, irreversible) of the bacterial functions.
See also Toxicity.

Labile : Readily biodegradable.

Methanogens : Bacteria which utilise volatile organic acids as substrate and produce methane and carbon dioxide.

Mineralisation : Microbial decomposition of an organic compound to inorganic constituents such as carbon dioxide, methane and water.

Organic load : Measure of the amount of organic matter fed to a digester.
Also: Organic Loading Rate (OLR). Units: kg COD/m^3/d or kg VS/m^3/d.

Recalcitrant : Resistant to microbial degradation.

Sludge : The general term applied to the accumulated solids segregated from waste water. A large portion of the sludge material in a digester consists of bacteria which are responsible for its decomposition and ultimately *mineralisation*.

Sludge Retention Time (SRT): Ratio between the reactor volume and the outgoing flow rate of solids. It determines which organisms can replicate and predominate and

what biomass inventory can be maintained (Speece, 1996). Units: hours or days.

Toxicity : Temporary (generally, non-lethal) adverse effect on bacterial metabolism. *See also* Inhibition.

Volumetric load : Measure of the amount of sludge fed to a digester.

Xenobiotics : (prefix; Greek *xenos*: foreign, stranger) synthetic organic chemicals or natural chemicals present in unnatural concentrations, which is 'foreign' to microrganisms and therefore they do not possess the enzymatic machinery to biodegrade it.

Table of Contents

Annexure D: Results

Annexure E: Laboratory scale results

List of Figures

List of Tables

List of Abbreviations

A list of abbreviations used throughout the report is provided in this Section.

AAT	: Anaerobic Activity Test.
ATA	: Anaerobic Toxicity Assay.
BMP	: Biological Methane Potential.
COD	: Chemical Oxygen Demand.
IC_{50}	: 50 %-Inhibitory Concentration
GC	: Gas-chromatograph.
HRT	: Hydraulic Retention Time.
OLR	: Organic Loading Rate.
SMA	: Sludge Methanogenic Activity.
SRT	: Sludge Retention Time.
VFA	: Volatile Fatty Acid

1. Introduction

1.1 WATER QUALITY IN S.A

South Africa is a region where water is a scarce resource, a situation that is exacerbated by increasing water demand due to economic and population growth. It has a low average annual rainfall of 500 mm, compared to the world average of 860mm. (Department of Water and Forestry, 1986). As a result, water across the country is unevenly distributed. It is also poorly served with natural lakes; therefore rivers are the most important source of water (DWAF, 1986). The river water quality has been impacted by the multiple uses of water. Although industry account for approximately 16 % of South Africa's direct water use, its impact is much higher because its effluents often contain toxic pollutants (Stander, 1997). The monitoring and management of water quality in river is thus vital for adequate long-term protection of South Africa's water resources.

1.2 LEGISLATION

In 1997 Department of Water and Forestry reviewed the existing 1956 Water Act. A new policy was proposed that integrated resource direct measures for protection, such as resource quality objectives, with source direct measures like effluent standards. It was proposed that there should be the development of new standards, which should be more flexible and possible, stricter than the existing standards. The preamble in National Water Act raised the following points:

- Recognizing that water is a scarce and unevenly distributed natural resources which occur in many different form, which are all part unitary, independent cycle.
- Recognizing that while water is a natural resource that belongs to all people, the discriminatory laws and practices of the past have prevented equal access to water and use of water resources.
- Acknowledging the national government's overall responsibility for and authority over the nation's water resources and their use, including the equitability allocation of water for beneficial use, the redistribution of water and international water matter.
- Recognizing that the protection of the quality of water resources is necessary to ensure the sustainability of the nation's water resources in the best interest of all water users.

- Recognizing the need for the integrated management of all aspects of water resources and where appropriate, the delegation of management function to a regional or catchments level so as to enable everyone to participate

1.3 INDUSTRIAL EFFLUENT TREATMENT

In light of the steady deterioration of water quality in rivers, the Department of Water and Forestry adopted a pollution prevention approach called **cleaner production** to control hazardous pollutant (Sacks 1997). Cleaner production is a continuous application of an integrated preventative environment strategy, applied to process, product and services to increase eco-efficiency and to reduce risk to human and environment. The promotion of **cleaner production** techniques might result in waste minimisation techniques being implemented: which will results in the identification of numerous small streams of high strength/or toxic liquid effluents. In anticipation of the move to **cleaner production** it is necessary to have pro-active strategies to deal with residual concentrates. These concentrated effluents can then be treated through biological, chemical or physical methods to reduce the pollution load in the fresh water. Currently, high strength/ or toxic industrial effluents are sent to landfill, or the marine outfall (Sutton, 1997). Marine discharge contaminates seawater and thus affects the aquatic system and co-disposal onto landfill sites may results into contamination of ground water by slippage especially in areas of high rainfall. Pre-treatment of these effluents prior to land filling is necessary because of dangers of reaction with other wastes and potential odor problems.

The organic contaminants in these effluents must be removed by appropriate treatment to render the water suitable for discharge to surface waters. **Anaerobic digestion** has been identified as one of the biological processes that can be applied to treat industrial wastewater (Speece, 1996). In anaerobic treatment the organic contaminant serve as the energy source (electron donor) in the reaction and sulphate or carbon dioxide as an electron acceptor. In the reaction the microbes metabolise the organic contaminant and utilize energy obtained there from, for cell replication and maintenance. The microbes convert some of the carbon in the organic to CO_2 and CH_4 under anaerobic conditions. These gases are subsequently stripped from the liquid phase.

Most effluents have shown to be toxic to aerobic biomass and there are numerous effluents, which may be toxic to the anaerobic biomass due to the presence of *xenobiotics* or recalcitrant

molecules. Fortunately, it has been shown that an acclimation associated with anaerobic bacteria can degrade many classes of toxic organic compound anaerobically provided the residence time is sufficiently long. The time required for *acclimation* depends on the substrate structure, the inoculum source and the environmental conditions (Speece, 1996). This degradation can be enhanced by addition of readily biodegradable organic substrate **(co-digestion/ *co-metabolism*)** (Cheng et al, 1996). The term cometabolism can be defined as the transformation of a non-growth substrate by growing microorganism in the presence of growth substrate or primary substrate, or by resting micro-organisms in the absence of growth substance (Criddle, 1993). Many cometabolic enzymes and cofactors are induced by the utilisation of the primary substrate: the secondary substrate is bio-transformed by these enzymes, but cannot be used by the biomass to support growth.The addition of a labile substrate can at times increase the capacity of an anaerobic digester as a labile substrate can overcame certain inhibiting pathways. This results in improved removal efficiencies.

Sacks, 1997, conducted a survey on anaerobic digesters in KwaZulu Natal for spare capacity. The performance efficiency of the anaerobic digesters was assessed by collating physical and operating data. Available capacity was evaluated in terms of hydraulic and organic load. Some digesters were found to be under-utilised and under performing. The conventional sewer system is only capable of diverting settleable solids and not soluble solids, to the anaerobic digestion system. Since these liquid effluents are trucked to a landfill site, it may be feasible to divert these effluents to the anaerobic digestion facilities at a conventional wastewater treatment works.

1.4 PROJECT OUTLINE

The main objectives of this project are the:

- Development of a screening procedure for the evaluation of highly toxic/high strength liquid effluents for co - disposal in conventional wastewater treatment digesters and,
- to investigate the feasibility of the co-digestion of toxic and/or high (organic) strength effluents with municipal effluent in the available capacity of existing digesting units, at wastewater treatment work-laboratory scale investigations

The concept of such a study is depicted in **Figure 1**: it is structured in the following steps:

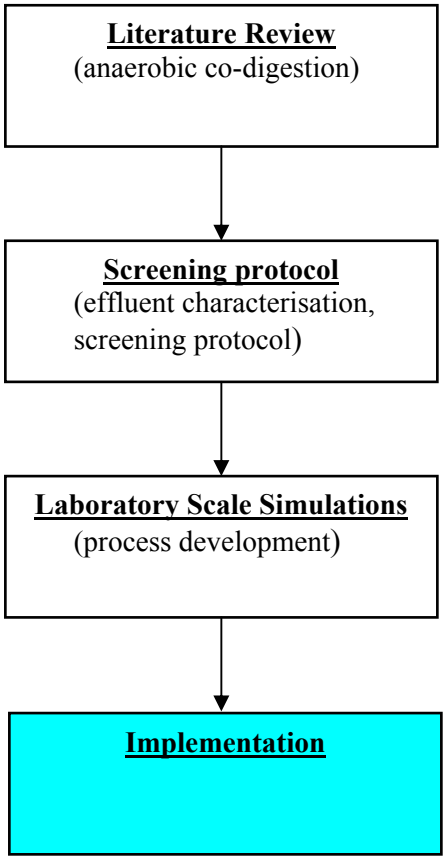


Figure 1 – Block diagram of a feasibility and implementation study for the process development for co-digestion of toxic effluent

- A review of literature was undertaken to gain familiarity with the fundamentals of anaerobic digestion such that the required technique for anaerobic test work could be mastered.
- The screening protocol was devised and four effluents classified as high strength and toxic were selected for the intended study.
- Preliminary assessment of the characteristics of the effluent, based on data available at the producer (i.e. pH, alkalinity and conductivity) was conducted and screening of effluents according to their biodegradability and toxicity potential was conducted, according to a specific protocol.
- Laboratory-scale reactor simulation i.e. investigate the feasibility of the co-digestion of toxic and/or high (organic) strength effluents with municipal effluent in the available capacity of

existing digesting units, at wastewater treatment work-laboratory scale investigations based on the data obtained from evaluation protocol was conducted

The final step is implementation of the co-digestion process, if the feasibility study was successful. This task is beyond the scope of this research. Monitoring the performance of the process is recommended, for a certain period of time after it has been implemented and in particular when the effluent has variable characteristics. This task is likely to be the responsibility of the operator; however, some guidelines will be given on how to conduct an efficient monitoring

1.5 THESIS OUTLINE

This thesis begins with a review of literature on the subject of anaerobic digestion. The information on the subject of anaerobic digestion microbiology, digesters and characterization of effluents is presented in **Chapter 2**. **Chapter 3** details the methodology of both the characterization of effluent using serum bottle study; start up and operation of CSTR lab-scale digesters used this study. In addition it describes how experimental information had been acquired and interpreted. **Chapter 4** contains the results and discussion of the experimental study. This section is divided into two parts: experiments conducted on the serum bottles and digester respectively. The thesis is concluded in **Chapter 5**. Summary of the experimental work is presented and the recommendations for future research are made.

2. Literature Review

2.1 ANAEROBIC DIGESTION

Anaerobic digestion is a process by which a wide variety of organic molecules can be converted into a gas rich in methane. In view of current problems, both in the protection of the environment and in the search for source of renewable energy, anaerobic digestion appear to be a favorable biotechnology process to treat an organic waste through bioconversion into energy (Sacks, 1997).

Anaerobic treatment is an effective method for complete treatment of many organic wastes, especially animal wastes and organic effluent from food processing industries. (Stafford *et al*. 1980). The organic substrate is degraded in the absence of oxygen to carbon dioxide and methane with only small amount of bacterial growth. Approximately 90 % of available chemical energy, in the form of organic material, is retained as methane production (McInnery *et al,* 1980). Apart from economical value of methane gas produced, anaerobic treatment has many advantages over aerobic treatment process, such as less biomass produced per unit of substrate utilized, high organic loading are possible as anaerobic treatment are not limited by oxygen transfer rate and the lower construction and operation cost compared with aerobic process.

Anaerobic digestion is commonly visualised as a biochemical chain reaction **(Figure 2)**, where the product of one process is the reactant of the next; however, in reality, it is a complex system of microbiologically mediated and physico-chemical sub-processes, with feedback loops of substrate and product regulation and inhibition. In fact, there is a relatively narrow range of environmental or operating conditions (e.g. pH and temperature) in which all the sub-processes of the digestion can function simultaneously.

2.2 ANAEROBIC MICROBIOLOGY

The process of sludge digestion is generally considered as a two-phase process, the non-methanogenic followed by a methanogenic phase. It is more accurate to describe the process of anaerobic digestion as being compromised of three discrete stages, hydrolysis and acid formation (the non-methanogenic phase), followed by methane formation (methanogenic phase). It is convenient to think of these stages as different trophic levels, and although all three stages occur simultaneously within a digester, the microorganisms involved at each stage are metabolically

dependent on each other for survival. For example, the methanogenic bacteria require the catabolized end product of the acid-forming bacteria. However the latter species would eventually

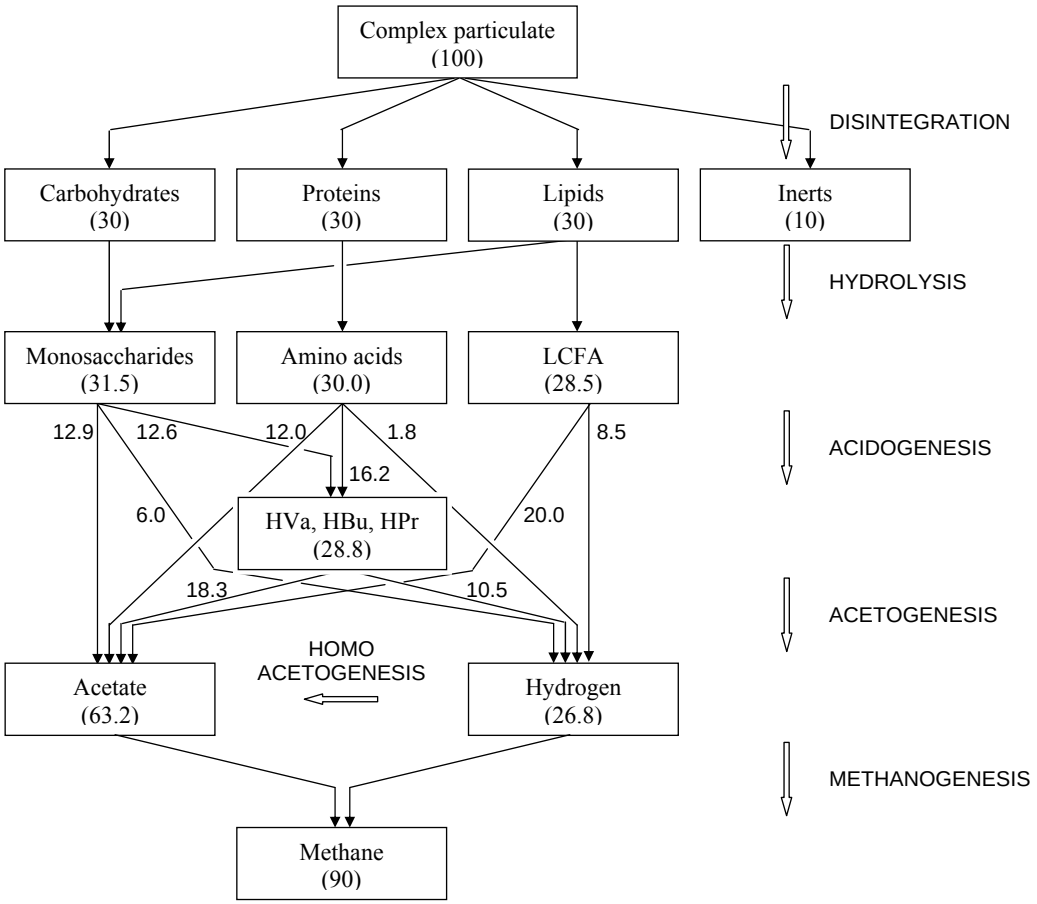


Figure 2 – Flow-diagram for the anaerobic degradation of a composite particulate material (from Batstone et al 2002).Valerate (HVa), Butyrate (HBu) and Propionate (HPr) are grouped for simplicity. Figures indicate COD fractions.

became inhibited by their own end-products if these were not degraded by the methanogenic bacteria (Gray, 1989).

In the first stage **hydrolysis**, the major substrates in the sludge are hydrolyzed to basic components; protein to amino acids, fats to glycerol and long-chain fatty acids and polysaccharides to mono- or disaccharides (Figure 2). Proteins are hydrolyzed to smaller units' polypeptide, oligopeptide or amino acids by extra-cellular enzyme called proteases, which are produced by a small proportion of the bacteria. Hydrolysis can be a slow process and can be rate

limiting in the fermentation particularly if the effluent contain particulate or large complex molecules in significant quantities. Dissolve compounds, generated in the liquification step, are taken up in the cells of fermentation bacteria and after **acidogenesis** are excreted as simple organic compounds such as volatile fatty acids, alcohols, lactic acid and mineral compounds such as carbon dioxide, hydrogen, ammonia and hydrogen sulphide gas. Acidogenic fermentation is carried out by a diverse group of bacteria most of which are obligates anaerobes. However some are facultative and can also metabolize organic matter via the oxidative pathway. This is important in the anaerobic sewage treatment, as dissolved oxygen might otherwise become toxic to obligate anaerobic organisms such as the methanogenes (van Haandel and Lettinga 1994).

The products of acidogenesis are converted into the final product for methane production: acetate, hydrogen and carbon dioxide (this stage is called **acetogenesis**). As indicated in **Figure 2**, a fraction of approximately 62 per cent of the COD originally present in the influent is converted into acetic acid and the remainder of electron donor capacity is concentrated in the formed hydrogen. Depending on the oxidation state of the original organic matter, the formation of acetic acid may be accompanied by the formation of carbon dioxide or hydrogen. This can be seen in the following reaction equations:

When $y < 2z\left(N_{el} < 4\right)$:

$$C_xH_yO_z + \frac{1}{4}\left(4x - y - 2z\right)H_2O \rightarrow \frac{1}{8}\left(4x + y - 2z\right)CH_3COOH + \frac{1}{4}\left(2z - y\right)CO_2 \qquad 1$$

When $y > 2\left(N_{el} > 4\right)$:

$$C_xH_yO_z + \left(x - z\right)H_2O \rightarrow \frac{x}{2}CH_3COOH + \frac{1}{2}\left(y - 2z\right)H_2 \qquad 2$$

In the mixture of different organic pollutant such as sewage, it is possible that both processes take place simultaneously, but generally more hydrogen than carbon dioxide is formed because the average number of electrons that are available for organic matter is generally higher than four per carbon atom. Consequently, the conversion of the influent organic matter into acetic acid is accompanied by formation of hydrogen (van Haandel and Lettinga 1994).

The final stage in anaerobic digestion is methane fermantation (**methanogenesis**) where the end product of acid fermantation are converted to gases, mainly methane and carbon dioxide, by several different species of obligates anaerobic bacteria. In the stage complete stabilization of substrate occurs and the end products are only gases. Methane is an ideal end product as it is non toxic, easily escapes from the sites of production with the use of separation process, is not very soluble, inert under anaerobic conditions, and can be readily collected and used as an energy source. In the overall anaerobic fermentation of carbohydrates to carbon dioxide and methane, equal volume of each gas are produced (Gray, 1989).

$$\left(C_6H_{12}O_6\right)_X \times xH_2O \rightarrow xC_6H_{12}O_6 \rightarrow 3xCH_4 + 3xCO_2 \qquad 3$$

This carbon dioxide evolved only escapes as gas, because, unlike methane, it is relative soluble in water. It also reacts with any hydroxide ion (OH^-) in the system to produce bicarbonate ions (HCO_3). The evolution of carbon dioxide gas is therefore a function of factors such as pH, bicarbonate concentration, temperature, and substrate composition. Anaerobic digestion and methane production are not unique to anaerobic digesters only, they also occur in natural environments including the digestive track of most animals, in the sediments of lakes and rivers and in estuaries and swamps, marshes and peat dogs (Gray, 1989).

2.3 ANAEROBIC DIGESTERS

2.3.1 Continuous stirred tank reactor (CSTR)

The **continuous stirred tank reactor** (CSTR) **(Figure 3)** contains a mechanical agitation system consisting of a vertical shaft with a number of impellors and number of baffles around the vessel perimeter. The impellor and baffle system provide an effective agitation system for the dispersion of the effluent. Mixing in anaerobic digester is advantageous as it eliminates scum and thermal stratification. It also provides good contact between the active biomass and the sludge (Pohland, 1992). The major disadvantage of complete mixing in the digester, in addition to the cost of mixing, is the need for a facility that will enhance the separation of the digester solids from the liquid phase.

For effective treatment this reactor design system requires an extended **hydraulic retention time** (HRT): it has no specific means of biomass retention, thus the **sludge retention time** (SRT) must be sufficiently high to permit biological conversion reaction to occur. The SRT and the HRT in this type of reactor are equal and tend to be protracted; high rate anaerobic treatment using the CSTR is therefore not possible. This system has however been utilized successfully for the stabilization of sewage sludge and the conversion of industrial wastewaters which contain high solid concentration such as crop residues (Morris, 1980). The CSTR reaction to unfavorable conditions has been reported to be unsatisfactory. A continuous mixed reactor with a STR of 10 days and no provision for recycle experienced failure if formaldehyde was introduced at 100mg/ℓ; the digester ceased to produce biogas after 4 days and had not recovered after 30 days (Lin Chou et al, 1979). CSTR systems are susceptible to malfunction upon shock loading and subsequent to the introduction of variety of toxic substance. Malfunction manifest itself in terms of reduced gas production, reduced degradation of organic material and simultaneous increase in acidity (Stronach et al, 1986)

CSTR systems are limited by the extended HRT's necessary for efficient waste treatment but their tolerance of high influent levels of suspended solids indicated their suitability for the stabilization of several type of wastewater.

2.3.2 Contact process

The **contact or recycle flocs process (Figure 3)** compromises a continuously-fed, completely mixed reactor stage followed by solid/liquid separation. A degasification step is frequently in the system design. The effluent is discharge from a settling device and the settled biomass returned to the digester vessel, where it is mixed with the incoming feed. (Stronach et al, 1986)

The bacteria in a contact reactor occur as suspended flocs and the system is maintained in suspension by mechanical stirring, gas sparging or recycle. Inert particle in the feedstock may act as media to convert the reactor to the carrier assisted contact process, but in general the bacteria must form flocs to remain in the system. Separation of flocs and treated wastewater occur in a separator assembly such as sedimentation tank (**Figure 3**) from which the suspended settled flocs are recycled to the reactor at moderate rates to prevent shear forces from disrupting the floc structure (Stronach et al, 1986)

In anaerobic design where flocs characteristics are important for operational performance environmental changes which tend to alter these characteristics is disadvantageous as process instability and often failure is the ultimate result. Organic overloading or large variation in loading and input of toxic substances are therefore liable to be less well tolerated in suspended floc process.

2.3.3 Up-flow anaerobic sludge blanket (UASB)

The **Up-flow anaerobic sludge blanket (UASB) (Figure 3)** are designed to treat low and medium strength wastewater at high volumetric loading rate and, therefore, at short hydraulic retention times. The most characteristic device of the UASB reactor is the phase separator. It is situated at the top of the reactor and divides it into the lower digestion zone and the upper settling zone (van Haandel and Lettinga 1994). No support medium is added to the reactor since the process is based on the immobilisation of the biomass in the form of sludge granules. Thus the success of the reactor depends on the formation of these highly flocculated granules. The granules allow the active biomass to be retained in the reactor, independent of the flow rate, thus maintaining a good conversion efficiency. The wastewater enters at the bottom of the digester and flow up through the bed of anaerobic granules sludge. The biomass of the sludge blanket convert the organic compounds to biogas.

At the top of the reactor the mixture of wastewater ,sludge and biogas is separated into its components by phase separator. The anaerobic granule is retained in the reactor and the effluent ,essentially free of suspended solids, is discharge (van Haandel and Lettinga 1994). Buffles placed beneath the apertures of gas collector units, operate as gas deflectors and prevent biogas bubbles from entering the settling zone where they would create turbulence and, consequently, hinder settling of the sludge particles (van Haandel and Lettinga 1994). Periodically, excess granular sludge can be removed and stored for use in other anaerobic treatment plant. The UASB system relies on the agitation brough about by biogas production since there is no mechanical mixing (Lettinda, 1995). The UASB is a simple processs to operate

2.3.4 Anaerobic attached –film expanded bed

The anaerobic attached –film expanded bed (AAFEB) is typical a cylindrical structure packed with inert supportive particle to about 10 % of its volume. The media may compromise sand,

gravel, anthracite or plastic (Henze et al, 1983) to which the anaerobic bacteria making up the biofilm is attached. The media particle, of slightly large diameter than those employed in the fluidized bed reactor (0.3-3 mm), are covered in the bio-film matrix and expanded by vertical fluid velocity which is generated by high degree of recycle (Stronach et al, 1986).

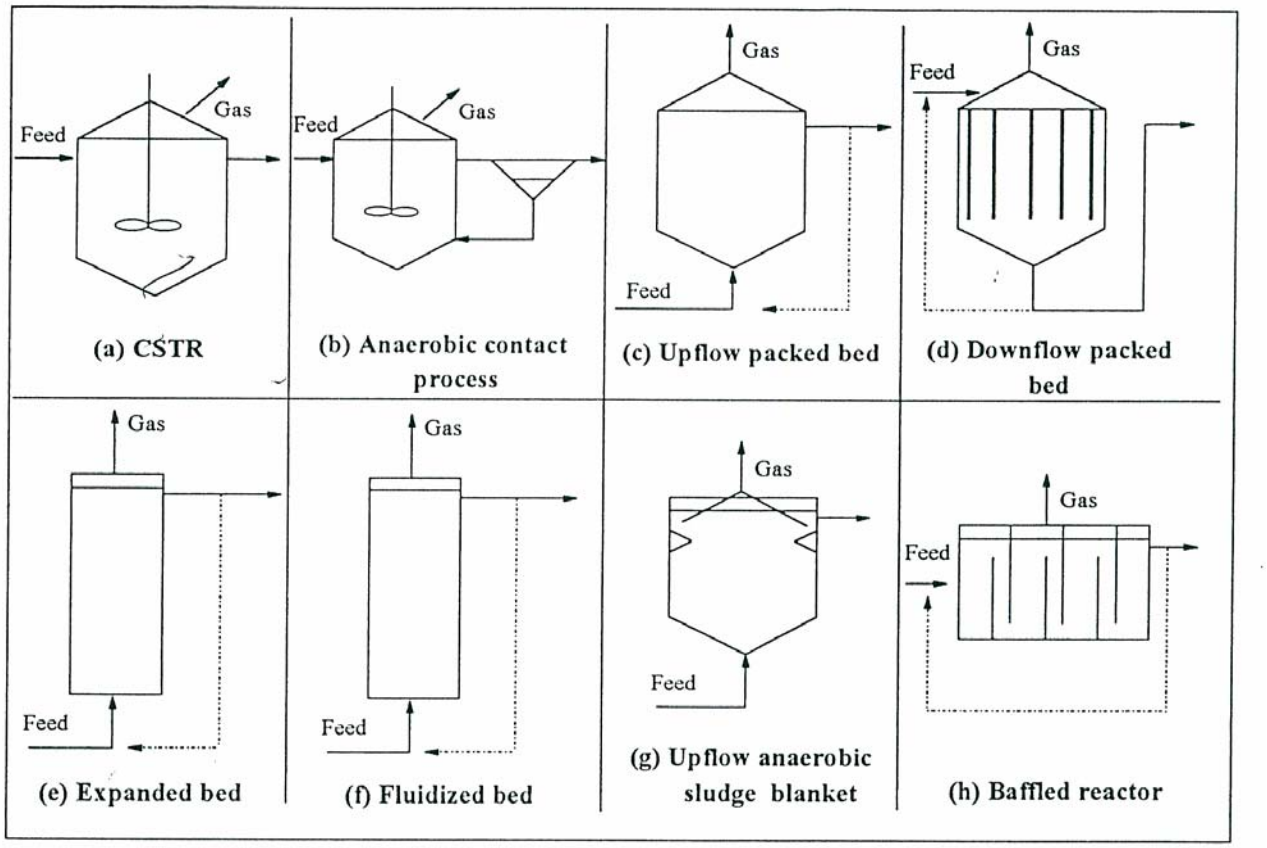


Figure 3: Schematic diagram of various anaerobic digester configuration (After: McCarty and Mosey, 1991; Speece, 1996

.

The expansion of the bed is sustained at a level at which each carrier particle retains its position relative to every other particle within the bed. A settled bed of particles under no external applied force will experience an initial expansion of 5 % when a fluid flow is applied upward through the bed; a more loosely –packed arrangement results (Cooper et al, 1981). The phenomenon of channeling may cause limited localized displacement but overall the media grain in stationary contact. Further expansion of the bed results from biomass growth and the generation of biomass

within the bed which accumulate in the interstitial spaces. If the upward flow to such an expanded bed were to increase to the point at which the trans-bed fall pressure was just equivalent to the weight of the media in bed (corrected for buoyancy in liquid), then the carrier particle would be free of such suspension in the upward fluid flow i.e. at the point of minimum fluidization, and any additional increase in the fluid flow velocity would result in the conversion of the expanded to the **fluidize bed.**

The energy input for expansion of the media in a AAFEB system is high, and in order to reduce it the density of the component carrier grain is required to be as low as possible but yet sufficient to permit rapid dis-entrainment from up-flowing liquid. Biomass growth on the carrier does increase overall particle density. The mixing characteristics of anaerobic reactor appears to have an effect upon operational performance: lithium tracer studies undertaken (Forster et al, 1982) indicated that the AAFEB exhibits a large degree of dispersion of substrate and mixing properties were defined as intermediate, with a bias toward good. The effectiveness of the expanded bed process may be accredited to a large surface area, to volume ratio made possible by use of small carrier media, the relative thin nature of bio-film minimizing difficulties and the large mass attached bacteria that can be maintained within the bed at high fluid velocities.

2.3.5 Anaerobic fluidized bed

The influent feed to the typical (**anaerobic fluidized bed) AFB** enters at the base of the reactor (**Figure 3)** The bacteria in the fluidized bed system attach to small diameter media which may be sand, activated carbon, garnet or glass beads (Lester et al, 1982 and Pohland et al, 1981). The high vertical velocity of the waste stream to be treated expands the bed to a point beyond which the net downward gravitational force is equaled by frictional drag. An extremely high degree of recycle is required and single carrier grains do not have fixed position within the bed but each particle tends to remain nonetheless within a restricted location.

Technical limitation of the conventional digesters – large volume of HRT's, intolerance of rapid temperature changes and high organic loading rates – are substantially reduced or eliminated by use of a fluidized bed configuration. The extremely high biomass concentration that maybe achieved through the use of small-diameter carrier media, in conjunction with the high rated of recycle typical employed in AFBs, enable this type of reactor to accommodate severe overload

conditions, alteration in influent composition and temperature changes with great efficiency (Stronach et al, 1986). The potential of AFB system for the conversion of high strength soluble industrial wastewater has been demonstrated, with low HRT's and thus high processing rates and high quality effluent being among the major advantages, in addition to reduced reactor volume and consequently much smaller land requirements than those necessary for conventional waste treatment process (Stronach et al, 1986).

2.3.6 Up-flow fixed /packed bed reactors

Up-flow fixed /packed bed reactors (Figure 3). The wastewater is passed through the medium particles resulting in a large proportion of neither the retained biomass nor being attached to the packing medium. This non-attached material is retained in the interstices between the medium particles partly by settling and partly through influence of physical contact with the medium (Pohland, 1992). Since the non attached biomass contribute significantly to the treatment activity in the up-flow fixed bed system, relatively low up-flow velocities are usually maintained to prevent washout of the material. New synthetic packing is large open structure with high void volume. The large voidage maximizes the available reaction volume and provide space for the accumulation of non-attached biomass (Pohland, 1992). A disadvantage of this system is blockage due to the excess biomass accumulation which ultimately leads to decrease retention capacity of the bed.

For wastewater with high concentrations of suspended solids, **down-flow fixed bed reactor (Figure 3)** may be preferable to the operation in the up-flow mode. They utilize ordered modular packing which provides a surface for the development of bio-film (Pohland, 1992). By operating in the downward mode, suspended solids and sloughed bio-film solids will be carried down with the liquid flow and out of the reactor. This efficiency removal of the suspended solids results in a process which retails only the attached microorganism. These systems are able to withstand several hydraulic conditions with only a slight reduction in treatment efficiency. Mixing in the downward system is provided by combination of both effluent recycle and the action of rising gas bubbles (Pohland, 1992, Sacks, 1997).

2.3.7 Anaerobic baffled reactor

The **anaerobic baffled reactor (ABR) (Figure 3)** is a simple rectangular tank which is divided into a number of equal volumes compartment by means of partitions from the roof and bottom tank (Gunnerson and Stuckey, 1986). The liquid flow alternate upward and downward between the partitions and on its passage the waste flows through an anaerobic sludge blanket (Gunnerson and Stuckey, 1986). Hence, the waste is in contact with the active biomass but, because of the design, most of the biomass is retained in the reactor. Due to its physical configuration, this type of reactor should be able to treat waste with high solids content (Gunnerson and Stuckey, 1986).

In principle, all phases of anaerobic digestion can proceed simultaneously (Lettinga, 1985). The sludge in each compartment will differ depending on the specific environmental conditions prevailing there and the remaining compounds or intermediate to be degraded. A stage reactor can provide higher treatment efficiency because more bio-refractory intermediate, such as propionate, will be in optimal environment for degradation ((Lettinga, 1985).The process stability is a distinct advantage (Sacks, 1997).

2.4 CHECKLIST OF REQUIRMENTS FOR OPTIMAL ANAEROBIC TREATMENT

In order for a microbial process to function effectively a number of conditions must be satisfied. Important environmental factors affecting anaerobic sewage digestion are temperature, pH, presence of essential nutrients and the absence of excessive concentration of toxic compounds in influent.

- Proper PH

The value and stability of the pH in an anaerobic reactor is extremely important because methanogenesis only proceeds at a high rate when the pH is maintained at neutral range. At pH value lower than 6.3 or higher than 7.8 the rate of methanogenesis is decreased. Acidogenic populations are significantly less sensitive to low or high pH values and hence acid fermentation will prevail over methanogenic fermentation, which may results in ‘souring’ of the reactor contents. (van Haandel and Lettinga 1994).

- Temperature

Anaerobic digestion like other biological processes strongly depends on temperature. With respect to the conversion rate of digestion process, there are maxima between 35 and 40 °C for

mesophilic range and at about 55 °C for the thermophilic range. The influence of temperature on the rate and extent of anaerobic digestion has been the subject of many investigations. Henzen and Harremoes (1983) evaluated the available data and concluded that the optimum range for mesophilic range is between 30 and 40 °C and the temperature below the optimum range the digestion rate decreases by about 11 % for each °C temperature decrease. The influence of temperature on anaerobic digestion is not limited to the rate of the process. The extent of anaerobic digestion is also affected, as found by O'Rourke (1968) and Van der Last (1991).

- Adequate Metabolism Time

  Two measures of time are involved. HRT defines the window of opportunity afforded the microbes to accomplish their task and SRT determines which organism can replicate and predominate within the system as well as what biomass inventory (biological safety factor) can be maintained. These two types of time affect the system in different ways. Microbial treatment necessitates adequate contact time (HRT) for metabolism to occur and is very much related to the nature if the substrate, which is reflected in the degree of difficulty encountered by biomass in metabolizing it. Simple low molecular weight VFA, sugar and alcohols can be metabolized within minutes but large complex or chlorinated molecules may require hours or even days. Adequate SRT must be maintained to allow the biomass to regenerate and accumulate to a satisfactory inventory concentration (Speece, 1996)

- Mass Transfer of Pollutant into the Microbial Consortia

  An imperative in the process design is to ensure adequate mass transfer between organic pollutant in the wastewater and the biomass responsible for its bioconversion by making possible both intimate contact and adequate exposure time. Suspended growth systems are characterized by biomass conglomerate sizes of about 1-5 mm nominal diameter. In such cases diffusion may not be significantly process limiting and adequate mass transfer may be provided. Attached growth system for cell immobilization can present problems in mass transfer if the biofilm thickness becomes excessive, however, or excessive accumulation of biomass cause short circuiting, but increased recycle rate can at least partially mitigate the limitations in mass transfer.

  Fluidized bed reactors have superior mass transfer characteristics especially with support media such as structured diatomaceous earth, granular activate carbon and porous, fused glass micro

beads. Anaerobic upflow filter require periodic biomass removal to preclude short circuiting and its attendant mass transfer limitation (Speece, 1996)

- Toxicity Accommodation

The anaerobic process can accommodate toxicity of various forms in industrial wastewaters and even biodegrade certain toxicant. Many toxicant are biodegradable only under anaerobic conditions e.g. carbon tetrachloride, tetrechloroethylene, formaldehyde, acrylate and cynide. The acclimation potential of anaerobic biomass too many toxicants can be realized if common sense and patient are employed to expose the biomass to relatively low concentrations first before ramping up the concentrations to the target range in the prototype. This procedure will accomplish two crucial functions: the biomass acclimation will be allowed to develop under favorable conditions, and the concentration of biodegradable toxicant within the reactor will be maintained at a significant lower level than that in the wastewater in question (Speece, 1996)

- Nutrients

In addition to fundamental requirements for macronutrients such as carbon and nitrogen, the inability of a great number of anaerobes to synthesize some essential vitamin or amino acid necessitates the supplementation of bacterial medium with certain specific nutrients for growth and activity. The minimum level of any essential nutrients, which is that concentration which will support a desired growth rate, must be maintained although a maximum level of nutrient requirements also exist above which substrate inhibition occur. The estimation of mineral requirements is particularly difficult due to the occurrence of cellular and non-cellular complexes (Cooney, 1981). Phosphate and sulfates can also exert catabolite repression of specific metabolic pathway, while not affecting growth.

Microbial nutritional requirements may also be interdependent: in the presence of low concentrations of potassium, for example, sodium may be able to satisfy some of potassium requirements. In addition nutrient not obligatory for growth, such as calcium, may nonetheless be needed for process stability (Stronach et al, 1986). Iron appears to be required in the highest concentrations relative to all other trace metals, while iron, cobalt, nickel and zinc are the most often reported as stimulatory. In addition there are surprisingly many references in literature citing stimulation of methanogenes by tungsten, manganese, molybdenum, selenium and boron (Speece, 1996).

- Carbon Source for Synthesis

  Since anaerobic systems are heterotrophis, except for the autotrophic methanogenes converting H_2 to methane, carbon source for synthesis of heterotrophic biomass comes from the organics in the feedstock. For autotrophinc hydrogen utilizers, the carbon source can be dissolved CO_2 in the reactor (Speece, 1996).

- Electron donor

  The electron donor that provides energy for biomass activity is the organic pollutant in the feedstock- the biodegradable COD (Speece, 1996)

- Electron acceptor

  Anaerobic systems operate in the absence of oxygen and utilize CO2 or sulphate as the electron acceptor. CO2 reduction results in CH4 production, sulfate reduction results in H2S production. The acid producing organisms are fermentative, oxidizing a fraction of the substrate and reducing the remainder (Speece, 1996)

2.5 CO-DIGESTION

The term co-digestion indicates the anaerobic digestion of two effluents, whereby a readily biodegradable stream is mixed with more recalcitrant one in order to enhance the digestibility of the latter. This results in improved removal efficiencies.

Some agricultural products processing industries generate wastewaters which can vary greatly in quantity and characteristics throughout the year but are generally characterized by a high organic content. The seasonal nature of these effluents makes the application of individual treatment units not feasible, due to the long start-up periods required by anaerobic digestion; not to mention that it can be prohibitively expensive for small-scale industries. For these reasons, effluents generated from various sources, either a single multi product processing company or multiple specialised small-size companies distributed in an area, are ideally suited for centralized co-digestion treatment works.

Olive mill or dairy wastewater are typical seasonal effluents in the Mediterranean area and contain high concentrations of lipids and carbohydrates respectively. An economically viable

option is reported by Gavala et al. (1996) and consists in co treating these effluents with piggery effluent, which is continually produced and is rich in proteins. The individual streams all have a high organic content (i.e. 44, 105 and 60 g COD/ℓ, piggery, olive mill and dairy wastewater respectively) and in combination give their own contribution to the mixture in terms of lipids, carbohydrates and proteins content. The Authors adapted three separate batches of anaerobic sludge to the three effluents, for a period of one year. They subsequently used piggery effluent adapted sludge as seed inoculum for three batch codigestion experiments, in which the olive mill and dairy effluents were digested with the piggery effluent. The results were used to estimate the kinetic parameters of a dynamic mathematical model describing the co-digestion process.

Farm animal manure is also becoming a major environmental issue for developed and developing countries. Huge amounts are produced in limited areas which then require urgent treatment and disposal solutions, because ammonia and greenhouse gases may cause air pollution and the improper application of nitrogen and phosphorous to land may result in eutrophication of surface water bodies and pollution of soils and groundwater. On a farm site, the biogas produced by anaerobic digestion of animal waste can be used as fuel for boilers, in replacement of natural gas: it can be burned in generators to produce electricity for on-farm use. The residual sludge is stabilized, as soil fertilizer.

Increasing cost of land filling and energy taxes on fossil fuels encourage the exploitation of renewable energy sources, thus making anaerobic digestion a highly competitive alternative for the treatment of animal manure (Salminen and Rintala, 2002). Angelidaki and Ahring (1997) studied the co-digestion of olive mill effluent (high concentrations of lipids, low concentrations of alkalinity and ammonia, and presence of polyphenols) with cattle manure, household waste or sewage sludge. The Authors conducted a preliminary screening to ascertain the feasibility and performance of the co-digestion, using a batch experiment in which the olive mill effluent with one of the readily biodegradable effluents was tested separately in a serum bottle test, at different dilutions of olive mill effluent with water. Subsequently, the manure and the household effluents were selected as preferential co-substrates for a laboratory-scale investigation. Conventional completely stirred anaerobic digesters were maintained at a hydraulic retention time of 13 d and at a temperature of 55 °C. During the start-up phase, which lasted two months, the labile effluents

only were fed to the digesters; thereafter, the olive mill effluent was added to the feed and different dilutions tested. The utilization degree of the olive mill effluent ranged from 55 to 75 %.

This study demonstrated that the high buffering capacity contained in the manure together with the content of several nutrients, makes possible to degrade an effluent which is lacking these compounds, without previous dilution –which would result in large effluent volumes– nor addition of chemicals, e.g. alkalinity or a nitrogen source –which is generally not economical nor environmentally desirable.

Güngor-Demirci and Demirer (2004) studied the co-digestion of cattle (53 g COD/ℓ) and broiler manure (12 g COD/ℓ) in batch reactors and specifically the influence of various conditions on the process performance, i.e. initial organic and solid content, digestion temperature and degree of acclimation of the seed inoculum. The batch reactor type was chosen because in agriculture, complex process configurations may result in technical and operational problems.

A study by Lin et al. (1998) reported a case of co-digestion of septage and landfill leachate. Septage and leachate were mixed at different ratios: as increasing fractions of septage were used, a marked improvement in the removal efficiencies of total COD was observed.

Co-digestion is frequently used for the co-disposal of urban solid wastes too. Peres et al. (1992) carried out a study aimed at treating primary sewage sludge and municipal solid waste. Results showed a total solids destruction of 54 to 57 % and a volatile solids destruction of 58 %.

Purcell and Stentiford (2000) investigated the co-digestion of biodegradable supermarket wastes and sewage sludge. Experiments were conducted in completely stirred reactors, operated at 35 °C. A total solids destruction of 28 % was reported for the control cells, while the co-digestion cells achieved significantly higher destruction of 54 %. The destruction of volatile solids in the co-digestion unit was again much higher than the control units, i.e. 63 % and 37 % respectively.

The main advantages of this approach have been outlined by Angelidaki & Ahring, (1998):

- it is expected to cost less than the separate treatments;

- it can improve the digestibility of a highly concentrated effluent (e.g. high organic content, or high solid fraction), by dilution with other wastes;

- it can counteract the presence of inhibitors (e.g. ammonia or *xenobiotic* compounds) or the lack of nutrients, by dilution; it can also favour the rise of *cometabolism*, which is often the only way to achieve detoxification of specific organic compounds.

2.6 CHARECTERISATION OF EFFLUENT: BIODEGRADABILITY, ACTIVITY, INHIBITION AND GAS MEASUREMENTS

Numerous methods have been developed over the past 30 years, since Van den Berg et al. (1974) illustrated a *manometric* method based on the measurement of the gas production to assess methanogenic activity.

Methanogenesis is often the rate limiting step of the entire process and since the quantification of gas flow rate is relatively easy to perform, most of the methods reported in literature monitor the **production of biogas**. These methods are referred to as *volumetric* or *manometric* methods, as the volume of biogas produced or the pressure increase due to gas production inside a close vessel are assessed, respectively. However, this same concept can be employed to assess activity or inhibition of individual metabolic steps preceding the methanogenic one, providing that they are rate limiting for the whole process. Other direct or indirect methods, targeting physico-chemical or microbiological parameter exist and have been investigated by various authors.

Biodegradability indicates the inherent property of a test material, which depends primarily on its molecular structure and refers to its susceptibility to undergoing a biologically mediated degradation. The extent of such degradation can be further referred to as (Battersby, 2000):

- *ultimate*, if the organic material is converted into inorganic by-products (associated with the specific metabolic process) that cannot be further biologically degraded; or
- *primary*, if the chemical structure of the parent compound is altered to an extent that results in the loss of a specific property, forming products that may also be biodegradable.

An organic material can also be referred to as *inherently biodegradable* when it is potentially biodegradable, if and only if specific actions are taken, such as pre-exposure of the inoculum to the substrate, increased test duration and/or higher microrganism-to-substrate ratios.

Biodegradability is generally expressed as the mass of the test substance converted within a given period of time, as compared to the theoretical mass that could be stoichiometrically converted to methane, i.e. based on the measured (or calculated) chemical oxygen demand of the test material

Activity indicates the inherent ability of a microbial group to undertake the degradation of the test material. It is measured as the specific rate of substrate consumption or product generation, referred to either the total biomass or the targeted microbial population. Bioassays have been established since the 1970s, that employ serum bottles and measure the pressure increase or the volume of liquid displaced by gas production and/or analyse the composition of the gaseous phase. Other more refined methods have recently been developed.

Activity can be assessed under non-limiting or limiting substrate concentration. The two approaches are equivalent if anabolism (i.e.: growth) and catabolism (i.e.: energy generation) are assumed to be coupled by a proportionality factor, as occurs in steady-state conditions, but the behaviour of the microbial culture in transient conditions may be more complicated. Biomass growth can influence the assessment, to an extent that also depends on the designated method of analysis: it generally induces a significant increase in the (absolute) rate of substrate consumption (relative to the steady-state value), that can be revealed by plotting the logarithm of the specific activity vs. time (Coates et al., 1996).

Inhibition indicates a detrimental effect that the test material exerts on the activity of a microbial population. The use of the terms *inhibition* and *toxicity* may generate some confusion; however, Speece (1996) remarks that inhibition denotes an impairment of the bacterial functions, that is generally irreversible; whereas, toxicity is a temporary, non-lethal adverse effect on bacterial metabolism.

Inhibition is assessed by comparison to a reference baseline activity, measured under optimal conditions, e.g. non-limiting substrate concentration; therefore, it is generally expressed as percentage reduction of the specific activity. Two *forms* of inhibition can be distinguished,

i.e.: short- and long-term, based upon the exposure time: for the purpose of assessing the fate of organic substances in the environment, the short-term inhibition is generally to be reported, although this does not necessarily imply that the compounds cannot be biodegraded, under appropriate conditions. The effect of exposure time on inhibition of methanogenesis has been investigated by many authors (Yang & Speece, 1986; Campos & Chernicharo, 1991).

The experimental techniques for assessing microbial inhibition do not differ in principle from the ones designed to measure microbial activity, although the test conditions may be different. If a batch system is used, the base activity and the inhibitory effect are separately assessed: in a first run, activity is measured at a given concentration of the reference substrate; subsequently, the same concentration of substrate is applied to a number of vials, that are each subjected to different concentrations of the inhibitory substance and the reduced activity is measured. If a continuous system is used, the culture is fed with the reference substrate at a constant load, until the gas production (or another performance indicator) is stable; thereafter the inhibitory substance is spiked to the reactor or added to the feed.

Although the term respiration strictly applies to a sequence of metabolic steps whereby aerobic or anoxic micro-organisms obtain energy and carbon using oxygen as the terminal electron acceptor, the term respirometer is widely used in anaerobic applications to designate a device whereby biodegradability or inhibition are assessed through the measurement of the biogas production. In the particular case of hydrogenotrophic methanogenesis, the consumption of hydrogen and carbon dioxide can be measured.

In a respirometer for anaerobic assays, gas production (or consumption) can be quantified either as volume, at constant (atmospheric) pressure or as pressure, at constant volume. Both *volumetric* and *manometric* techniques may use manual or automated devices. Early applications are reported by Valcke & Verstraete (1983) and by Shelton & Tiedje (1984), respectively.

2.5.1 Volumetric methods

In a volumetric respirometer, the volume of biogas produced is displaced into an external container for measurement, prior to discharge. This may be a lubricated syringe in which the piston expands to balance the overpressure generated inside the reactor to the atmospheric

pressure. The needle of the syringe is inserted into the cap of the reactor (Owen et al., 1979) or else the syringe can be used as reactor itself (Cohen, 1992). The same two set-ups can be automated.

In a different arrangement, the biogas produced inside the reactor moves into a suitable external vessel, containing a barrier solution and displaces an equivalent volume of liquid, which can be manually or automatically measured. A Mariotte flask or a *eudiometer* (ISO 11734, 1995) is widely used for the manual measurement. The former consists of a closed vessel with an inlet tube inserted deep into the barrier solution and an outlet tube at the same level: this ensures that a constant head is maintained throughout the duration of the test, and therefore a constant discharge per unit volume is produced. The latter consists of a metered gas collecting pipe, mounted on top of the reactor and of a reservoir tank; biogas production pushes the barrier solution towards the reservoir tank thus altering the two levels. After the reading, the reservoir tank is lowered to reset the levels, i.e. to bring the overpressure back to the atmospheric value.

An automated device for the measurement of the volume displaced, typically consists of a small column containing the barrier solution; a sensor for the detection of the level and a three-way solenoid valve: the barrier solution is pushed inside the column until it reaches a pre-set level, where the sensor actuates the valve that discharges the excess biogas and resets the system. Some automatic volumetric methods may be considered as mixed volumetric/manometric systems where the pressure is allowed to increase to a pre-set level until a valve is opened which allows the gas to be discharged.

Since methane is hardly soluble in water whereas a considerable fraction of carbon dioxide can dissolve (Stumm & Morgan, 1996), it is preferable that the latter is confined either in the gaseous phase (by using an acidic or saline barrier solution) or in the liquid phase (by using an alkaline barrier solution): correspondingly, the volume displaced will account for a mixture of the two components (in a definite ratio) or for methane only. It is more convenient to use an alkaline solution which enables to directly measure the sole methane fraction, as this is directly related to the COD conversion and thus makes mass balances easier to be carried out. A possible drawback arises when the amount of sludge tested is relatively small and/or exhibits low specific methanogenic activity, and when the gaseous composition in the headspace of the vessel changes during the test (James et al., 1990).

2.5.2 Manometric methods

In a manometric respirometer, the biogas produced is confined into the bioreactor and hence generates a proportional overpressure. An early manometric respirometer for anaerobic applications was derived from the Warburg respirometer (Umbreit et al., 1964), which makes use of a differential manometer. The measurement of the pressure build-up can be automated by using a pressure transducer permanently fitted to the bioreactor (Concannon et al., 1988). When multiple bioreactors are run simultaneously, a more viable option consists in using a single very sensitive transducer and a rotating unit with multiple inlets that sequentially connects each vessel to the transducer (Cohen, 1992). The results obtained on a similar device were validated against the conventional gas-chromatographic measurement of the biogas composition (Angelidaki et al., 1998).

The output of a pressure transducer can be extremely accurate but the procedure requires particular care: the pressure increase, the temperature and the gas-to-liquid volume ratio can have a strong influence on the assessment. The main drawback of dealing with a pressure increase in a closed system is that the solubility of the gas components changes, depending on the particular physico-chemical properties; this in particular affects the carbon dioxide in solution, which in turn affects the pH. James and co-workers (1990) indicate that for a biogas mixture CH_4:CO_2 of 3:1, errors can be as high as 1 % and 30 % for methane and carbon dioxide respectively (up to 100 % for the latter, if bicarbonate is taken into account). Angelidaki et al (1998) report that, under poor mixing and low biogas production conditions, super saturation of the liquid with carbon dioxide is likely to occur.

To avoid large increase of the pressure, the gas accumulated inside the vessel should be regularly vented. Variations in temperature create background fluctuations that reduce the sensitivity of a pressure transducer (Suflita & Concannon, 1995). The inaccuracy of the assessment that would result from disregarding dissolved carbon dioxide can be counteracted by comparing the initial dissolved inorganic carbon (DIC) concentration to the final one (ISO 11734, 1995): this allows a more accurate mass balance to be performed. It is also recommended that the sludge be washed prior to use, in order to lower its initial DIC, with a carbonate-free solution and flush it using pure nitrogen.

3 Methods

This chapter describes the method (serum bottle method) used to make up the experimental screening protocol in details. It outlines the experimental conditions, procedure and method of data acquisition and analysis. This chapter also describes the method used to investigate the feasibility of the co-digestion of toxic and/or high (organic) strength effluents with municipal effluent in the exceeding capacity of existing digesting units, at wastewater treatment work-laboratory scale investigations.

3.1 SCREENING METHODS

Anaerobic degradability and inherent toxicity of an effluent must be evaluated prior to loading into a digester, in order to prevent digester failure. Bioassay technique for measuring degradability, as well as the presence of inhibitory substance, could resolve anaerobic treatment problems (Speece, 1994). Bioassay techniques facilitate the evaluation of wide range of variables (Owen et al., 1979).

The proposed screening protocol is illustrated in **Annexure A**. The protocol consists of a sequence of assays which employ the serum bottle methodology. A first step of the procedure is aimed at rapidly estimating whether the effluent is potentially toxic to the methanogenic biomass and in what concentration. The second step is a more extensive screening, aimed at precisely characterising the toxicity of the effluent, the extent of biodegradation that can be achieved, as well as at establishing whether a potential for adaptation of the biomass exists upon exposure. If the sample passes the screening stage, the same serum bottle method will be used to conduct a series of batch co-digestion experiments aimed at evaluating a convenient volumetric ratio between the test material and the readily biodegradable substrate. Finally, a laboratory-scale co-digestion trial could simulate the full-scale process, thus enabling the selection of appropriate operating conditions for the start-up of the full-scale implementation.

This screening protocol is based on the methodology by Owen et al. (1979). The methodology describes the technique for measuring biodegradability and toxicity of material subjected to anaerobic treatment, they are termed Biological Methane Potential (BMP) assay and Anaerobic Toxicity Assay (ATA) respectively. The bioassays are relatively simple and could be undertaken without the need for sophisticated equipment. Biochemical methane potential is a measure of

substrate biodegradability and is determined by monitoring cumulative methane production from which is anaerobically incubated in a chemically defined medium. The anaerobic toxicity measures the adverse effect of a compound on predominantly methanogenic substrate i.e. in the presence of a labile substrate, which therefore must be supplemented in all the bottles (Owen et al., 1979). Both tests comprise *i*) a control set, which enables to quantify the *baseline* conditions and *ii*) a number of test units, in which the microrganisms are exposed to a variety of conditions. To ensure the reproducibility of the determination, each unit is done in replicates (generally, three).The composition of each set varies depending on the type of test to be conducted and is summarised in **Table 1**.

Table 1 – Set-up of the serum bottles assays.

Type of assay		Sludge	Medium	Sample	Objective of the test
BMP	sterile control	No	Yes	No	(optional) to verify the existence of non-biological degradation potential.
	control units	Yes	Yes	No	To quantify the baseline gas production, due to e.g. residual organic matter in the sludge.
	- test units	Yes	Yes	**Substrate** (1)	To assess the biodegradability of the substrate and possible influence of substrate concentration.
ATA	control units	Yes	Yes	Substrate (2)	To quantify the 'reference' activity, in the absence of the inhibitory test substance.
	test units	Yes	Yes	Substrate (2) +(1) **Test compound**	To assess the inhibitory effect of the test compound at different concentrations.

In bold, the parameter which is varied in each test.

(1) If the test substance is suspected to be recalcitrant and to require a co-substrate to be effectively biodegraded, both species must be present.

(2) Reference, readily degradable substrate: e.g. acetate, propionate, etc.

3.1.1 Anaerobic Activity Test.

The procedure outlined here was established in the course of this project, by progressively refining the methodology of Owen and co-workers (1979). Although the principle underlying a serum bottle test is very simple, several factors of seemingly little importance may in fact

influence the outcomes. The generalised procedure which applies to both a biodegradability and a toxicity test is presented, since its believed that *i*) the key variable which is measured in both cases is the microbial activity and that *ii*) similar information can be obtained from either test. Therefore, the term Anaerobic Activity Test (AAT) could be used, to unify the two concepts. A serum bottle AAT **(Table 2)** comprises two control sets (which allow for the quantification of the baseline e.g. endogenous activity of the seed inoculum and of the reference activity, respectively) and a number of test units, in replicates, in which the anaerobic sludge is exposed to a variety of conditions. The activity is assessed at varying concentrations of the test material

Table 2 – Set-up of the serum bottle AAT.

Set	**Composition**	**Objective**
Control-A	▪ Seed inoculum ▪ Reference substrate	To quantify the 'reference' activity, in the absence of the inhibitory test substance.
Control-S	▪ Seed inoculum (only)	To quantify the baseline gas production, due to e.g. residual organic matter in the sludge.
Test units	▪ Seed inoculum ▪ Reference substrate ▪ Test material	To assess the inhibitory effect of the test material at different concentrations and its biodegradability.

Several aspects were taken to account *prior* to performing a serum bottle test:

- Test duration

The common practice is to say that a biodegradability test should last at least 30 d, or until the biogas production becomes negligible, whereas a toxicity assay should last 7 d. It is proposed that both assays are continued until the microbial activity becomes negligible: this enables to observe slow biodegradation processes as well as to discriminate between acute and permanent inhibitory effects. It is therefore proposed that the main quantity to be monitored is microbial activity, i.e. the rate of biogas production.

- Total liquid volume in a vial (or: **headspace-to-liquid volume ratio**)

  When the test materials are diluted, relatively large liquid volumes may be necessary to obtain a sufficient concentration of the organic matter. At the opposite end, if the test materials have a high strength, large liquid volumes are also necessary to lessen the concentration of organic matter. Low gas-to-liquid volume ratios enhance the sensitivity of the determination, as a higher overpressure is generated for a given amount of biogas produced. However, large liquid volumes leave little space to the biogas produced to expand. This imposes that the manual release of the gas is sufficiently frequent, to prevent the pressure from building up to an extent that enhances the solubilisation of carbon dioxide and may ultimately cause a drop in pH, which can be detrimental to the activity of methanogens.

- Frequency of pressure re-equilibration

  This influences primarily the physico-chemical equilibrium within and between the liquid and the gas phases; and also the long-term resistance of the seal. If the rate of gas production between two subsequent re-equilibrations is too high, the pressure might exceed the resistance of the rubber septum causing it to burst, or even just swollen to an extent that gas may leak through previous punctures. Since the rate of gas production is a function of the inherent activity of the biomass, of the amount of biomass present and of the amount of organic substrate supplemented, these three variables should be carefully chosen in order to ensure a gas production which can be handled.

- Inoculum type and concentration

  The volume of sludge used to seed a serum bottle and its inherent specific activity will affect the rate of gas production and in turns the frequency of pressure re-equilibration, as discussed above. Speece (1996) stated that a cardinal rule to conduct a biodegradability assay is to use the seed sludge that has been acclimated to the test materials. However, in the first attempt of assessing biodegradability, the inoculum will inevitably be un-acclimated to that particular test material; thereafter, the same sludge could be re-exposed to the test material: to verify if acclimation has occurred or not. However in a batch process, the use of large inoculums or of a highly active inoculum can prevent the accumulation of volatile fatty acids that would negatively affect the activity of methanogens.

- Substrate (or, test material) concentration

Excessively low or high substrate concentrations are equally detrimental to the accuracy of a serum bottle test: in the former case, the activity displayed by the biomass will be low and the inherent inaccuracy of the manual gas volume determination may be of the same order of magnitude of the gas production; in the latter case, the high rate of biogas production will have the consequences that have been discussed above. Also, at high substrate concentrations, the availability of nutrients and other 'growth factors' may become limiting. If the concentration of substrate is too high, it may even become inhibitory to the biomass (e.g. resulting in a fast production of volatile acids, which inhibits methanogenesis). The **substrate-to-biomass ratio** has proven to be a crucial factor in batch conditions, which influences the kinetics of the process (Moreno et al., 1999). The proposed guideline for the set of the serum bottle AAT is in **Table 3.**

Table 3 – Tentative guidelines for the set-up of an AAT.

Parameter	**Units**	**Value or interval**
Headspace-to-total volume ratio (V_H/V_{TOT})	-	0.2 - 0.6
Substrate (preferably: acetate)	g COD/ℓ	3 - 6
Organic matter (i.e. substrate + test material)	g COD/ℓ	< 30
Solids (i.e. seed inoculum)	g VS/ℓ	10 - 20
Substrate-to-biomass ratio (S/X)	-	0.2 - 0.3

- Number of units

To verify that the outcome of an AAT is reliable, it is recommended that three replicates per unit are prepared. If more accurate insight in the progression of the reaction is desired, a larger number of replicates must be prepared, so that some vials can be sacrificed at given intervals and their content analysed, e.g. to measure the residual COD. It is recommended that all determinations are conducted at least in triplicate, to calculate a meaningful average and standard deviation of the measurements: therefore, three additional replicates should be considered for each unit to sacrifice.

3.1.2 Experimental procedure

The following materials are needed before starting an activity test:

- Substrate

The proposed protocol **(Annexure A)** was used to assess the amenability of four industrial effluent to be anaerobically (co)digested. Size and distillery effluent, which are classified as high strength were sampled from Frame (New Germany, Pinetown) and Illovo in (Merebank, Durban) respectively. Scour effluent classified as toxic was sampled from Dyefin Textiles (Pinetown) and synthetic dye also classified as toxic was prepared in the laboratory. Substrates were characterised by measuring the organic content (gCOD/ℓ) to optimally set up the units **(Table 4)**

Table 4 – Characteristics of substrates and seed inoculum

Characteristics (g/ℓ)	**Inoculum (Test 1)**	**Inoculum (Test 2)**	**Size**	**Distillery**	**Scour**	**Synthetic Dye**
COD	0.4±1	0.2±1	75	120	4	12
TS	43±2	39±2	-	-	-	-
VS	30±1	24±2	-	-	-	-

- Mineral salts solution

A defined solution containing trace elements, minerals and vitamins was prepared according to Owen et al (1979) with some modification. The stock solutions for preparation of the nutrient medium are presented in **Appendix C (Table 4).**

- Inoculum

Inoculum or seed biomass for all assays was sampled from the primary anaerobic digester at Umbilo Sewage Purification Works. This biomass was stored for a short period at 4°C. The inoculum was characterised by measuring solids concentration i.e. volatile solids (g VS/ℓ) and organic content (gCOD/ℓ):ideally, the residual substrate in the inoculum should be negligible compared to the substrate added, so that its contribution to the biogas production is small; Similar determinations should be performed at the end of the test: solids and COD concentration; other

parameters, such as the concentration of some metabolites or end products, would provide additional data for conducting accurate mass balances.

A serum bottle AAT comprises a 'control' set –which allows for the quantification of background conditions– and a number of 'test' units, in which the anaerobic sludge is exposed to a variety of conditions. All units are generally carried out in replicates, to ensure the repeatability of the results. The composition of each set varies depending on the type of test to be conducted. An activity is assessed at varying concentrations of the substrate (for a biodegradability test) or of the test material (for a toxicity test).

The general procedure for conducting a serum bottle assay (AAT) originally developed by Owen et al (1979).is summarised hereafter:

- The vials are over-gassed with CO_2 N_2 balance (40:60) %) at a flow rat of 0.5mℓ/min for 15min.
- All the components i.e. substrate, inoculums, nutrient medium and sodium acetate are poured into the vials, ideally handling the sludge under strict anaerobic conditions.
- The headspace is flushed with CO_2:N_2 balance at 0.5mℓ/min for 15min.
- The vials are then sealed, with rubber septa and aluminium crimps, and stored at the appropriate temperature of 37°C.
- After equilibration for 1h, the gas volumes ere zeroed to ambient pressure with a glass syringe.
- Thereafter, the excess pressure generated by the microbial activity is periodically re-equilibrated and the volume of gas produced is measured using a gas glass syringe (Section 2); gas composition should also be determined by gas chromatographic analysis, to enable accurate mass balances to be carried out.

The main outputs of a serum bottle assay (AAT) are the *biodegradability* of the test material and/or the *inhibitory effect* of the test material on the methanogenic biomass.

3.1.2 Toxicity Test

The Anaerobic Activity Test assay was carried out to evaluate the potential toxicity of the effluents under study (i.e. size, scour, distillery and synthetic dye) to anaerobic micro-organism. In the anaerobic toxicity assay, the initial gas production is of significance since the toxicity of the

effluent is reflected in the reduced initial rate of gas production. At the start of the test, acetate is added in excess as the substrate for acetoclastic methanogenes, which are the most sensitive group of bacteria in the anaerobic consortia.

The standard serum bottle procedures outlined **above** was followed in preparing assay bottles. Three sets of controls were prepared for each *test* which are termed *group A*, *group B,* and *group C:*

-*Group A* contained only the sludge (40 mℓ): it provided the background gas production, hence activity, due residual organic matter and/or endogenous decay.

-*Group B* contained the sludge (40 mℓ), supplemented with acetate (3.5 g/ℓ) to trigger the methanogenic activity of the biomass.

-*Group C* contained only the sludge (40 mℓ), acetate (3.5 g/ℓ) and the NMS (10 mℓ). *Group B* and C were aimed at evaluating the necessity of NMS when conducting the serum bottle test.

Tests sets aimed at evaluating the effect of the test materials on the methanogenic activity of the sludge, biodegradability of test material and acclimation potential, contained sludge (40 mℓ), acetate (3.5 g/ℓ), NMS (10 mℓ), and test material at varying volumes. Three levels of concentration were prepared for each *test*. *Test 1* contained 9 %, 23 % and 41 % v/v, T*est 2* contained 1 %, 5 % and 9 % v/v of the test material. Each group consisted of three replicates.

Table 5 – Composition of the serum bottles for the toxicity test on industrial effluents.

Constituents		**Controls**			**Size effluent**			**Distillery effluent**			**Scour effluent**		
Acetate	g	0.35	0	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
NMS	mℓ	10	0	0	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0	0	0	5	15	35	5	15	35	5	15	35
Parameters													
Total volume	mℓ	50	40	40	55	65	85	55	65	85	55	65	85
Organic m.	g COD/ℓ	5.5	0	6.8	7	17.7	32	11	28	49	0.36	0.92	1.64
Solids	g VS/ℓ	24	30	30	22	18	14	22	18	14	22	18	14
S-to-X	-	0.2	0	0.2	0.6	1.2	2.5	0.7	1.8	3.8	0.2	0.3	0.3
Ratio v/v	%	-	-	-	**9**	**23**	**41**	**9**	**23**	**41**	**9**	**23**	**41**

All groups contained the seed inoculum, acetate as substrate and NMS: therefore, their gas production was compared to that of *group C* of the controls. **Table 5** and **6** report the quantities of

each components which were put into the vials, in the section *constituents*; and some *parameters* of interest which characterise each group of replicates

Table 6 – Composition of the serum bottles for the toxicity test on industrial effluents (II).

Constituents		**Size effluent**			**Distillery effluent**			**Scour effluent**			**Synth. dye effluent**		
Acetate	g	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
NMS	mℓ	10	10	10	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0.5	2.5	5	0.5	2.5	5	0.5	2.5	5	0.1	0.25	0.5
Parameters													
Total volume	mℓ	51	53	55	51	53	55	51	53	55	50	50	51
Organic m.	g COD/ℓ	0.76	3.9	7	1.18	5.71	10.9	0.04	0.2	0.4	0.02	0.06	0.12
Solids	g VS/ℓ	19	19	18	19	19	18	19	19	18	19	19	19
S-to-X	-	0.3	0.5	0.7	0.3	0.6	0.9	0.3	0.3	0.3	0.3	0.3	0.3
Ratio v/v	%	**1.0**	**4.8**	**9.1**	**1.0**	**4.8**	**9.1**	**1.0**	**4.8**	**9.1**	**0.2**	**0.5**	**1.0**

(note: the composition of the control units was identical to the one reported in Table 5)

3.1.3 Biodegradability Test

Anaerobic Activity Test was conducted to measure substrate biodegradability and is determined by monitoring cumulative methane production from a sample which is anaerobically incubated in a chemically defined medium (Owen et al. 1979). Several biodegradability assays were carried out to determine the biodegradability of the effluents under study i.e. size, distillery, scour and synthetic dye and to simulate the combined digestion of two industrial effluents, namely the distillery and size /size and scour/ distillery and synthetic dye effluents, in the attempt of verifying whether the performance of the process benefits from the simultaneous presence of the two test materials.

The standard serum bottle procedure outlined above was followed in preparation of the assay bottles. The composition of the vials used is reported in **Table 7-Table 10**. The set-up for test 1 and test 2 comprised of:

- one control group, to quantify the baseline gas production in the absence of external substrate;
- three groups with the size effluent as external substrate, at increasing concentrations;

- three groups with the distillery effluent as external substrate (the same increasing concentrations were used);
- three groups at increasing concentration with the 1:1 (v/v) mixture of size and distiller (test 1 only).
- three groups with the 1:2 and 2:1 (v/v) mixture of size and distillery (test 2 only).

Table 7 – Composition of the serum bottles for the biodegradability test on industrial effluents (Test 1).

Constituents		**Controls**	**Size effluent**			**Distillery effluent**			**Mixed**		
NMS	mℓ	10	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0	0.5	2.5	5	0.3	1.6	3.6	0.2+0.3	0.8+1.3	1.6+2.5
Parameters											
Total volume	mℓ	50	51	53	55	50	52	54	50	52	54
Organic m.	g COD/ℓ	xxx	0.75+	3.8+	7.27+	0.79+	3.87+	7.46+	0.79+	3.84+	7.39+
Solids	g VS/ℓ	19.2	19.0	18.3	17.4	19.1	18.6	17.9	19.1	18.4	17.7
Vol. Ratio	-	-	-	-	-	-	-	-	**1:1**	**1:1**	**1:1**

Table 8 – Composition of the serum bottles for the biodegradability test on industrial effluents (Test 2).

Constituents		**Controls**	**Size**			**Distillery**			**Mixed (1:2)**			**Mixed (2:1)**		
NMS	mℓ	10	10	10	10	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0	2.5	5	10	1.5	3	6	0.8+1	1.7+2.1	3.3+4	1.7+0.5	3.4+1	6.6+2
Parameters														
Total V	mℓ	50	52.5	55	60	51.5	53	56	51.8	53.8	57.3	52.2	54.4	58.2
Organic m.	g COD/ℓ	Xxx	3.8	7.3	13	3.8	7.5	14	3.8	7.4	13.7	3.9	7.4	13.2
Solids	g VS/ℓ	19.2	18	17.4	16	18.6	18.11	17.14	18.8	18.5	18	18.5	17.9	16.9
Vol. Ratio	-		-	-	-	-	-	-	**1:2**	**1:2**	**1:2**	**2:1**	**2:1**	**2:1**

The set up for test 3 and 4 comprises of the following:

- one control group to quantify the baseline gas production in absence of external substrate
- two groups with each substrate at increasing concentration (i.e. size, scour, distillery and synthetic dye)

- two groups at increasing concentration with mixtures of size: scour and distillery: synthetic dye at ratios of 1:1, 1:2, 2:1.

Table 9 – Composition of the serum bottles for the biodegradability test on industrial effluents (Test 3).

Constituents		**Control**	**Size**		**Scour**		**Mixed (1:1)**		**Mixed (1:2)**		**Mixed(2:1)**	
NMS	mℓ	10	10	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0	2	1	40	20	1+19.5	0.5+10	0.7+27	0.3+13	1.4+13	0.7+6.7
Parameters												
Total V	mℓ	50	52	51	90	70	70.5	60.5	77.7	63.3	64.4	57.4
Organic m.	g COD/ℓ	xxx	3	1.5	1.8	1.1	2.2	1.3	2.1	1.3	2.4	1.4
Solids	g VS/ℓ	19.2	18.4	18.8	10.6	13.7	12.4	15.07	14.8	18.1	13.6	15.8
Vol. Ratio	-	-	-	-	-	-	**1:1**	**1:1**	**1:2**	**1:2**	**2:1**	**2:1**

Table 10 – Composition of the serum bottles for the biodegradability test on industrial effluents (Test 4).

Constituents		**Control**	**distillery**		**Syn. Dye**		**Mixed (1:1)**		**Mixed (1:2)**		**Mixed(2:1)**	
NMS	mℓ	10	10	10	10	10	10	10	10	10	10	10
Sludge	mℓ	40	40	40	40	40	40	40	40	40	40	40
Effluent	mℓ	0	1	2	10	20	0.5+5	1+10	0.32+7	0.6+13	0.64+3	1.3+7
Parameters												
Total V	mℓ	50	51	52	60	70	55.5	61	57.32	63.6	53.64	58.3
Organic m	g COD/ℓ	xxx	2.4	4.6	2	3.4	2.16	3.9	2.1	3.8	2.2	4.1
Solids	g VS/ℓ	19.2	18.8	18.46	16	13.7	16.84	15.00	17.8	16.6	17.3	15.7
Vol. Ratio	-	-	-	-	-	-	**1:1**	**1:1**	**1:2**	**1:2**	**2:1**	**2:1**

3.2 THE LABORATORY SCALE METHOD

The objective of this experimental phase: the laboratory-scale, was to feed a digester in a continuous mode with a mixture comprising a labile and a recalcitrant effluent in appropriate proportions, for a period of time sufficient to assess the performance of the process under a variety of operating conditions. This clearly requires that the entire system is technically reliable and that the performance of the process is preliminarily evaluated under reference conditions against which the effectiveness of the co-digestion option will then be evaluated.

For the system to be reliable, the set-up is to be verified, particularly the reliability of the device that measures the biogas flow rate and the functionality of the feeding and sampling systems. One should also elaborate a monitoring and controlling strategy that enables the reliable detection of the conditions of the process and the effective control of potentially dangerous situations (e.g. incoming acidification).

Therefore it was decided to start-up a completely stirred reactor, inoculated with anaerobic sludge extracted from an operating full-scale anaerobic digester (Stronach et al,1986), and to feed it with one of the labile effluents, i.e. the size or the distillery effluent, for a certain period of time. This first phase would have provided the reference conditions for the final assessment of the effectiveness of the co-digestion process but would have also enabled us to optimise the setup and the monitoring program. Thus the whole operation was divided in three phases:

Batch condition: The seed sludge is discontinuously supplemented with an easily degradable substrate, typically acetate, to promote a stable methanogenic activity prior to the addition of more complex substrates that would generate acidic intermediates. This is done manually by spiking an appropriate dose of the substrate in the digester e.g. daily, based on the knowledge of the SMA of the seed sludge –ideally this is to be assessed in advance with a serum bottle activity test. This phase does not differ in principle from a serum bottle activity test in that it allows verifying the reliability of the gas measuring device and gas tightness of the digester, through mass balances, based on the assumption that the cumulative volume of methane generated must be equivalent to the amount of acetate fed.

Semi-continuous (or fed-batch) conditions. The actual test effluent is used as the sole source of organic matter to feed the sludge; an appropriate volume of effluent is to be selected that will not

result in an organic (or toxic) shock for the biomass and sufficient nutrients must be provided (e.g. by diluting the effluent with a NMS prior to feeding the reactor). The rationale of the semi-continuous regime is to give the biomass a sufficient period or time (reaction time) to degrade the organic substrate, and overcome any transient event, under pseudo-batch conditions before the continuous feeding operation. This phase is obviously a step forward in the direction of a continuous operation in that the micro-organisms are basically working in batch conditions (like in a serum bottle test) but are periodically subjected to a dose of substrate and therefore can operate in a limited period of time, between two subsequent dosages.

Continuous feeding. Once the biomass has been exposed to the substrate and has proved able to degrade it, the influent can be fed to the digester continuously. Under these conditions, the parameters of interest are the organic loading rate (OLR) and hydraulic retention time (HRT). The ultimate objective is to optimise the performance of the process, i.e. maintaining the desired organic removal efficiency at the highest possible OLR (hence lowest possible HRT). Thereafter a co-digestion exercise can be started in which the recalcitrant effluent is added to the feed (e.g. by 'splitting' the OLR set in the previous phase between the labile and the recalcitrant components of the mixture in appropriate proportions) and the performances are evaluated (and possibly optimised) against the performances of the digestion of the labile substrate in isolation.

The full experimental plan could not be completed in the course of this project, due to a variety of technical problems and to the unexpectedly long duration of the semi-continuous phase. Two digesters that treated size and distillery effluents were started and operated in batch and semi-continuous mode. Co-digestion exercise could not be performed due to time constrains.

3.2.1 Equipment Description

A 5 ℓ-and 2 ℓ volume flasks were used to set-up a completely stirred laboratory-scale reactors, to undertake a long-term (co)digestion experiment using size and distillery effluent as test materials. The laboratory-scale set-up is depicted in **Figure 4**. The glass flask was sealed with a rubber cork in which two holes accommodated the gas outlet (**a**) and the deep-end pipe (**b**) which was used to feed the reactor as well as to sample the mixed liquor. Continuous mixing was provided by a magnetic stirrer (**c**); the gas outlet was equipped with a gas sampling port (**d**), a compensation

volume (**e**) and was connected to the gas meter. The gas sampling port (**d**) consisted of a stainless steel T-joint, which housed a 9.5 mm rubber septum (Supelco) in which gas-lock syringe can be inserted to take gas samples. The compensation volume consisted of a 1 litre Tedlar bag (Supelco). It's normally used on larger scale set-ups to collect gas samples and transport them. The Tedlar bag was used to provide a variable volume headspace which enabled a constant pressure to be maintained inside the reaction vessel during the liquid sampling and feeding operations: when a liquid sample is withdrawn, gas is drawn into the reactor and the bag deflates; reversely, when the feed is pumped into the reactor, the gas is pushed back into the bag which inflates. Both operations leave the gas meter unaffected.

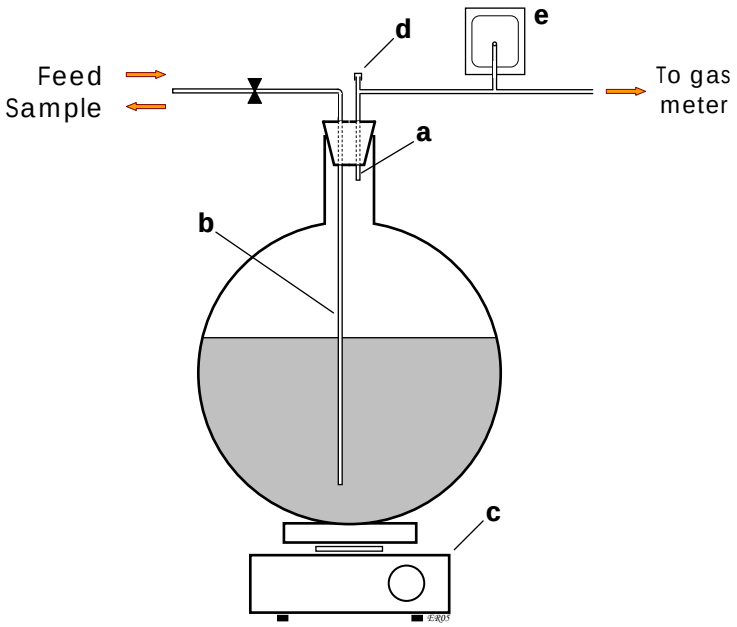


Figure 4 – Schematic of the laboratory-scale completely stirred reactor.

The gas measuring device consisted of a plastic U-shaped tube (diameter 45 mm), filled with acidified water (H_2SO_4 1 %) to the level of the minimum level probe (next section).

3.2.2 Reactor Start-up and Operation: Distillery effluent

Three litres of sludge were withdrawn from the anaerobic reactor at the (Umbilo) wastewater treatment plant and immediately transferred in a 5 ℓ glass vessel that was immediately closed with a rubber cap. No further manipulation of the sludge was done, to prevent any possible

contamination with air. The reactor was placed in a temperature controlled room, maintained at 31 ± 2 °C throughout the experimental period. The experimental period can be subdivided into two phases i.e. batch phase and semi-continuous phase

▪ phase no. 1 (day 0-11): the reactor was maintained in a batch-fed mode: acetic acid (glacial, 35.4 %) was spiked daily in the attempt of enhancing the methanogenic group; the last four days of operations, the distillery effluent was also added, at increasing volumes **(Table 11)** to evaluate whether it had a significant impact on the 'baseline' methanogenesis (maintained by the spikes acetic acid). The anaerobic process was closely monitored through gas production and composition: readings of the gas counter and GC analysis of gas samples were done several times, after the addition of the reagents.

Table 11 – Feed composition during the fed-batch experiment.

Day No.	Acetic acid mℓ (g COD)	Distillery effluent mℓ (g COD)	Nutrients and minerals mℓ
1-5	5 (x)	0	0
6	7.5 (x)	0	100
7	5 (x)	20 (2.4)	0
8	5 (x)	40 (4.8)	0
9	5 (x)	80 (9.6)	0
10	5+5 (x)	80+80 (19.2)	0

▪ phase no. 2 (day 12-95): the reactor was switched to a semi-continuous mode, which consisted in the daily extraction of a liquid sample, immediately followed by the addition of an equal volume of feed. During this phase, not only gas production and composition were constantly monitored, but also pH, alkalinity, VFA concentration and COD were measured daily (with few exceptions); solids were quantified on a weekly basis and off-line activity tests using serum bottle were carried out. The retention times and the loading rates were periodically changed, in order to assess the performance of the system. This semi-continuous phase comprised a few distinct periods (**Table 12)**

- day 12-16: a 100 % distillery influent was fed at a flow rate of 0.1 ℓ/d;
- day 17-41: the raw distillery (effluent) was mixed to a concentrated nutrients and mineral stock solution (10 x) to give a 1:1 diluted influent, that was fed at a flow rate of 0.2 ℓ/d, to maintain the same organic loading rate;

- day 42-95 the effluent was mixed to the concentrated NMS to give a 1:3 diluted influent, that was fed at a flow rate of 0.1 ℓ/d.

Table 12 – Feed composition during the semi continuous experiment.

Day No.	Effluent (mℓ/d)	NMS (mℓ/d)	Flowrate (ℓ/d)	Feed (%)	HRT (d)	OLR (gCOD/ℓ/d)
12-16	100	0	0.1	100	34	3.4 ± 0.1
17-41 [(a)]	100	100	0.2	50	18	3.4 ± 0.2
42-69 [(b)]	25	75	0.1	25	35	1.0 ± 0.1
70-75	50	50	0.1	50	35	1.9 ± 0.1
76-95 [(c)]	50	50	0.1	50	35	2.5 ± 0.2

[(a)] On days 28 and 29, 5 mℓ of acetic acid and 95 mℓ of NMS were used.

[(b)] On days 66 and 69, tap water instead of NMS was used, in the attempt of limiting the excessive alkalinity that seemed to accumulate in the digester.

[(c)] The feed included Glucose (2 g) and Yeast extract (1 g), in the attempt of improving activity and favouring the growth of biomass.

3.2.3 Reactor Start-up and Operation: Size Effluent

The sludge that was withdrawn daily from the Reactor–A as part of the operating procedure was kept active and when it was about 1.5L was poured into a 2L glass flask and used to start Reactor-B which was used for digestion experiments of size effluent. The experienced gained from start-up and operating of Reactor-A was applied in the start-up of this reactor. The batch experimental phase that was applied in Reactor-A was omitted in the reactor as it was believed that the sludge was active enough since it was extracted from a well and active reactor-A. The reactor was started in a semi-continuous mode, which consisted of daily extraction of sludge, immediately followed by the addition of an equal volume of feed. During the whole experimental phase the reactor was monitored closely by measuring gas production and composition, pH, alkalinity, VFA concentration and COD. The solids were measured weekly and activity test was conducted offline by using serum bottles. This semi-continuous phase has 5 distinct phases **(Table 13)**:

day 1-29: equal volumes of 20mℓof size and NMS were fed daily into the reactor resulting in 0.04L/d flowrate.

day 29-55: 80 mℓ/d of feed which consist of 50% size effluent and 50% NMS was fed into the reactor.

day 56-59: the hydraulic retention time was increased to 10d by feeding 160ml/d of feed made of 80 mℓ of size and 80 mℓ nutrient medium.

day 60-67: The reactor was not fed during this period (in a attempt of improving the conditions as its was overloaded in the previous phase)

day 68- 78: The loading rate was decreased to 2gCOD/ℓ/d by feeding 80 mℓ of feed consisting of 50 % size and 50 %NMS.

Table 13 – Feed composition during the semi-continuous experiment.

Day No.	Effluent (mℓ/d)	NMS (mℓ/d)	Flowrate (ℓ//d)	Feed (%)	HRT (d)	OLR (gCOD/ℓ/d)
1-29	20	20	0.04	50	38	1
30-55	40	40	0.08	50	19	2
56-59	80	80	0.16	50	10	4
60-67	0	0	0	0	n/a	0
68-78	40	40	0.08	50	19	2

3.2.4 Monitoring/Operating Parameters

The following operating conditions were monitored, which ensure a sufficient (if not optimal) performance of the process. This in turns translates into the necessity of closely monitoring the process, to identify the 'performance indicators' which are more sensitive and convey the greater information.

The indicators which are commonly used are (**Table14)**:

- the gas flow rate, or better the flow rate of methane (ℓ/d);
- the gas composition, i.e. the fraction of carbon dioxide and methane in the headspace (%);
- the methanogenic activity (ℓ CH_4/g VSS/d);
- the COD removal efficiency (%) or the COD concentration (g/ℓ) in the effluent;
- the solids concentration (g VSS/ℓ) in the effluent;
- the alkalinity and/or the VFA concentration (mol/ℓ) or their ratio; and
- the pH value of the liquor.

▪ Readings of the volume of gas produced and analysis of the gas composition were done at least once a day.

▪ Feeding and sampling was done manually using a 50 mℓ plastic syringe.

▪ COD, solids concentration, alkalinity, VFA concentration and pH were determined as discussed in the next section, all on a daily basis except solids that were measured weekly.

▪ The methanogenic activity of the sludge was evaluated weekly by means of an off-line AAT (in duplicate): an aliquot of mixed liquor (30 to 50 mℓ) was poured into a serum bottle, immediately after extraction; flushed with a 1:1 N_2:CO_2 gas mixture for one minute, then sealed; no additional substrate nor NMS were added and the SMA on the residual substrate (i.e. distillery or size) was evaluated for the two subsequent weeks.

The determination of other parameters, e.g. hydrogen concentration in the liquid and/or in the gas phase, VFA speciation, etc., would certainly assist in obtaining a better monitoring and control of the process, but established methods were not available and would certainly not routinely be available on site at a wastewater work.

Table 14 – Operational parameters which can be used as indicators of performance and/or as early warnings of an incipient upsetting condition.

Quantity	**Indicator**	**Warning** (*)
Gas (or methane) flow rate	✓	✓
Gas composition	×	✓
SMA	✓	(✓)
COD concentration	✓	×
Solids concentration	✓	×
Alkalinity, VFA concentration	×	✓
pH value	✓	(✓/×)

(*) A parameter suitable to give an early warning must *i*) be sufficiently sensitive to the characteristics of the influent and *ii*) respond fast enough to enable the operator to intervene on the characteristic of the feed *before* the upsetting conditions become irreversible.

3.3 ANALYTICAL METHODS

Analysis included the determination of solids, chemical oxygen demand (COD), pH, volatile fatty acids and alkalinity, biogas volume and compositions.

3.3.1 Determination of the gas volume and composition

Gas volume was measured manually with a glass syringe or automatically using a liquid displacement device. The former method was used for the serum bottle assays; the latter, for the laboratory-scale digesters.

The composition of a digester gas was analysed by GowMac gas chromatography equipped with thermal conductivity detector (TDC) which could detect methane, carbon dioxide and nitrogen. The carrier gas used was helium. A packed column was used for the separation of the gases. The characteristics of the GC and the calibration procedure are detailed in **Annexure C**. Biogas sample was drawn from a serum bottle or digester with a 100μℓ precision syringe, and immediately injected into the gas chromatograph.

3.3.2 Determination of the gas volume for serum bottle assays

Gas volume sampling and removal during incubation were performed with graduate glass syringes of sizes10, 20, 50 and 100 mℓ fitted with disposable needle. The sample syringe was initially flushed with the OFN gas and lubricated with distilled water. The syringe is then inserted through the rubber septum into the headspace and held vertically for measurement. Volume determinations were made by allowing the syringe plunger to move and equilibrate between the bottle and the atmospheric pressure. Readings were verified by drawing the plunger past the equilibration point and releasing to ensure that the plunger return to the to the original equilibration volume (Owe et al, 1979). The syringe full of gas was removed for wasting after each measurement.

3.3.3 Determination of the gas volume for laboratory--scale digesters

A liquid displacement (also termed: *volumetric*) device was constructed in our laboratory and mounted on a rack which can host eight units; each of those in connected to an analogue counter

which enables to semi-automatically record the volume of biogas produced in the respective digester. The measuring unit is depicted in **Figure 5** and consists of a U-tube (**a**), filled with an appropriate barrier solution; a solenoid three-way electro valve (**b**) and a three-level electrical sensor (consisting of an earth probe and a minimum and maximum level sensors; **c**) which triggers the valve to release the excess pressure.

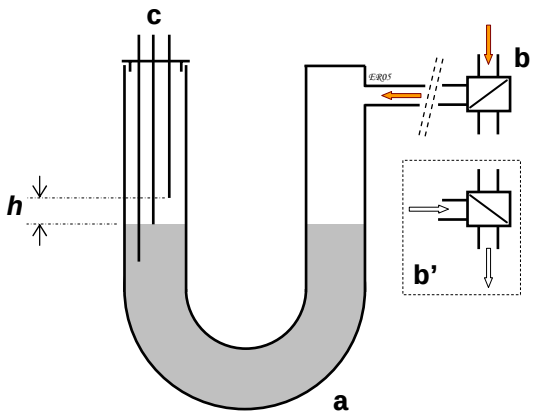


Figure 5 – Volumetric device to measure the volume of biogas produced in a digester

The principle of the measuring device is the following:

- the biogas produced in the digester enters one arm of the tube, through the solenoid valve (which is in the state **b**), causing the level of the barrier solution in the other arm to rise;
- when the level in the second arm comes into contact with the maximum level sensor, the electrical circuit triggers the valve, changing its state to **b'**;
- the excess biogas that was 'trapped' into the tube is pushed back through the valve and vented to the atmosphere;
- the state of the valve is restored to **b**, when the level of the barrier solution in the second arm loses contact to the minimum level sensor.

When the solenoid valve is opened/ triggered, a signal is passed to an analogue recorder which registers a count.

The volume of gas measured ($10^{-3}\cdot m\ell$) for each opening of the electro valve (i.e. per count on the analogue counter) can be calculate as:

$$v = \frac{\pi}{4}\cdot D^2 \cdot h \qquad (4)$$

where:

D is the diameter (mm) of the tube; and

h the distance (mm) between the minimum and maximum level sensors.

An acidified (e.g. H_2SO_4, 1 N) or alkaline (e.g. NaOH, 1 N) solution can be used as barrier solution. The former prevents the solubilisation of carbon dioxide. Therefore the entire volume of the biogas produced is measure: the analytical determination of the gas composition is imperative, in order to quantify the amount of COD actually converted to methane. A more accurate option is the use of an alkaline solution which enables the direct measurement of the volume of methane produced. The sensitivity of the device can be tuned to the desired level by adjusting the minimum and maximum levels, or clearly using tubes of smaller or larger diameters.

3.3.4 Determination of chemical oxygen demand (COD)

Differences in chemical oxygen demand at the beginning and at the end of the experiment period provide an indication of biodegradability. The reduction in COD is an important parameter as it indicates the extent of organic degradation. The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by strong chemical oxidant. It is based on a closed (titrimetric method) or open reflux method, or on a calorimetric method (Standard Methods, 1995). The open reflux method which is suitable for wide range of waste was used for all COD analysis. This method is adopted from Standard Methods, 1995. The COD reagents were prepared as described in Table 15.

Table 15 – Preparation of COD reagents

Reagent	**Method**	**Volume (mℓ)**	**Mass (g)**
Potassium dichromate (0.0147)	$K_2CR_2O_7$ (dried at 103°C for 2h) was placed in a 1ℓ volumetric flask It was diluted to the mark with distilled water		12.259
Sulphuric acid reagent	Ag_2SO_4 powder was added to 1kg concentrated H_2SO_4,Left to stand for 2d to dissolve the Ag_2SO_4		5.5
Ferrous ammonium sulphate (0.25)	$Fe(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in 400 mℓ distilled water in a 1ℓ volumetric flask Concentrated H_2SO_4 was added Cooled to room temperature	20.2	40.0
Ferrion indicator	*o*-phenanthroline was placed in a 100 mℓ volumetric flask FeSO4.7H2O was added		1.485 0.695

The sample to be analysed is diluted in a volumetric flask, to reach a COD concentration included within the detectable range of the method, i.e. not exceeding 900 mg/ℓ. A 10 mℓ aliquot of the diluted sample is placed into a 250 mℓ refluxing flask. To this, a small amount of mercuric sulphate, several glass beads and 15 mℓ of sulphuric acid reagent are added. A 5 mℓ aliquot of potassium dichromate solution (0.0417 M) is added. The flask is then attached to the condenser, cooling water turned on and the mixture is refluxed for 2 h. A blank consisting of 10 mℓ distilled water, instead of the sample, is refluxed in the same way. The samples are cooled and diluted with 80 mℓ of distilled water. Thereafter, they are titrated with ferrous ammonium sulphate solution (FAS) using ferroin indicator.

The COD of the sample is evaluated as follows:

$$COD = \frac{(A - B) \times M \times 800}{mLsample} \tag{5}$$

Where:

A is the mℓ of FAS used for blank

B is the mℓ of FAS used for samples

M is the molarity of FAS

The molarity of FAS (ferrous ammonium sulphate solution) is determined by standardizing against $K_2CR_2O_7$ solution as follows:

10mℓ of $K_2CR_2O_7$ is diluted to 100mℓ then 30mℓ of H_2SO_4 is added and cooled. The solution is titrated with ferrous ammonium sulphate titrant following the addition of 2 drops of ferroin indicator. Molarity of FAS solution is calculated as follows:

$$M = \frac{V_{K2Cr2O7}}{V_{FAS}} \times 0.25 \tag{6}$$

Where:

$V_{K2Cr2O7}$ is the volume of 0.0417M $K_2Cr_2O_7$

V_{FAS} is the FAS used in titration.

3.3.5 Determination of solids

- Total solid measurement: The Standard Method was applied (American Public Health Association, 1985) for measuring total solids in sample. An evaporation dish (crucible) is placed in a muffle furnace at 550-600 °C for approximately 60 min. The dish is cooled in a desiccator, weighed and stored until used. A well-mixed sample is transferred to the weighed dish and evaporated to dryness in a drying oven at approximately 105 °C. The dish is then cooled in a desiccator and re-weighed. The difference in weight represents the total residue. (**Table 16, equation 7)**
- Volatile solids: The residue is then ignited in a muffle furnace pre-heated to 600 °C for 60 min. The dish is partially cooled in air and then transferred to a desiccator for final cooling. The dish is weighed once completely cooled. The loss of weight on ignition is reported as the total volatile residue. (**Table 16, equation 8).**
- Total and volatile suspended solids: A filter is prepared by vacuum filtration of Whatman No 1 filter. The filter is washed with three times successive 20mℓ aliquots of distilled water. The vacuum is applied until all traces of water are removed and the washing is discarded. The filter is removed and placed on a drying dish and then dried at 103 to 105°C for 1h. It is then stored in a desiccator and weighed immediately before use. A 50 mℓ well-mixed sample is vacuum filtered through prepared filter. It is washed with three successive 10mℓ aliquot of distilled water. The vaccum is applied until all traces of liquid are removed. The filter paper is removed and placed on a drying dish. Its dried at 103 to 105 °C for 1 h., cooled in a desiccator and weighed (American Public Health Association, 1985). The mass of total suspended solids was calculated as **Table 16, equation 9.**
- Volatile Suspended Solid: The residue is then ignited in a muffle furnace pre-heated to 600 °C for 60 min. Cool in a desiccator to balance temperature and weight. The dish is weighed once completely cooled. The loss of weight on ignition is reported as the total volatile suspended solid. (**Table 16, equation 10).**

Table 16 – Determination of the solids concentration.

Parameter	Formula	
Total solids	$TS = \frac{W_{105} - W_0}{M}$	(7)
Volatile solids	$VS = \frac{W_{105} - W_{600}}{M}$	(8)
Total Suspended solids	$TSS = \frac{W^*_{105} - W^*_0}{M}$	(9)
Volatile suspended solids	$VSS = \frac{W^*_{105} - W^*_{550}}{M}$	(10)
▪ W denotes the weight (g) of the crucible or the filter (indicated by the superscript *). ▪ M denotes the size of the sludge sample, as volume (ℓ) or mass (g). ▪ the subscripts 105, 550 and 600 indicate the temperatures; 0 denotes the tare.		

3.3.6 Determination of the pH

The pH plays a major role in the anaerobic biodegradation. It influences the activity of the micro-organisms which are active within certain, narrow pH ranges. Anaerobic digestion occur in the pH range of 6.0 to 8.3, however methanogenes have a pH optimum value between 7 and 8 while acidogenes have a lower optimum range. If the pH of waste is outside the optimum range and if the buffering capacity of the system is not sufficient, the anaerobic process will be inhibited. This will lead to under-estimation of the methane potential

Measurement of pH have been generally done *off-line*, using a pH electrode (Toledo series 400), manually immersed into the sample of sludge or effluent. Care must be taken to insure that the pH determination of a sample is a true measure of the pH in the system by measuring the sample as soon as possible. If the sample is allowed to stand exposed to air, carbon dioxide will escape causing the pH to increase.

3.3.7 Determination of the alkalinity and volatile acids concentration

Alkalinity is a measure of the capacity of a solution to neutralise acids and is primarily due to the salts of weak acids (Sawyer & McCarty, 1995). It controls the pH and thus is a measure of the capacity of an aquatic system to buffer the pH in presence of unbalanced acids (Speece, 1996). Alkalinity is comprised of different species of the salts of weak acids, so it is conventionally expressed as its $CaCO_3$ equivalence.

Example of calculation: $CaCO_3$ equivalent of acetic acid.

- The *equivalent weight* of a chemical species is defined as: $\mathrm{EW} = \frac{\mathrm{MW}}{\mathrm{valence}}$, where MW is the molecular weight.
- The *$CaCO_3$ equivalent* is measured as the ratio between the equivalent weight of CaCO3 and that of the species.

$\mathrm{EW(HAc)} = \frac{60}{1} = 60$; $\mathrm{EW(CaCO_3)} = \frac{100}{2} = 50$ → $CaCO_3$ equivalent of acetic acid $\frac{50}{60} = 0.83$

Anaerobic digesters operate optimally at neutral pH conditions in which bicarbonate is the primary constituent, therefore bicarbonate alkalinity is significant. It is crucial to distinguish between *bicarbonate* and *total* alkalinity. The former refers to the total alkalinity minus the VFA equivalent alkalinity (due to neutralised VFA): however, VFA alkalinity is transient since the VFA concentration varies and therefore cannot be consistently relied upon. A conservative approach is generally adopted of ignoring the VFA alkalinity and monitors the bicarbonate fraction only.

A *two-point titrimetric* method was developed by Anderson &Yang (1992) which enables a precise determination of bicarbonate alkalinity and total volatile fatty acids concentrations. A modification of this method was followed throughout this project

The following steps were followed when measuring the VFA:

i. collects the sample in a container which minimises the liquid surface exposed to air, e.g. a cylinder;

ii. measure pH immediately after sampling, directly in the container: stirring is not necessary and by pouring the liquid into another container would favour the stripping of carbon dioxide;

iii. pour the known volume of sample for alkalinity and VFAs determination into a beaker and stir at a very low speed, only sufficient to thoroughly distribute the titrating solution in the entire volume;

iv. use a concentrated rather that diluted titrating solution in order to make the process fast. In our experience, a 0.5 mol/ℓ solution is ideal;

v. titrate with acid solution slowly to pH 5.1 and record the volume used.

vi. titrate further to pH 3.5, record the volume of acid solution used for titrating

The following spreadsheet based on the two-point titration method developed by Anderson & Yang (1992) to determine the alkalinity and VFA concentration was implemented to process titration data

Input data	*Symbol*	*Units*
▪ Volume of the sample of mixed liquor	V	mℓ
▪ Concentration of the titrating solution	c	mol/ℓ
▪ Initial pH	pH_0	-
▪ Initial volume of titrating solution (*)	v_0	mℓ
▪ Volume of titrating solution dosed to reach pH 5.1	v_1	mℓ
▪ Volume of titrating solution dosed to reach pH 3.5	v_2	mℓ

(*) It denotes the volume of acid added to the sample prior to starting the titration, e.g. to inactivate the biological activity.

The following constants will be used:

$H_0 = 10^{-pH_0}$; $H_1 = 10^{-5.1}$; $H_2 = 10^{-3.5}$

$K_1 = 6.6 \cdot 10^{-7}$ $K_2 = 2.4 \cdot 10^{-5}$

Calculate the following:

$A_i = \frac{H_i - H_0}{K_2 + H_i}$; $B_i = \frac{H_i - H_0}{K_1 + H_i}$; $C_i = \frac{v_i \cdot c}{V} \cdot 1000$ (i = 1, 2)

Output	*Formula*	*Symbol*	*Units*
▪ Bicarbonate alkalinity	$\frac{C_1 \cdot A_2 - C_2 \cdot A_1}{B_1 \cdot A_2 - B_2 \cdot A_1}$	BAlk	mol/ℓ
▪ Dissociated VFA	$\frac{C_2 \cdot B_1 - C_1 \cdot B_2}{B_1 \cdot A_2 - B_2 \cdot A_1}$	dVFA	mol/ℓ
▪ Total VFA	$dVFA \cdot \frac{H_0 + K_2}{K_2}$	tVFA	mol/ℓ

4. Results and Discussion

4.1 TOXICY ASSAY

These assays were a sort of 'hybrid' between a biodegradability and toxicity test but they were started as a pure toxicity test and it was realized that interesting results could be obtained, on a long-term test, concerning the actual biodegradability of the substrates. Then it was decided that the two tests can be unified into one and called Anaerobic Activity Test (AAT).

Two sets of AAT were conducted which are termed *test 1* (i.e. concentrations ranging from 9 to 41 % v/v) and *test 2* (with concentrations ranging from 1 to 9 % v/v). A factor that was not taken into account in *test 1* was the organic load and consequently the substrate-to-biomass ratio. Particularly for the size and distillery effluents, which have a high organic content **(Table 5**), the lowest volumetric ratio of 9 % corresponded to COD concentrations as high as 7 and 11 g/ℓ and to substrate-to-biomass ratios of 0.6 to 0.7, which are double the corresponding values in the control units. This might have caused a biased outcome of the *test 1* results. For this reason it was decided to conduct an additional *test 2* with lower concentrations.

4.1.1 Test 1

The average results of the three control groups are reported in **Figure 6(a).** Error bars are not shown, for clarity; however, the variation coefficient has constantly been in the range 1-6 %. This indicates a very good reproducibility between replicates. The background gas production (i.e. on the residual organic matter present in the sludge which is represented by the controls with sludge only) is low, thus indicating that the outcome of the test can be trusted in that no external organic substrate has likely affected the gas production. The addition of nutrients and mineral supplement to the sludge does not seem necessary in this case, possibly because the sludge was taken from a well operated full scale plant and likely contained those elements in excess (Stronach et al,1986). The difference in the ultimate gas production of the control with and without nutrients is statistically non-significant, as it falls in the range of variability of the results. A possible effect of the addition of nutrients is however visible if one considers the activity of the sludge. The control with nutrient had higher activity (54 g COD/g VS/d) and the higher conversion of initial COD

added as acetate **(Table 17**). The equilibrium gas composition in the control group 1 and group 2 approximately 67 %:27 % CH_4:CO_2.

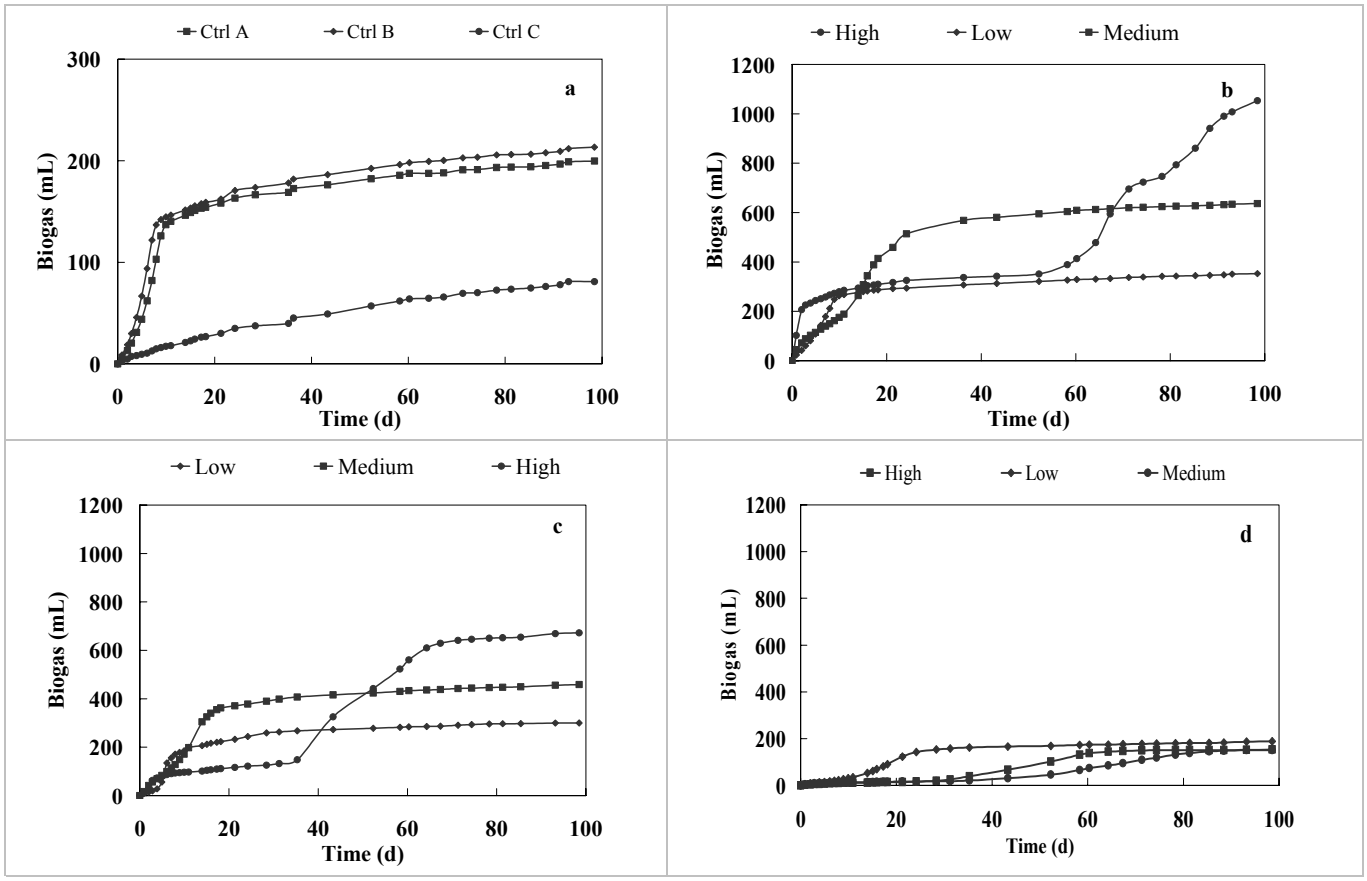


Figure 6 –Average gas production in the control units (a), in the units with size effluent (b), with distillery effluent (c) and with scour effluent (d).

The results for size effluent are presented the same way as the controls **(Figure 6(b))**. The variation coefficient between replicate were very small ranging for 1 to 6 % indicating excellent reproducibility between replicates. Low concentration has a lag of about 4 d and the curve reaches the plateau early (after 10 d) and the corresponding gas composition is constant thereafter: This indicates that the biodegradable material was exhausted. The pattern of gas composition shows carbon dioxide concentration is highly increased for the first two days **(Annexure D),** which is rapidly compensated thereafter. The maximum methanogenic activity is higher than the control which, suggest that size is stimulatory to anaerobic biomass **(Table 17**). At medium concentration the lag phases increases to 8 d and the gas production curve reaches the plateau much later, well

after 40 days. The maximum activity (84 mg COD/g VS/d) took longer to reach but still higher than the control **(Table 17)**. The highest concentration had a lag of about 50 d. The continued gas production show that size was still metabolizing and the test was stopped before the gas production reaches plateau. The gas composition profile shows that carbon dioxide fraction reaches values as high as 90-95 % with the first two days; this must have inhibited the methanogenic biomass for a long time. The maximum activity reached was lower than that of the control (52 mg COD/g VS/d). It's clearly that size does not inhibit the biomass but concentrations higher than 17 g COD/ℓ may cause overloading.

The outcome of the distillery test units are in **Figure 6**. Reproducibility among replicates was also good with variation coefficient ranging from 1 to 5 %. Low concentration had lag phase of 7 d and within 20 days gas production levels of. The gas composition also remained constant thereafter. The maximum activity was higher than of the controls (**Table 17**), which suggest size to be stimulatory to anaerobic biomass at this concentration. The medium concentration had much longer lag phase of 35 d, which suggest gradual acclimation to the potential inhibitory substrate. The gas production also reaches the plateau much later (after 70 days). The maximum activity was much less than that of the control which shows that the biomass was inhibited by distillery at this concentration. At high concentration the gas production plots reaches the plateau early and did not show any significant recovery. The gas composition profile shows that the concentration of carbon dioxide was almost 100 % for the first 20 days, inhibiting any methanogenic activity **(Annexure D)**. The concentration of methane gradually increase thereafter, indicating some methanogenic activity occurring. However the volume of gas that was produced in this period was very small, with zero methane concentration. This suggests that at high concentration (49 g COD/ℓ) distillery effluent inhibits methanogenic activity.

The reproducibility among scour units is slightly poorer than in the other cases. The variation co-efficiency reached 10 % for high concentration for toward the end of the experimental period. The biomass was progressively inhibited by scour effluent concentration from 9 to 40 % (v/v) (**Figure 6).** At medium and high concentration, not only was the rate of methane generated retarded but the ultimate quantity of gas produced was low. Low concentration had a lag phase of 11 d and reaches the plateau early (after 20 d incubation). Medium and high concentration

requires 35 to 45 d to actively produce gas. Activity was very low in all concentration compared to the control.

The tests units with synthetic dye did not produce any appreciable gas during the incubation period therefore no results are discussed. It was concluded that the biomass is completely inhibited by synthetic dye at the concentration tested i.e. 9, 23 and 41 % (v/v) and therefore much lower concentration should be tested (0.2, 0.5 and 1 % (v/v).

Table 17 – Summary of the results of the serum bottle assay on industrial effluents. (test 1)

	Parameter	Units	Group 1	Group 2	Group 3
Controls	Ultimate CH_4 fraction	%	65±2	38±2	66±1
	Ultimate CH_4 volume	mℓ	133±0	27±1	120±3
	COD-to-CH_4	%	**100±0**	n.d.	**95±3**
	Max SMA	mg COD/g VS/d	54±4	≈ 0	49±1
	Lag-phase	d	0	0	0
Size	Ultimate CH_4 fraction	%	66±1	66±0	≈ 58 [(a)]
	Ultimate CH_4 volume	mℓ	221±7	406±10	≈ 587
	COD-to-CH_4	%	**65±2**	**62±0**	**≈ 44**[(a)]
	Max SMA	mg COD/g VS/d	98±1	84±3	≈ 52
	Lag-phase	d	**≈ 4**	**≈ 8**	**≈ 50**
Distillery	Ultimate CH_4 fraction	%	66±1	56±2	44±2
	Ultimate CH_4 volume	mℓ	275±5	340±10	110±5
	COD-to-CH_4	%	**66±2**	**29±1**	**≈0**
	Max SMA	mg COD/g VS/d	69±2	32±1	16±1
	Lag-phase	d	**≈ 7**	**≈ 35**	n.d.
Scour	Ultimate CH_4 fraction	%	67±1	69±1	68±1
	Ultimate CH_4 volume	mℓ	115±5	95±5	90±3
	COD-to-CH_4	%	**≈0**	**≈0**	**≈0**
	Max SMA	mg COD/g VS/d	22±2	7.5±2	9±1
	Lag-phase	d	**≈ 11**	**≈ 43**	**≈ 35**

Group A, B and C indicates the low, medium and high concentrations respectively (for the test units, with the industrial effluents); and the controls with nutrients and acetate, sludge only and without nutrients respectively

4.1.2 Test 2

The average results of the three groups of controls are presented the same as in ***test 1.*** The variation co-efficient was low; ranging between 1-5 %, indicating good reproducibility between the results. The outcome of ***test 2***, is comparable to ***test 1*** if one considers the control units results

(**Table 18),** and any difference between results in these units is statistically non-significant (±5 %) as it falls in the range of variability of the results. A discussion similar to test 1, still apply in terms of gas production and the effect of the addition of the nutrient medium. The equilibrium gas composition was approximately 67:27 %, CH_4:CO_2.

The results for tests 2 were also good with variation coefficient ranging from 1-3 % indicating good reproducibility among replicates. The summary of the results is in **Table 18**. The volume of gas increased with increasing concentration indicating concentration effect of size. Size concentration from 1 % (v/v) to 9 % (v/v) stimulated the activity of the biomass since they had activity higher than that of the control (**Figure 7**). All concentrations had a lag of 2 days and reached plateau at around the same time (10 days) and their corresponding gas volume was constant thereafter. This indicates that the biodegradable substrate was depleted.

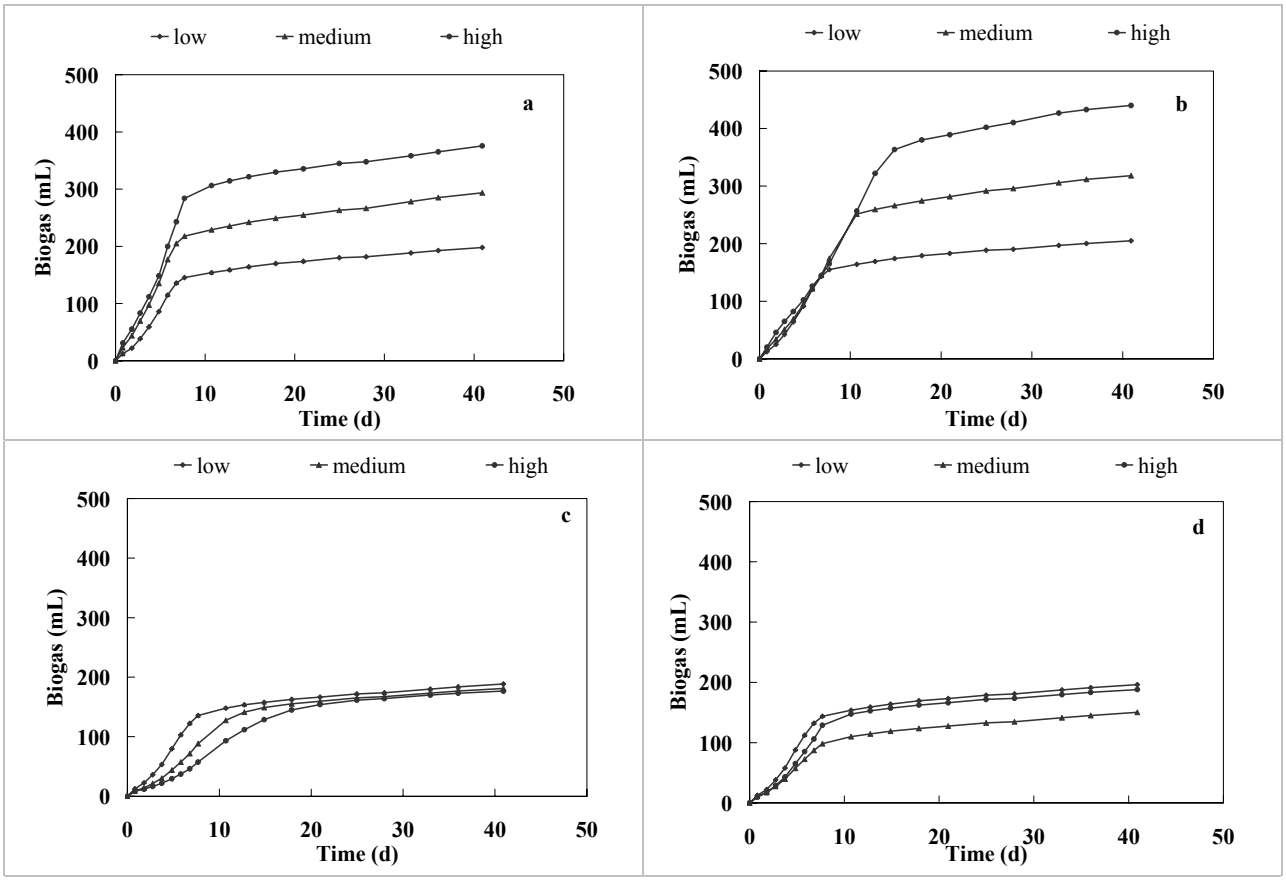


*Figure 7 – Average gas production in the units with size effluent (**a**), with distillery effluent (**b**), with scour effluent (**c**) and with synthetic dye (**d**).*

Distillery effluent had similar effect with size where concentration from 1 to 9 % stimulated the activity of the biomass **(Figure 7**). All concentrations had short lag phase and reached the plateau early (after 7, 10, and15 days respectively). There was no noticeable gas volume change hereafter. Detailed summary of the results is in **Table 18.**

There was a progressive inhibition of methane production as the scour concentration was increased from 1 to 9 % (v/v) **(Figure 7)**. Both a lag in the onset of gas production and a decrease in activity were observed as the concentration was increase (**Table 18)**. Low concentration has a lag phase of 2 days, reached the plateau after approximately 8 days and the gas composition was constant thereafter. The activity of the sludge (45 mg COD/g VS/d) is not significantly affected by this concentration. The intermediate concentration took 4 days to actively produce gas and reaches the plateau after 12 days. The activity of the sludge is decreased by 40 % in this concentration. High concentration had a lag phase of 8days and reaches the plateau after 20 days. The activity if the sludge was decreased by 60 % compared to the control.

The concentrations of synthetic dye tested were very low in terms of g COD/ℓ and % v/v compared to the other effluents; this could have caused the biased outcome of the results. As its can be seen from the table of results there is no significant different between the results of the controls units and of synthetic dye units as its falls in the range of the variability if the results.

Table 18 – Summary of the results of the serum bottle assay on industrial effluents (test 2).

	Parameter		Group A	Group B	Group C
Controls	Ultimate CH_4 fraction	%	67±1	23±2	66±2
	Ultimate CH_4 volume	mℓ	112±4	14±1	112±4
	COD-to-CH_4	%	**103±2**	n.d.	**101±2**
	Max SMA	mg COD/g VS/d	50±2	≈ 0	41±2
	Lag-phase	d	0	0	0
Size	Ultimate CH_4 fraction	%	63±1	63±2	64±2
	Ultimate CH_4 volume	mℓ	120±5	136±5	215±3
	COD-to-CH_4	%	**55±1**	**78±1**	**66±2**
	Max SMA	mg COD/g VS/d	51±2	74±2	107±1
	Lag-phase	d	2	2	2
Distillery	Ultimate CH_4 fraction	%	66±1	67±2	68±1
	Ultimate CH_4 volume	mℓ	126±2	150±2	270±4
	COD-to-CH_4	%	**57±1**	**72±1**	**67±2**
	Max SMA	mg COD/g VS/d	56±2	64±2	67±3
	Lag-phase	d	2	3	4
Scour	Ultimate CH_4 fraction	%	61±2	62±1	64±1
	Ultimate CH_4 volume	mℓ	113±1	93±2	108±5
	COD-to-CH_4	%	**20±2**	**<0**	**<0**
	Max SMA	mg COD/g VS/d	45±2	30±2	20±1
	Lag-phase	d	3	4	8
Synthetic dye	Ultimate CH_4 fraction	%	64±1	64±1	63±0
	Ultimate CH_4 volume	mℓ	120±2	117±6	117±3
	COD-to-CH_4	%	**>100**	**>100**	**>100**
	Max SMA	mg COD/g VS/d	52±1	45±3	45±1
	Lag-phase	d	0	0	0

Group A, B and C indicates the low, medium and high concentrations respectively (for the test units, with the industrial effluents); and the controls with nutrients and acetate, sludge only and without nutrients respectively.

- Estimation of inhibitory concentration

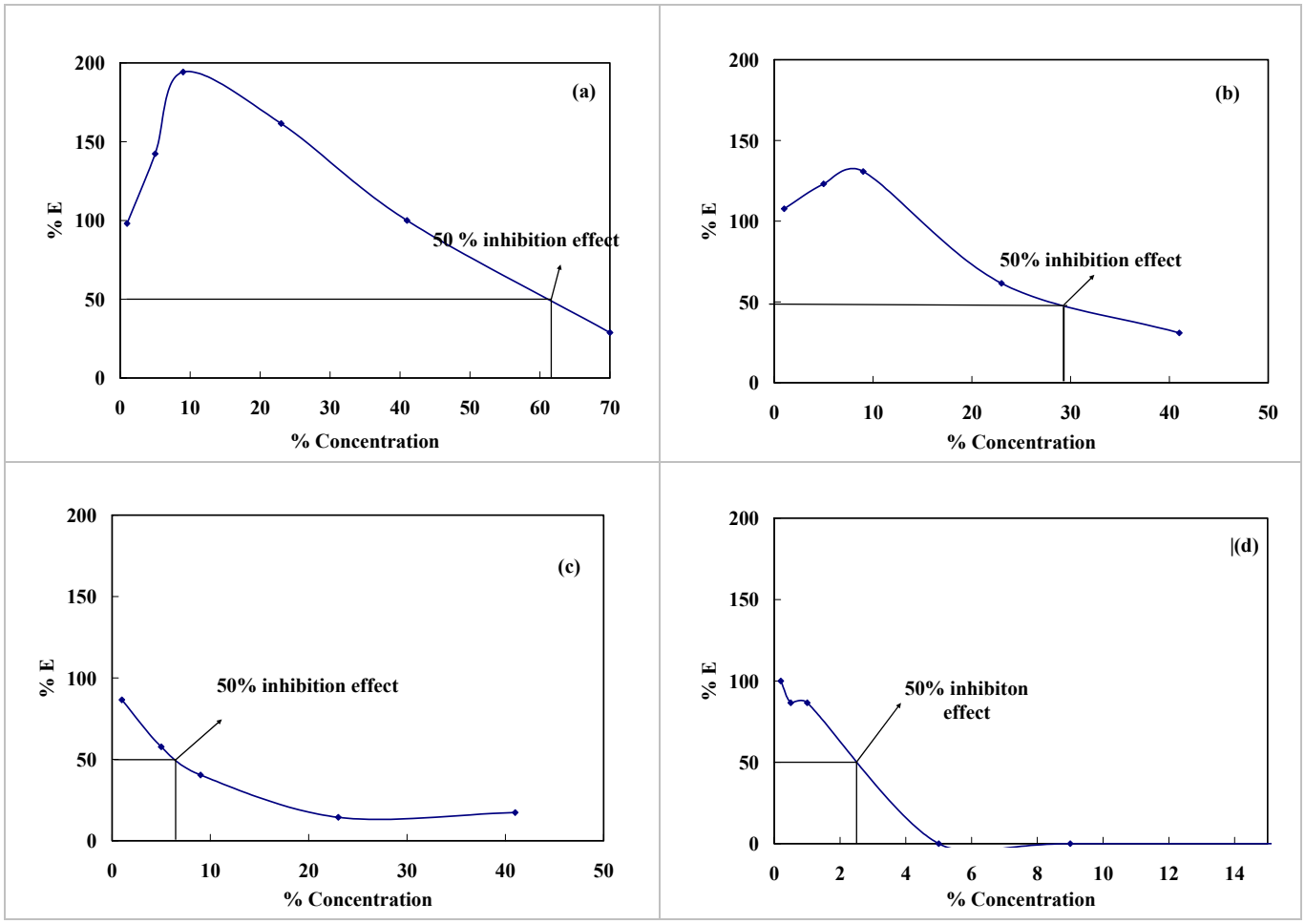


*Figure 8 – Estimation of IC_{50} on industrial effluents (test 1and test2 combined). **(a)** size **(b)** distillery **(c)**scour **(d)** synthetic dye effluents*

Toxicity measures how harmful a substance is and depends on 2 factors: dose (the amount of potentially harmful substance) and exposure time (Young and Tabak, 1993). The most common measure of toxic effect is 50 % level. This refers to the concentration of material which results in 50 % inhibition effect. The inhibition effect under 50 % is considered mild and likely to recovery by suitable biomass acclimation (Mrafkova et al, 2003). Inhibition concentrations were estimated for test materials by calculating inhibitory effect (equation 11) for each concentration tested for each test material.

Calculate the inhibition effect as:

$$E = \frac{SMA_{t.m.}}{SMA_{ref}} \qquad 11$$

Where:

$SMA_{t.m.}$ is the activity in the test unit; and

SMA_{ref} is the baseline activity, in the control unit.

As mentioned above, this 'effect' is concentration-dependent and can be conveniently described through a inhibition curve which plots the relationship between %concentration (v/v) of the test material and %inhibition **(Figure 8)**: the value IC_{50} is found on the horizontal axis in correspondence to I=50

IC_{50} for size effluent could not be estimated properly as all the concentration tested had inhibition effect higher than 50 %, but by interpolation of result obtained, 70 % concentration of size was estimated to results in 29 % inhibition effect. IC_{50} for size effluent was then estimated at 62 % concentration (v/v) which corresponds to about 52 g COD/L. Its should be noted that concentrations of 23 % and 41 % (v/v) had lag phase of 8 and 50 d respectively which were not considered in the calculation of inhibition effect, therefore it was decided to use concentrations lower than 23% (v/v) for further investigations. IC_{50} for distillery was estimated at 28 % concentration (v/v), which corresponds to about 34 g COD/L. It should also be noted that a lower concentration of 23% (v/v) had a lag of 35 d, therefore lower concentration should be i.e. <23% (v/v) should be used for optimal process performance even though IC_{50} is 28 % concentration (v/v). Scour effluent IC_{50} was estimated at about 7 % concentration (v/v) and synthetic dye at 2.3 % concentration (v/v) respectively.

- Comparisons

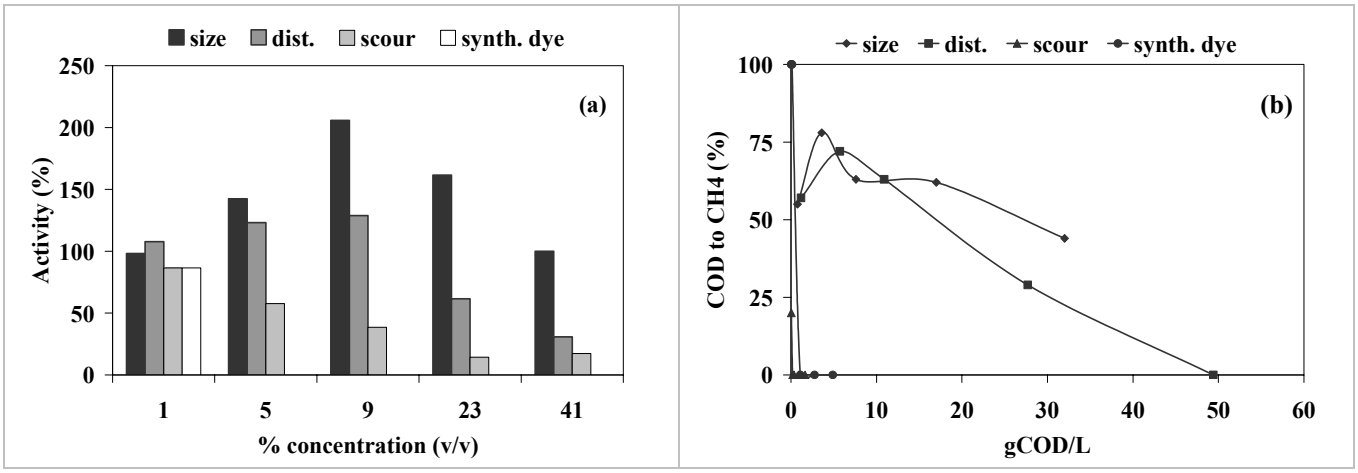


Figure 9 – Percentage Sludge Methanogenic Activity (a) and fraction of COD converted to methane vs. initial COD (b), for the different industrial effluents tested.

Figure 9 summarizes the activity and biodegradability estimates. **Figure 9a** compares the activity of test materials at all concentration to that of a control (with acetate and nutrients). Size effluent is slightly inhibitory at the highest concentration tested (32 g COD/ℓ) and it greatly enhance activity at the lower concentration. The distillery effluent is also slightly inhibitory at 28 g COD/ℓ and completely inhibitory at 49 g COD/ℓ concentration and it also enhance activity at low concentration. The scour and synthetics dye effluent are clearly inhibitory at all concentration tested.

Figure 9b shows the fraction of COD converted to methane i.e. biodegradability of the test material. Both size and distillery effluent shows an unexpected behaviour, where by the 3.6 g COD/ℓ and 5.71 g COD/ℓ concentration appears to be more efficiently converted into methane than the lowest concentration .Thereafter a clear pattern in which the fraction of COD converted to methane decreases as the initial concentration increases is seen **(Figure 9b).** This is a useful indication for a digestion trial: since the higher the load, the higher in the temporary carbon dioxide production and the lower in the fraction ultimately converted to methane, a load not exceeding 15 g COD/ℓ/d for both effluents should be maintained. The scour was found to be

recalcitrant at all concentrations. Synthetic dye is 100 % degradable at concentration of 0.12 g COD/ℓ and lower and highly inhibitory at concentration higher than1.1g COD/ℓ

Conclusions

- Extended periods of zero methane production are not necessarily indicative of destruction of methanogenic viability and hence not indicative of process failure. With size and distillery effluents at 32 g COD/ℓ and 28 g COD/ℓ a lag phase of 50 and 43 days were observed with rapid return to gas production. Scour effluent concentrations of 0.9 g COD/ℓ and 1.6 g COD/ℓ resulted in 43 and 35 days of no gas production. This confirms that methanogenes have a large capacity for acclimation to toxicity exposure given sufficient time (Speece, 1996). The effect of exposure time on inhibition of anaerobic biomass has been investigated by various authors. Yang & Speece (1986); noted that when methanogenes were first exposed to 2.5 mg/ℓ chloroform; gas production almost ceased, requiring about a week recovery. Repeated exposure of the same dose of chloroform showed no reduction in rate of activity demonstration the potential for acclimation and even biotransformation of methanogenes. Bhattacharya & Parkin (1994); reported that upon acclimation the inhibition coefficient increased 5-fold for Nickel toxicity

- The magnitude of decrease in methane production was found to be function of substrate type, concentration and hence substrate to biomass ratio. Results showed that all four substrate decreased the rate of methane production with increasing substrate concentration. Although no detailed experiments were conducted to verify the importance of substrates to biomass ratio, but if one considers the results of size and distillery effluents, which have a high organic content **(Table 1**), the lowest volumetric ratio of 9 % corresponded to COD concentrations as high as 7 and 11 g/ℓ and to substrate-to-biomass ratios of 0.6 to 0.7, which is double the corresponding values in the control units. This resulted in prolonged lag phase and decrease in rate of methane production.

4.2 BIODEGRADABILITY ASSAYS

These assays were aimed at assessing the biodegradability of the four selected industrial effluents (i.e. size, distillery, scour and synthetic dye) using the conventional Owen method (1979) and at simulating the co-digestion between effluents in an attempt to verify whether the digestion performance benefits from simultaneous presence of the two substrates. In this type of test in addition to the usual parameters that have influence on the quality of the results (i.e. the organic load and volatile solids), the volumetric ratio between the two substrate must also be taken into consideration.

Four tests were conducted: *Test 1* and *Test 2* aimed at biodegradability testing of size and distillery only and co-digestion between the two effluents at three organic loads (i.e. 0.75, 3.8 and 7.4 g COD/ℓ) and three volumetric ratios of 1:1, 1:2 and 2:1 between effluents. *Test 3* aimed at biodegradability testing of size and scour only and co-digestion between the two effluents at two organic loads for each effluent (i.e. 3 &1.5 g COD/ℓ for size and 1.8 &1.1 g COD/ℓ for scour) and three volumetric ratios between effluents i.e. 1:1, 1:2 and 2:1 respectively. *Test 4* aimed at biodegradability testing of distillery and synthetic dye and co-digestion between the two effluents at two organic load (i.e. 2.4 & 4.6 for distillery and 2 & 3.4 for synthetic dye) and three volumetric ratios between effluents i.e. 1:1, 1:2 and 2:1 respectively.

4.2.1 Test 1

The cumulative biogas production in the control units (background gas production) reached a value of approximately 50 mℓ **(Figure 10)**. This value is significantly higher than the one reported in the previous tests (AAT 1 and AAT 2): this might be interpreted by larger concentration of residual organic matter in the sludge sample (this was collected at the same wastewater treatment plant but few months later). The average net biogas production for the test units is presented in **Figure 10.** Data for these tests are in **Appendix D.** A general remark is that all groups showed a high reproducibility between the replicates, with the standard deviation never exceeding 6 % **(Table 19).**

Secondly, unlike the previous two tests with the same test materials, the concentration effect is much clearer, i.e. the gas production is progressively higher at increasing concentrations of the test material. Furthermore, no significant lag-phase can be reported in any of the cases studied, as

can be observed in terms of methanogenic activity, **(Appendix D)** which invariably started off at the highest value and gradually decreased to the endogenous/background value after 10-15 days. This is certainly due to an optimal choice/selection of the organic and solids concentration

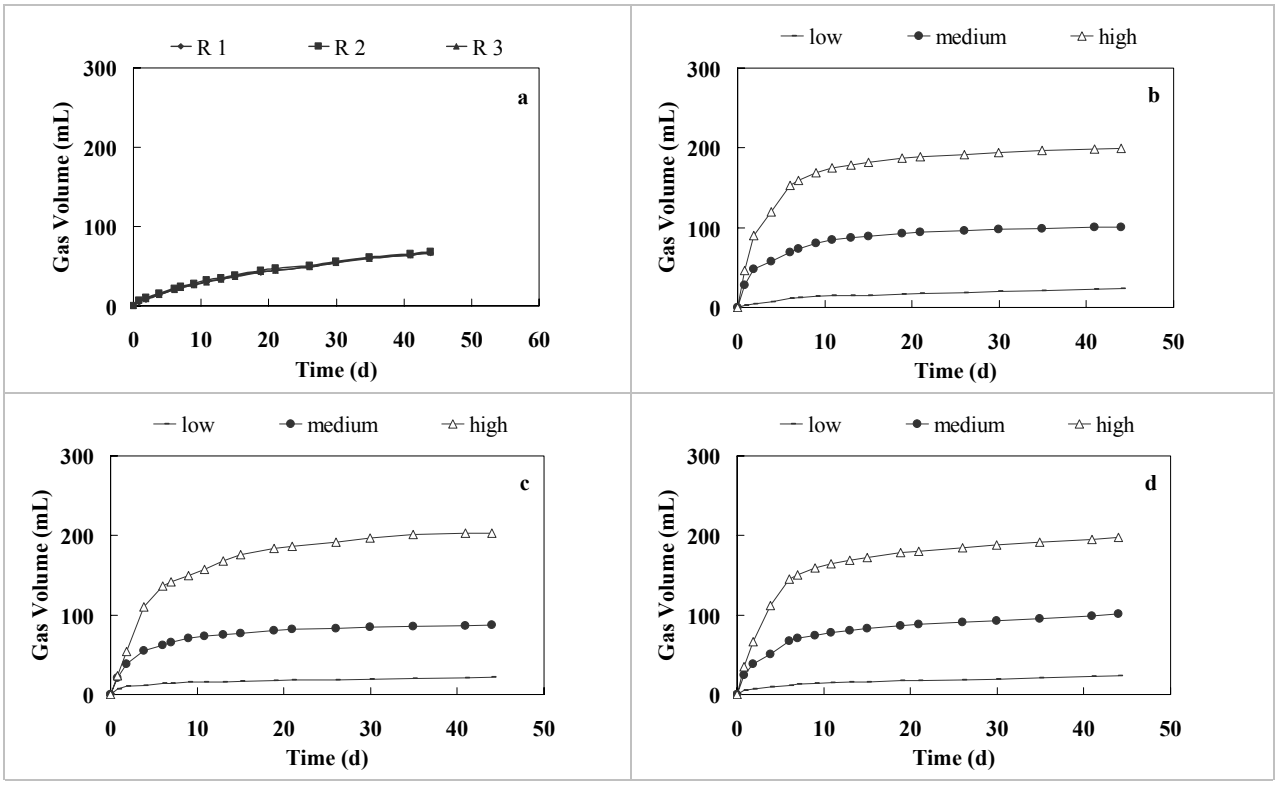


*Figure 10 – Gas production in the control units (**a**) and average net gas production in the units with size (**b**), distillery (**c**) and the mixture (**d**).*

This is further substantiated by the progression of the gas composition: none of the concentrations levels tested caused an initial increase in the carbon dioxide fraction, thus indicating that the initial amount of organic substrate supplemented was not (sufficient) to result in an overload to the methanogenic biomass.

The outcome of this co-digestion test is summarised in **Table 19**. The lowest concentration level is possibly too low to be really meaningful in terms of biodegradability, but it seems to indicate that both test materials are fully biodegradable at 0.75 g COD/ℓ. More realistically, the concentration levels of 0.75 g COD/ℓ in size and 0.79 g COD/ℓ in distillery indicate biodegradation in the range of 88±5 and 94±2 % respectively.

The main output of interest is the fraction of the initial COD converted to methane: this quantity is plotted against the initial substrate concentration in **Figure 11a** shows how the extent of biodegradation of the test materials varies with the concentration. The results of the two types of test, i.e. in which the test material is the only external substrate and in which it is mixed with another substrate, could in principle be presented in two ways: one assumes the concentration of the *total* organic matter as the 'independent variable', the other looks at the contribution of each one of the test materials separately, i.e. assumes the *partial* COD concentration deriving from one of the two and neglects the other one.

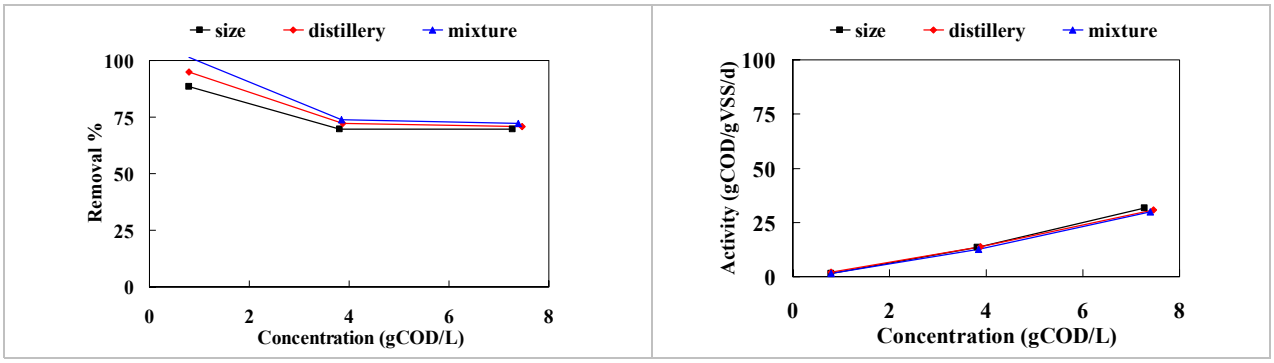


Figure 11: (a) Removal % vs. Concentration and (b) Activity vs. Concentration

The first approach is to interpret as follows: if we *split* the *total* COD between the two test materials based on a given volumetric ratio, how does the fraction of COD convert to methane change? If a given COD concentration results in a higher volume of methane produced than that produced by the two test materials separately, one would conclude that the use of the mixture is preferable in terms of performance of the process.

The second approach would be interpreted as follows: if we take a given concentration of organic matter, how does the volume of methane produced change if no additional substrate is present (i.e. the test material is present alone) or if the other test material is also present (resulting in additional COD being supplemented)? in this case, for a given COD concentration one should compare the volume of methane produced by the one test material in isolation, to that portion of the total volume of methane produced when both test materials are present which is due to the test material alone, since in the second test the overall volume of methane will most likely be larger.

The two approaches are possibly equivalent and both have been taken into consideration to analyse the data of this AAT.

▪ The first approach is immediate. The result is presented **in Figure11 a**: at each COD level tested, the percentage of biodegradability (denoted as 'removal') is plotted. The results show that although the difference between the size and the distillery effluents is small, the biodegradability of the mixture is higher than the corresponding curves of the two test materials in isolation: this seems to suggest that the use of both in combination *slightly* improves the performance of the process.

Table 19 – Summary of the results of the serum bottle assay on industrial effluents (BMP1).

	Parameter		**Group A**	**Group B**	**Group C**
Size	Ultimate CH_4 fraction	%	36±1	47±1	52±0
	Net ultimate CH_4 volume	mℓ	14±1	55±1	110±1
	COD-to-CH_4	%	**88±5**	**69±1**	**69±1**
	Max SMA	mg COD/g VS/d	1.5±0	13±1	31±1
	Lag-phase	d	0	0	0
Distillery	Ultimate CH_4 fraction	%	38±0	51±0	58±0
	Net ultimate CH_4 volume	mℓ	15±1	57±0	112±2
	COD-to-CH_4	%	**94±2**	**72±1**	**70±1**
	Max SMA	mg COD/g VS/d	2±0	14±1	30±1
	Lag-phase	d	0	0	0
Mixture	Ultimate CH_4 fraction	%	37±0	49±1	54±0
	Net ultimate CH_4 volume	mℓ	16±0	58±2	114±1
	COD-to-CH_4	%	**101±0**	**74±0**	**72±1**
	Max SMA	mg COD/g VS/d	1.46±0	13±0	28±0
	Lag-phase	d	0	0	0

Group A, B and C indicate the low, medium and high concentrations respectively.

▪ The second approach needs further elaboration of the data and requires that if a concentration c_1 and a concentration c_2 are respectively tested for the two materials in isolation, a concentration equal to the sum (thereof) is tested when the two are used in combination (the volumetric ratio of the co-digestion experiment will obviously equal c_1:c_2). In the test presented/discussed here,

these circumstances are applicable to one case only, i.e. we compare the two intermediate concentrations of Size and Distillery, to the highest concentration of the mixture as follows:

	Units	Size effluent	Distillery effluent	Mixture
Concentration	g COD/ℓ	3.80	3.87	7.39 (1:1) [(a)]
Net volume of methane	mℓ	55	57	114

[(a)] The mixture contained 3.51 and 3.63 g COD/ℓ of Size and Distillery effluent respectively, as one can calculate from Table 7.

The volume of methane that one would expect to be produced by the mixture of the two test materials is calculated as follows:

- V* = 55 .(3.51/3.8)+57 (3.63/3.87)=104

This would agree to the conclusion that has been drawn based on the first approach, as the actual volume of methane produced is *slightly* more than the than theoretically expected. [V*]

Conclusions

- A pragmatic conclusion is that the experimental evidences gained from this experiment are not sufficiently conclusive to support one or the other hypothesis, i.e. whether the digestion of the test materials in association is a better option or not. A broader range of concentrations (and volumetric ratios) should be examined, if a more reliable and specific response was needed for these two materials.
- On a different level of interpretation, this exercise has demonstrated the feasibility of performing a co-digestion test using the serum bottle technique.

4.2.2 Test 2

As concluded in the previous test, the results were not sufficiently conclusive as only one volumetric flow rate was tested. A broader range of volumetric ratios (2:1 and 1:2) is tested here. The cumulative gas production curves over the 45 d period are shown in **Figure 12.** The volumes are corrected against those produced by the controls. The three concentrations are presented such that any concentration effect could be identified. The similarity between replicates was good with standard deviation ranging from between 1 % and 6 %. There was no lag phase with all concentration tested, which could also be seen in **Annexure D** (SMA figure) where the activity

started off high then decreasing thereafter. This could be due to optimum selection of organic and solid concentration **(Toxicity Assays, Chapter 4, Section 4.1.2)**.

Yang and Speece, 1985 investigated the effect of biomass concentration on substrate utilization. Equal concentrations of same substrate were administered in three reactors containing various biomass concentrations. The ratio of substrate to biomass will be lower in reactor with high bacteria inventory, therefore the distribution of substrate per unit weight if organism would be proportionally decreased. It should be noted that when 2.5mg/ℓ of cyanide was administered to all three reactors, gas production in the process which contained the higher biomass concentration recovered fast. This is similar to our case where the reduced organic load resulted in higher bacteria inventory and thereafter higher efficiency. This is further substantiated by gas composition (**Annexure D**) where the ultimate methane composition is reached within 7 d.

The results are presented in **Table 20**. The ultimate degradation of both material decreases with increasing concentration. This demonstrates the concentration effect in both materials which show that at higher concentration the materials could be inhibitory to biomass. Both materials are readily biodegradable at 3.8g COD/ℓ. Distillery showed to be more readily degradable than size, giving a better conversion of COD to methane in the concentrations tested **(Figure 13).** The two approaches, described in **section 4.2.1**, were used to analyze the effect of having two external

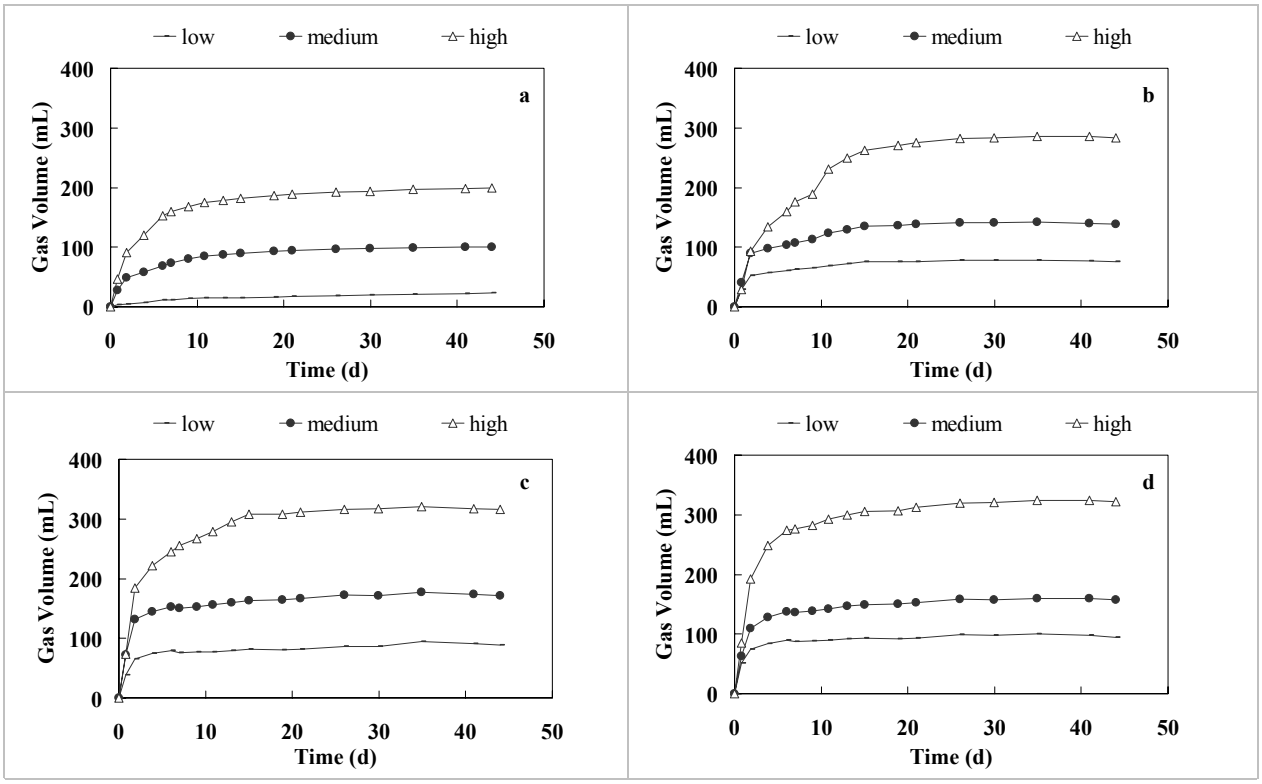


Figure 12 – Average net gas production in the units with size (a), distillery (b) and the mixtures 1:2(size: distillery) (c).and 2:1(size: distillery)(d)

substrates i.e. size and distillery mixed in one reactor (co-digestion effect). The results obtained are in **Figure 13**. Both mixtures were better converted to methane compared to the individual substrates. The mixture with the volumetric flow rate of 1:2 (size: distillery) is preferable in terms of process performance as it had highest COD removal in the concentration tested compared to the other mixture and individual substrate. The second approach applies to one case only. The two lowest concentration of size and distillery are compared to the intermediate concentration of both mixtures i.e. both volumetric rates if 1:2 and 2:1 as follows.

	Units	**Size effluent**	**Distillery effluent**	**Mixture1[(a)]**	**Mixture[(b)]**
Concentration	g COD/ℓ	3.8	3.8	7.4	7.4
Net CH4 volume	mℓ	45	50	107	96

[(a)] Mixture1 is size to distillery mixture to the ratio of 1:2 which contained size of 2.54gCOD/L and distillery of 4.61g/COD/L and [(b)] Mixture2 is size to distillery mixture to the ration of 2:1 which contain size of 5.18gCOD/L and distillery of 2.28gCOD/L as it can be calculated from ***Table 8***

The volume of methane expected to be produced by mixtures was calculates as:

$$V_A(mixtureA) = 45\left(\frac{2.45}{3.8}\right) + 50\left(\frac{4.61}{3.8}\right) = 90.7$$

$$V_B(mixtureB) = 45\left(\frac{5.18}{3.8}\right) + 50\left(\frac{2.28}{3.8}\right) = 91.34$$

These volumes agree to the conclusion reached when using the first approach as the actual volumes produced are slightly more than the theoretical expected. The mixture of size: distillery (2:1) had COD to CH_4 conversion of 61 % which was slightly more than the theoretical expected of 57.4 %. The mixture of size: distillery (1:2) had COD to CH_4 of 68 % which was more that the theoretical expected of 57.4 %, process performance improved by 10.6 %. Therefore it can be concluded that the use of distillery and size mixtures improves the process performance with size: distillery (1:2) being more favorable.

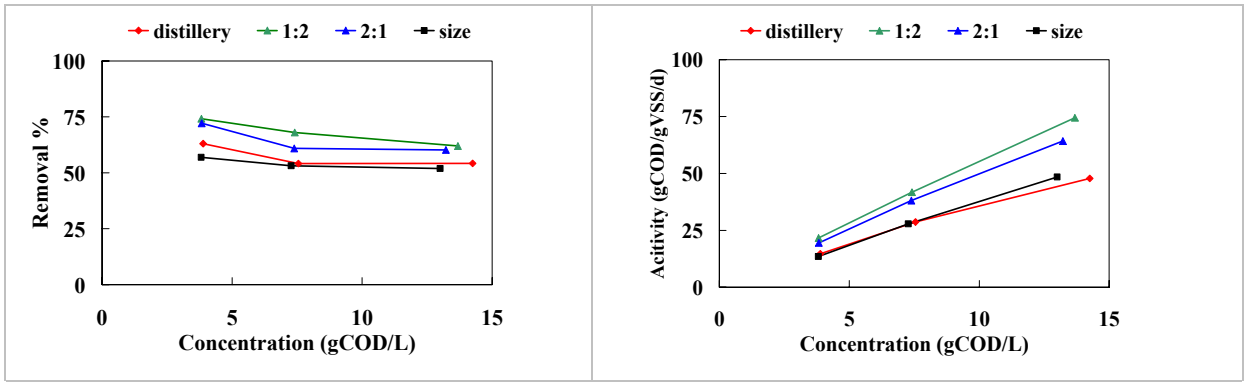


Figure 13: (a) Removal % vs. Concentration and (b) Activity vs. Concentration

Table 20 – Summary of the results of the serum bottle assay on industrial effluents (BMP2).

	Parameter		Group A	Group B	Group C
Size	Ultimate CH_4 fraction	%	45±1	50±0.5	53±2
	Net ultimate CH_4 volume	mℓ	45±2	84±2	160±5
	COD-to-CH_4	%	**70**	**69**	**51**
	Max SMA	mg COD/g VS/d	14	28	48
	Lag-phase	d	0	0	0
Distillery	Ultimate CH_4 fraction	%	43±1	49±1	51±1
	Net ultimate CH_4 volume	mℓ	50±1	86 ±1	170±2
	COD-to-CH_4	%	**73**	**68**	**54**
	Max SMA	mg COD/g VS/d	15	29	50
	Lag-phase	d	0	0	0
Mixture (1:2) (1size: 2distillery)	Ultimate CH_4 fraction	%	44±1	49±1	53±1
	Net ultimate CH_4 volume	mℓ	58±2	107±2	190±5
	COD-to-CH_4	%	**74**	**68**	**62**
	Max SMA	mg COD/g VS/d	22	42	74
	Lag-phase	d	0	0	0
Mixture (2:1) (2 size: 1distillery)	Ultimate CH4 fraction	%	46±2	50±2	52±1
	Net ultimate CH4 volume	mℓ	58±1	96±1	190±2
	COD-to-CH4	%	**72.1**	**61**	**60.25**
	Max SMA	mg COD/g VS/d	19	38.	64.
	Lag-phase	d	0	0	0

4.2.3 Test 3 (Size and Scour)

The net gas production plots for all samples are in **Figure 14**. The gas produced was subtracted against those produced by the controls, which explains the negative gas production in some samples. The negative gas production is an indication of biomass inhibition by toxic substrate. The reproducibility of the results between replicates was good with variation co-efficient ranging from 0.5 to 0.05 for all replicates.

As expected high volumes of biogas were produced in size samples, with gas production stabilizing at ca 80 mℓ after 30d for high concentrated samples, and at ca 38 mℓ after 20d for low concentrated samples (**Figure 14a**). Both concentrations of the scour samples tested produced very little amount of biogas, of which when subtracted against those produced by the controls,

negative values of gas production were found (**Figure 14b).** This agrees with the conclusion reached on the previous test that scour effluent is toxic to anaerobic biomass. This little amount of gas production is also an indication of acclimation potential of scour dye to anaerobic biomass (Speece, 1996).The mixture of size and scour (ratio 1:1) hardly produced any gas with the high concentration stabilizing at ca 5 mℓ. The lower concentration proved to be toxic to biomass as shown by the negative gas volume (**Figure 14c**). The mixture of ratio (1:2) size to scour was even more toxic to the biomass, as shown by the negative gas production in both concentrations tested.

The final mixture tested of ratio: (2size:1scour) was the best combination in terms of gas production. The high concentration stabilized early after 5d at ca. 20 mℓ. The low concentration had a lag of approximately 20d, indicating acclimation potential of biomass to the inhibitory component of the mixture. The acclimation potential of anaerobic biomass to many toxicants can be realized if common sense and patience are employed to expose the biomass to a relatively low concentration first before ramping up the concentration to the target range in the prototype (Speece, 1996). Yang et al (1980) illustrated the acclimation of biomass to chloroform. When methanogenes were introduced to 2.5 mg/ℓ chloroform, biogas production almost ceased requiring about a week recovery. Repeated exposure to the same dose of chloroform showed no reduction in the rate of activity, demonstration the potential for acclimation and even biotransformation of methanogenes. Unfortunately for this study acclimation of biomass was never conducted but these results shows that with acclimation better performance in the digester could have been realized. Gas production was very low stabilizing around 10 mℓ after 50d.

The results are in **Table 21**. The volume of methane produced by each sample was calculated through a step by step mass balance as follows:

$$\Delta_{CH,T} = f_{CH,T}.(\Delta v_t + V_t - \Delta t) - f_{ch,t} - \Delta t - V_t - \Delta t \qquad 12$$

Where:

- f_{CH} indicates the molar fraction of methane (at time t and $t - \Delta t$ respectively

$-V_t - \Delta t$ is the headspace volume of $t - \Delta t$ (mℓ) (for simplicity it can be considered contain throughout the test, unless a fed batch regime is adopted.

ΔV is the volume of gas produced in the interval (mℓ) ($t - \Delta t, t$)

From the methane volume, the ultimate degradation of each compound or mixtures tested was calculated. Size is highly degradable at both the concentration tested at 88% for low and 74% for high concentrations (**Table 21**). Scour is toxic and non-degradable in anaerobic biomass with 0% conversion of COD to methane for both concentrations tested. The maximum activity reached by high concentration of scour effluent tested was only 1±0.5 mg COD/g VS/d and low concentration was zero, which shows that the biomass activity was inhibited. The mixture with ratio of 1:2 of (size: scour) showed to be inhibitory to anaerobic biomass with zero COD to CH_4 conversion and zero SMA for both concentrations tested.

The low concentration mixture of (1size:1scour) has a conversion of COD to CH_4 of 19±0.5 and activity of 1.09 mg COD/g VS/d while the high concentration had had zero conversion of COD to methane and activity of only 0.8mg COD/g VS/d. This suggests this mixture is inhibitory to anaerobic biomass as degradation percentage is less than 50%. The mixture of (2size:1scour) had high degradation percentage in both concentrations tested compared to the other mixtures tested but not high enough to qualify as degradable (less than 50%). The conclusion that can be drawn from the pattern of degradability of the mixtures is: the degradability of the mixtures increases with the increasing amount of the most degradable compound in the mixture. Similar results were obtained by Lin et al (1998) where they investigated co-digestion of septage and landfill leachate. Septage and leachate were mixed at different rations: as increasing fraction of septage were used, a marked improvement in the removal efficiencies of total COD was observed.

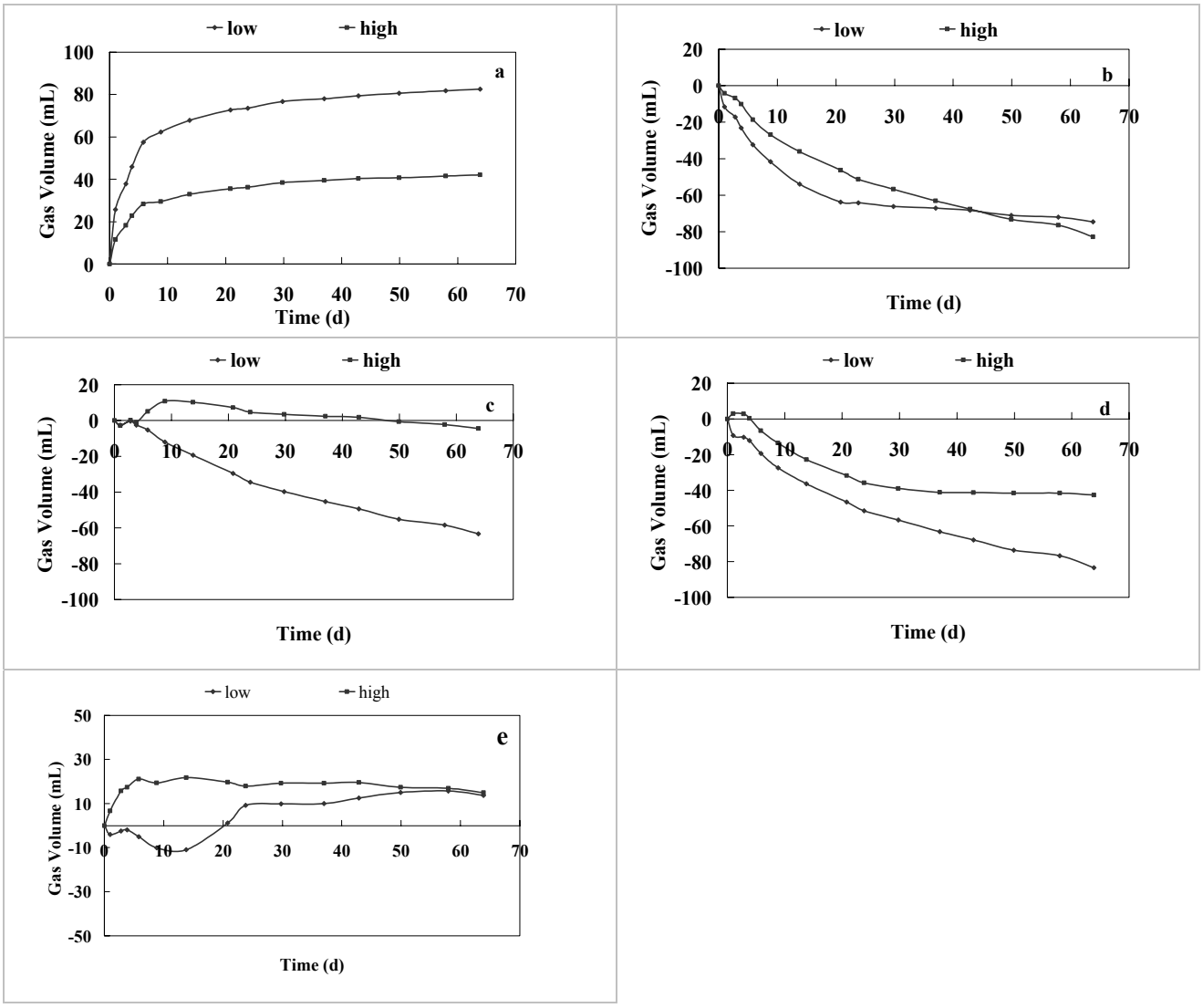


*Figure 14 – Average net gas production in the units with size **(a)**, scour**(b)** and the mixtures 1:1 **(c)**, 1:2(1size: 2 scour)**(d)** and 2:1(2 size: 1scour)**(e)***

The two approach described in **section 4.2.1** was applied to analyze data. The first approach results are presented in **Figure 15**; at each COD level tested the percentage of biodegradability is plotted. The results show that size is highly degradable and scour in non-degradable (with it curve flat on the x-axis). The corresponding curves of the combination of mixtures are in between the two isolated compounds. This suggests that the use of the combine substrates improves the process performance *slightly* in terms of the biodegradability of scour effluent. The second approach which requires further elaboration of data as described in previous section was not applicable to any of the data set available.

Table 21 – Summary of the results of the serum bottle assay on industrial effluents (BMP3).

	Parameter	**Units**	**High**	**Low**
Size	Ultimate CH_4 fraction	%	50±1	48±1
	Net ultimate CH_4 volume	mℓ	47±1	25±2
	COD-to-CH_4	%	**74.36**	**87.63**
	Max SMA	mg COD/g VS/d	12.09±0.5	5.52±0.1
	Lag-phase	d	0	0
scour	Ultimate CH_4 fraction	%	50±1	10±2
	Net ultimate CH_4 volume	mℓ	0	0
	COD-to-CH_4	%	**0**	**0**
	Max SMA	mg COD/g VS/d	1±0.5	0±0.5
	Lag-phase	d	n/a	n/a
Mixture (1:2) (1size:2scour)	Ultimate CH_4 fraction	%	10±2	36±2
	Net ultimate CH_4 volume	mℓ	0	0
	COD-to-CH_4	%	**0**	**0**
	Max SMA	mg COD/g VS/d	0	0
	Lag-phase	d	n/a	n/a
Mixture (2:1) (2 size: 1scour)	Ultimate CH4 fraction	%	44±2	44±1
	Net ultimate CH4 volume	mℓ	11±2	9±2
	COD-to-CH4	%	**17.85**	**28.48**
	Max SMA	mg COD/g VS/d	1.4±0.5	2.74±0.5
	Lag-phase	d	20d	0
Mixture (1:1) (1size: 1scour)	Ultimate CH4 fraction	%	15±2	44±1
	Net ultimate CH4 volume	mℓ	0	6
	COD-to-CH4	%	**0**	**19.47**
	Max SMA	mg COD/g VS/d	0.8±0.6	1.09±0.4
	Lag-phase	d	n/a	6

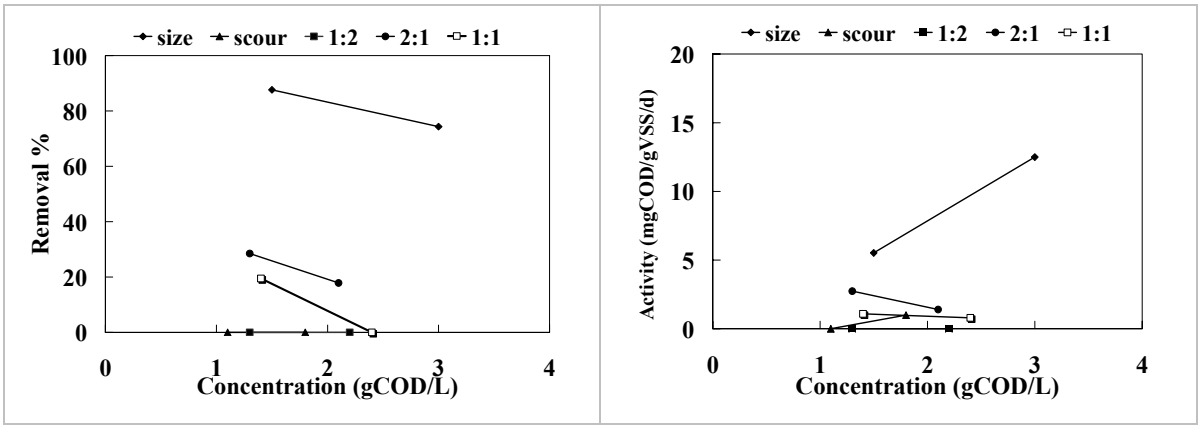


Figure 15: (a) Removal % vs. Concentration and (b) Activity vs. Concentration

4.2.4 Test 4 (distillery and synthetic dye)

The net cumulative gas production plots are shown in **Figure 16**. The negative values in some plots were obtained because the controls produced higher volume of gas than the experimental culture. The reproducibility between replicated was good with variation co-efficient ranging from 1 -5%.

Distillery effluent produced high volume of gas with high concentration stabilizing at ±80mL after 20d and low concentration stabilizing at 60mL after 20d of incubation. This suggests that the degradable component of substrates was depleted after 20d. Both concentrations of synthetic dye tested had negative gas production which shows that it toxic to anaerobic biomass. The low concentration sample of mixture (1:1) had a lag of ca 15d then stabilized at 17mL after 37d while the high concentration mixture had a lag of ca 51d. This prolonged lag phase show acclimation potential of biomass to the inhibitory component in the mixture. The continued gas production plots in high concentration suggest that the mixture was not exhausted during the incubation period. Both concentration of mixture (1distillery:2synthetic dye) had negative gas production which suggest that the mixture is inhibitory to the biomass. The low concentration mixture of (2distillery:1synthetic dye) had no lag, stabilized early after 9d at 26mL and high concentration had a lag of 20d stabilizing at 50mL after 50d. The lag shows acclimation of biomass to the potential inhibitory substrates.

The results are in **Table 22**. The volume of methane produced and the percentage COD converted to methane was calculated as described in the previous sections. Distillery effluent had COD

conversion of 86% and 60% for high and low concentrations respectively. This shows that distillery effluent is highly degradable. Both concentrations of synthetics dye tested were found be non degradable with 0% conversion. The mixture of (1dististillery:1synthetic dye) was found to be semi degradable with conversion of 24% and 15% for low and high concentrations respectively, while the mixture of (1distillery: 2synthetic dye) is non degradable with 0% conversion for both concentrations. The mixture of (2distillery:1synthetic dye) had better conversion compared to the other two, with conversion of 41 and 39% for low and high concentration respectively.

The two approach described in **section 4.2.1** were also applied here. The main output of the first approach is the fraction of the initial COD converted to methane: this quantity is plotted against the initial substrate concentration in **Figure 17a,** to show if and how the extent of biodegradation of the test materials varies with the concentration. The results show that distillery is highly degradable whereas synthetic dye and mixture of (1distillery:2synthetic dye) are non- degradable. The other two mixtures are semi-degradable with (2distillery:1synthetic dye) mixture being the best in terms of process performance as it had higher conversion of COD to methane. The second approach which requires further elaboration of data as described in **section 4.2.1** was not applicable to any of the data set available.

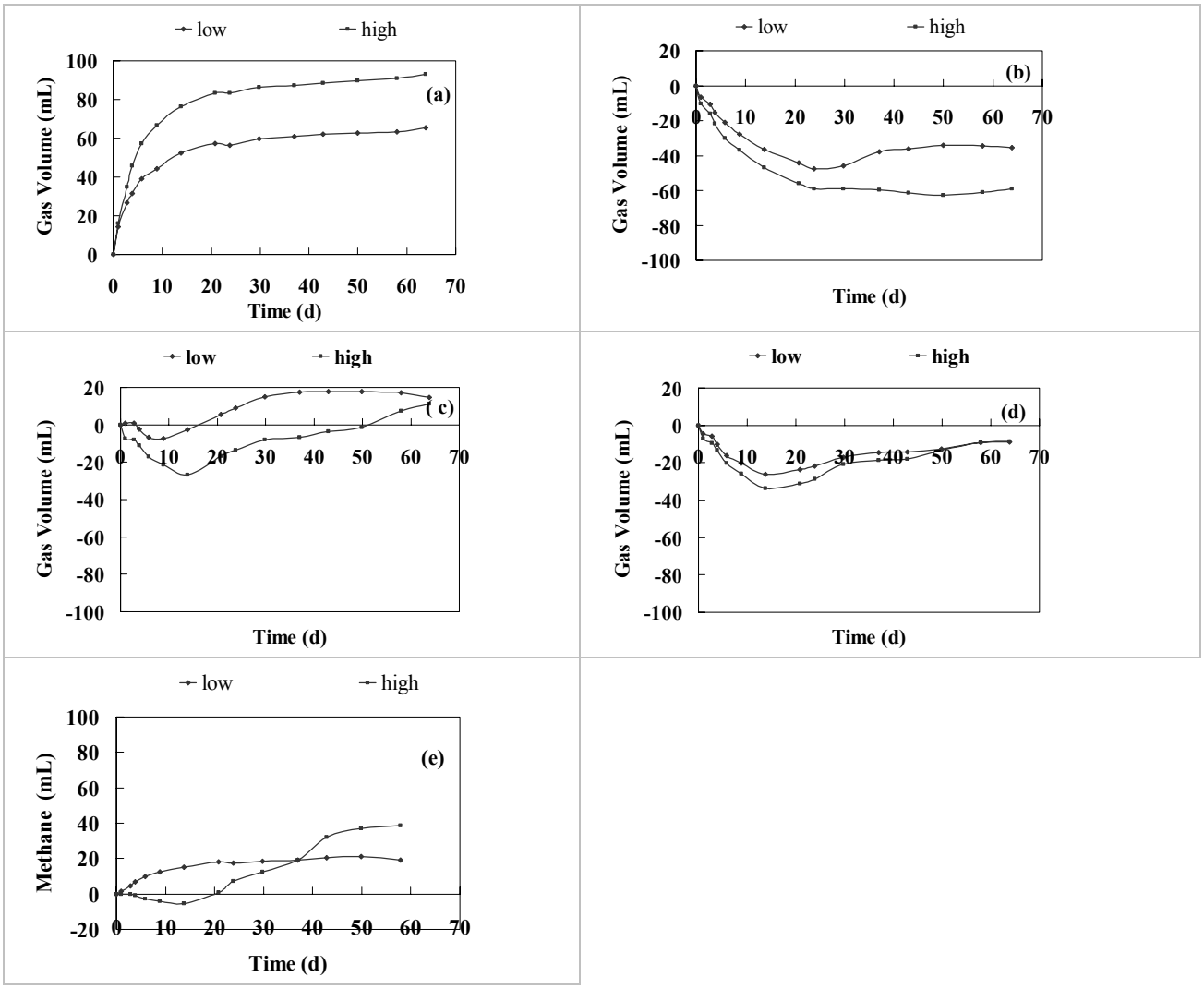


*Figure 16 – Average net gas production in the units with distillery **(a)**, synthetic dye **(b)** and the mixtures 1:1 **(c)**, 1:2 (d) 2:1 (distillery: synthetic dye)*

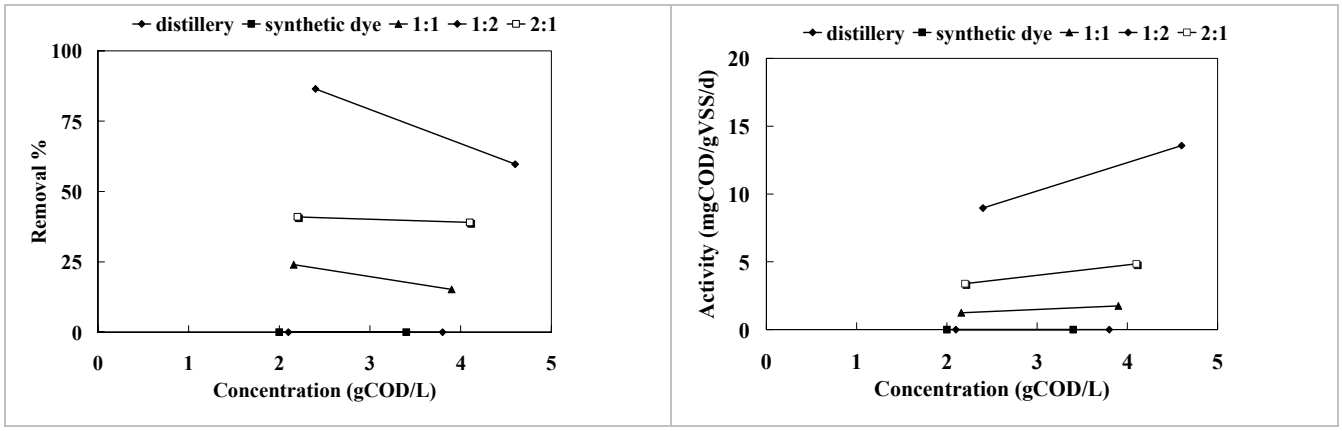


Figure 17: (a) Removal % vs. Concentration and (b) Activity vs. Concentration

Table 22 – Summary of the results of the serum bottle assay on industrial effluents (BMP4).

	Parameter		**low**	**high**
Distillery	Ultimate CH_4 fraction	%	50±1	51±1
	Net ultimate CH_4 volume	mℓ	40±1	56±2
	COD-to-CH_4	%	**86**	**60**
	Max SMA	mg COD/g VS/d	8.97	13.57
	Lag-phase	d	0	0
Synthetic Dye	Ultimate CH_4 fraction	%	36±1	33±2
	Net ultimate CH_4 volume	mℓ	0	0
	COD-to-CH_4	%	**0**	**0**
	Max SMA	mg COD/g VS/d	1.09	0
	Lag-phase	d	n/a	n/a
Mixture (1:2) (1Distil:2Synth.)	Ultimate CH_4 fraction	%	43±2	47±2
	Net ultimate CH_4 volume	mℓ	0	0
	COD-to-CH_4	%	**0**	**0**
	Max SMA	mg COD/g VS/d	0.8	1.13
	Lag-phase	d	n/a	n/a
Mixture (2:1) (2Distillery:1Syn.D)	Ultimate CH4 fraction	%	48±2	54±1
	Net ultimate CH4 volume	mℓ	18±2	39±2
	COD-to-CH4	%	**41**	**39**
	Max SMA	mg COD/g VS/d	3.39	4.85
	Lag-phase	d	20d	0
Mixture (1:1)	Ultimate CH4 fraction	%	51±2	46±2
	Net ultimate CH4 volume	mℓ	14.5	11.4
	COD-to-CH4	%	**24**	**15.2**
	Max SMA	mg COD/g VS/d	1.75	1.85
	Lag-phase	d	52	20

4.3. SUMMARY OF THE FINDINGS ON SERUM BOTTLE STUDY

One of the main outputs of this project is the evaluation of a protocol that enables the conduction of screening tests aimed at assessing the biodegradability and inhibitory potential of an industrial effluent (under anaerobic conditions).

Functional to this primary objective is the evaluation of the reliability of the method that we recommend as the preferential tool to conduct the screening, i.e. the serum bottle method. A method is reliable when it is able to picture the phenomenon targeted as close as possible to the reality. This concept of reliability implies two other concepts, i.e. the accuracy and the reproducibility of the method which apply to two distinct levels.

The accuracy deals with the ability of replicates of the same conditions to give a consistent response: it is therefore evaluated by comparing the replicate units within a single experiment. On the other hand, the reproducibility deals with the ability of independent tests to respond consistently under the same conditions.

So far it has been demonstrated that each activity assay was inherently accurate, in that the variation (both in the volume and in the composition of gas produced) between the replicate units within the same experimental group was generally very small, with only few exceptions. The reproducibility of the serum bottle method can be appraised by comparing the response of independent activity tests on the same test material under homogeneous 'boundary conditions'. This is the objective of this section.

The biodegradability potential of the size and distillery effluents was repeatedly assessed through the activity tests described earlier in this chapter. The output of these tests is summarised in **Figure 39**.

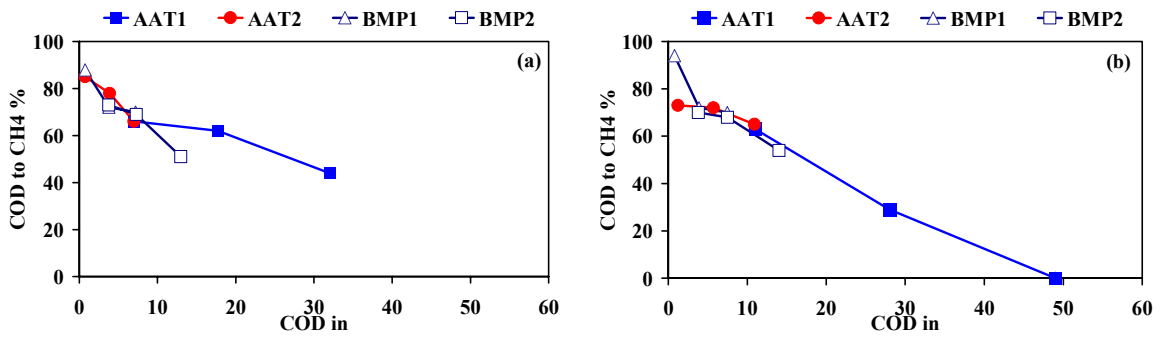


Figure 18 – Comparison of the biodegradability potential for the size (a) and distillery effluents (b), assessed in four serum bottle activity tests performed.

Not all the experiments conducted were homogeneous: AAT 1 and AAT 2 were conducted according to the methodology of a combined activity and biodegradability test proposed in Annexure B, i.e. an external reference substrate was supplemented along with the test materials; BMP 1 and BMP 2 were conducted without external substrate as pure biodegradability tests. More importantly, an excessively large amount of acetate was supplemented in tests No 1 and No 2 which most likely resulted in the inhibition of the methanogenic biomass. Therefore a comparison is possible within the two groups separately. The values of biodegradability for both the size and the distillery effluent obtained in AAT 1 and AAT 2 matches quite well. In fact, one concentration level only was replicated in the two tests (7 and 11 g COD/ℓ respectively) and the corresponding estimates are sufficiently close for the distillery effluent and almost identical for the size effluent.

The reproducibility of tests BMP 1 to BMP 2 for both size and distillery effluents are very high since the values of biodegradability calculated at 3.8 and 7.2 g COD/ℓ are matching almost perfectly. The biodegradability of both effluents gradually declines with the concentration. In conclusion, the serum bottle method seems to be sufficiently reproducible although a quantitative assessment of this property (similar to the variation coefficient of the replicates, for the assessment of the accuracy) was not possible due to a generally poor homogeneity of the different tests performed. The summary of the results is presented in **Table 22 (a) and (b).**

It should be noted that the methodology itself is the output of the project. Therefore the purpose of this summary is not to present a conclusive set of data which is inevitably of limited interest, rather to show the type of results that one could expect from the screening protocol developed in this project.

The general conclusion reached by conduction of co-digestion biodegradability tests is to increase the ratio of the most biodegradable effluents in the combination until the desired/ optimal COD removal efficiency is achieved. Three combinations of effluents were tested for co-digestion option; Size: distillery, Size: scour and distillery: synthetic dye. Size: distillery, were the best combination in terms of process performance. The COD removal percentage was constantly high on all volumetric ratios tested, ranging from (60 to 80 %). Although no significant improvement was noted compared to treating waste separately as the process improved by ±4 %, but the possibility of treating two effluents simultaneously should not be overlooked as its cost less than

separate treatment (Angelidaki & Ahring, 1998).

Scour and synthetic dye have shown to be non-biodegradable and toxic to anaerobic biomass. Co-digestion of these effluents with biodegradable effluents i.e. size and distillery improved the process performance remarkably. The degradability of distillery: synthetic dye mixture (2:1) was approximately 40 % and of size: scour mixture (2:1) 30 %. Unfortunately it was difficult to compare our finding to those of other researchers as there is no published work on co-digestion of these effluents. Generally success had been reported in this field (co-digestion). Angelidaki and Ahring, 1997 investigated co-digestion of olive oil mill effluent (OME) with munure .OME could be degraded to biogas (COD reduction up to approx. 80%) with dilution with water (at a ratio of 1:5 ;OME: water) and addition of urea as nitrogen supplement (Tsonis & Grigoropoulos ,1993). However addition of water results in large effluent volume and addition of chemicals is not economically and environmentally desirable. Batch experiments were prepared using digested manure as inoculum. OME was added undiluted and in 1:2, 1:2.5 and 1:5 (OME: manure). Results show that OME could be degraded without dilution (77 %) by co-digestion with manure.

Gregor et al, 2008 studied the co-digestion of organic waste of domestic refuse and municipal sludge in full scale experiments. In European countries organic waste of domestic refuse has been the source of food for domestic animals. As a food source it was recognized as a possible source of pathogen hazards (Knapel, 2000) and it's was therefore banned for such used. This caused increased quantities of organic waste which are disposed in landfill. Such handling is prohibited by decree on waste and pollution (Environmental Protection Act). The alternative offered is the processing by co-digestion. Organic waste of domestic refuse was added to the mesophilic digester with 20 days HRT to increase the OLR by 40 %. The experiments resulted in virtually complete degradation of organic waste. Degradation increased from 71 % to 81 %.

Based on our results and results of other researchers we can conclude that co-digestion is an option that is worth considering when dealing with toxic and high strength effluents.

Table 23(a): Summary of serum bottle results: BMP (Individual Substrate)

	Parameter	BMP 1			BMP 2			BMP 3		BMP 4	
Distillery	COD in	0.79	3.87	7.46	3.8	7.5	14	-	-	2.4	4.6
	COD to CH4 %	94	72	70	70	68	54	-	-	86	60
Size	COD in	0.75	3.8	7.2	3.8	7.3	13	3	1.5	-	-
	COD to CH4 %	88	72	70	73	69	51	74	88	-	-
Synthetic	COD in	-	-	-	-	-	-	-	-	2	3.4
Dye	COD to CH4 %	-	-	-	-	-	-	-	-	0	0
Scour	COD in	-	-	-	-	-	-	1.8	1.1	-	-
	COD to CH4 %	-	-	-	-	-	-	0	0	-	-

Table 23(b): Summary of serum bottle results BMP (Co-digestion)

Size : Distillery				
1.1	COD in	0.79	3.84	7.4
	COD to CH4%	101	74	72
1:2	COD in	3.8	7.4	13.2
	COD to CH4 %	74	68	62
2:1	COD in	3.9	7.4	13.2
	COD to CH4%	72	61	60.3
Size: scour				
1.1	COD in	2.2	1.3	
	COD to CH4%	0	19.47	
1:2	COD in	2.1	1.3	
	COD to CH4%	0	0	
2:1	COD in	2.4	1.4	
	COD to CH4%	17.8	28.3	
Distillery: synthetic Dye				
1.1	COD in	2.16	3.9	
	COD to CH4%	24	15.2	
1:2	COD in	2.1	3.8	
	COD to CH4%	0	0	
2:1	COD in	2.2	4.1	
	COD to CH4%	41	39	

4.4. LABORATORY SCALE INVESTIGATIONS

These experiments were conducted primarily to start-up and maintain a stable anaerobic digestion process, in which the selected industrial effluents can be effectively converted into methane and also to provide data which could be used for the start-up of the pilot plant. The results obtained from the serum bottles provided the detailed information regarding a safe set of operating parameters which should be used as a starting point for a large scale investigation.

The AAT in **section 4.1** showed that:

- The distillery and size effluent were not inhibitory to the methanogenic biomass, at concentrations as high as 28 g COD/ℓ and 32g COD/ℓ respectively*;* in fact the methanogenic activity is greatly enhanced. Concentrations exceeding these limits seem to negatively affect the process;
- Approximately 30 % and 45 % (for initial concentrations of 28 and 32 g COD/ℓ of size and distillery respectively) of the organic material contained in the effluent are effectively converted to methane; and
- An adaptation period, estimated in 7 to 30 days, might be needed before an un-adapted biomass could effectively degrade both effluents.
- Since the un-acclimatized biomass was used, very low concentrations were used in order to bypass the adaptation period.

4.4.1 Co digestion of distillery effluent

- <u>Batch phase</u>

During the batch phase, the only variables monitored were the gas production and composition. Initially, the digester was fed with acetic acid (glacial, 35.4 %): a single dose of 5 mℓ was spiked daily in order to favour the (start up) of methanogenesis. The dose of 5 mℓ had been chosen based on a previous experience on serum bottle tests, in which doses of 30 to 50 µℓ of acetic acid (or ethanol) were spiked into vials containing 50 mℓ of seed sludge: that procedure was proven to be successful in maintaining a stable methanogenic activity for long period of times (results not included in this report), without affecting the composition of the biogas produced.

A relatively constant biogas flow rate was maintained for the first five days (3.5±0.6 ℓ/d; **Figure 18a)** and the gas composition seemed to be reaching an equilibrium **(Figure 19a)**.The following day, a larger dose of acetic acid was added together with 100 mℓ of concentrated NMS, in the attempt of supplementing the culture with nutrients and minerals should these have been depleted for the degradation of the previous doses. No apparent improvement in the biomass activity was detected: this was assumed as an indication of relatively good health of the micro-organisms. It was therefore decided that it was safe to add a dose of the test material to the feed.

The following three days, single doses of acetic acid mixed to increasing volumes of the distillery effluent were added once a day. On day 4, two doses were added approximately 12 h apart. As this was believed to be a delicate phase, the headspace of the reactor was sampled frequently, immediately after the feed had been added. This procedure allowed to identify a consistent trend in the composition of the biogas **(Figure 19b)**, which clearly indicates that the distillery effluent undergoes a relatively fast fermentation which results in mostly carbon dioxide being produced. van Haandel and Lettinga (1994); states that the main product of fermentation process are volatile fatty acids, lactic acid and mineral compounds such as carbon dioxide, hydrogen, ammonia and hydrogen sulphide gas. The sudden shift in methane and carbon dioxide was gradually resumed within a day and the general trend seemed promising **(Figure 19b)** as the methane fraction was increasing.

When the distillery effluent was fed to the reactor, the volume of gas produced increased remarkably. However, this increase was not quite linear: this seems to indicate that a load of 6 g COD/ℓ/d might be too high to maintain stable operation. This indication should be regarded as qualitative only, since the number of data available is too small. Although it had been a relatively short experiment, it was concluded that a well-active methanogenic biomass was able to cope with organic loadings as high as 3-6 g COD/ℓ/d. It is clear that the distillery effluent (as proven by serum bottle test) is readily, even if not completely, biodegradable, as it induces a fast acidification that is gradually compensated by methanogenesis. Throughout the experiment, it has been noticed that CO2 consistently drops after the spike of substrate. Therefore a semi continuous mode was started.

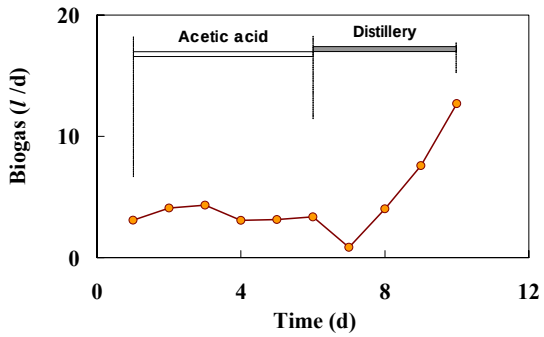


Figure 19 – Batch phase (I): biogas production

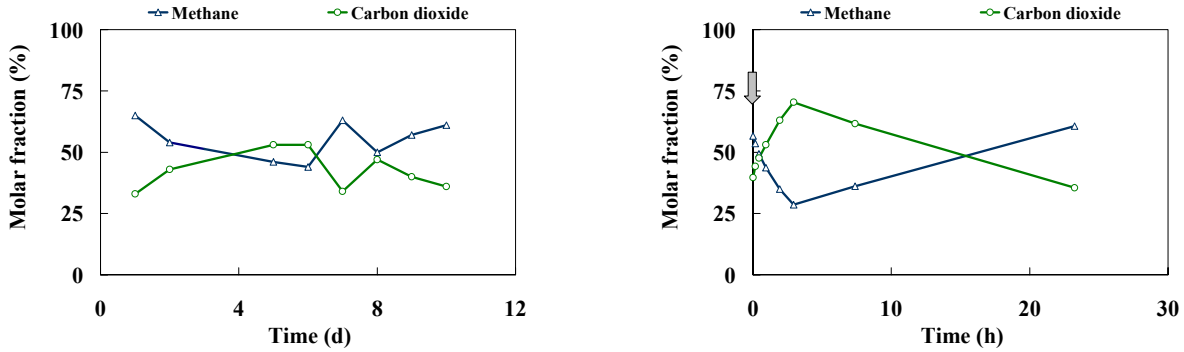


Figure 20– Batch phase (II): biogas composition throughout the experimental period (a) and after a spike of distillery effluent (40 mℓ on day 9) (b).

- Semi continuous phase

The biomass concentration (as volatile suspended solids) was relatively stable during the experimental period **(Figure 20d)**: although the first two determinations seem to indicate a gradual reduction in biomass, the following two indicate a gradual improvement.

first period: Initially, the distillery effluent was fed to the reactor undiluted, i.e. no additional NMS was supplemented. The marked decrease in the volume of biogas produced and, to a smaller extent, the increase in the carbon dioxide fraction in the headspace **(Figure 20c):** indicated that the biomass was possibly not coping with that load rate. The other candidate warning indicators: pH and alkalinity were decreasing whereas the concentration of VFA was increasing. However, more than the individual parameter, the marked drop of the alkalinity-to-VFA ratio clearly indicates that the acidity of the liquor was increasing: the trend was to some extent anticipated by the finding of the batch phase but was judged as concerning for a safe start up of the digester.

Anderson and Yang (1992) concluded that the concentration of VFA, sulphate, nitrogenous compounds and COD have a profound effect in alkalinity demand for pH control in an anaerobic treatment. Therefore, in order to provide the biomass with the means of counteracting the acidity generated by the fermentation of the organic matter in the distillery effluent, as well as with the nutrients and minerals that, during continuous operations would have been depleted, it was decided to make up a synthetic feed comprising 50 % of distillery and 50 % of a concentrated NMS.

second period: A concentrated NMS was prepared and fed to the digester at a rate of 0.1 ℓ/d, which would have resulted in an in-reactor concentration approximately equivalent (of the same order of magnitude) to that used in the serum bottle AAT. For a period of 24 days, the same hydraulic regime was maintained with few exceptions at a retention time of 18 d and organic loading rate of 3.4 g COD/ℓ/d **(Figure 20b).** The pH value of the mixed liquor was relatively stable until the end of this second period, when it gradually fell out of a range of safe operation (indicated by the two horizontal lines, in (**Figure 21a)**. The pH of the environment within a reactor must range between 6.5 and 8.2 respectively (Brock & Madigan, 1991). Methanogenic series are rate controlled by the slowest member in the process. In case of anaerobic process the slowest process is characterised by accumulation of substrate built-up found just prior to the rate controlling step. The slowest members of the consortia are often the propionic and acetic acid utilizers, so that an accumulation of these organic acids can overwhelm the reserve bicarbonate alkalinity. Such malfunction may cause the drop in pH which can have drastically adverse impact upon entire consortia (Speece, 1996).

This was one of the factors which suggested the reduction of the loading rate (third period). The marked change in pH, between day 27 and day 30, was caused by the change in the composition of the feed that was decided in the attempt of improving the performance of the digester in terms of COD removal:

- two single doses of 5 mℓ of acetic acid were added on days 28 and 29;
- the normal feed was used the following day; and
- NMS only was fed for two days, with no distillery: this transient resulted in a local change in retention time, not indicated in **Figure 20b**.

Thereafter, the normal feed was resumed, until the end of this experimental period. The general worsening of the performance of the process is clearly illustrated by the trend in alkalinity and VFA concentration **(Figure 20a)**, that reach values of approximately 100 and 250 mmol/ℓ respectively. Even more evident is the trend in their ratio, as illustrate in **Figure 21a**.

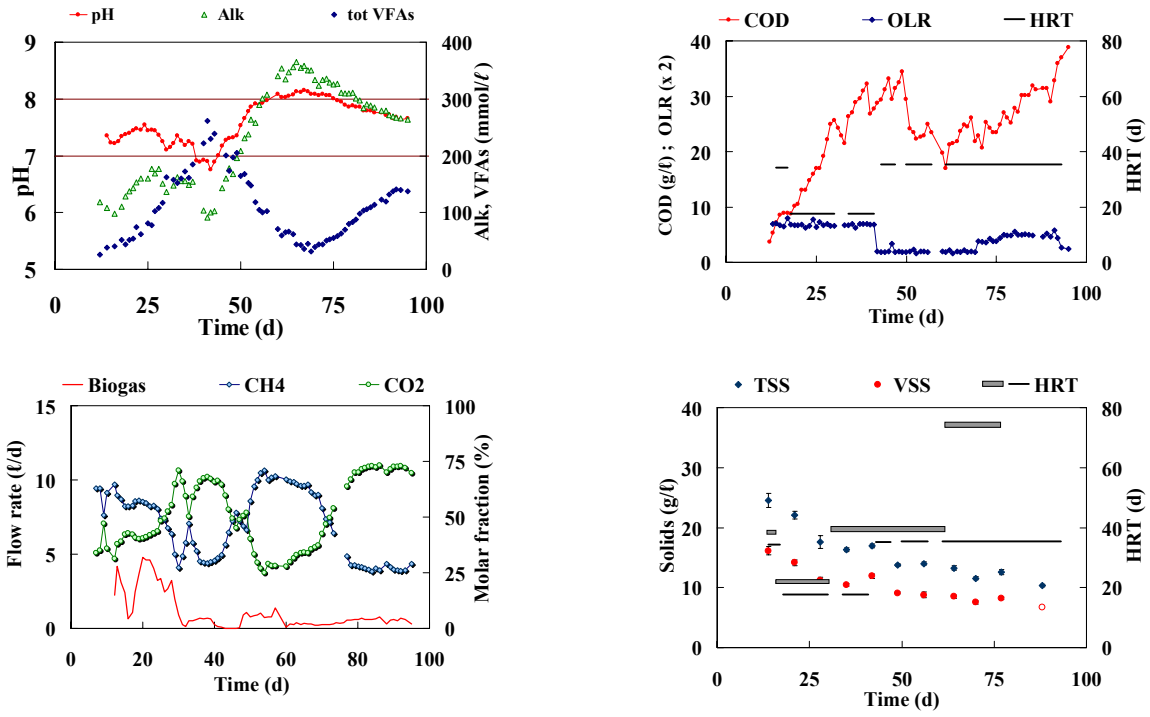


*Figure 21 – Semi continuous phase (I): progression of the pH value, alkalinity and VFA concentration (**a**); in-reactor COD concentration against HRT and OLR (**b**); biogas flow rate and composition (**c**); Solid and HRT(**d**).*

The transient and particularly the (interruption) of the supply of distillery effluent seemed to have an immediate beneficial effect on the gas composition in the headspace (**Figure 20c**), which however vanished as soon as the normal feed was resumed. Thereafter, both the biogas flow rate and composition indicate relatively stable conditions which can probably be regarded as the steady-state conditions for that particular regime **(Figure 20c)**. From day 38, the pH value started decreasing noticeably; alkalinity and the VFA concentration mirrored the behaviour of pH: this indicated that the reactor was (suddenly) accumulating acids, suggesting that the methanogenic biomass was not able to cope with the organic load.

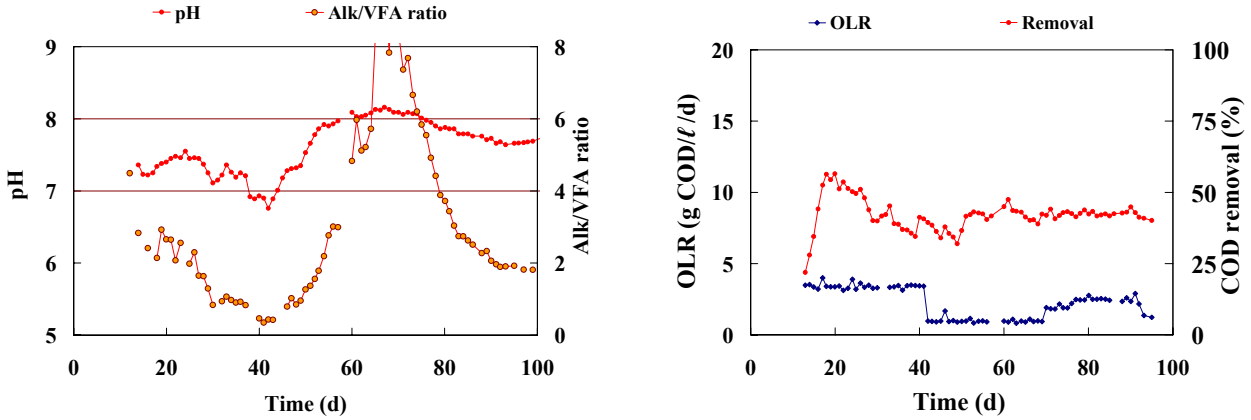


*Figure 22 – Semi continuous phase: progression of the pH and the alkalinity-to-VFA ration (**a**); organic loading rate against cod removal percentage (**b**).*

third period: As the organic load was decreased from 3.4 to 1 g COD/ℓ/d on day 41, all indicators resumed a healthy trend. The pH value steadily increased to the initial values and beyond, to values as high as 7.90. Similarly, alkalinity and VFA concentration respectively increases to approximately 250 and decreases to 100 mmol/ℓ **(Figure21a).** The COD concentration eventually inverted its increasing trend, after day 49 it's started to decrease **(Figure 21b)**. The most remarkable change can be noticed in the composition of the headspace: the fraction of methane gradually but steadily increased to values as high as 71 % and remained constant after that day. The biogas flow rate also stabilised. Clearly as both the COD concentration in the effluent decreased and the methane fraction in the gas increased, the efficiency of COD conversion increased **(Figure 22b).** As the conditions (measuring parameters) indicated that the reactor has reached steady state after 27 d operation in this OLR, it was then increased to 1.9 ± 0.1 g/COD/ℓ/d by increasing the volume of distillery to 50mℓ/d and decreasing NMS to 50mℓ/d thereby keeping the HRT the same.

The pH continued its healthy trend but alkalinity started to decrease steadily, while VFA's increased mirroring the trend. The gas composition and flow rate followed the same trend: methane fraction dropped to 30±2 % and the gas flow rate did not double as it was expected when the load was doubled, but remained at 0.4±0.1 ℓ/d. Due to bad reactor performance from d76 till end the reactor was spiked with 2 g of glucose and 1 g yeast in an attempt of improving and favoring the growth of biomass. This increased the OLR to 2.5 ± 0.2 g/COD/ℓ/d. This did not improve any condition in the reactor as the pH started to drop gradually but remained within the safety operating range. The alkalinity and the VFA concentration mirrored the behavior of the pH:

alkalinity continued to drop while the VFA increased. This indicated that the acidity in the reactor was increasing, which does favor methanogenes. The gas composition also became worse: methane fraction dropped to 26±2 %. The gas flow rate increased to 0.93±0.2 ℓ/d: this did not signify any improvement in reactor performance as the concentration of methane in the headspace did not improve. As the condition in the reactor did not change it was concluded that the optimal feed rate for digestion of distillery effluent was 1 g COD/ℓ/d.

The ultimate objective of the process is to effectively convert the organic carbon of the feed into methane. The efficiency of this process is generally measured in terms of COD removal efficiency as follows:

$$\eta_I = 1 - \frac{COD_{out}}{COD_{in}} \qquad 13$$

where COD_{in} and COD_{out} denote the COD concentrations in the feed and in the effluent respectively. However, this approach is correct when the system has reached a steady-state and not before one (or more) retention times have passed, as the efficiency defined according to **equation 13** does not account for the dilution effect (function of HRT) that occurs in a completely stirred digester. A mass balance on the COD is actually a better approach in that it compared the *total* amount of organic matter fed, to the *total* amount degraded, as:

$$\eta_{II}\ \frac{COD\ deg\ raded}{CODfed} = \frac{\sum C_{in,t} \cdot V_{in,t} - \left[C_{out,t} \cdot V_R + \sum \left(C_{out,t} \cdot V_{out,t}\right)\right]}{\sum \left(C_{in,t} \cdot V_{in,t}\right)} \qquad 14$$

where:

$C_{in,t}$ and $C_{out,t}$ denote the COD concentration in the feed and in the effluent respectively, at time *t*;

$V_{in,t}$ and $V_{out,t}$ denote the volume of feed , at time *t*; and

V_R is the reactor volume.

Ideally, the fraction of COD degraded should be stoichiometrically converted into methane and therefore the efficiency evaluated according to **equation 14** should equal the following alternative definition:

$$\eta_{III}\,\frac{\mathrm{CODtoCH_4}}{\mathrm{CODfed}} = \frac{\sum \mathrm{M_{ch,t}}}{\sum\left(\mathrm{C_{in,t}}\cdot \mathrm{V_{in,t}}\right)} \qquad 15$$

where $\mathrm{M_{ch,t}}$ is the amount of methane (in g COD) produced at time *t*.

Possible reasons for the efficiency (**14 vs. 15**) not being equal are:

a) the COD measured analytically is not accurate nor representative of the actual organic matter in the mixed liquor
b) the quantification of the methane produced is not accurate. In fact, the volume of methane produced is calculated through a mass balance which is based on a number of parameter that must be determined with sufficient accuracy for the overall mass balance to be accurate.

The curves obtained for each one of the three approaches is reported in **Figure 22a**. As anticipated, **equation 13** largely overestimates the actual efficiency in the early stages, but becomes more accurate as the system approaches a steady-state. However, after day 41, the change in the feed flow rate and organic load prevented a true steady-state to be reached and maintained and consequently the efficiency defined according to **equation 13** is different from the other curves. The curves obtained from **equations 14** and **15** show a sufficient similarity (are sufficiently close to one another), which suggests that although neither of the COD concentration or the methane volume is very accurately determined, a reasonably estimate of the actual efficiency in removing the COD may be obtained from either curve.

COD mass balance was calculated to provide assessment of the accuracy of the experiments from the following equation:

$$Feed = COD_{effluent} + COD_{methane} + COD_{in-reactor} \qquad 16$$

Figure 22b show that the curve obtained from the **equation 16** and feed are sufficiently close to one another at the beginning which indicate that the measurement of the COD and gas production were accurate up to approximated 50d. After which various problems in the lab were experienced with regards to the gas measurement system of the reactors.

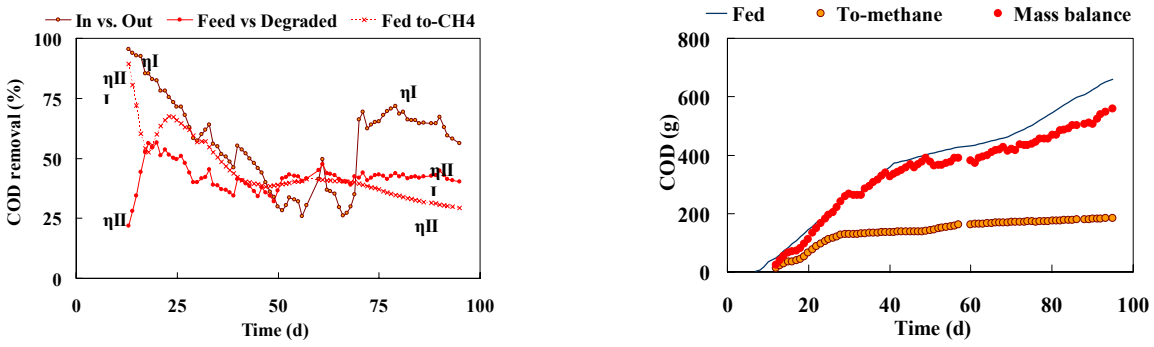


Figure 23 – Semi continuous phase (I) COD removal efficiency (a).COD balance(b)

4.4.2 Co-digestion of size effluent

From the onset the reactor was fed with a synthetic feed containing 50 % size and 50 % nutrient medium. The feed was mixed with nutrients in order to provide the biomass with means of counteracting the acidity generated by fermentation of the organic matter of size effluent, as well as with nutrient medium and minerals that would have been depleted during the continuous operation. The biomass concentration (as volatile suspended solids) was relatively stable during the entire experimental period **(Figure 23d).**

first period: Initially the reactor was feed with 20 mℓ of size mixed with 20 mℓ NMS at a flow rate of 0.04ℓ/d at HRT of 38 d for a period of 29 d. The pH was low at the beginning (6.92) but its gradually increased reaching a peak of (8.05) after 29 d, which was a little above safe operating range by 0.5 (safe operating range is indicated by two horizontal lines in **Figure 23(a)**. This increase was not considered as a major problem as other parameters were stable i.e. alkalinity and VFA's. The gas production rate was initially high at the beginning (for the first four days) at a rate of 1.5±0.5 ℓ/d then it's gradually decreased stabilizing at 0.5±0.1 ℓ/d thereafter. The gas composition also indicated a relatively stable condition (methane composition was 55±5 % and carbon dioxide was 45±5 %) which can probably be regarded as the steady state condition for this feeding rate regime. As the conditions (measuring parameters) indicated that the reactor has reached steady state in this HRT (38d) the HRT was decreased to 19days by doubling OLR to 2gCOD/ℓ/d.

second period: The second phase of (19d HRT) lasted 25 d. The pH was stable throughout this phase ranging between 7.89 and 8.02 (above safe operating range by 0.2). The optimal pH for

anaerobic digestion is between pH 6.5 and 7.5. At value below 5 and in the excess of 8.5 growth is inhibited (Stronach et al,1986).Alkalinity and VFA's were stable for first 15 d at 200±10 and 30±10 meq/ℓ then alkalinity increased sharply thereafter stabilizing at 230±15 meq /ℓ and VFA decreasing to the range of 10±5 meq/ℓ. The gas production rate doubled from the rate of 0.5±0.1ℓ/d in phase1 to the rate of 1±0.5ℓ/d. This was expected as the OLR was increased from 1 g COD/ℓ/d in the first period to 2 g COD/ℓ/d (second period).The gas composition was also consistently stable in this phase with CH_4 at 55±5% and CO_2 at 45±5 %. Both the biogas flow rate and composition indicate relatively stable conditions which can probably be regarded as the steady-state conditions for this phase, therefore the OLR was increased to 4gCOD/ℓ/d.

third period: During third phase the reactor performed well for first 5days. The pH was a little above safe operating line but stable (8.22). The alkalinity and VFA's were also stable at 220±10 and 10±5meq/ℓ respectively **(Figure 23a).** The methane fraction in the headspace steadily decreased to 45±5 % and remained stable thereafter. The gas production rate was a little inconsistent ranging from 2 to 0.55ℓ/d which was below the expected rate of 2±0.5ℓ/d at this OLR of 4 g COD/ℓ/d. On day 6 the marked decreased in methane fraction (21 %) in the headspace raised concerned that the biomass was not coping on this load therefore feeding was stopped for a period of 8 d (to give the biomass time to recover from overload). During this phase no noticeable improvement was noted in terms of gas fraction (CH4 fraction was 22±4 %) and gas production rate (0±0.5ℓ/d)

Feeding of the reactor was resumed at a smaller load of 2gCOD//d in the hope that gas production and composition will resume. This final phase with the feeding rate of 2gCOD/ℓ /d lasted 16d. The gas production rate in this phase was inconsistent at 05±0.5 mℓ/d. The gas composition in the headspace did not improve (methane fraction was 16±2 %), which suggested that the activity of the methanogenes was inhibited. This was also confirmed by marked decrease in alkalinity and increase in VFA's **(Figure 23c).** Because of the time constrains these results were considered enough for the purposes of this project. From these results it was concluded that the maximum load of size effluent which could be digested in anaerobic digester is 2gCOD/ℓ/d.

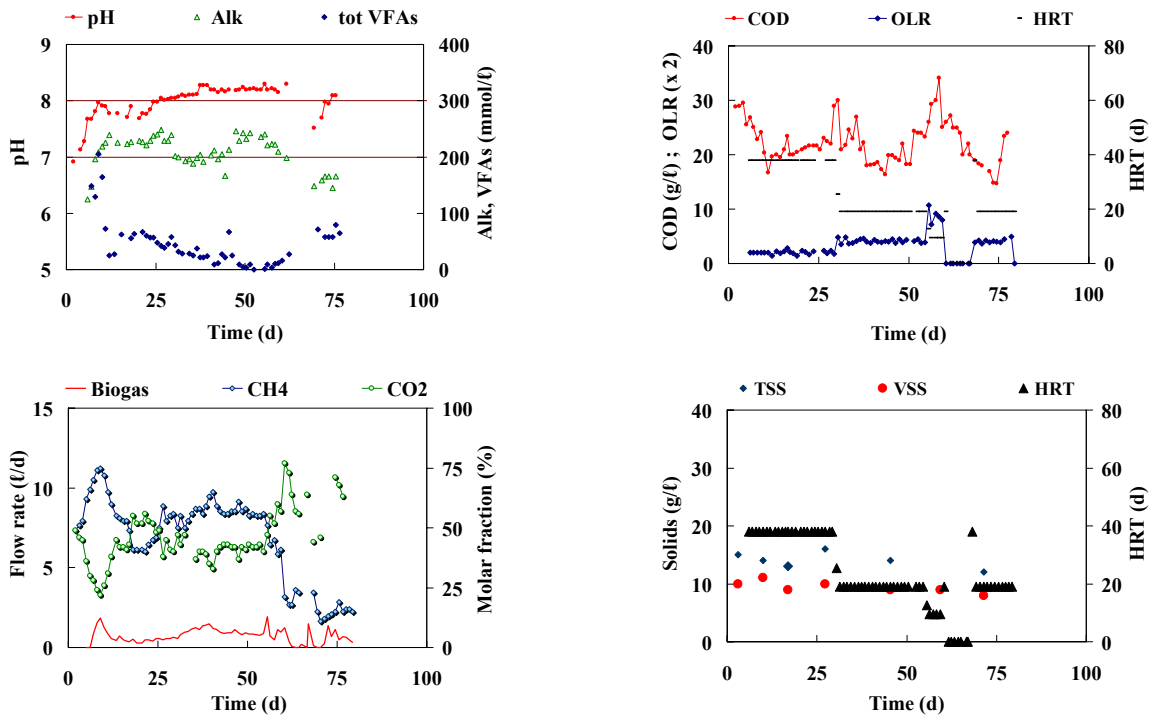


Figure 24 – Semi continuous phase (I): progression of the pH value, alkalinity and VFA concentration (a); in-reactor COD concentration against HRT and OLR (b); biogas flow rate and composition (c); Solids and HRT (d).

The ultimate objective of the process is to effectively convert the organic carbon of feed into methane. The efficiency of the process is generally measured in terms of COD removal by using equation **(13), (14)** and **(15).** The curves obtained for each one of the three approaches is reported in **Figure 24a**. As anticipated, **equation 13** largely overestimates the actual efficiency in the early stages, but becomes more accurate as the system approaches a steady-state. The curves obtained from **equations 14 and 15** are sufficiently close to one another, which suggests that although neither of the COD concentration or the methane volume is very accurately determined, a reasonably good estimate of the actual efficiency in removing the COD may be obtained from either curve.

Figure 24b show the COD mass balance obtained from the **equation 10**: feed curve and the mass balance are close enough to one another to conclude that the measurement of gas production and COD concentration were accurate.

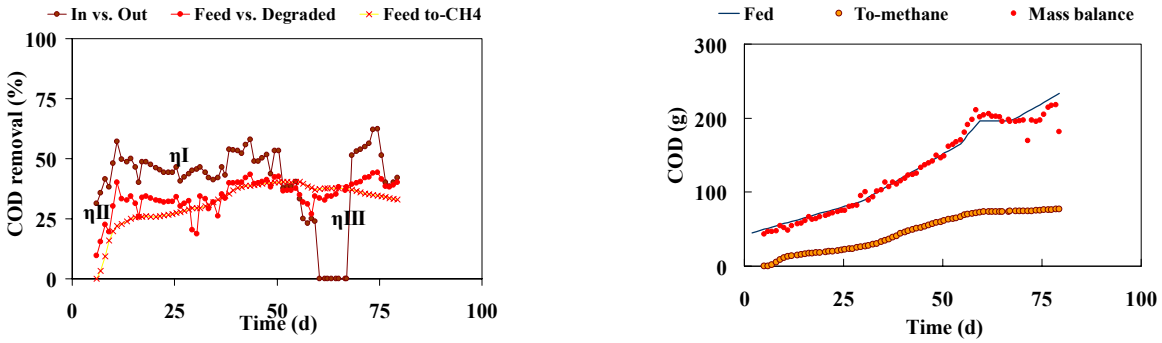


Figure 25 – Co-digestion of size (a) COD removal efficiency (b).COD balance

Conclusion

As a preliminary investigation on the feasibility of a laboratory-scale digestion and co-digestion of the industrial effluents, this exercise was certainly successful in that it enabled us to identify a number of issues that would be of great help for a follow-up study. These can be summarized as follows.

- The best option of monitoring variable, given the facilities in our laboratory, is the composition of the gas phase: this determination can be reliably undertaken within 10 min from the extraction of the gas sample and unlike alkalinity or VFA concentration does not require the extraction of a liquid sample.
- The liquid displacement gas measuring device is not reliable. We have not been able to conclusively identify the problem, but this is surely a key issue that one will have to address prior to starting any larger-scale and/or long-term investigation, as the determination of the volume of gas generated is crucial for conducting mass balances.
- Distillery effluent can definitely be regarded as partially biodegradable and does not inhibit the methanogenic biomass, except indirectly and for a limited period of time due to a rapid degradation of the organic matter to acidic intermediates. In this respect, the main risk to avoid is organic overloading.
- Size effluent can be regarded as biodegradable and does not inhibit biomass but care should be taken not to overload the reactor.
- The addition of a NMS to the feed seemed to be beneficial in that it helped avoiding the depletion of alkalinity. Whereas the addition of a more readily degradable complex substrate such as glucose has not proven very successful as it can promote the acidogenic activity of the

sludge rather than the methanogenic activity, thus further enhancing the conditions of substrate inhibition for the methanogens.

- As a general procedural recommendation, one should maintain a feeding regime for a sufficiently long period of time of at least one retention time, unless the stability of the digester was at risk. This will ensure a set of meaningful data that cannot be obtained if the operating conditions are varied frequently.

4.4.3 Assessing the sludge methanogenic activity

The SMA (sludge methanogenic activity) test can be used for determination of optimal operation conditions for anaerobic reactor and hence the organic feed concentration. Three fundamental operating conditions were defined by Ince et al (1995) in a study of laboratory scale up-flow anaerobic sludge blanket reactor. Operating condition (i) corresponded to an actual methane production (AMP) rate in the digester of 60 % of potential methane production (PMP) rate of sludge using SMA test, thus resulting in high operating stability and an excellent COD removal. Operating condition (ii) was identified as being 80-100 % of PMP rate, resulting in lower COD removal and stability depended on the available alkalinity. Operating condition (iii) took place at excessive organic loading rate (i.e. where the AMP rate in the digester is greater than the PMP rate) resulting in an irreversible imbalance in the sequential stages of anaerobic biodegradation. De Zeeuv (1984) stated that at the beginning of the start-up of the new reactor, the specific activity of seed sludge together with the amount of sludge present determines the permissible initial organic loading rate.

The methanogenic activity of the sludge is an ideal indicator of the performance of a digester, because it indicates how efficient the conversion of the organic matter to methane is. In general terms, it is defined (**equation 17**) as the ratio between the rate of methane production (ℓ of methane/d) and the amount of biomass present in the system (g VSS, or g VS).

$$SMA = \frac{R_{CH}}{X_0} \qquad 17$$

where:

R_{CH} is the *net* rate of methane production; and

X_0 is the biomass initially present in the vial.

R_{CH} is calculated as the first derivative of the net volume of methane (Step 4)

The former variable can be easily determined in a semi-continuous way, by measuring the biogas flow rate (ℓ of gas/d), e.g. using a liquid displacement or an equivalent system, and by analytically determining the gas composition, i.e. the molar fraction of methane. Although online indirect techniques exist to measure solids concentration (e.g. flow cytometry), these are also very expensive and therefore not viable. On the other hand, it is generally not feasible to measure solids off-line too often. Therefore the assessment of the methanogenic activity of the sludge is also necessarily a discontinuous process variable.

For practical purposes, the following options are equivalent:

- to estimate a daily value for SMA, using the measured methane flow rate and the last measured solids concentration; or to predict a daily solids concentration, based on a number of previous measurements of solids concentration (a simple regression model is sufficient); or
- to calculate a value for SMA in correspondence to the measurement of solids concentration, using the current measured flow rate; or an average methane flow rate.

An alternative method can be to use an aliquot of the sludge withdrawn from the digester, as seed inoculum for a serum bottle AAT. No external substrate should be supplemented, nor NMS: the sludge must be poured into the vial, purged with a nitrogen-carbon dioxide mixture (or equivalent gas) and immediately sealed. Our experience indicates that purging for 1-2 minutes is sufficient to thoroughly flush the headspace. The methanogenic activity of the sludge can be assessed immediately after one day, according to the mass balance in equation 18.

$$\Delta v_{CH,t} = f_{CH,t} \cdot (\Delta v_t + V_{t-\Delta t}) - f_{CH,t-\Delta t} \cdot V_{t-\Delta t} \qquad 18$$

where:

f_{CH} indicates the molar fraction of methane (at time t and $t-\Delta t$ respectively);

$V_{t-\Delta t}$ is the headspace volume at time $t-\Delta t$ (for simplicity, it can be considered constant throughout the test, unless a fed –batch regime is adopted); and

Δv is the volume of gas produced in the interval $(t-\Delta t, t)$.

The advantage of this method of assessing off-line the methanogenic activity of the digester is that a number of vials are now available which 'clone' the digester on a smaller scale and may be

used for further testing. The procedure that was used in this research comprised the following steps.

- Activity assessment: day 0 to day 1.

- Biodegradability assessment 1: day 1 until the gas production levels off. The biodegradable fraction of the test material can be calculated as the rate between the ultimate gas production and the initial COD (which is equal to the COD measured in the 'mother' digester prior to withdrawal). This is valuable information in that it can suggest whether an increase in the retention time of the digester would significantly improve the removal efficiency.

- Activity assessment: until a constant activity is displayed. An aliquot of acetic acid is spiked daily to evaluate the methanogenic activity under 'optimal' conditions. A volume of 30 μℓ of acid 35 % in 30-50 mℓ of sludge does not affect the chemistry of the system to an irreversible extent. An accurate mass balance during this phase can provide very useful indications on the actual degradation process. Ideally the acetate fed should be stoichiometrically converted to methane. Should an excess of methane be detected, this would suggest that the presence of the readily biodegradable substrate allows the further degradation of the test material **(Table 24**, day 36). Moreover, this phase prepares the sludge for the next one, by creating a baseline optimal activity.

- Biodegradability assessment 2: the availability of small scale 'clones' of the digester enables to investigate changes in the operating conditions without distressing the digester. For example, by spiking a dose of the test material and NMS or a dose of a labile substrate, conditions of nutrients deficiency of or co-substrate requirements could be identified, respectively.

4.4.3.1 Reactor A: Distillery effluent

Six AAT were conducted on the sludge of the laboratory-scale digester-A, according to this procedure. The results are summarised in **Table 24.**

Table 24 – Summary of the results of the off-line activity tests.
Units are (g/ℓ) for COD and VSS; (mg COD/g VSS/d) for SMA and (%) for the recovery η.

Test [(a)]	Initial conditions		1st period		2nd period		3rd period	
	COD	VSS	SMA	η	SMA	η	SMA	η
day 15	8.6±0.2	16±1	24±2	≈ 30 %	52±8 [(b)]	> 97 %	87±5	> 70 %
day 22	13.1±0.4	14±0	17±2	≈ 18 %	26±2	40-50	-	-
day 36	29.0±1.2	10±0	13±1	≈ 10 %	93±12	> 115 %	18±2	≈ 19 %
day 43	29.4±0.0	12±0	29±4	13-20 %	68±9	100 %	17±3	14-22 %
day 50	29.5±1.2	9±0	68±0	≈ 19 %	79±4	67-72 %	-	-
day 84	30.2±0.9	9±0	20±2	≈ 7 %	-	-	-	-

[(a)] It indicates the day the AAT was started, with reference to the 'mother' digester.
[(b)] Activity reached a first level at 61±4 and few days later it dropped to 45±1. The value reported in the Table is an overall average.

The sludge methanogenic activity at the operating conditions was evaluated as the average of the first one or two values for each duplicate vial. These estimates, throughout the experimental period, are plotted in **Figure25 a**: the initial slow decrease in the methanogenic activity mirrors the general slow worsening of the performance of the digester, subsequently, a remarkable almost three-fold increase in activity was observed, which indicates that the digester is eventually approaching a steady operation. Between day 50 and day 84, no off-line AAT was conducted due to various technical problems in the laboratory. In the same period, the organic loading rate of the digester was gradually increased. This resulted in a general worsening of the performance of the process, which is reflected in the severe drop in the methanogenic activity

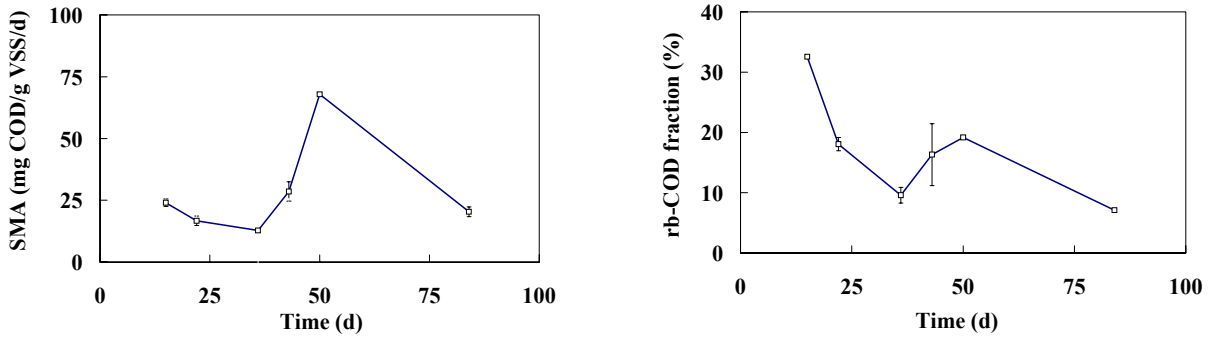


Figure 26 – Semi continuous phase (II): methanogenic activity (a) and rbCOD fraction (b) evaluated by off-line AAT.

A somewhat similar trend was obtained for the estimated in-reactor residual biodegradable COD. This is calculated, as biodegradability assessment, by comparing the ultimate volume of methane produced at the end of the first period, to the initial COD content at the time of the withdrawal. In the first period of operation of the digester **(Figure25 b)**, the rb-COD was as high as 30 %. Thereafter it remained in the interval 10-20 % (the last measured value is approximately 7 %). A relatively high rb-COD certainly indicates that the operating conditions (particularly the OLR) are possibly causing a stress to the biomass which is not able to effectively degrade the organic matter that is fed to the reactor. However, a low value of rb-COD alone should not be interpreted as a sign of improving performance of the process. If we look at the last value measured, the low rb-COD is associated with a low value of SMA: this is rather indicative of a highly overloaded biomass.

The *optimal* sludge methanogenic activity calculated for the second AAT (day 22) is very low compared to the other values calculated and the recovery of the acetate at the end of the second period is only 40-50 % **(Table 24)**: this possibly indicates that the sludge was highly inhibited. As per the procedure, the addition of acetic acid was suspended for one day between the second and the third experimental period. When the latter phase was due to start, it was noted that a considerable gas production had occurred, so the addition of the mixture of the distillery effluent and the NMS was postponed. Such 'residual' gas production was observed for the following ten days: this clearly prevented from undertaking the third phase, but it further substantiated the hypothesis that the sludge was previously inhibited. In fact, while not fed, both units produced a volume of methane equivalent to 1.3-1.4 times the amount of the residual acetate at the time the spikes were stopped (data not shown), therefore accounting for a portion of the residual COD from the distillery effluent.

A similar conclusion (i.e. of an overloaded digester) can be drawn looking at the acetate recovery of the second period of the AAT started on day 36 **(Table 24)**: the mass balance, in excess of 115 % suggests that the addition of acetate might have enhanced the degradation of the residual effluent.

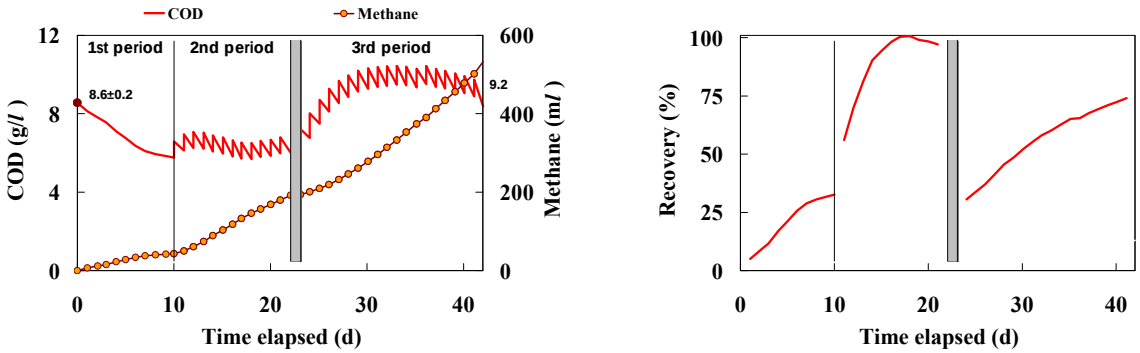


Figure 27 – Off-line AAT (day 15): calculated COD concentration vs. measured methane production (a) and COD recovery (b) for the three periods.

The AAT started on day 15 completed the three stages, i.e. on the residual organic matter (batch mode), on acetic acid and on distillery effluent (fed-batch mode).The results are plotted in Figure 25: the curve of the COD concentration is obtained through a mass balance on the methane produced from the initial measured concentration; the curve of the COD recovery is also obtained through a mass balance **(equation 3)**, with reference to the substrate of that particular period, i.e. for the first period, the residual COD; for the second period, the acetic acid added; and for the third period, the distillery effluent added. At the end of the test, the volume of the mixed liquor, pH, alkalinity, VFA concentration, COD and solids were determined.

- The final volume was 58 mℓ, against an expected value of 59.4 mℓ, resulting in an error of 2.4 %.
- pH, alkalinity and VFA concentration were 7.79; 214 mmol/ℓ and 14 mmol/ℓ respectively.
- The final COD was 9.2±0.0 g/ℓ, against an expected value of 8.4 g/ℓ.
- The solids concentration was as follow: TSS 16±0 g/ℓ; VSS 10±0 g/ℓ. A considerable amount of solids was also found attached to the internal wall of the vial. Small glass beads were poured into the empty vial and 30 mℓ of distilled water added; the vial was capped and vigorously shaken, until most of the biofilm was cut off the wall. This portion of solids was determined separately and yielded the following results: TSS 0.11 g and VSS 0.03 g, which account for the 11.9 and 5.2 % of the total and volatile suspended solids respectively.

4.3.3.2 Reactor B: Size effluent

AAT were conducted on the sludge of laboratory scale **digester-B** according the procedure mentioned in **section 4.3.3**. The biodegradability assessment i.e. the last step in the procedure was

not conducted on these AAT test for reactor A. The sludge methanogenic activity at operating conditions was evaluated as the average of first period of the procedure i.e. biodegradability assessment1. These estimates throughout the experiments are on **Figure 27a**. For the first 33d of the operation of the reactor an improvement was seen in terms of SMA which indicated that the reactor was reaching steady state. From thereon the reactor organic loading rate was increased to 2gCOD/ℓ/d for the next phase (day 30-55) this is seen in slow decrease in methanogenic activity. Furthermore the organic loading rate of the reactor was increased to 4gCOD/ℓ/d which generally caused the worsening of the reactor performance with activity decreasing to zero. This was an indication of the overload in the reactor.

Table 25 – Summary of the results of the off-line activity tests.
Units are (g/ℓ) for COD and VSS; (mg COD/g VSS/d) for SMA and (%) for the recovery η.

Test [(a)]	Initial conditions		1st period		2nd period	
	COD	VSS	SMA	η	SMA	η
day 12	19.5±1.2	10±1	13±2	≈ 6 %	25±1	≈ 24 %
day 21	21.3±1.2	9±0	12±2	≈ 3%	46±3	≈ 26 %
day 32	23.1±0.6	6±0	54±1	≈ 10 %	116±10	≈ 45 %
day 38	20.09±0.6	8±0	47±4	≈ 16 %	65±5	≈ 62 %
day 45	19.3±0.6	8±0	30±0	≈ 8 %	22±5	≈ 49 %
day 52	23.3±0.1	5±0	43±2	≈ 5 %	15±5	≈ 33 %
Day 61	27.3±1.2	5±0	0±0.2	≈ 0%	5±1	≈ 1 %
Day 69	17.0±0	4±0	3±1	≈ 0%	-	-

[(a)] It indicates the day the AAT was started, with reference to the 'mother' digester

This behavior is mirrored in the estimated in-reactor residual biodegradable COD. This is calculated from the first step in the procedure (biodegradability assessment 1), by comparing the ultimate volume methane produced at the end of the first period to the initial content at the time of withdrawal. At the first period it was low, 6 % decreased to 3 %, this generally indicated good reactor performance. At the third period, it increased to 10 % but this did not decrease the activity of the digester (54 g COD/g VSS/d). During the fourth phase it increased to 16 % while the activity of the reactor started to drop (47 mg COD/g VSS/d), this was the indication that the reactor was starting overloading. At fifth period the in-reactor residual biodegradable COD started to decrease, until it reached zero in the last phase. This behavior is not an indication of the digester performing well but, an indication of the overload in the digester. This is also seen in the decrease of SMA, which confirms that the biomass was inhibited. This generally means there is high COD in the biomass which cause inhibition (low activity) therefore it cannot be degraded.

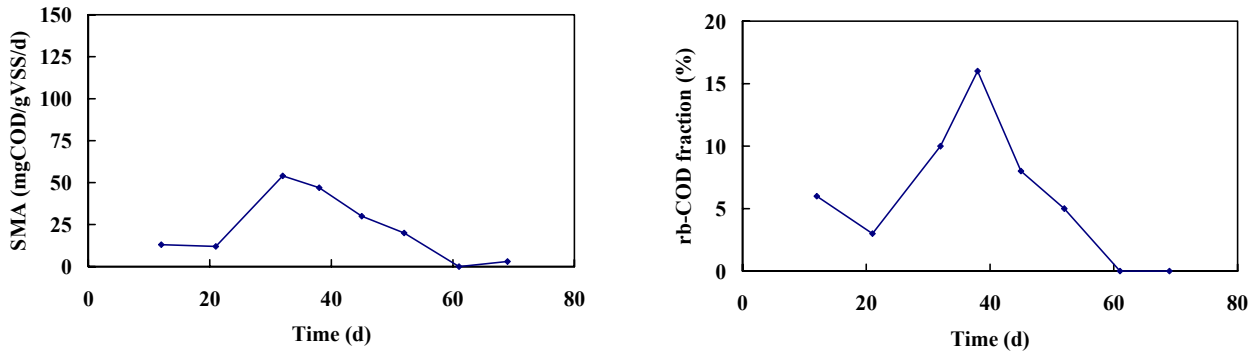


Figure 28 – Semi continuous phase (II): methanogenic activity (a) and rbCOD fraction (b) evaluated by off-line AAT.

The *optimal* sludge methanogenic activity calculated in the second phase i.e. activity assessment followed the similar trend of the first phase (biodegradability assessment) with improvement in terms of numbers. From day 38 (2^{nd} period) both the SMA and in-reactor residual biodegradable COD steadily decreased from 65 mg COD/g VSS/d to 5 mg COD/g VSS/d and 62 % to 1 % respectively. This confirms the results of the first period which indicated that the reactor was overloaded during this phase.

Conclusions

An off-line AAT aimed at assessing the actual and the optimal methanogenic activity of the sludge and the residual biodegradable organic matter is certainly not a viable option for monitoring the performance of the digester, since it lasts several days. Nonetheless, being a relatively inexpensive test it could become of help in the developing phase, as an investigation/research tool.

On the other hand, if it was used merely to estimate the activity under the operating conditions, a reliable estimate can be obtain within 24 hours and it could therefore be performed regularly

5. Conclusions and Recommendations

From this investigation, the following can be concluded

Overview:

- The promotion of cleaner production techniques will results in the identification of numerous small screams of higher strength liquid effluent. In anticipation of the move to cleaner production it is necessary to have pro-active strategies to deal with residual effluents. Survey conducted by Sacks, 1997 identified the available digester capacity in KZN region which can accept high strength effluents.
- Anaerobic digestion has shown to have the potential to treat effluent of this nature. Acclimation associated with anaerobic bacteria can degrade many classes of toxic organic compounds, furthermore this degradation can be enhanced by the addition of labile substrate (co-digestion).The primary objective of this project was to propose an experimental approach whereby a generic effluent can be assessed for its amenability prior to being co-digested**.** The experimental approach will necessarily have to address the two key properties/characteristics, which determine/influence the behaviour of a test material in respect to an anaerobic digester: these are its toxicity and its biodegradability.
- A very detailed protocol is discussed in **(annexure A).** Other authors proposed similar concepts, e.g. a protocol to determine the effect of toxic organic chemicals (Young and Tabak, 1993) or co-digestion experiments on serum bottles (Angelidaki and Ahring, 1997). However, our approach is original in that it proposes a wider picture and provides the operator with a set of methodologies to apply which will ensure that the results he obtains are reproducible and very accurate. This should be regarded as the major scientific output of this project.
- The technical tools that are instrumental to the execution of the protocol are the serum bottles method, to conduct anaerobic activity and co-digestion tests; and a laboratory-scale set-up which enables a close simulation of a full-scale application.

Summary of experimental findings and conclusion:

- The "hybrid" between a toxicity and biodegradability test which originated as pure toxicity test was developed. This method we called it Anaerobic Activity Test(AAT) which enables to evaluate both the toxicity and biodegradability of the test material on the same test by simple monitoring the process much longer/ until gas production levels off. Moreover co-digestion of the substrate with another labile substrate (acetate) was also evaluated on this same test. Effluents were assessed for toxicity and degradability using the serum bottle method of Owen et al (1979). These assays facilitated the determination of whether the loading of substrate into an anaerobic digester would be detrimental to its operation and provided the information on the volumes and concentration of an effluent that could be treated effectively. Materials balances provided an indication of the efficiency of the digestion process in the serum bottles.

- The batch test i.e. AAT should run until the gas production levels off or as long as possible in order to allow for acclimation of micro-organism to toxic substrates. These methods are simple to apply and there is no more time consuming than the standard analytical procedure e.g. COD determination. No specialized equipment is necessary apart from gas chromatography for gas composition analyses.

- The protocol **(Annexure A**) has been used to assess the amenability to be anaerobically co-digested of four industrial effluents, i.e. size and distillery effluents which are classified as high strength and scour and synthetic dye effluents classified as toxic. Effluent was characterized by determination of COD, pH and solids. Size had a neutral pH of 6.5 and high COD content of ca. 120 g COD/ℓ. Distillery effluent was acidic at pH of 5.5 and the COD content was lower then that of size but still high at ca. 80g COD/ℓ. Both effluents had negligible solid content of ca. 0.3 g VSS/ℓ. The scour effluent had high pH (11) and COD content of 4g COD/ℓ. The synthetic dye had a pH of 8 and the COD of 12g COD/ℓ.

- From the biodegradability and toxicity assays the following conclusions were drawn. The size and distillery effluent were found to be degradable at 32 g COD/ℓ and 16 g COD /ℓ concentrations respectively. Concentrations higher than these stipulated above were found inhibitory. Scour effluent was found to be recalcitrant at all concentration tested and synthetic dye was 100 % degradable at 0.12 g COD/ℓ and lower and highly inhibitory at concentration higher than 1.1 g COD/ℓ.

- Co-digestion experiment were undertaken between effluents i.e. size + distillery, size + scour, distillery + synthetic dye in an attempt to verify whether the digestion performance benefits from simultaneous presence of the two substrates. The ratios between the effluents were 1:1, 1:2, 2:1. The presence of two mixtures in the case of size and distillery had better methane production compared to individual substrate i.e. size or distillery separate. The mixture with volumetric flow rate ratio of 2:1 (size: distillery) was preferable in terms of process performance as it had highest COD removal compared to the other mixtures /ratios and individual substrates. The mixture of size and scour (2:1) had highest degradation % compared to other ratios but not high enough to qualify as degradable (less than 50 %). The mixture of distillery and synthetic dye had the same pattern with ratio of 2:1 giving the best COD conversion. The pattern than can be drawn from the degradability of mixtures is: the degradability of mixtures increase with the increasing amount of the most biodegradable compound/effluent in the mixture.
- The results obtained from the serum bottles tests provided the detailed information regarding the safe operating parameters which should be used during the starting point for the larger scale investigation i.e. lab-scale investigations. The lab scale investigations were conducted primarily to validate screening and monitor how the digestion progresses and also to provide data for future project i.e. pilot plant investigation. Other effluents i.e. scour and synthetic dye and their co-digestion mixture were excluded from the lab-scale investigations since they were found to be non-biodegradable i.e. their COD conversion was less the 50 % in the screening protocol. Due to time constrains and other technical difficulties in the laboratory, the co-digestion of size and distillery mixture trials were not conducted on the laboratory scale.
- The results obtained from the lab-scale digestion trials showed that the best organic loading rate for distillery effluent in terms of reactor performance and stability was 1.0g COD/ℓ with efficiency of about 45 %, and for size was 2.0g COD/ℓ with an efficiency of 40 %. The efficiencies obtained in both effluents trials could be greatly improved by acclimation; however these results showed that the digestion of these effluents on the bigger scale is possible.

Based on the above conclusions, the following is recommended:

- Serum bottle method: to be used much more widely, as *routine* tool for monitoring state of laboratory-scale reactors.
- A large Master Culture should be set up, and constantly maintained over the years to provide seed inoculum for various applications (e.g. serum bottles): identify a working and maintenance plan that requires minimal labour but guarantees the performance.
- A simple software application should be designed and implemented which should assist in the continuous monitoring of the AAT serum bottle and laboratory scale units hence improve the quality of experimental output. The software should have data logging application and processing: this could be used to generate a 'baseline' for continuous research material (e.g. for student, teaching, etc).
- A small laboratory-scale (e.g. PVC) completely stirred reaction vessel should be designed and constructed and be permanently placed in the temperature controlled room and connected to the monitoring software. The automatic acquisition of pH and gas volume data is the minimum requirement in the lab-scale unit; and the actuation of feeding and sampling pump(s). All other discontinuous work will be undertaken manually, when necessary.

6. References

Alberto Rozzi & Enrico Remigi, (2004*) Methods for assessing microbial activity and inhibition under anaerobic conditions: a literature review.* Reviews in Environmental Science and Bio/Technology 3: 93-115**.**

Anderson G. K. and Yang G. (1992). *Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration*. Wat. Environ. Res., **64**: 53-59.

Angelidaki B& Ahring K.(1997). *Co-digestion of olive mill wastewaters with manure, household waster or sewage sludge*. Boidegration **8**: 221.-226, 1997.

Angelidaki I., Schmidt J.E., Ellegaard L and Ahring B. K. (1998). *An automated system for simultaneous monitoring of gas evolution in multiple closed vessels*. J. Microbiol. Meth., **33**: 93-100.

Battersby N. S. and Wilson V. (1988). *Evaluation of a serum bottle technique for assessing the anaerobic biodegradability of organic chemicals under methanogenic conditions*. Chemosphere, **17**(12): 2441-2460.

Bhattacharya S. K. And Parkin G.F, "*Toxicity of Nickel in Methane Fermantation System: Fate and Effect on Process Kinetics"* Int'l. Conf. On Innovative Biological Treatment of Toxic wastewater., Arlington. VA, 1986.

Brock T.D. and Madigan M.T. (1991) *Biology of Microorganism*. 6th edition.Prentice-Hall International, Inc, USA.

Campos C. M. M. and Chernicaro C. A. L. (1991). *The use of the SMA-test for measuring toxicity in anaerobic sludges*. Wat. Sci. Tech., **24**(12): 103-111.

Cheng, J, Suidan, M.T., and Venosa, A.D. (1996): *Anaerobic Biotransformation of 2, 4-Dinitrotoluene with Ethanol as Primary Substrate*. Water, Science & Technology **36**: 271-278

Coates J. D., Coughlan M. F. and Colleran E. (1996). *Simple method for the measurement of the hydrogenotrophic methanogenic activity of anaerobic sludges*. J. Microbiol. Methods, **26**: 237-246.

Cohen A. (1992). *Effects of some industrial chemicals on anaerobic activity measured by sequential automated methanometry*. Wat. Sci. Tech., **25**(7): 11-20.

Concannon F., Reynolds P. J., Hennigan A. and Colleran E. (1988). *Automated measurement of the specific methanogenic activity of anaerobic digestion biomass*. Biochem. Soc. Trans., **17**: 425.

Cooney C.L. (1981) *Growth of microorganism*. In: Rehm H-J, Reed G (eds) Biotechnology, a comprehensive treatise in 8 volumes, 1: microbial fundamentals. Verlag Chemie, Weinheim-Deerfield Beach, Florida Basel, pg 73

Cooper P.F., Wheeldon D.H.V. (1981) *Complete Treatment of sewage in a two fluidized bed system*. In: Cooper, Atkinson B (eds) *Biological fluidized bed treatment of water and wastewater*. Ellis Horwood, Chichester, p 251

Criddle C.S. (1993). "*The Kinetics of Cometabolism*" Biotechnology Bioengineering 41: 1048-1056.

De Zeeuv W. (1984). *Acclimation of anaerobic sludge for USAB reactor start-up*. Ph. D. thesis. Wageningen Univ., The Nertherlands

Decree on the input of dangerous substance and plant nutrients into the soil, Official Journal of Republic of Slovenia, NO, 68/96

Department of Water and Forestry (1986). *Management of Water Resources of the Republic of South Africa*. CTP Book Printers. Cape Town

F. van Knapel, *Control of trichinellosis by inspection of farm management practice*, Veterinary Parasitology: Volume 93, Issue 3-4, 1 December 2000, Pgs 385-392.

Gavala H.N., Skiadas .IV., Bozinis N.A. and Lyberatos G. (1996). *Anaerobic codigestion of agricultural industries wastewaters.* Wat. Sci. Technol., 34(11): 67-75.

Georgiou D., Melidis P., Aivasidis A. and Gimouhopoulos K. (2002). *Degradation of azo-reactive dyes by ultraviolet radiation in the presence of hydrogen peroxide.* Dyes Pigments, 52: 69-78.

German Buitron, Gloria Moreno, Mario E. Garcia and Jaime Moreni,.(2004) *Effect of co-substrate, biomass and sulphate concentration on the performance of a control strategy used to determine the anaerobic phase length of an anerobic/aerobic SBR degrading p-nitrophenol.* Anaerobic digestion 10th world Congress, Monreal, Canada. Proceedings Vol. 1,pp. 59-64.

Gray N.F. (1989) *Biology of Waste Water Treatment*, Oxford University Press,United States

Gregor D. Zupancic, Natasa Uranjek-Zevart, Milenko Ros (2008). *Full-Scale Anerobic Co-digestion of Organic Waste and Municipal Sludge- Enlargement of Renewable Energy*. Bioamss and Bioenergy , Volume 32, Issue2, pg 162-167.

Gunnerson C.G. and Stuckey, D.C (1986) *Anaerobic Digestion: Principle and Practices for Biogas System* (Report No 5.) UNDP Project Management

Henzen M. and Harremoes P.. (1983) *Anaeobic treatment of waste water in fixed film reactors-a literature review*. Water Science and Technology, **15**, 1

Ince O., Anderson G.K. and Kasapgil B. (1994). *Control of organic Loading Rate using the specific methanogenic Activity Test During Start up of an Anaerobic Digestion* Sytem. Wat.Res. Vol. **29**, No1, pp. 349-355, 1995

ISO 11734 (1995). *Water quality - Evaluation of the ultimate anaerobic biodegradability of organic compounds in digested sludge - Method by measurement of the biogas production*. International Standard (First Edition 15-12-1995).

James A.,Chernicaro C. A. L.and Campos C. M. M. (1990). *The development of a new methodology for the assessment of specific methanogenic activity*. Wat. Res., **24**(7): 813-825.

Letting, G. (1995). Anaerobic digestion of wastewater treatment system, *Antonie van Leeuwenhoel* **67**:3-28

Lin Chou W, Speece RE, Siddiqi RH (1979) *Co-digestion of leachate and septage in an anaerobic digester.* Biotechnol. Bioeng Symp 8:391

McInnery,M.L., Bryant, M.P., and Stafford, D.A. (1980). *Metabolic stages and energetics of microbial anaerobic dogestion. In Anaerobic digestion,* (ed D.A. Stafford,B.I. Wheatley and D.E Hughes) pp91-8,Applied Science Publishers,London.

Morris JE (1980) *The digestion of crop residues- an example from the Far Eas*t. In: Stafford DA, Wheatley BI, Hughes DE (eds) First Int Synp on Anaerobic Digestion, Cardiff, 17-21 Sept 1979. Applied Science Publishers, London pg 289.

Mrafkova L., Goi D., Gallo V., Colussi I., (2003) *Preliminary Evaluation of Inhibitory Effect of Some Substances on Aerobic and Anaerobic Treament Plant Biomassess.* Chem. Biochem. Eng Q. **17** (3) 243-247

O'Rourke J.T. (1968) *Kinetics of anaerobic treatment at reduced temperature*. PhD Thesis, Stanford University, Stanford.

Owen W. F., Stuckey D. C., Healy J. B., Young L. Y. and Mc Carty P. L. (1979). *Bioassay for monitoring biochemical methane potential and anaerobic toxicity*. Wat. Res., **13**: 485-492.

Peres, C.S., Sanchez, C.R., Mutumol, C.& Schmidell, W (1992) *Anaerobic biodegradability of organic component of municipal solid wastes*. Water Science and Technology, **25**, 7, 285-293

Pohland E.G., Verstraete W., Cohen A., Goma G (1981) *Process Design. In 2nd Int Symp on Anaerobic Digestion*, Sep 6-11, Travemunde, Germany

Pohland F.G. (ed))1992) *Design of Anerobic Process for the treatment of Industrial and Municipal Waste.* Technomic Publishing Company, In. Pennsylvania. 1-14.

Purcell, B.E. & Stentiford, E.I (2000). *Co-treatment: fuelling recovery of organic wastes*. Waste Management, August 2000, pp. 32-33

Sacks J. (1997) *Anaerobic Digestion of High Strength or Toxic Organic Effluents*. MSc Eng Thesis. Department of Chemical Engineering, University of Natal, Durban, South Afica

Salminen E.A. and Rintala J. (2002). *Anaerobic digestion of organic solid poultry slaughterhouse waste* – a

Sawyer L.L and McCarty P. L. (1995). *Chemistry for environmental engineering*. McGraw Hill.

Schmidt J.E., Ellegaard L and Ahring B. K. (1998). *An automated system for simultaneous monitoring of gas evolution in multiple closed vessels*. J. Microbiol. Meth., **33**: 93-100

Shelton E. A. and Tiedje J. M. (1984). *General method for determining anaerobic biodegradation potential*. Appl. Environ. Microbiol., **47**(4): 850-857.

Speece R. E. (1996). *Anaerobic biotechnology for industrial wastewaters*. Archae Press, Nashville (TN, USA).

Stafford,D.A., Wheatley,B.I., and Hughes,D.E.(1980) *Anaerobic Digestion.*Applied Science Publishers,London.

Standard Methods (1995). 19th Ed. - Edited by APHA, AWWA & WEF, United Book Press, Inc.

Stander, G.J. (1997) *Our Water Supply and water environment pollution problems*. Chemical Processing SA **4** (4):13-14

Stronach S.M., Rudd T., Lester JN (1986) *Anaerobic Digestion Process in Industrial Wastewater Treatment*, Springer-Verlag Berlin Heidelberg, Germany.

Stumm W. and Morgan J. (1996). *Aquatic chemistry: chemical equilibria and rates in natural waters*. Wiley Interscience, New York, USA.

Suflita J. M. and Concannon F. (1995). *Screening test for assessing the anaerobic biodegradation of pollutant chemicals in subsurface environments*. J. Microbiol. Methods, **21**: 267-281.

Sutton C. (1997) Survey of Industrial Effluents produced in Kwa-Zulu Natal. BSc Thesis, Natal Technikon, South Africa.

Tsonis S. P. and Grigoropoulos S. G (1993) *Anaerobic treatability of olive oil mill wastewater.* Wat.Sci. Tech 28:34-44
*ultraviolet radiation in the presence of hydrogen peroxi*de. Dyes Pigments, 52: 69-78.

Umbreit W. W., Burris R. H. and Stayffer J. F. (1964). *Manometric techniques*. 4th edition Burgess. Berks.

Valcke D. and Verstraete W. (1983). *A practical method to estimate the acetoclastic methanogenic biomass in anaerobic sludges*. J. Wat. Poll. Contr. Fed., **55**: 1191-1195.

Van der Last, A.R.M. (1991) *Anaerobic treatment of settled sewage with the EGSB and FB process*. Report Agricultural University of Wageningen, Department of Water Pollution Control (in Dutch)

van Haandel A.C. and Lettinga G. (1994) *Anaerobic Sewage Treatment* , John Wiley and Sons LTD, England

Van den Berg L., Lentz C. P., Athey R. J. and Rooke E. A. (1974). *Assessment of methanogenic activity in anaerobic digestion apparatus and method*. Biotechnol. Bioeng., **16**(11): 1459-1469.

Yang J. and Speece R. E. (1986). *The effects of chloroform toxicity on methane fermentation*. Wat. Res., **20**: 1273-1279

Yang, J., R. E. Speece, G.F Parkin, J. Gossett and W. Kocher, (1980) *The response of Methane Fermantation to Cynide and chloroform* Prog. Wat. Tech. IAWPRC., Vol. 12, p. 977-989.

Young J.C. & Tabak H.H. (1993) *Multilevel protocol for assessing the fate and effect of toxic organic chemical in anaerobic treatment processes*. Wat. Environ. Res. 65(1): 34-35

Annexure A: Experimental protocol for screening on industrial effluents

The primary output of this project was to propose an experimental approach whereby a generic effluent can be assessed for its amenability to being co-digested. The experimental approach will necessarily have to address the two key properties, which influence the behaviour of a test material in respect to an anaerobic digester: these are its toxicity and its biodegradability.

- Toxicity denotes a detrimental effect that the effluent exerts on the activity of a microbial population. It indicates the impact of the effluent on the baseline (i.e. prior to the co-digestion trial) performance of the digester. That is to say:
  "*if we feed this effluent (under certain conditions) to a given, well operating anaerobic digester, how will the overall performance of this system worsen, as a consequence of this additional stream?"*

  The following conditions have to be ensured: the gas flow rate must not be dramatically reduced, as methane is the primary economical output of the process; the stabilisation of the waste (e.g. the extent of the COD removal) needs to be maintained, as this is a prerequisite that the legislation imposes for the final discharge (and disposal of residues); and the overall stability of the digester must not be compromised, as rescuing a failing digester or starting-up a new digester can be very expensive and time-consuming. However, a reasonable reduction in the process performance might be agreed upon, as the combined treatment will counterbalance the specific losses.

  Methanogenesis is generally the most sensitive stage of the entire process and certainly this is the case for high strength organic effluents, which may be relatively easily converted to acidic intermediates but thereafter cause a stress overload for methanogens. Further, methanogenesis is immediately related to the production of methane. It is therefore obvious that the general practice is to address methanogenic toxicity.

- Biodegradability indicates the inherent property of a test material, which depends primarily on its molecular structure and refers to its susceptibility to undergoing a biologically mediated degradation. It is related to the actual conversion of the test material to degradation products (or intermediates) which will likely pose a lesser concern in terms of final disposal.

That is to say:

"*if we feed this effluent (under certain conditions) to a given, well operating anaerobic digester, to what extent will the biomass succeed in degrading its organic constituents to intermediates and ultimately to methane?"*

Since the research focused on organic effluents, we adopted the approach whereby if the organic content of the test material is reduced during the anaerobic digestion process and the extent of this reduction is reflected in a stoichiometrically equivalent output of methane, this is sufficient to conclude that the test material had undergone a certain degree of biodegradation.

Limits of the protocol: By no means, this protocol is intended to be a comprehensive and a rigorous instrument which enables to assess the fate of the test material, simply because other forms of toxicity may in fact be induced by particular chemical species contained in the test material that not necessarily are limited to inhibiting the methanogenic biomass. Similarly it may happen that the degradation intermediates and/or the undegradable fractions of the test material are in fact equally or even more environmentally dangerous than the original effluent and although it may be seen that the test material is degraded to some extent, the disposal issue persists.

Other more specific analytical tools exist and generally require a more in-depth study of the degradative pathway of the test material. However these circumstances may be anticipated by a preliminary knowledge of the general composition of the effluent to be treated and possibly of the industrial process that generates it. This step is also (indispensable).Nevertheless, it is believed that the protocol illustrated in this Section can be a reliable decision support tool for high organic strength effluents: purposely, a relatively simple and inexpensive method such as the serum bottle method has been adopted, since it is believed that it is sufficiently simple and accurate to enable the characterisation of a test material in a relatively short period of time and without a great amount of laboratory work.

Table A3 contains a list of the material needed to perform the experimental assessment described hereafter.

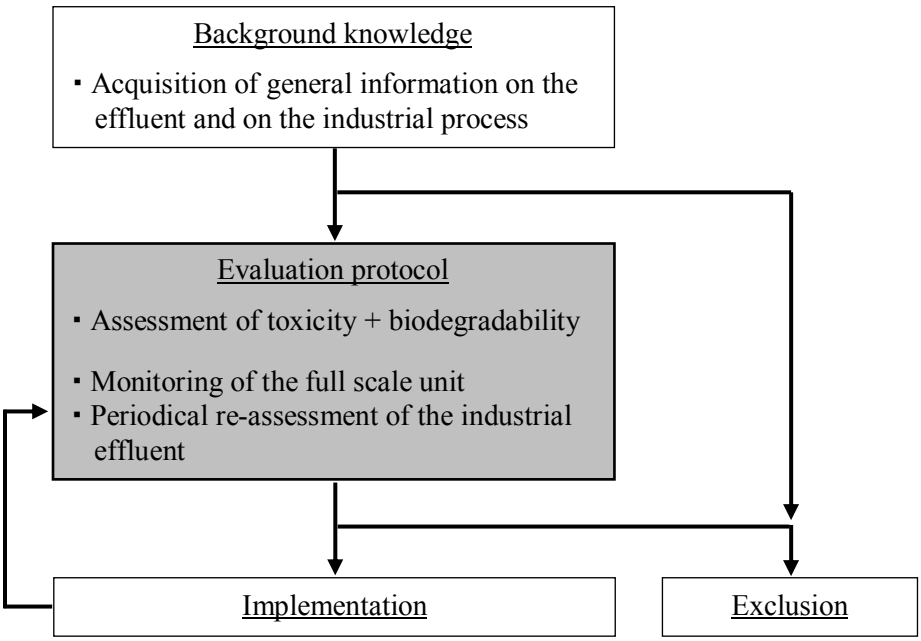


Figure A1 – Flow diagram of the Protocol for the screening of high strength industrial effluents

This discussion of concepts can be visualised as in **Figure A1**, which summarises the general approach of this research. Prior to undertaking the experimental assessment, the operator of the co-digestion facility should acquire sufficient information, regarding the general characteristics of the effluent under study and of the process that generates it. This preliminary step should enable the selection of high strength effluents, which do not pose other more specific and possibly dangerous threats to health and environment, than their high content in organic matter, salts, colour, detergents, etc.

The evaluation protocol serves the double function of initial assessment of the industrial effluent to investigate the feasibility of the co-digestion option for its disposal, as well as continuous monitoring tool of both the actual performance of the full scale application and the periodic reassessment of the effluent, should its make-up and characteristics vary in time, due to changes in the production line.

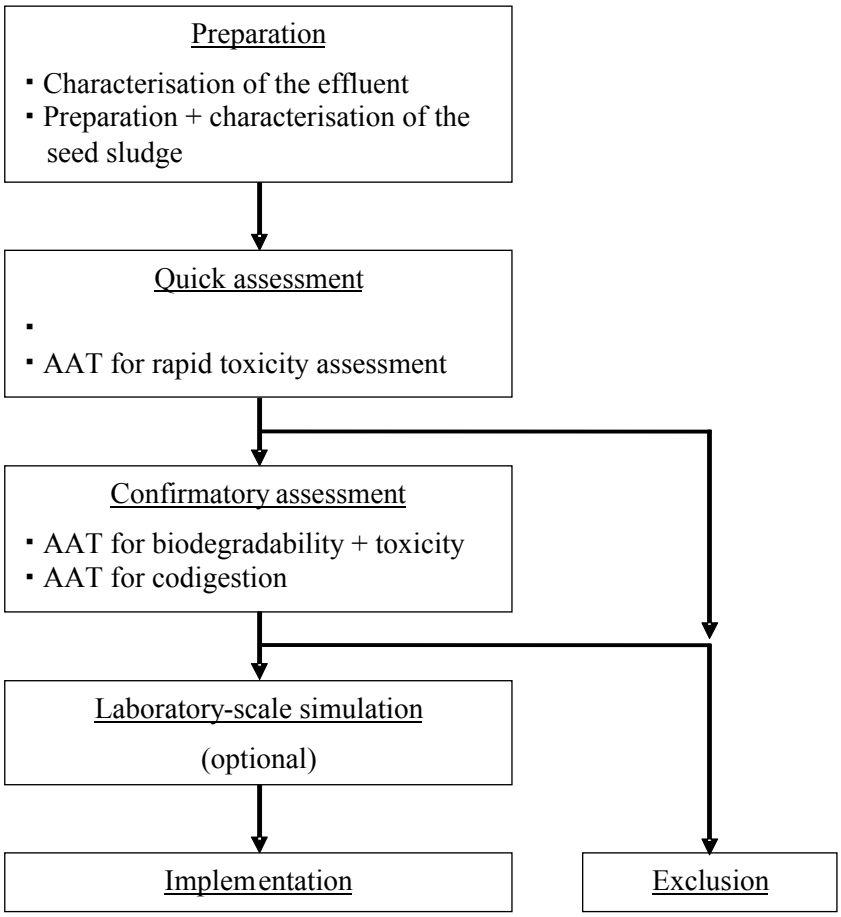


Figure A2 – Experimental stage of the Protocol for the screening of high strength industrial effluents.

A.1 THE EVALUATION PROTOCOL

The assessment of the (methanogenic) toxicity and of the biodegradability of the effluent under study (the test material) consists of the following steps **(Figure A2).**

A.1.1 Characterisation of the test material and preparation of the seed sludge.

The test material will be characterised in terms of its organic content (as COD), pH and alkalinity and solids concentration (total and volatile solids).

For specific applications additional parameters, that may be relevant to the anaerobic degradation process and yet relatively simple and inexpensive to obtain, can be determined: e.g. it might be of interest to assess to what extent the colour can be removed through the anaerobic (co)digestion of the test material.

The characterisation of the effluent must be carried out within the shortest time possible after collection, in order to minimise alterations that may occur due to the incorrect handling of the effluent or to (bio) chemical or physical degradation processes that may intervene. Once the characterisation is completed, the test material should be stored at low temperature (4 °C), in the dark, in a sealed container.

A generic, i.e. unadapted, sludge must be used for the small-scale assessments (which include the Quick and the Confirmatory stages; **Figure A2**), in order to *objectively* assess the toxicity and the biodegradability of the test material in a manner which will be independent from the specific application and therefore comparable to other similar assessments as well as to evaluate a possible adaptation of the biomass to the test material upon long-term exposure. The unadapted sludge could be sampled at a wastewater works and maintained for a certain time on a readily biodegradable substrate, e.g. acetate.

For the Laboratory-scale simulation, a well operating digester will be used in order to accelerate the start-up phase. The sludge used must be characterised in terms of its solids content and have a low residual organic content. This can be done by subjecting it to a period of starvation, prior to starting the assessment.

A.1.2 Quick toxicity assessment

The objective of this first stage is to characterise the test material as potentially inhibitory or readily biodegradable and to identify a concentration range to be extensively investigated in the following step.

A first AAT will be conducted by using three values of concentration of the test material. The indications which can be given here are inevitably qualitative, as the actual concentrations will vary from case to case taking into account primarily the strength of the test material and also the technical viability of a particular dilution.

A very high strength effluent will have to be significantly diluted, so that the highest concentration level does not exceed the value reported in **Table A1;** a higher COD concentration is likely to negatively affect the performance of the biomass and should therefore be avoided *at this stage*;

An excessive dilution of the test material may not be of practical interest at the real scale, because it would not meet the rate of production of the test material itself and/or would require storage capacity that might be not available. This is clearly a more volatile limit that the former one, but it should similarly be avoided, *at this stage*.

Tentatively, we could take as the *high*, *intermediate* and *low* concentrations the values of 50, 25 and 10 % v/v respectively (these volumetric ratios obviously refer to the aliquot of liquid volume available for the test material). It is important to stress that discretion should be applied in ensuring the primary objective which must always be that the outcome of the AAT is meaningful.

This AAT will comprise **15 units** (6 controls, three containing the seed inoculum only and three supplemented with the reference substrate; and 3 replicates per concentration level: **Table A2**) and should be monitored for not less than 7 days. Based on the outcome of this test, the next step will be set-up, according to the following reasoning.

Table A1 – Tentative guidelines for the set-up of an AAT.

Parameter	**Units**	**Value or interval**
Headspace-to-total volume ratio (V_H/V_{TOT})	-	0.2 - 0.6
Substrate (preferably: acetate)	g COD/ℓ	3 - 6
Organic matter (i.e. substrate + test material)	g COD/ℓ	< 30
Solids (i.e. seed inoculum)	g VS/ℓ	10 - 20
Substrate-to-biomass ratio (S/X)	-	0.2 - 0.3

a) If no significant inhibition is visible, even at the high concentration level: the test material is likely to be readily biodegradable with no or negligible harm for the digestion process. In the following step, higher concentrations may be investigated or else the current test extended for a longer time.

b) If the low concentration level shows a significant inhibition: the test material is likely to result in a strong impairment of the digestion process, unless the biomass would be able to adapt upon exposure. In the following step, if lower concentrations are still technically of interest/viable, lower concentration ranges could be investigated in order to identify a concentration range of low toxicity.

A.1.3 Confirmatory assessment

This experimental step is aimed at characterising the biodegradability and/or the toxicity of the test material: the former will be expressed as the fraction of the organic matter content of the test material which is converted to methane. The latter, will be given as inhibition curve and, if possible, as 50 % inhibition concentration (IC_{50}). In order to define a meaningful inhibition curve (or segment), three or four data point are required.

Further, if the test material shows to be inhibitory in the early stage of the incubation, the test period can be extended until signs of biomass adaptation are seen. In our experience, we believe that if after 60 to 90 days, no gas production has occurred; we can conclude that no biomass adaptation is feasible.

Should the test material result amenable to be digested, two further objectives could be achieved at this stage: *i*) the simulation of the co-digestion treatment and *ii*) the identification of a set of 'safe' operational parameters to start-up the laboratory-scale experiment or to directly implement the same treatment option on a full scale.

This AAT will comprise **15 units** (3 controls, with the seed inoculum and the readily biodegradable substrate; 3 controls, with the seed inoculum and the test material; and 3 replicates per concentration level) and should be monitored *until the methanogenic activity decreases to zero*.

A second level of AAT could be started specifically aimed at testing different volumetric ratios between the test material and the designated readily biodegradable effluent (it is assumed that such effluent that will possibly be used in the co-digestion treatment has been identified, as for instance the targeted digester unit on site is currently treating that effluent. It is beyond the scope of this protocol to actually screen various options of readily biodegradable effluents, unless specifically required).

A.1.3.1 Data interpretation

An anaerobic activity test will provide the Sludge Methanogenic Activity (SMA) of the seed inoculum, which indicates the (maximum) activity attainable, i.e. the rate of substrate uptake (g COD/g VSS/d), and the Biodegradability (B) of the test material, which indicates the COD fraction which is actually converted to methane (g COD/g COD).

A tentative value for the Organic Loading Rate to apply to a laboratory-scale digester can be estimated as:

$$OLR = \frac{SMA}{B} \cdot X \qquad \text{(A 1)}$$

Where X is the concentration of solids in the reactor (g VSS/ℓ).

A.1.4 Laboratory-scale simulation

This phase is aimed at verifying the findings of the screening phase, i.e. the extent of biodegradation, the inhibitory effect to the methanogenic biomass, the potential for adaptation and the optimal volumetric ratio effluent-to-substrate, and at simulating the full-scale co-digestion process. It is crucial that the operation of the laboratory-scale unit is closely monitored, to enable the optimisation of the co-digestion performance. The composition of the biogas is relatively easy to determine and is a very sensitive parameter that could serve as main indicator of the process performance.

A.1.5 Monitoring

Once the co-digestion process has been implemented at the full-scale, the same methodology underlying the *screening* protocol can be used to off-line monitor the performance of the process and to periodically re-assess the characteristics of the industrial effluent.

A serum bottle AAT can be carried out at regular intervals (e.g. monthly) on a sludge sample withdrawn from the digested and incubated in the vials with no addition of other components. During a first period, the activity of the sludge is monitored until the residual organic substrate is depleted. Thereafter a dose of reference substrate is spiked into the vials daily (e.g. 30 µℓ of acetic acid) and the activity monitored until it levels off. The determination of the *optimal* methanogenic activity on acetate, as opposed to the *actual* methanogenic activity on the mixed substrate inside the digester, enables to semi-quantitatively estimate the active methanogenic biomass which is a most direct indication of the health of the digester.

The periodical re-assessment of the industrial effluent is a necessity to ensure that its characteristics do not significantly deviate from those initially assessed, as the result of changes in the production line. The set-up described for the AAT of the Confirmatory Assessment of the protocol (**Table A2**) will be used; conditions close to the actual conditions in the co-digestion facility (e.g. effluent concentration, substrate-to-biomass ratio, etc.) will also be used as reference. Should the response be significantly different to the initial one, the option of diverting the effluent until an extensive assessment has been completed must be considered.

Table A2 – Minimal guidelines for the protocol, detailing the number of tests and the time needed to complete the experiment.

▪ Characterisation of the test material (Overall time: 2 days) Perform the following analytical determinations. - COD: 1 to 3 determinations, with 4 replicates each (time: 2 days) - Alkalinity and VFA: 1 determination, with 3 replicates (time: negligible) - Solids: (total and volatile, unless otherwise needed) 1 determination, with 4 replicates (time: 1 day) ▪ Preparation of the seed sludge (Overall time: 1 day) The following steps are to be undertaken. - Collect the sample of the sludge from the plant (or from a laboratory-scale reactor). - Characterise the sample. As per 'Characterisation of the test material', except for the item *Solids*, where total and volatile suspended solids are to be preferred. - Our experience indicates that the sludge sample should not exceed the 30-50 g TS/ℓ, to ensure that a representative sample can be obtained.
▪ **Stage 1** Quick toxicity assessment (Overall duration: 8 days) Perform 1 test, using 15 vials. - preparation of the test, e.g. preparation of the Nutrient and Mineral salts Solution (1 day). - set-up of the test: add all components to the vials; purge with inert gas for 1-2 min; seal the vials; re-equilibrate after 1 hour and GC determination of the gas composition at time zero (1 day). - monitoring the test: every day, determine the gas volume and composition (recommended at least 2 replicates per gas sample; 0.75 day). - analytical determinations at the end of the test: pH, COD, Alkalinity and VFA; as per 'Characterisation of the test material', to be conducted on one replicate per group of vials (2 days)
▪ **Stage 2** Confirmatory assessment - AAT (Overall duration: 30 to 90 days) Perform 1 test, using 15 vials. Follow the same indications of the 'Quick toxicity assessment': monitor the test every day, until the conditions reach equilibrium; thereafter, once every 3 days or every week. ▪ Confirmatory assessment – co-digestion (Overall duration: unknown) Perform 1 test, using 15 vials. Follow the same indications of the 'Quick toxicity assessment'. When the methanogenic activity decreases to zero, the operational mode can be switched to fed-batch by spiking one dose of the substrates, for a follow-up. The duration is unknown, because it is function of the biodegradability of the test material (combined with the readily biodegradable substrate), but unlikely less than a week.
▪ **Stage 3** (optional) Laboratory-scale simulation (Overall duration: variable) The set-up should resemble the actual configuration of the co-digestion facility. Otherwise, use a basic completely stirred vessel (e.g. 3 to 5 litre-volume). Feeding and effluent collection can be done manually. A variable volume headspace (e.g. a gas sampling bag) can prevent gas losses and air contamination during these operations. It is imperative to determine the biogas flow rate (a basic liquid displacement device can be used). Perform the following determinations: - daily: pH, alkalinity and VFA, COD, gas volume and composition; - weekly: solids concentration.

Maintain the same operating conditions for approximately 1 HRT.

Table A3 – List of material required to execute the Protocol for screening industrial effluents.

- Temperature controlled room (or equivalent device, e.g. thermostated cabinet)
  The assays are ideally to be conducted in a mesophilic temperature range, i.e. at 35 °C, and the temperature must be maintained constant throughout the test.
- Material to conduct anaerobic activity tests using the serum bottle method (Annexure B)
  Glass vials (125 mℓ), rubber septa and aluminium crimps (Separation Pty Ltd.), glass syringes (various sizes, e.g. 2, 10 and 50 mℓ); glass syringe (100 µℓ) for gas sampling.
- Gas-chromatograph, equipped with a column capable of detecting carbon dioxide and methane.
- Nutrients and mineral salts stock solution (Annexure E).
- Apparatus for the determination of COD, solids, alkalinity and VFA (Annexure E).

Annexure B: Execution of an Anaerobic Activity Test

A step-by-step guide for the execution of an AAT is illustrated hereafter.

Step 1: Prepare the following components:

- Nutrients and minerals stock solution (NMS): refer to **Section C.1.**
- Healthy, active and homogenised sludge (the seed inoculum). This must be characterised in terms of its solids content (as volatile or volatile suspended solids) and organic content (as COD).: refer to **Section 3.3**
- Test material: this is to be characterised in terms of its pH, alkalinity and organic content (as COD): refer to **Section 3.3**
- Organic substrate (for toxicity assays, only), either as concentrated stock solution or pure reagents. Acetate, propionate or a mixture thereof are commonly used. The type of test can be slightly modified, i.e. from a strictly batch mode to a semi-continuous mode; and pure acetic acid or ethanol can also be used.

Step 2: Equilibrate all components to the working temperature.

Step 3: Pour the components into the vials.

- Ideally, the components should be anaerobically transferred into the vials. However, it has been demonstrated that by manipulating them with care, i.e. trying to minimise the exposure to air and thoroughly flushing the vials with oxygen free gas for 1 to 2 min prior to sealing, is sufficient to leave the anaerobic biomass almost unaffected.
- If the substrate (for toxicity tests) or the test material are in solid form (e.g. powder), it might be easier to add them first. In all other cases, they should be added immediately before sealing the vials.

Step 4: Flush with an oxygen free inert gas.

- A nitrogen and carbon dioxide mixture would be ideal in that it removes residues of oxygen while establishing a CO2 partial pressure above the liquid. If such a mixture is not available, nitrogen can be used to over gas the headspace, but must not be passed through the liquid as it would strip carbon dioxide, thus altering the equilibrium of inorganic carbon.
- Over gas for 5 min at low flow rate; then, immediately seal with butyl rubber septa (20 mm x 3 mm, Separations Pty Ltd.) and aluminium crimp (20 mm, Separations Pty Ltd.).

Step 5: Store at the working temperature, in the dark and motionless.

Step 6: Re-equilibrate the headspace, after 1 to 2 h after preparation.

- As the pressure in the serum bottles might have changed as a consequence of the temperature change and of gas/liquid equilibria adjustment, it should be zeroed to atmospheric pressure.
- Puncture each septum with a needle and vent the excess gas that might have accumulated.
- Although the value does not have to be recorded as part of the AAT, quantifying the volume of excess gas could provide useful indications of biological activity. Clearly, all units containing the same volume of liquid are expected to exhibit the same volume of excess gas. Differences from this behaviour could be due to:

  i) a prompt start-up of the microbial degradation process, resulting in the immediate production of gas: wasting the excess gas in this circumstance would neglect a portion of microbial activity;

  ii) an onset of physico-chemical reactions, in the presence of a test compound which contains volatile fractions; or

  iii) non-homogeneous conditions across and/or within each unit; this is indicative of an inaccurate set up of the test: the measurement of the excess gas might help in the identification of the faulty units, which should be discarded.

Step 7: Determine the gas composition, after re-equilibration.

- Although one can assume the initial composition of the headspace, based on the composition of the mixture used to spurge the vials, a measured value is certainly preferable. Moreover, knowing the actual gas composition could help in understanding the circumstances discussed in Step 6.

Step 8: Record all information, including the starting time on a log sheet

Step 9: Constantly monitor the progress of the test.

- As mentioned earlier, this consists in the periodical determination of both the volume of gas produced and the composition of the headspace. The gas produced should always be wasted to prevent an excessive increase of pressure; unless it is too small to be accurately quantified: in this latter case, it can be re-injected into the vials, so that the pressure can gradually build up to a detectable level.
- Due to the temporary increase in pressure between two subsequent determinations, a fraction of the gas produced remains dissolved in the liquid. The release of the excess gas will bring the pressure back to the atmospheric pressure. However, this will happen too rapidly for the gas/liquid equilibrium to re-establish and the excess dissolved fraction will likely remain undetected. Manually shaking the vials will favour gas/liquid re-equilibration. It is suggested that

two measurements of the gas volume be performed, initially on the undisturbed vial, then after shaking. In this way the conditions closed to equilibration will be established, as the 'driving force' will be from the liquid to the gas phase. Shaking the vial before releasing the excess gas would enhance the internal overpressure to an extent that could make it practically impossible to accurately measure the volume of biogas produced, as the excess pressure could force the gas to escape past the plunger. Under particular conditions, this excessive overpressure could also cause a surge of foam that would result in sludge entering the needle and possibly obstructing it.

- A syringe of suitable volume should be used to ensure that the measurement is sufficiently accurate: clearly, if the production is large, a small-volume syringe which is potentially very sensitive, would result in either a loss of gas (due to the leakage past the plunger) or in the necessity of performing numerous repeated insertions of the needle, that would damage the resistance of the butyl rubber. Conversely, a large-volume syringe would not be sufficiently accurate to quantify a small gas production, due to its low sensitivity. These circumstances can be anticipated, and avoided to some extent, when preparing the serum bottles and the dilutions.
- The syringe should be lubricated with deionised water, prior to the measurement. The reading of the gas volume (or the last one, in case of multiple punctures) should be done by holding the syringe horizontally, in order to minimise the effect of the weight of the plunger, and by gently twirling it, thus allowing a free movement. Readings are verified, by drawing the plunger past the equilibrium point and released, to verify that it returns to the original equilibrium volume.
- Gas composition should be determined immediately after withdrawal from the headspace. The volume should be small enough to leave the headspace pressure unaffected. With a headspace volume of 50 to 100 ml, a sample of 40 µl would alter the pressure by 4 to 8 mbar. For accurate mass balances, this volume should be recorded and accounted for.
- The frequency of the re-equilibration (and determination of gas volume and composition) should be adjusted to the progress of the reaction. Typically, in the early stages of the process, when the gas production is stronger (and the gas composition changes rapidly), frequent determinations would enable the accurate monitoring of the degradation.

Step 10: Stop the test when the gas production has levelled off and the gas composition is constant.

- If long-term information is required (e.g. when the potential adaptability of the micro-organisms to the test compound is investigated), the test can be continued further. Clearly, the frequency of the manual re-equilibration may be reduced, e.g. once per week, until signs of resumed activity

are noted. If no follow-up is needed and the bottles can be opened, the following determinations should be done, to provide quantitative data for mass balances:

-pH, alkalinity and volatile acids concentration;

- COD concentration; and

- solids concentration.

B1: ANAEROBIC ACTIVITY TEST (DATA INTEPRETATION)

The objective of a biodegradability test is to answer the question:

"*to what extent is the test material biodegradable?"*

This translates into the necessity of quantifying the portion of the organic matter contained in the test material that undergoes microbial degradation, i.e. which is converted into methane.

This is done by comparing the initial COD added in the serum bottle (COD_0) to the amount of methane produced in the course of the test ($V_{CH,\infty}$), as:

$$B \approx \frac{V_{CH,\infty}}{COD_0} \qquad \text{(B.1)}$$

Suitable units must be used, by converting COD into methane (or vice versa), using the equivalence factor:

$$1 \text{ g COD} = 0.350 \ \ell \ CH_4 \text{ at STP} \qquad \text{(B.2)}$$

Note that the extent of biodegradation *B* can be affected by the concentration of the test compound (or, by the COD-to-biomass ratio): typically, at relatively high concentrations, the activity of the micro-organisms might be inhibited due to excess substrate, whereas at low concentrations, the substrate might be not sufficient to promote the maximum activity.

For this reason (and not existing a clear reference prescription on this matter;

Müller & Frommert 2004), it is recommended to assess more than just one level of concentration.

The objective of a toxicity test is to answer the question:

"*how does the test material affect the methanogenic activity of the biomass?"*

This translates into the necessity of comparing the methanogenic activity of the sludge exposed to the test material to the activity in the absence of the test compound.

The reason why acetate or other *direct* precursors of methane, e.g. propionate, are preferred to e.g. glucose as substrates is that the drop in pH which follows the initial break down of the substrate could result in a transient reduction in methanogenic activity, that would hide the actual effect of the test material, i.e. it would be interpreted as inhibitory effect, although the test material was not inhibitory or would worsen the true inhibitory effect of the test material and even make it irreversible, although it was in fact only temporary.

The effect of the test material is quantified as:

$$E \approx \frac{A_{t.m.}}{A_{ref}} \qquad \text{(B.3)}$$

Where:

$A_{t.m.}$ is the methanogenic activity in presence of the test material; and

A_{ref} is the methanogenic activity on the reference substrate.

Clearly, *E* is a function of the concentration of the test material, so that generally the effect of a compound ranges from *no-effect*, at low concentrations, to *full inhibition*, at high concentrations. This effect-to-concentration relationship is formally described by an inhibition curve. For technical applications, more than in the entire curve, we are interested in the quantity EC_{50}, defined as the concentration of the test material which results in a 50 % reduction in the methanogenic activity as compared to the reference activity.

B.2 INTERPRETING BIODEGRADABILITY TEST

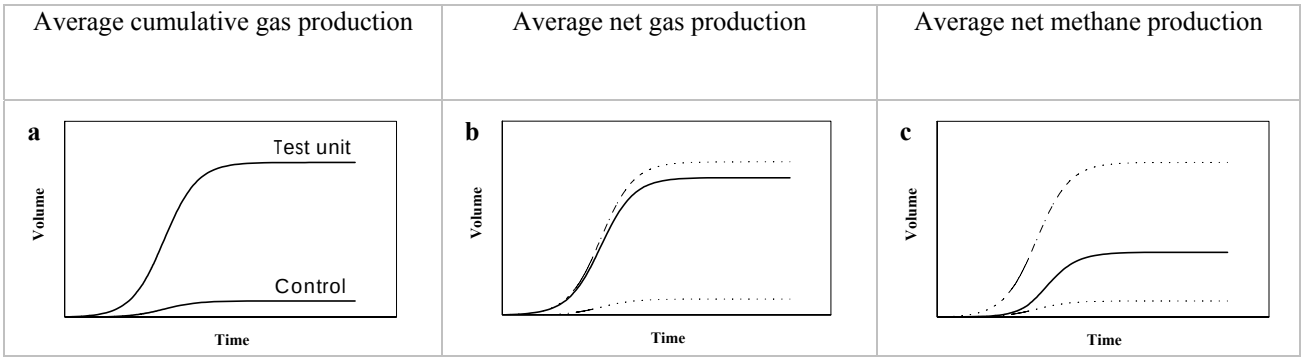


Figure B1 – The outcome of a biodegradability test.

A guide for interpreting the outcome of an AAT aimed at assessing the biodegradability of the test material is illustrated hereafter.

i) Plot the cumulative gas volume for the control and for the test units **(Figure B1).**

ii) Calculate the range of uncertainty of the above curves by calculating the average and standard deviations of each data point. Ideally, the variation coefficient should not exceed 5-10 %. If one of the replicates is clearly out of range, it must be disregarded in the further calculations.

iii) Calculate the volume of methane, through a step-by-step mass balance, as follows:

$$\Delta v_{CH,t} = f_{CH,t} \cdot (\Delta v_t + V_{t-\Delta t}) - f_{CH,t-\Delta t} \cdot V_{t-\Delta t} \quad \text{(B.4)}$$

where:

f_{CH} indicates the molar fraction of methane (at time t and $t-\Delta t$ respectively);

$V_{t-\Delta t}$ is the headspace volume at time $t-\Delta t$ (for simplicity, it can be considered constant throughout the test, unless a fed –batch regime is adopted); and

Δv is the volume of gas produced in the interval $(t-\Delta t, t)$.

iv) Calculate the *net* volume of methane produced in the test unit, i.e. the methane generated by the conversion of the biodegradable fraction of the test material, by subtracting the volume of methane measured in the control units to the one measured in the test units (**Figure B1**). Ideally, the gas production in the control should be small compared to the one of the test material to make the determination reliable.

Four cases are possible, which are schematically represented in **Figure B1:**

- the test material is readily biodegradable (**1**);
- the test material is biodegradable after a lag-phase (**2**);
- the test material is inhibitory in the initial phase of incubation (**3**); or
- the test material is inhibitory throughout the entire period of incubation (**4**).

v) Calculate the ultimate biodegradation (or: biodegradability) of the test material, as:

$$B = \frac{V_{CH,\infty}}{V_{CH,theory}} \quad \text{(B.5)}$$

where:

$V_{CH,theory} = f_T \cdot 0.350 \cdot COD_0$; and

f_T is the temperature correction factor, from STP to the working conditions.

vi) To complement the interpretation of the results, one can calculate (and plot) the Sludge Methanogenic Activity (SMA) in g COD/g VS/d (or: g COD/g VSS/d)

$$SMA = \frac{R_{CH}}{X_0} \quad \text{(B.6)}$$

where:

R_{CH} is the *net* rate of methane production; and

X_0 is the biomass initially present in the vial.

R_{CH} is calculated as the first derivative of the net volume of methane (Step 4)

The growth rates of anaerobic micro-organisms are low, so that in many applications it can be assumed negligible. However, over long periods such those of biodegradability assay, it can actually result in a sensible increase in biomass concentration. Therefore, the use of the *initial* biomass concentration in **equation B6** would overestimate the SMA of the later stages of the process. If the solids concentration is measured at the end of the test (preferably, as volatile suspended solids), one can assume a linear increase between the initial and the final values, to obtain a more accurate estimate through **equation B6.**

B.3 INTERPRETING TOXICITY TEST

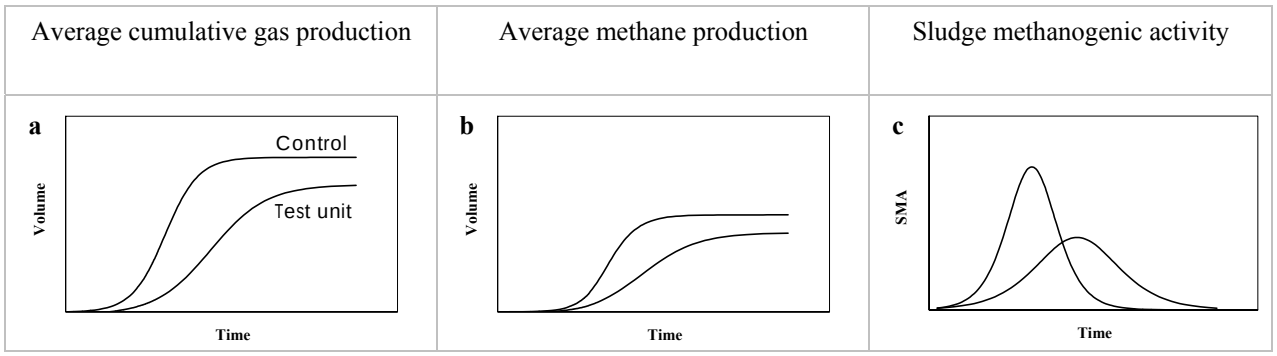


Figure B2 – The outcome of a toxicity test.

A guide for interpreting the outcome of an AAT aimed at assessing the toxicity of the test material is illustrated hereafter.

i) Plot the cumulative gas volume for the control and for the test units Figure B2).

ii) Calculate the range of uncertainty of the above curves by calculating the average and standard deviations of each data point. Ideally, the variation coefficient should never exceed 5-10 %. If one of the replicated is clearly out of range, it must be disregarded in the calculations.

iii) Calculate the volume of methane, according to **equation B4**

iv) Calculate the Sludge Methanogenic Activity, according to **equation B6**

Note that the SMA calculated for a toxicity assay is the rate of methane production in the test units and is conceptually different from the one calculated for a biodegradability assay, which was

based on the *net* methane production. The reason being that in a toxicity assay we are interested in the microbial activity, "in presence of" the test material, whereas in a biodegradability assay we want to *isolate* the effect of the test material from a baseline.

Four cases of inhibition are possible, which are schematically represented in Figure B3:

- the micro-organisms adapt immediately to the test material, although activity is affected (**1**);
- the micro-organisms require a period of adaptation, before a certain degree of activity is resumed (**2**);
- after a period of adaptation, the micro-organisms are no longer affected by the presence of the test material (**3**); or
- the micro-organisms show no ability to adapt to the test material, throughout the entire period of incubation (**4**).

v) Calculate the inhibition effect as:

$$E = \frac{SMA_{t.m.}}{SMA_{ref}} \qquad (B.7)$$

where:

$SMA_{t.m.}$ is the activity in the test unit; and

SMA_{ref} is the baseline activity, in the control unit.

As mentioned above, this 'effect' is concentration-dependent and can be conveniently described through an inhibition curve which plots the relationship between concentration of the test material and inhibition. This latter is defines as:

$$I = 1 - E \qquad (B.8)$$

*Figure B3 – Possible patterns of net methane production, in an AAT for the assessment of biodegradability (**a**, adapted from: Battersby & Wilson, 1988) and of activity curves in an AAT for the assessment of toxicity (**b**).*

Annexure C: Materials and methods

C.1 MINERAL SALT SOLUTION

The nutrients and minerals solution (NMS) that was used for the experiments presented in this report, contained trace elements, minerals and vitamins, according to Owen et al. (1979) with some modifications. The stock solutions for the preparation of the medium are presented in Table C1

Table C1 – Stock solutions for the preparation of the minerals and nutrients medium.

Stock solution	**Composition**	**Concentration (g/ℓ)**
S2	Resazurin	1
S3	$(NH_4)_2HPO_4$	26.7
S4	$CaCl_2 \cdot 2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2 \cdot 6H_2O$	120
	KCl	86.7
	$MnCl_2 \cdot 4H_2O$	1.33
	$CoCl_2 \cdot 6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2 \cdot 2H_2O$	0.18
	$Na_2MoO_4 \cdot 2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2 \cdot 4H_2O$	370
S6	$Na_2S \cdot 9H_2O$	500
S7	Biotin	0.002
	Folic acid	0.002
	Pyridoxine hydrochloride	0.01
	Riboflavin	0.005
	Thiamin	0.005
	Nicotinic acid	0.005
	Panthotenic acid	0.005
	p-aminobenzoic acid	0.005
	Thioctic acid	0.005

The vitamins *p*-aminobenzoic acid and thioctic acid were unavailable, therefore were omitted from stock solution S7.

The method for the preparation of the medium is illustrated hereafter.

1. Add 1 ℓ of deionised water to a 2 ℓ Pyrex vessel.
2. Add the following stock solutions: S2 (1.8 mℓ), S3 (5.4 mℓ) and S4 (27.0 mℓ).
3. Add deionised water up to a volume of 1.8 ℓ.
4. Boil for 15 min whilst flushing with oxygen-free nitrogen (1 ℓ/min).

5. Cool to room temperature.
6. Add the following stock solutions: S7 (18.0 mℓ), S5 (1.8 mℓ) and S6 (1.8 mℓ).
7. Add sodium bicarbonate ($NaHCO_3$: 8.4 g) in powder form.
8. Flush with oxygen-free nitrogen until pH stabilises around 7.1.
9. Autoclave at 121 °C, for 30 min.
10. Store at 4 °C until use.

C.2 GAS-CHROMATOGRAPHIC DETERMINATION OF GAS COMPOSITION

The biogas composition was analysed with a GOW MAC 350 gas chromatograph, equipped with a thermal conductivity detector (TCD) which was used to detect nitrogen, methane and carbon dioxide. A Haysep D stainless steel column (L 4 m, ID 2.2 mm, OD 3.2 mm) was used for the separation, with the following conditions:

▪ Conditions for the GOW MAC 350 and TCD:

- Column oven: 25 °C
- Detector: 25 °C, 100 mA
- Filaments: 40 °C
- Injection port: 40 °C

The carrier gas was Helium at a flow rate of 24.5 mℓ/min. The residence times of nitrogen, methane and carbon dioxide were approximately 1.05, 1.39 and 2.30 min respectively. Gas samples were withdrawn from the serum bottles or laboratory-scale reactors by inserting the needle of a gas-lock syringe (100 µℓ) through the butyl rubber septum (GR-2 9.5 mm, Supelco) and withdrawing 40 to 60 µℓ of headspace gas. The peak area was recorder with a Varian 4270 integrator with the attenuation set at 1.

The settings for the integrator were as follows.

1. Switch power on.
2. Press the DIALOG key.
3. Enter a FILE NAME if desired, and then press ENTER.
4. Set the following parameters:

TT	TF	TV
0.01	AZ	1
0.01	CS	0.5
0.01	PM	1
0.01	AT	1
5	ER	5

5. At the next prompt, press ENTER to exit.
6. Press ENTER, to END DIALOG.

7. Press PRINT FILE to display programme code

The calibration of the gas chromatograph was performed by using the pure gases (UHP, Afrox Scientific). Gas samples of given volumes were withdrawn by inserting the gas-lock syringe into a butyl rubber septum positioned on the outlet of the gas bottle. The pressure on the regulator was set to 16 ℓ/min and 0.35 ℓ/min for carbon dioxide and methane respectively.

Sets of five to six volumes were injected (ranging from 10 to 60 $\mu\ell$), in 3 to 5 replicates.

The detailed calibration procedure was as follows:

▪ Calibration procedure:

- Set up the sampling line. Make sure that the integrator and GC settings are correct.
- Check the septum seal on the sampling line: as a precaution, the septum should be changed every 30 35 injections, to prevent gas leaks through the injection port.
- Look at the reading on the pressure gauge and ensure that there is no fluctuation.
- Record the ambient temperature and the gauge pressure.
- Withdraw a sample of the gas *i* (*i* = N_2, CH_4 and CO_2) of defined volume ($v_{i,j}$, *j* = 10, , 60 $\mu\ell$): lock the syringe until the point of injection.
- Inject the gas sample into the GC and wait for integrator analysis.
- Record the retention time (RT) and Area ($A_{i,j}$) of the gas with the highest area percentage.
- Repeat steps 5 to 7, for all the *j* volumes of the first gas, from the lowest to the highest volume.
- Replicate steps 5 to 7, a number of times sufficient to generate a statistically significant set of data for the first gas (five replicates should suffice).

1. Repeat steps 5 to 9, for the other gases.

The raw data for the GC calibration on methane and on carbon dioxide are reported in **Tables C.2** and **C3**

The respective calibration curve are plotted in **Figures C.1** and **C.2**: the data points (diamonds) are the average values of each set of replicates (column *Ave*, in **Tables C.2** and **C.3**); the solid lines are the linear regressions on the average values (the respective slopes and intercepts are indicated under the Figures, as *calibration factor* and *detection limit*, respectively); the dotted lines represent a sort of confidence intervals for the calibration curve and are calculated for each data point as $\mathrm{Ave} \pm 2 \cdot \mathrm{StDev}$.

As one can see, the determination coefficient of the regression is very high ($R^2 \geq 99.9\%$) and the detection limits very low ($v_0 \leq 5\ \mu\ell$).

Table C 2 – Data for the calibration curve of METHANE.

Volume	Area					Ave	StDev	VC
(μℓ)	1st	2nd	3rd	4th	5th			(%)
10	4183	4150	4495	4269	4343	4288	138	3
20	10557	11244	11047	11380	11320	11110	333	3
30	15299	16226	15939	17284	17316	16413	877	5
40	22471	22565	22609	23659	23800	23021	651	3
50	29938	29481	29579	30229	30209	29887	347	1
60	36069	35810	36004	36624	36724	36246	404	1

Table C 3. – Data for the calibration curve of CARBON DIOXIDE.

Volume	Area					Ave	StDev	VC
(μℓ)	1st	2nd	3rd	4th	5th			(%)
10	6196	5755	5763	5269	5421	5681	359	6
20	15015	14192	13920	13866	14088	14216	465	3
30	21677	23511	22056	21035	21687	21993	925	4
40	29753	31234	31949	29617	29354	30381	1142	4
50	41226	40265	38024	37575	37632	38944	1688	4
60	47785	45063	n.p.	n.p.	n.p.	46424	1925	4

n.p., not performed

Ave, average

StDev, standard deviation

VC, variation coefficient: VC = StDev/Ave

For mass balances purposes, the number of moles of each component (n_i), in a biogas sample, can be calculated from the gas law:

$$P_i \cdot V = n_i \cdot R \cdot T \qquad (C.1)$$

where:

P_i is the partial pressure (bar) of each constituent,

V is the total volume (ℓ),

R is the gas coefficient: 0.0821 ℓ·bar/(mol·K),

T is the temperature (K).

The partial pressure of a component is calculated as:

$$P_i = (P_{atm} - P_w) \cdot \chi_i \qquad (C.2)$$

where:

P_{atm} is the atmospheric pressure: 1.013 bar,

P_w is the water vapour pressure (bar),

χ_i is the molar fraction of each constituent (mol/mol).

The water vapour pressure was calculated as (Batstone et al., 2002):

$$P_w = 3.225 \cdot 10^9 \cdot e^{0.05393 \cdot T} \quad \text{(C.3)}$$

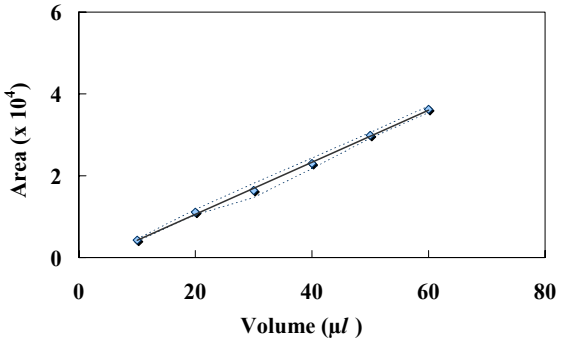


Figure C1 – Calibration curve for methane.

Calibration factor: **636** Area/µℓ (R^2 99.90 %)

Detection limit: **3** µℓ

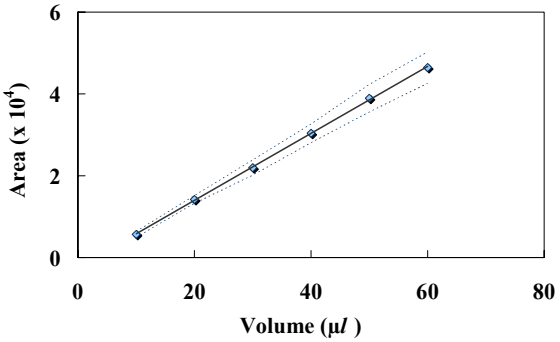


Figure C2 – Calibration curve for carbon dioxide.

Calibration factor: **818** Area/µℓ (R^2 99.97 %)

Detection limit: **3** µℓ

Annexure D: Results

This Section presents the detailed results of the first and second test conducted on the industrial effluents, i.e. size, distillery, scour and synthetic dye. Refer to **Chapter 4** for an extensive discussion. In the following pages, the outcomes of the three control groups and of each test material are reported separately. Four sets of plots are presented, i.e. the biogas production curves; the methane production curves; the gas composition curves and the methanogenic activity curves. Unlike in Chapter 4, where average values were plotted, the plots show each replicate individually, to evaluate the reproducibility of the results. The rationale of maintaining the same scale for all the homologue plots have been privileged to facilitate the comparison between groups, despite the fact that in some instances details might disappear

D.1 TOXICITY TEST 1

Control units		
Sludge, acetate and NMS	Sludge only	Sludge and acetate

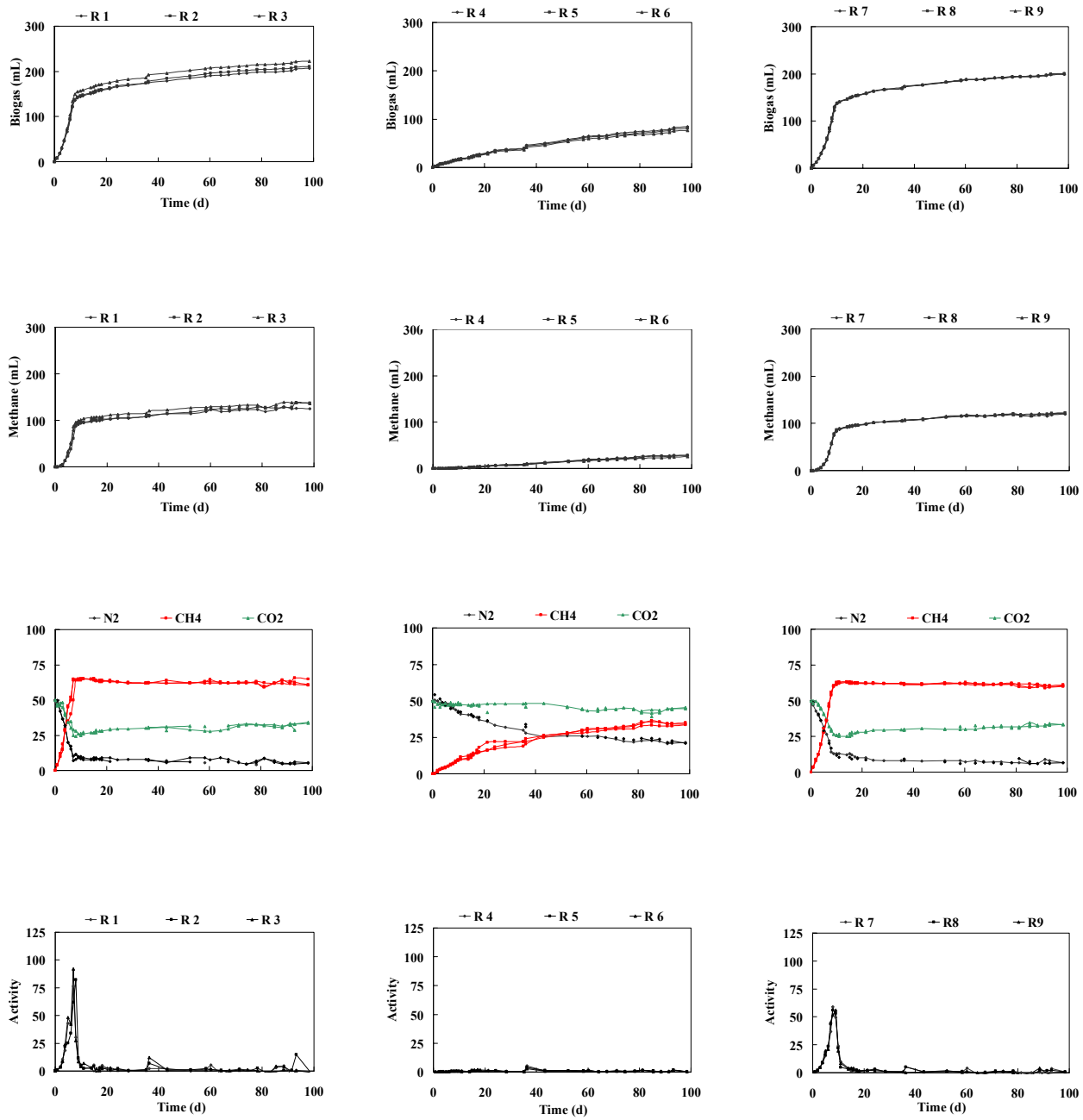


Figure D 1 -(a)Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for control units

Test compound: Size effluent		
Low	Medium	High

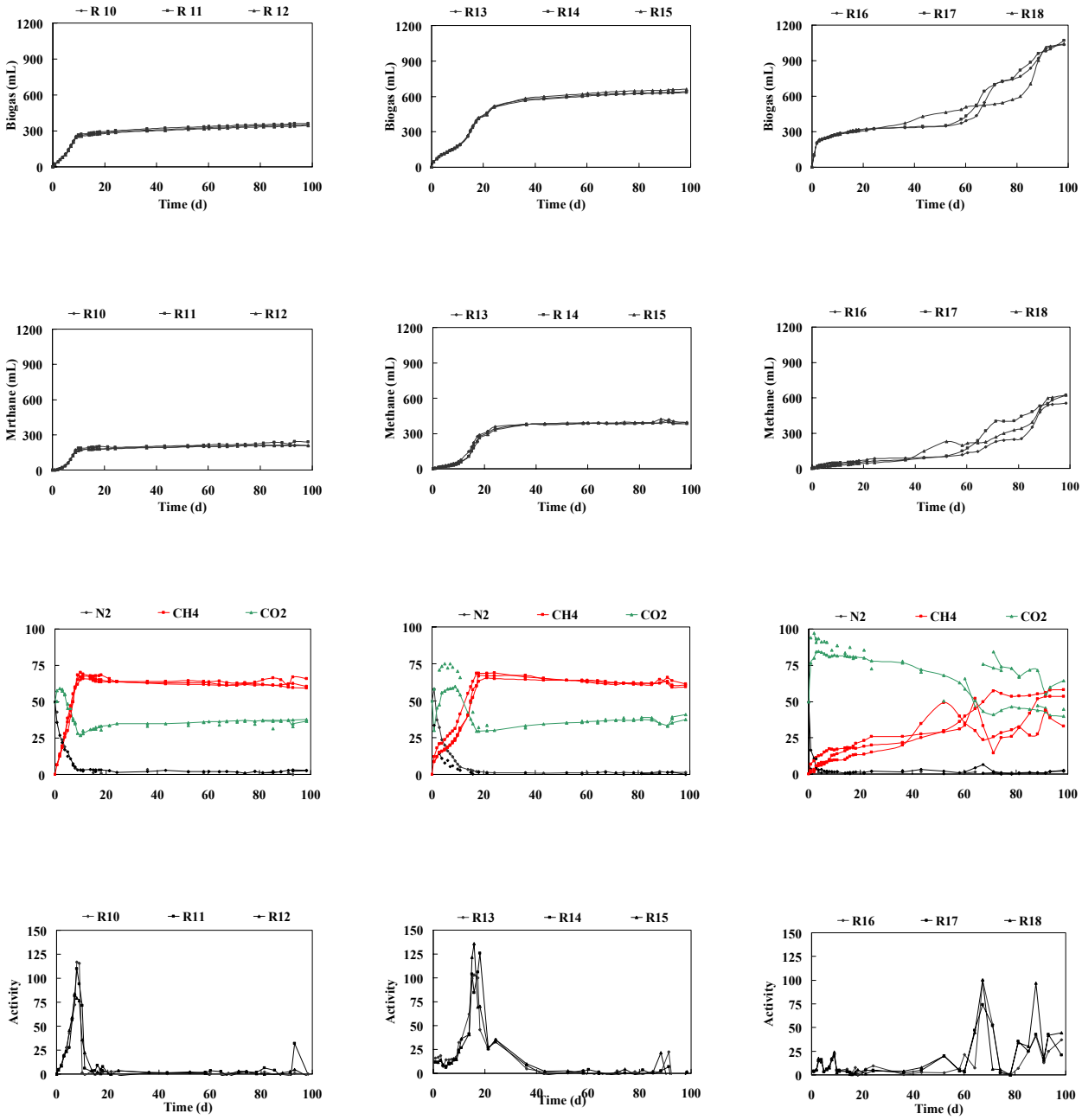


Figure D 2 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity. For size units

Test compound: Distillery effluent		
Low	Medium	High

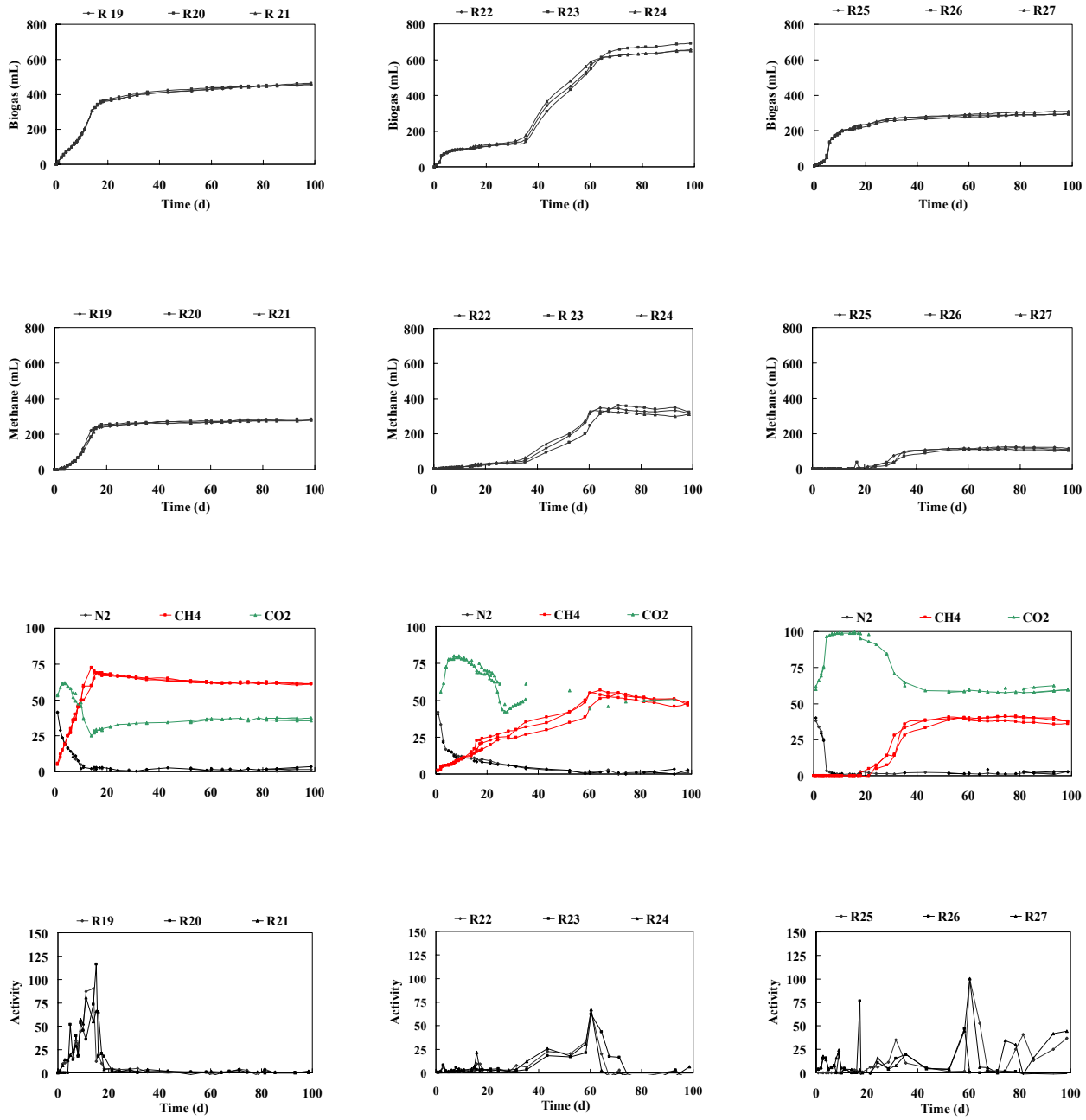


Figure D 3 -(a)Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for distillery units

Test compound: Scour effluent		
Low	Medium	High

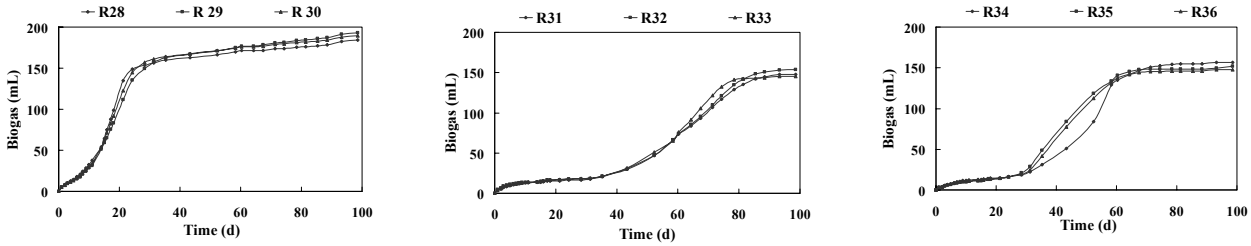
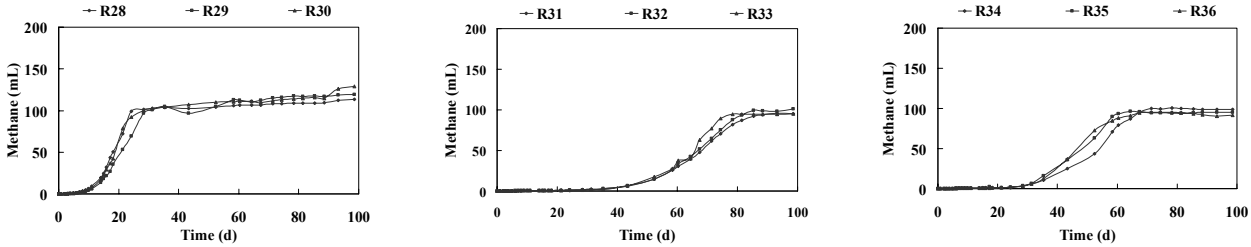
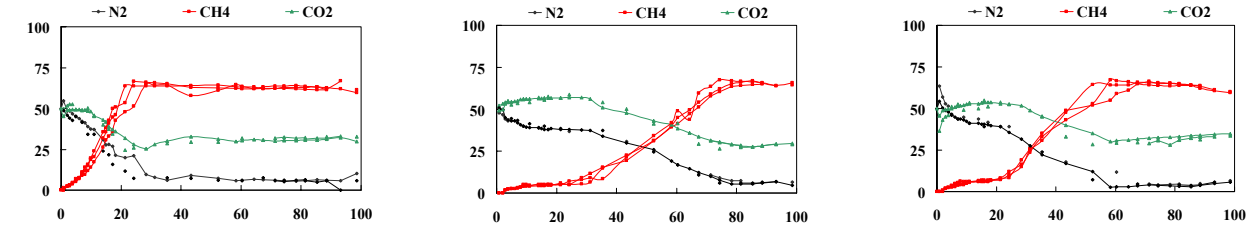


Figure G.1 – Biogas production.





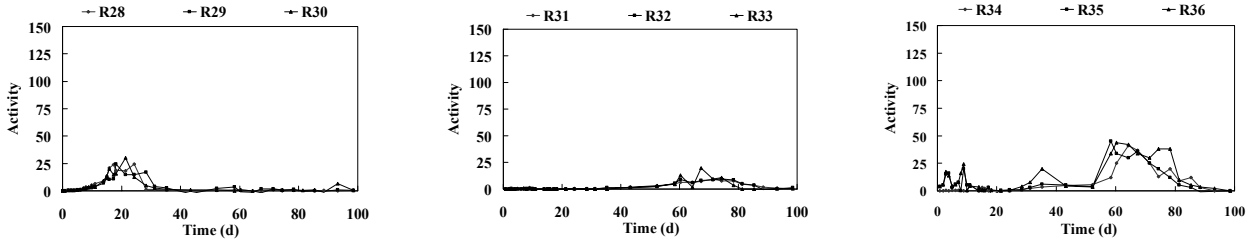


Figure D4 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for scour units.

D.2 TOXICITY TEST 2

Control units		
Sludge, acetate and NMS	Sludge only	Sludge and acetate

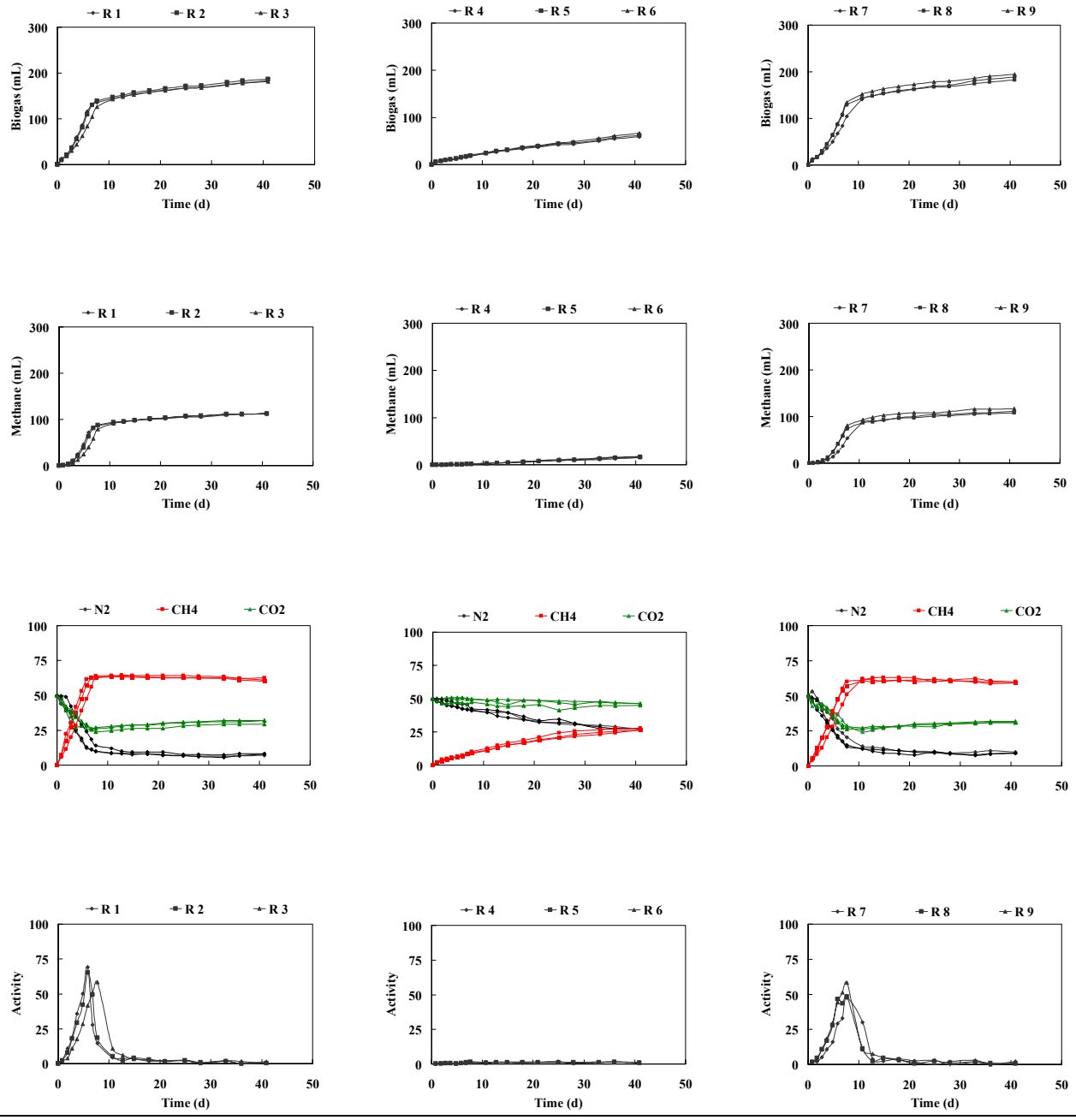


Figure D 5 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for control units.

Test compound: Size effluent		
Low	Medium	High

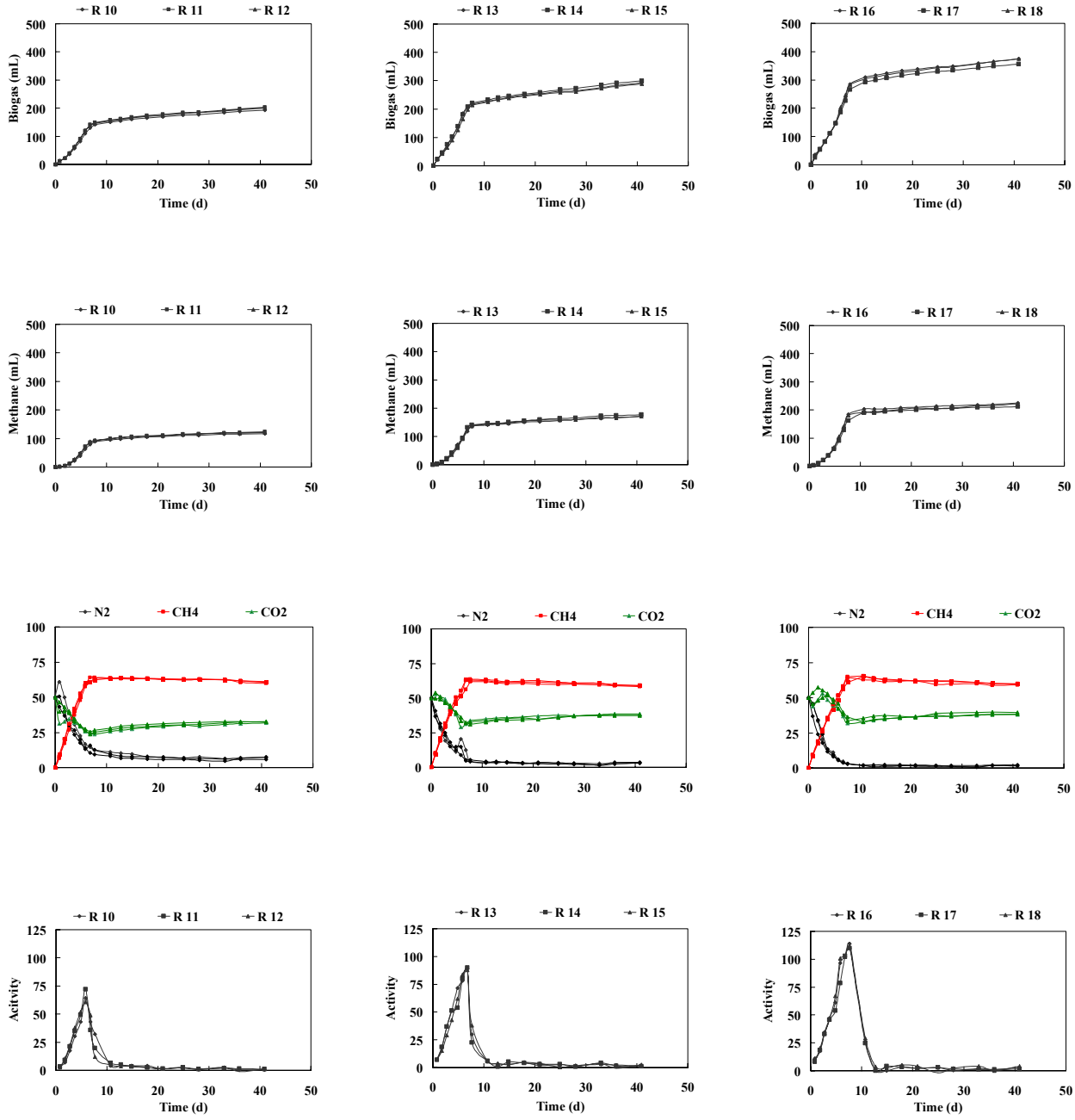


Figure D 6 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for size units.

Test compound: Distillery effluent		
Low	Medium	High

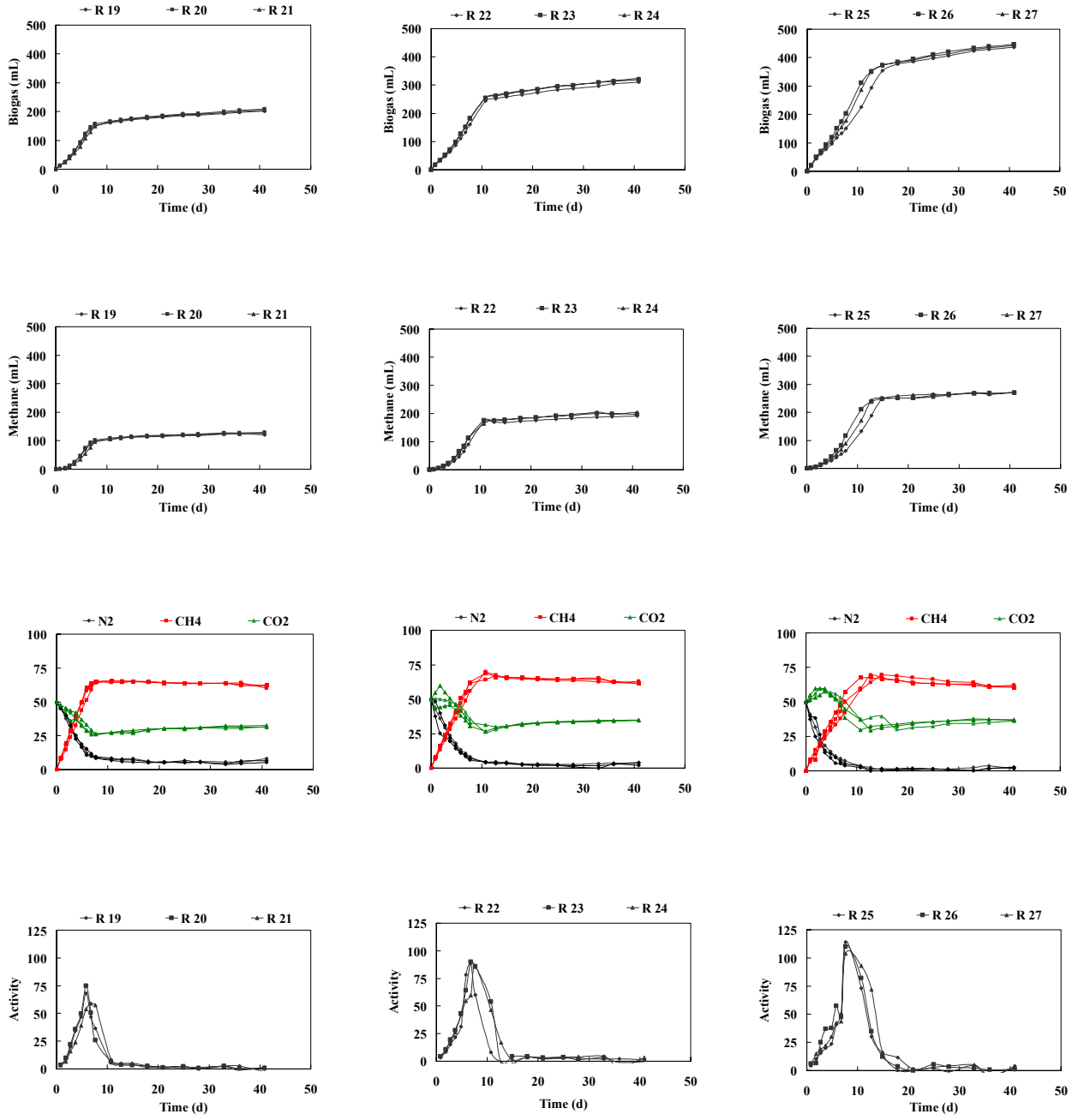


Figure D 7 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for distillery units.

Test compound: Scour effluent		
Low	Medium	High

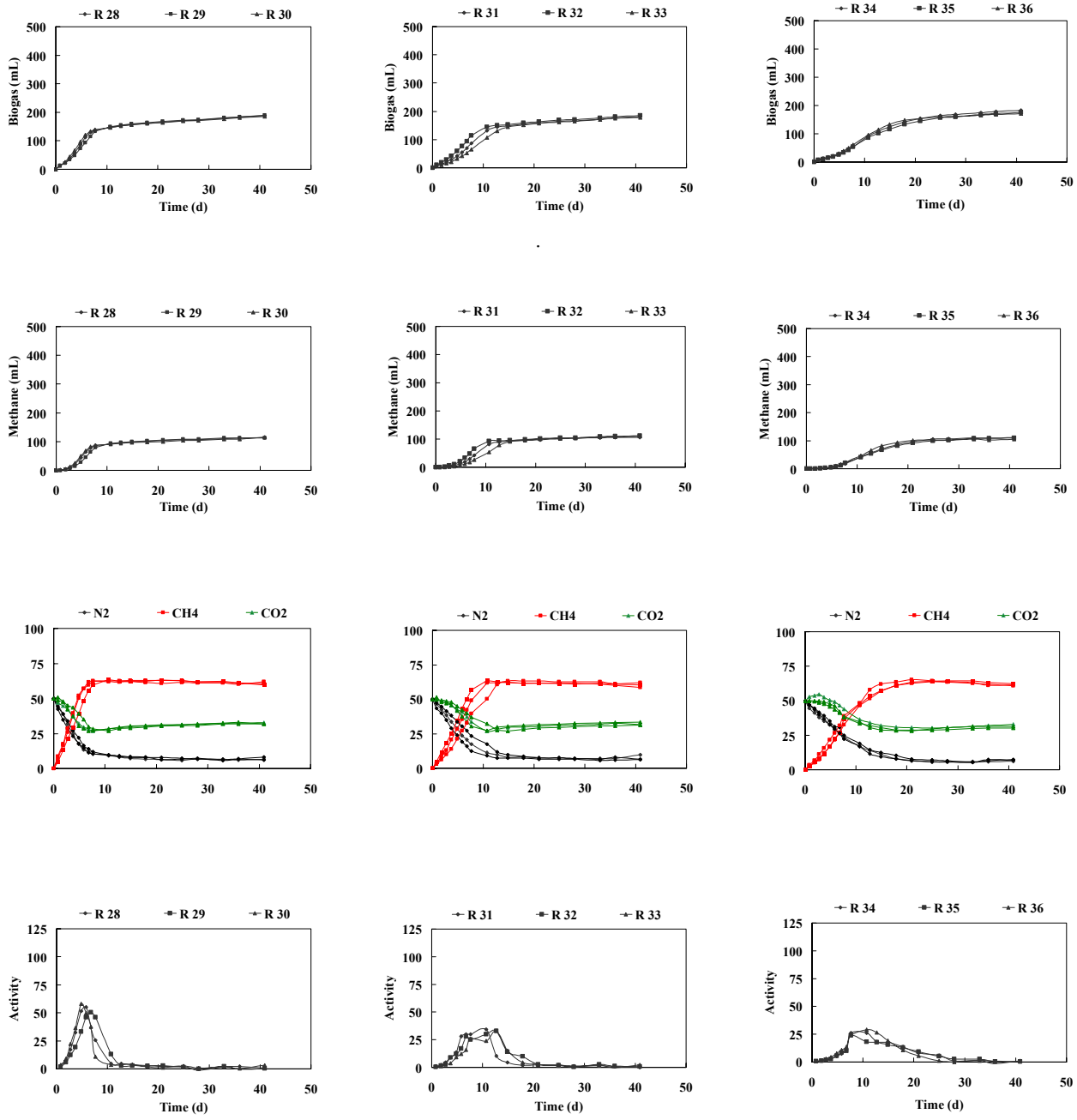


Figure D 8 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for scour units.

Test compound: Synthetic dye effluent		
Low	Medium	High

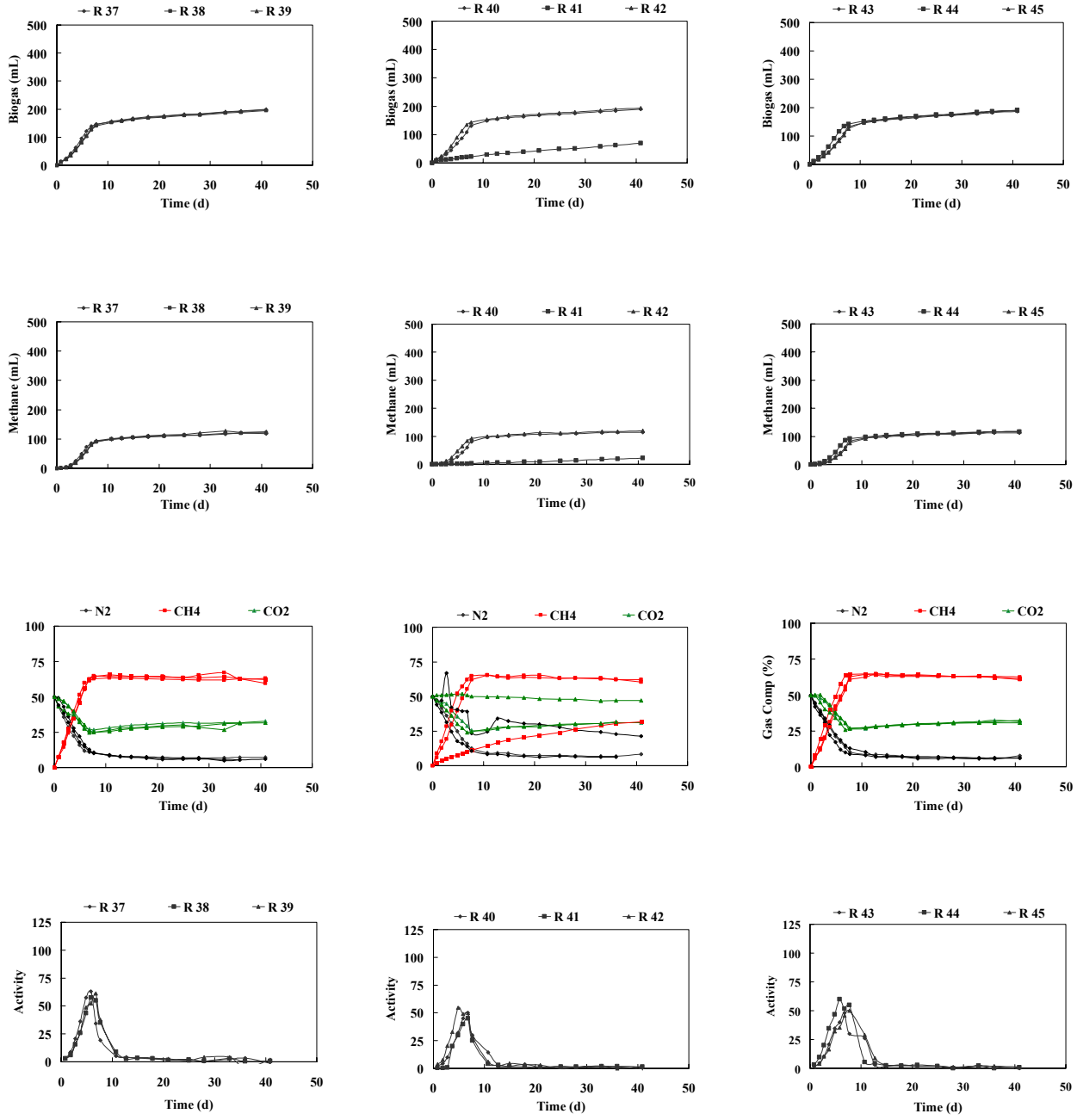


Figure D 9 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for synthetic dye units.

D.3 BIODEGRADABILITY TEST 1

The same template described in the previous Section has been adopted.

Control units

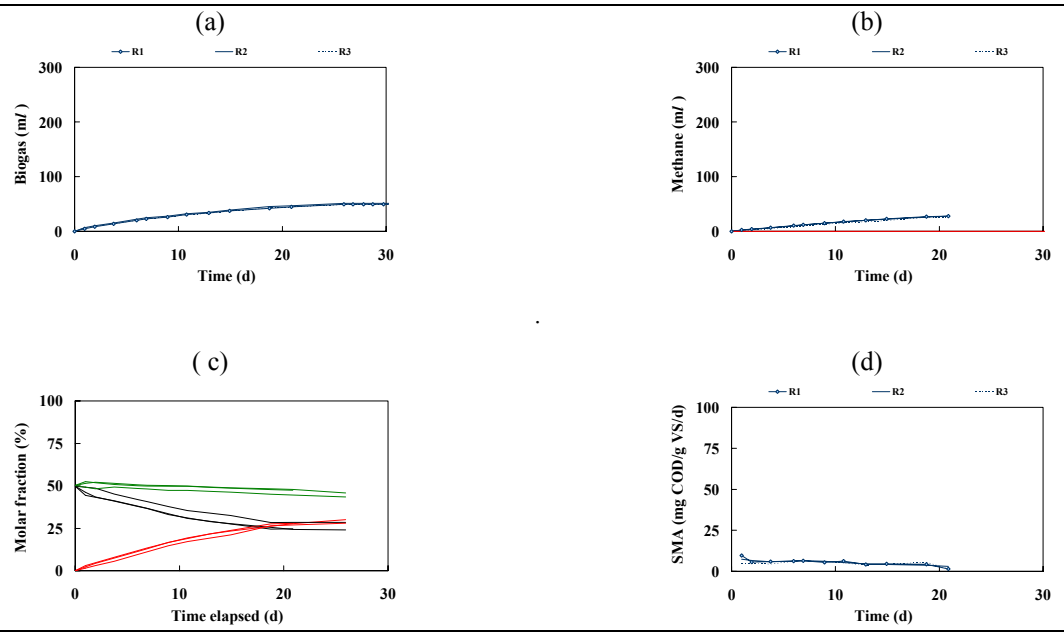


Figure D 10 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for control units.

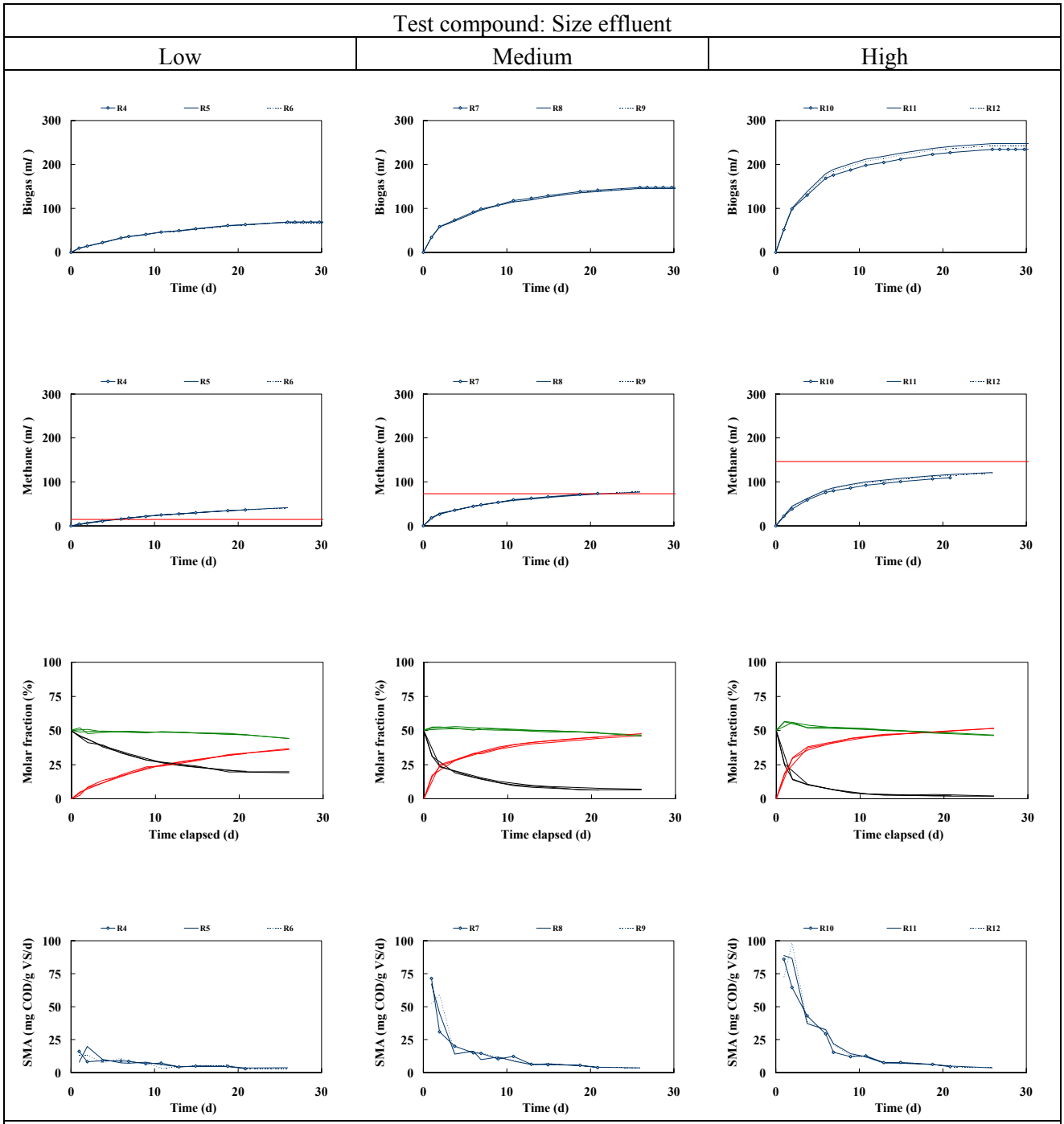


Figure D 11 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for size units.

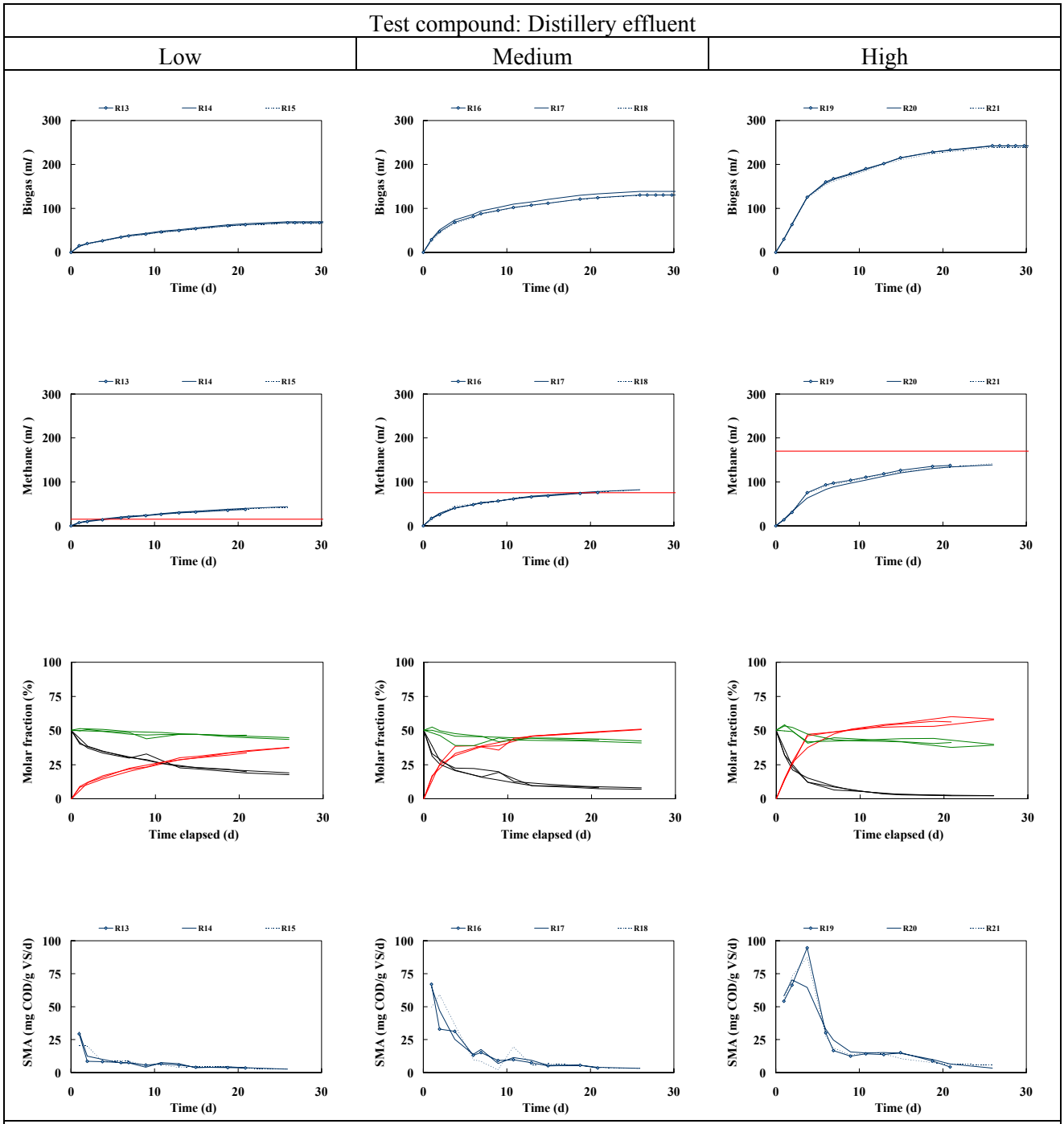


Figure D 12 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for distillery units...

Test compound: Mixture		
Low	Medium	High

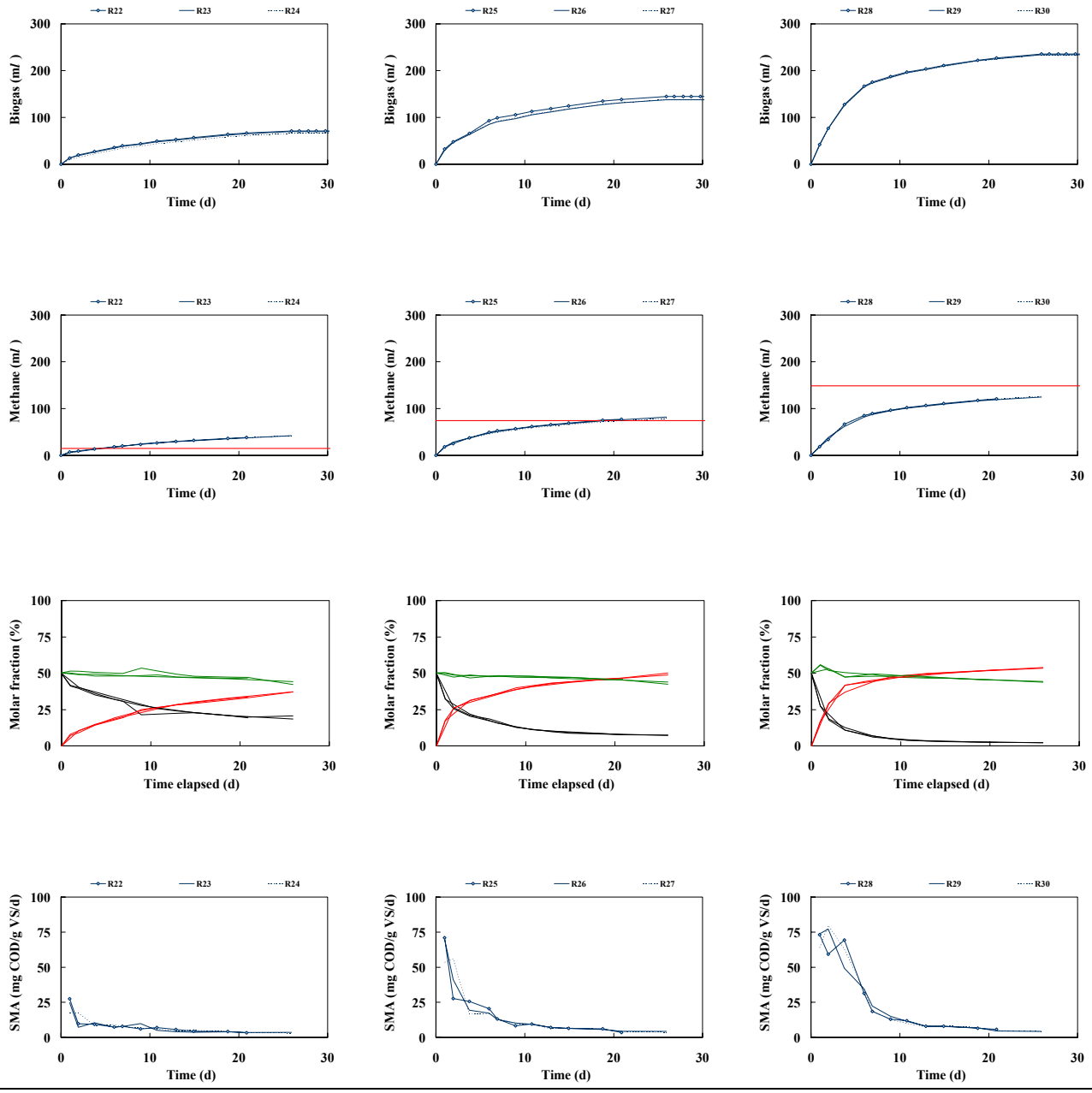


Figure D 13 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for mixture units

D.4 BIODEGRADABILITY TEST 2

Control units

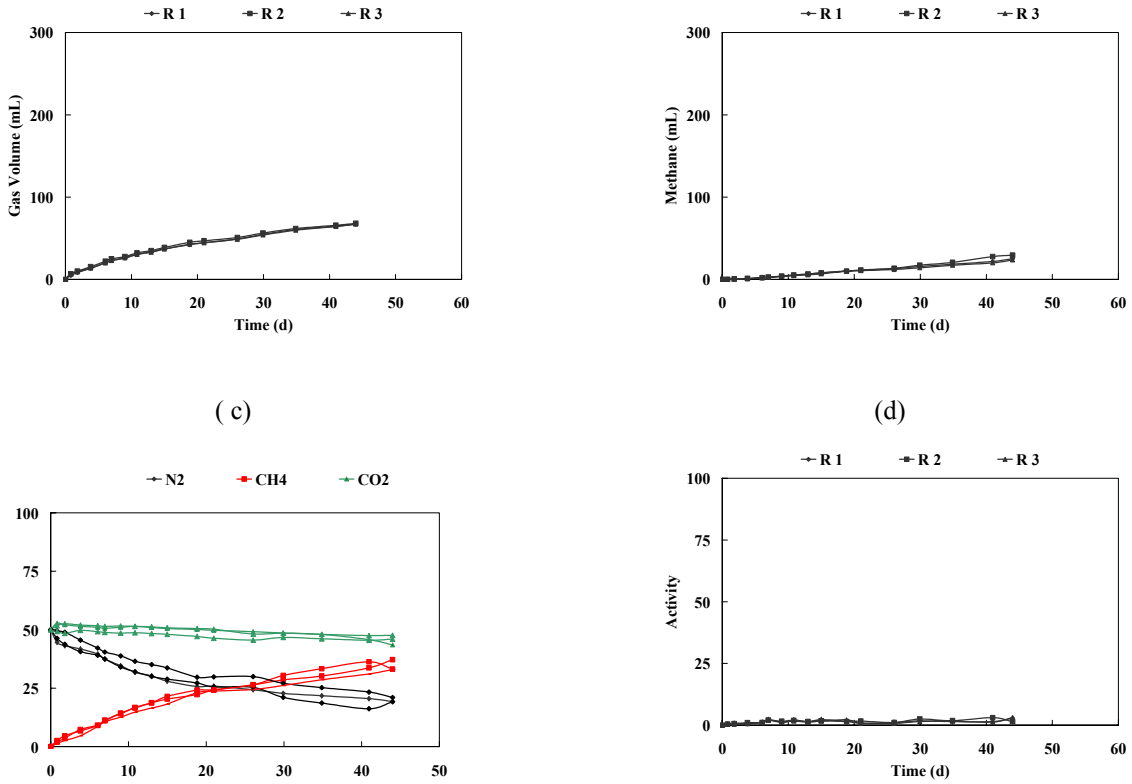


Figure D 14 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for control units.

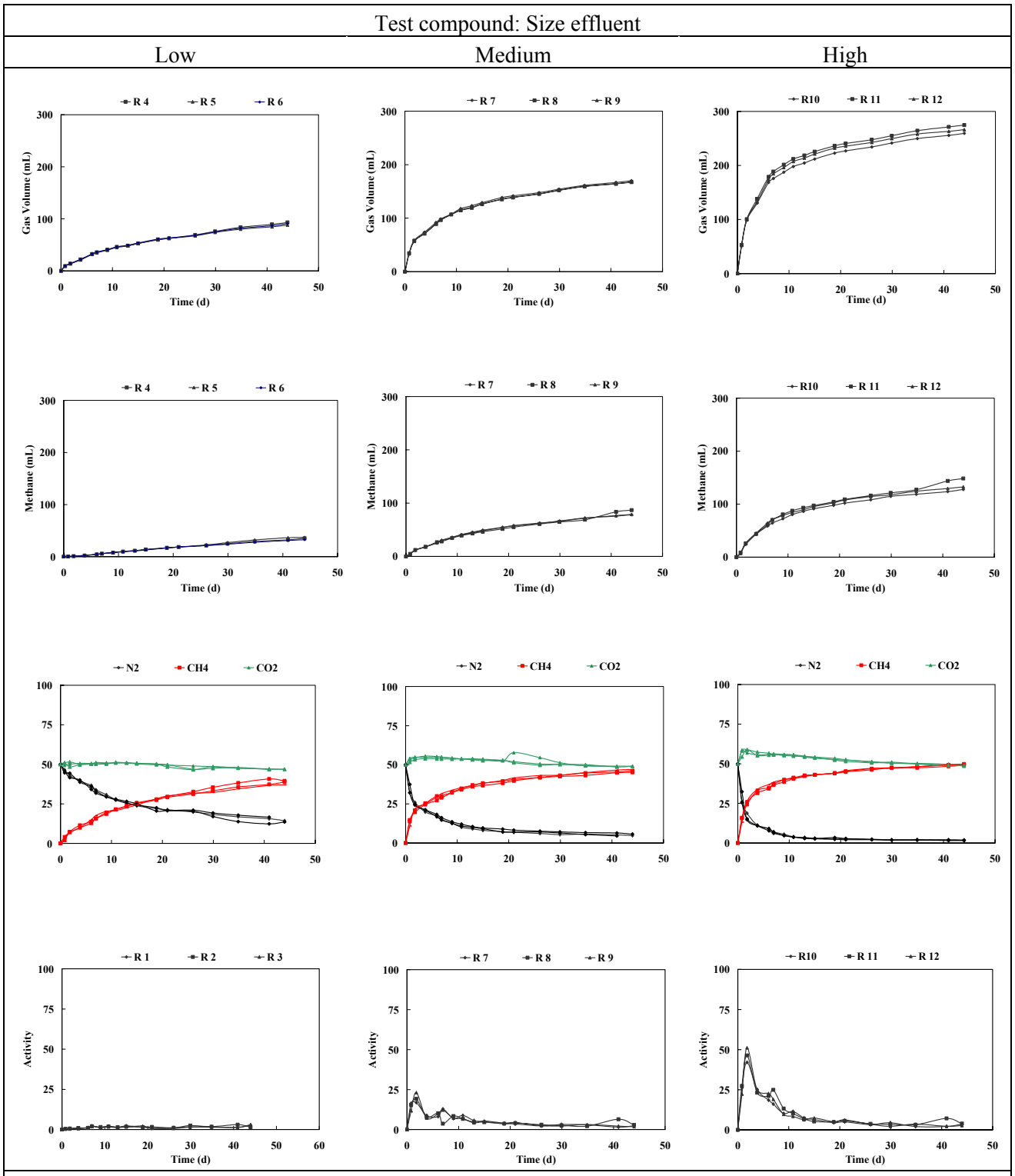


Figure D 15 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for size units.

Test compound: Distillery effluent		
Low	Medium	High

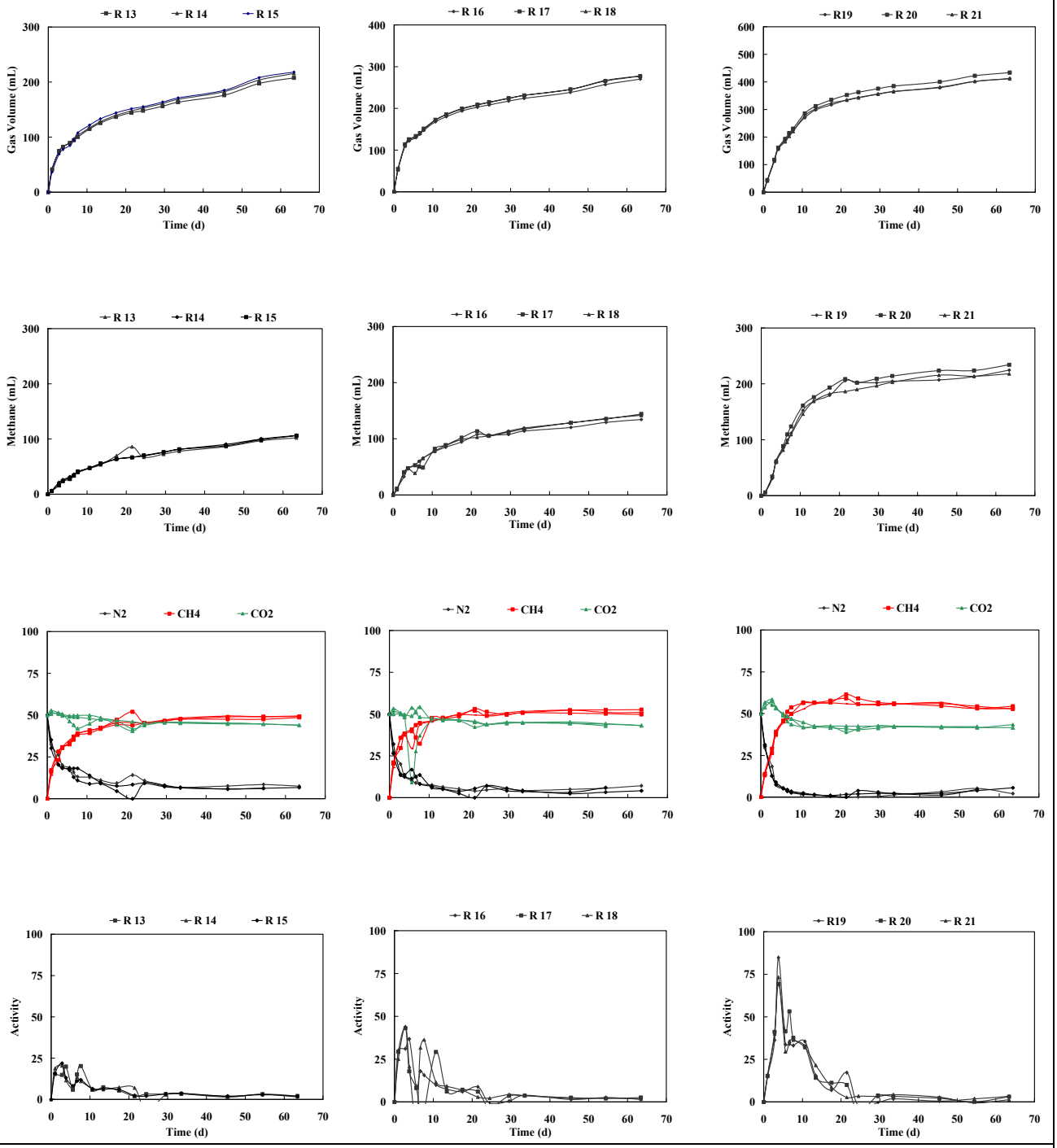


Figure D 16 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for distillery units.

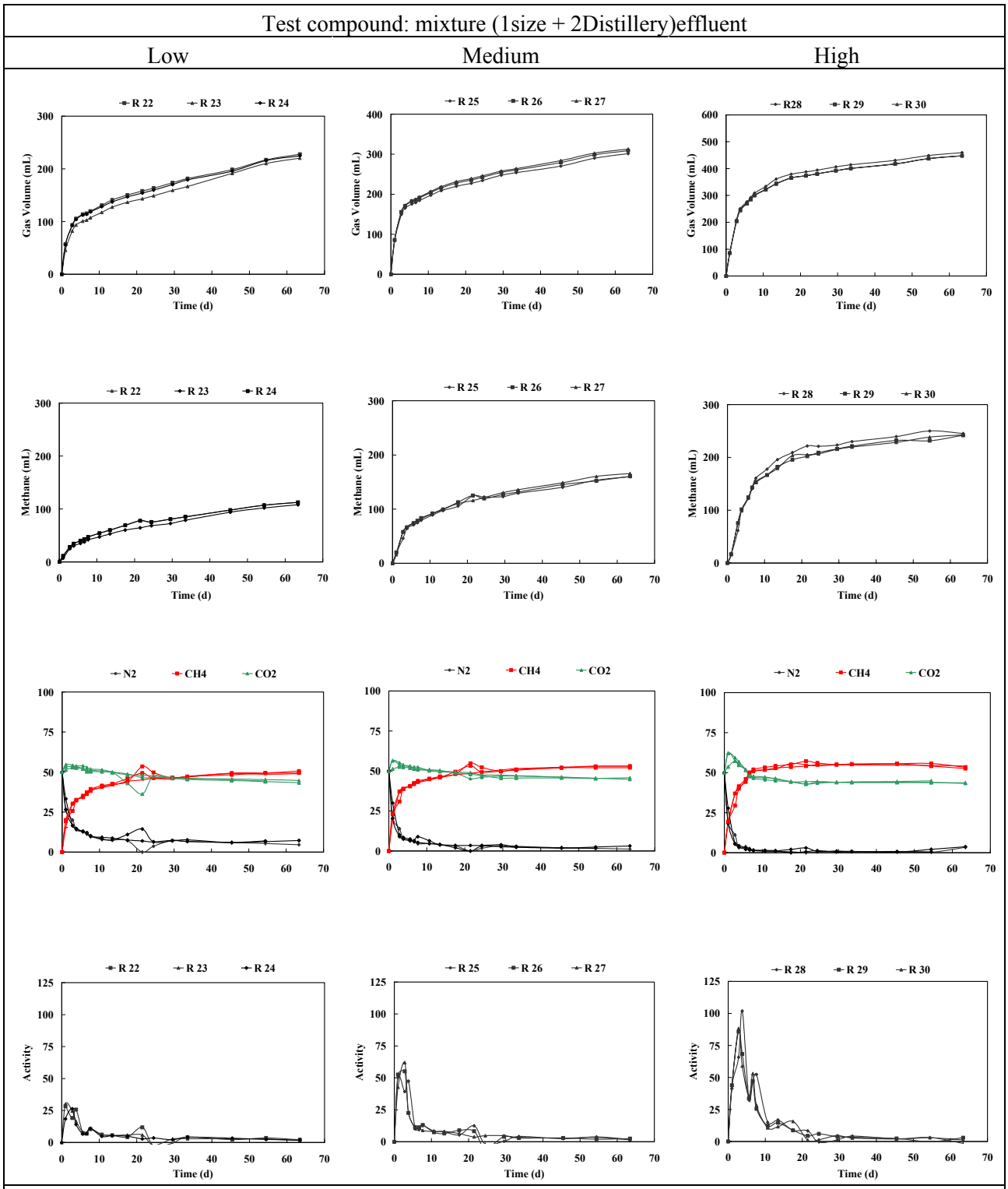


Figure:D 17 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for(1size +2distillery) mixture units..

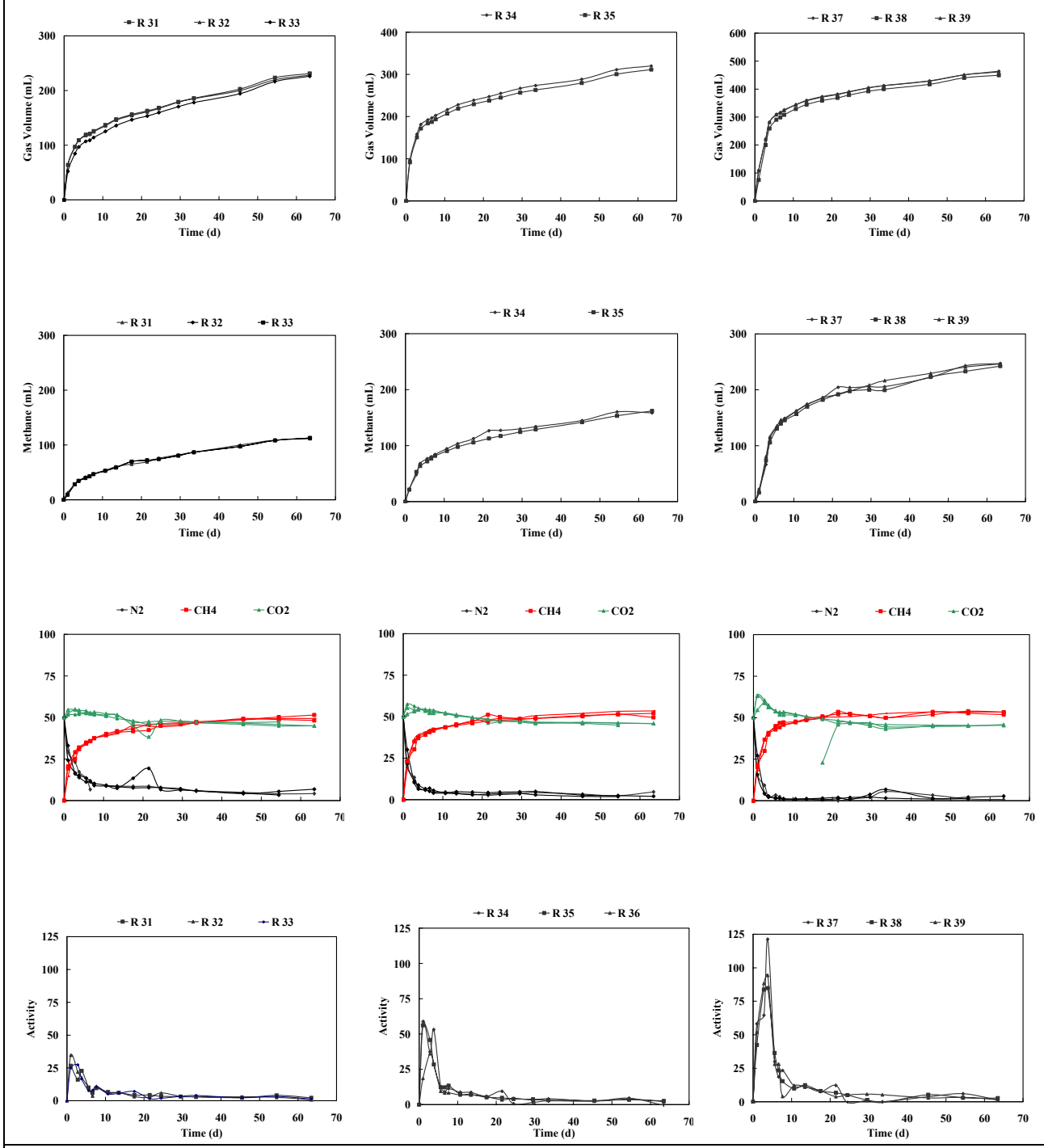


Figure: D 18 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for (2size+1distillery) mixture units..

D.5 BIODEGRADABILITY TEST 3

Control units

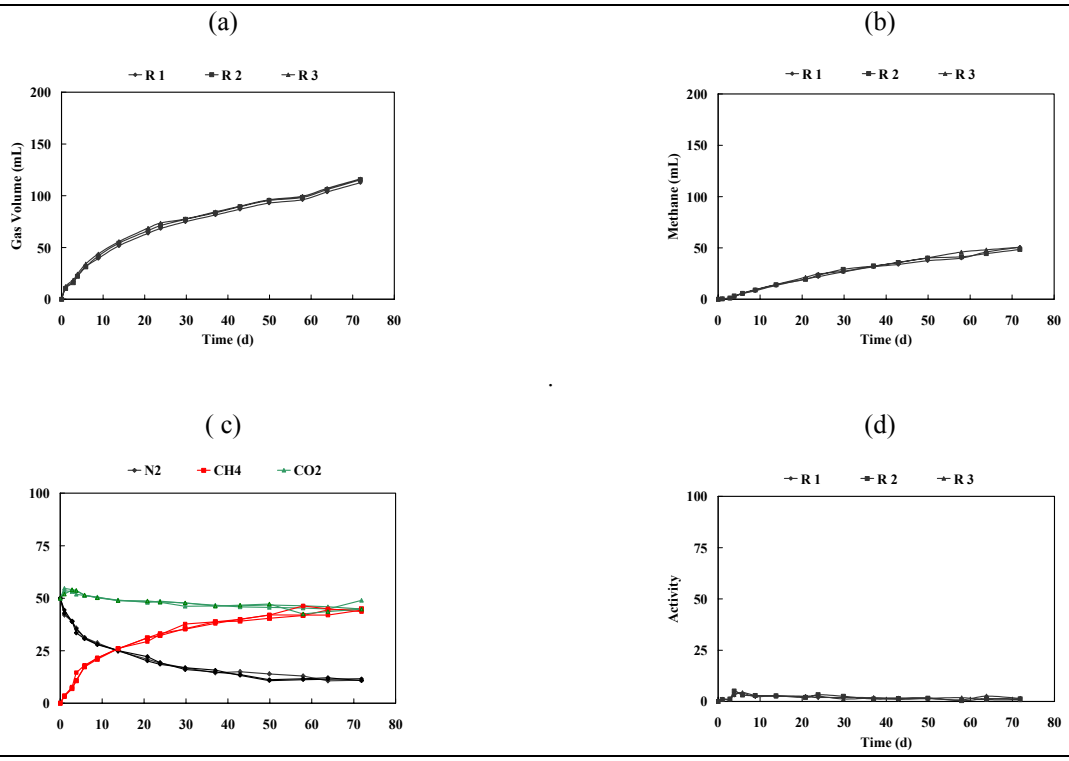


Figure:D 19 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for control units.

Test Compound :Size	
Low	Medium

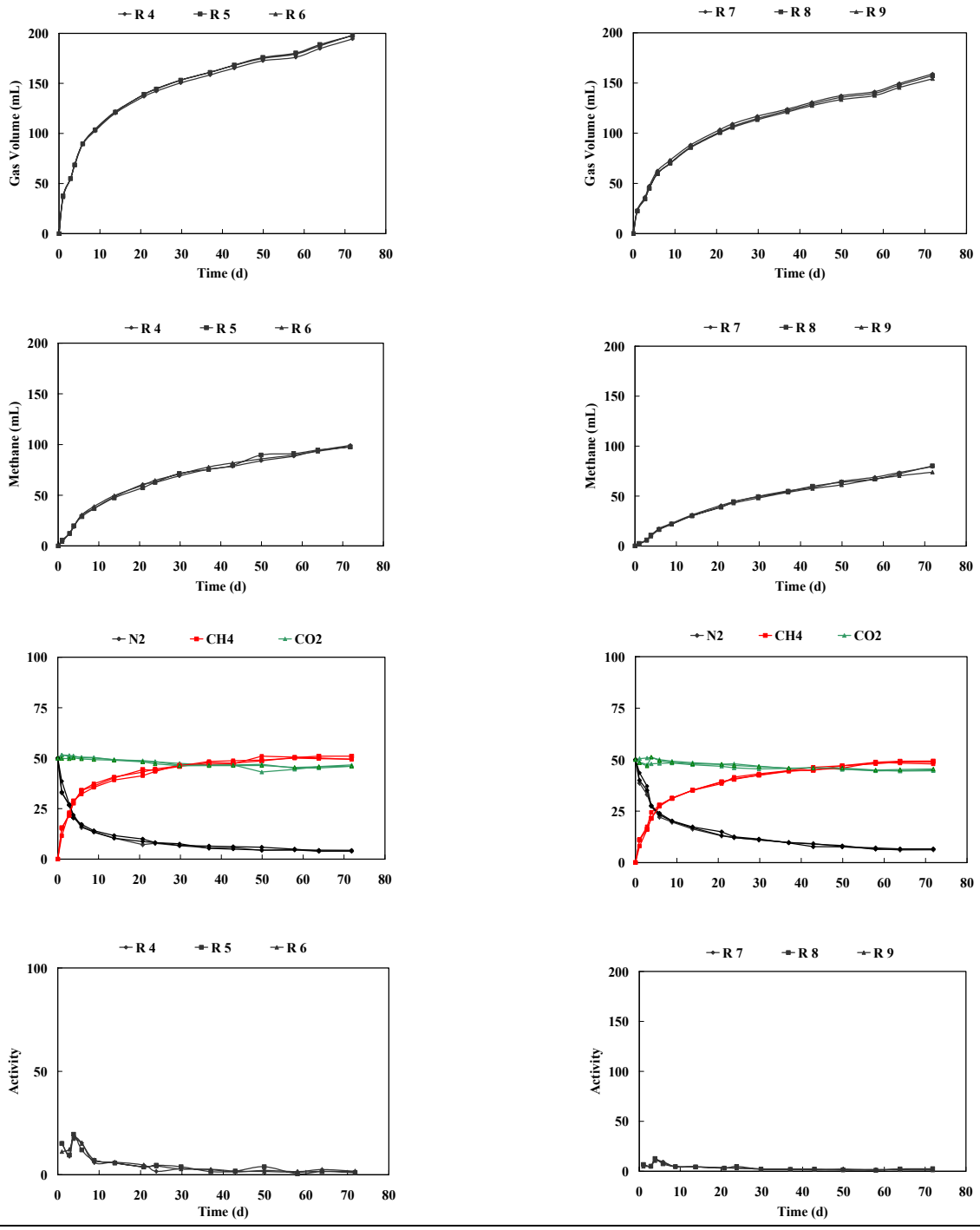


Figure: D 20 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for size units.

Test Compound :Scour	
Low	Medium

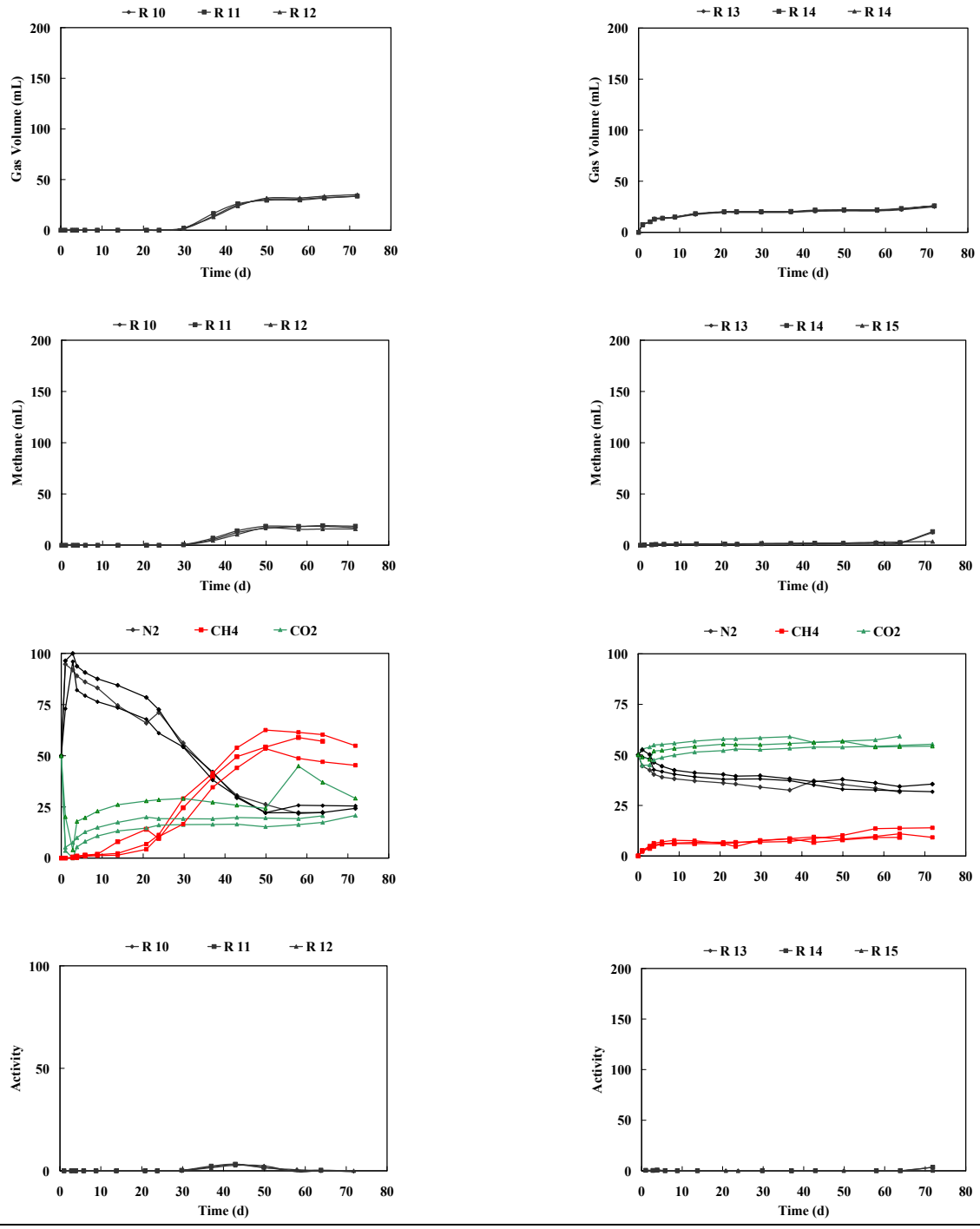


Figure: D 21 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for scour units.

Test Compound :(1size +1 scour) mixture	
Low	Medium

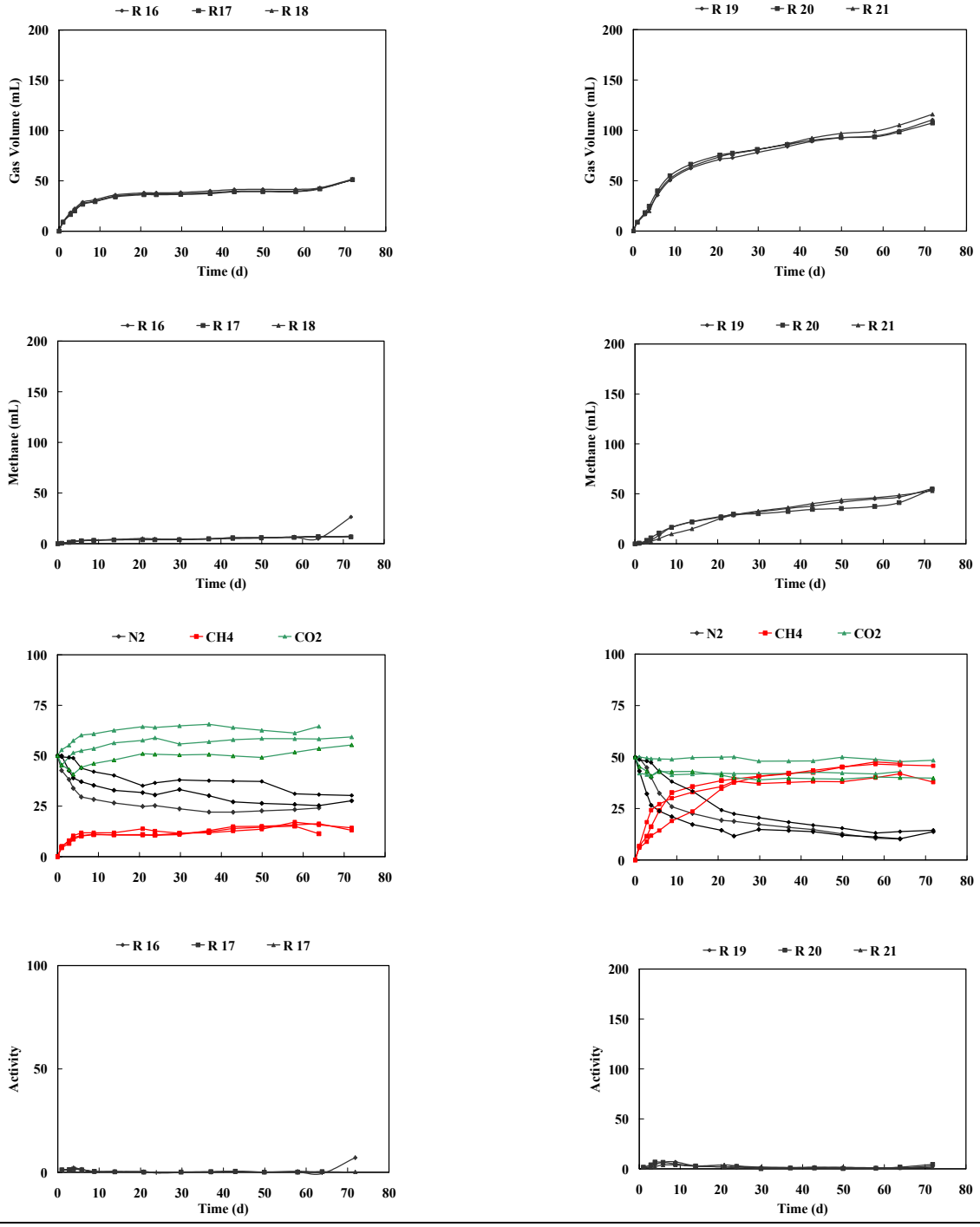
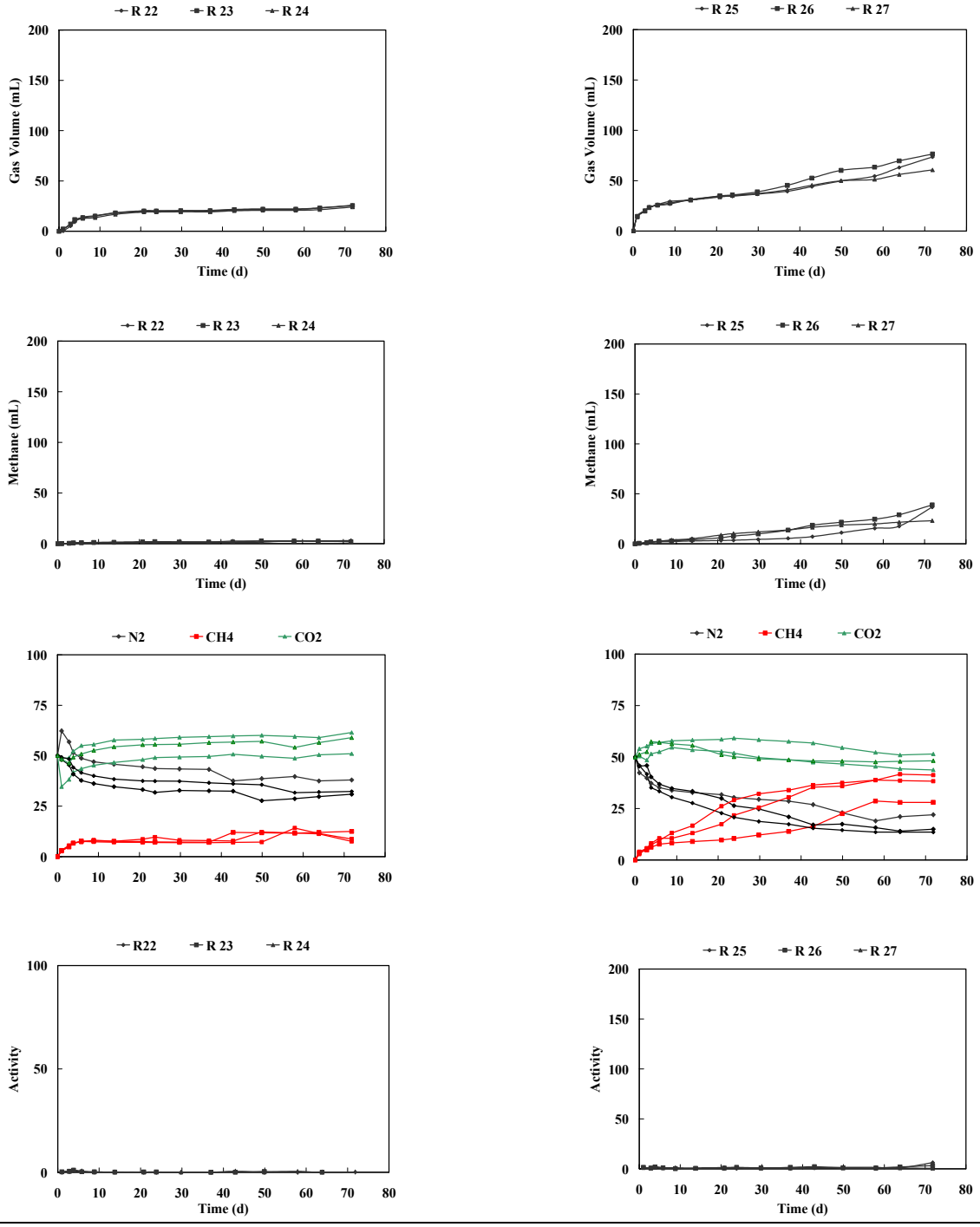


Figure: D 22 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for(1size +1 scour) mixture units..

Test Compound :(1size +2 scour) mixture	
Low	Medium



Figured: D 23 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for(1size +2 scour) mixture units..

Test Compound :(2size +1 scour) mixture	
Low	Medium

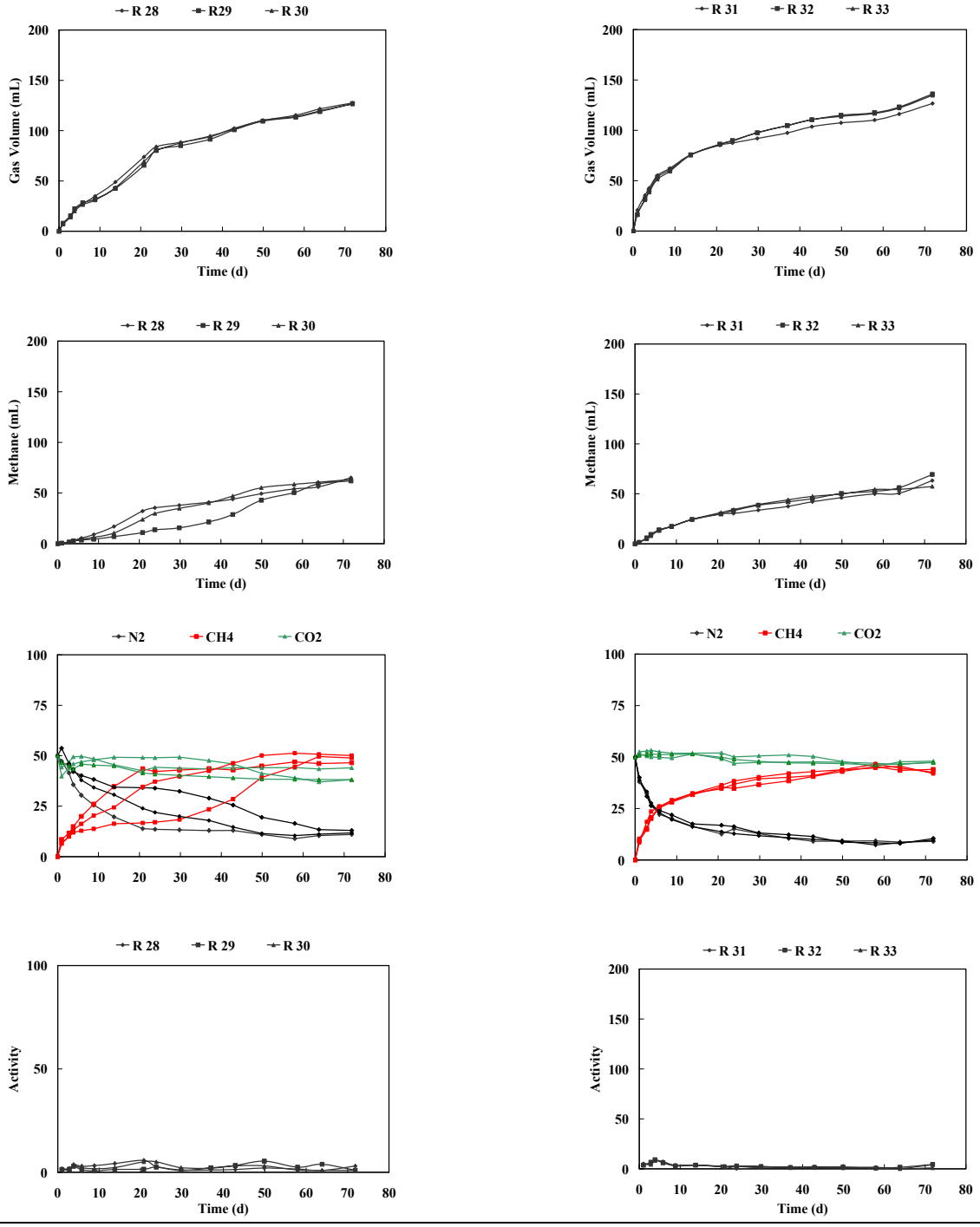


Figure: D 24 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for(2size +1 scour) mixture units..

D 6 BIODEGRADABILITY TEST 4

Test Compound :distillery	
Low	Medium

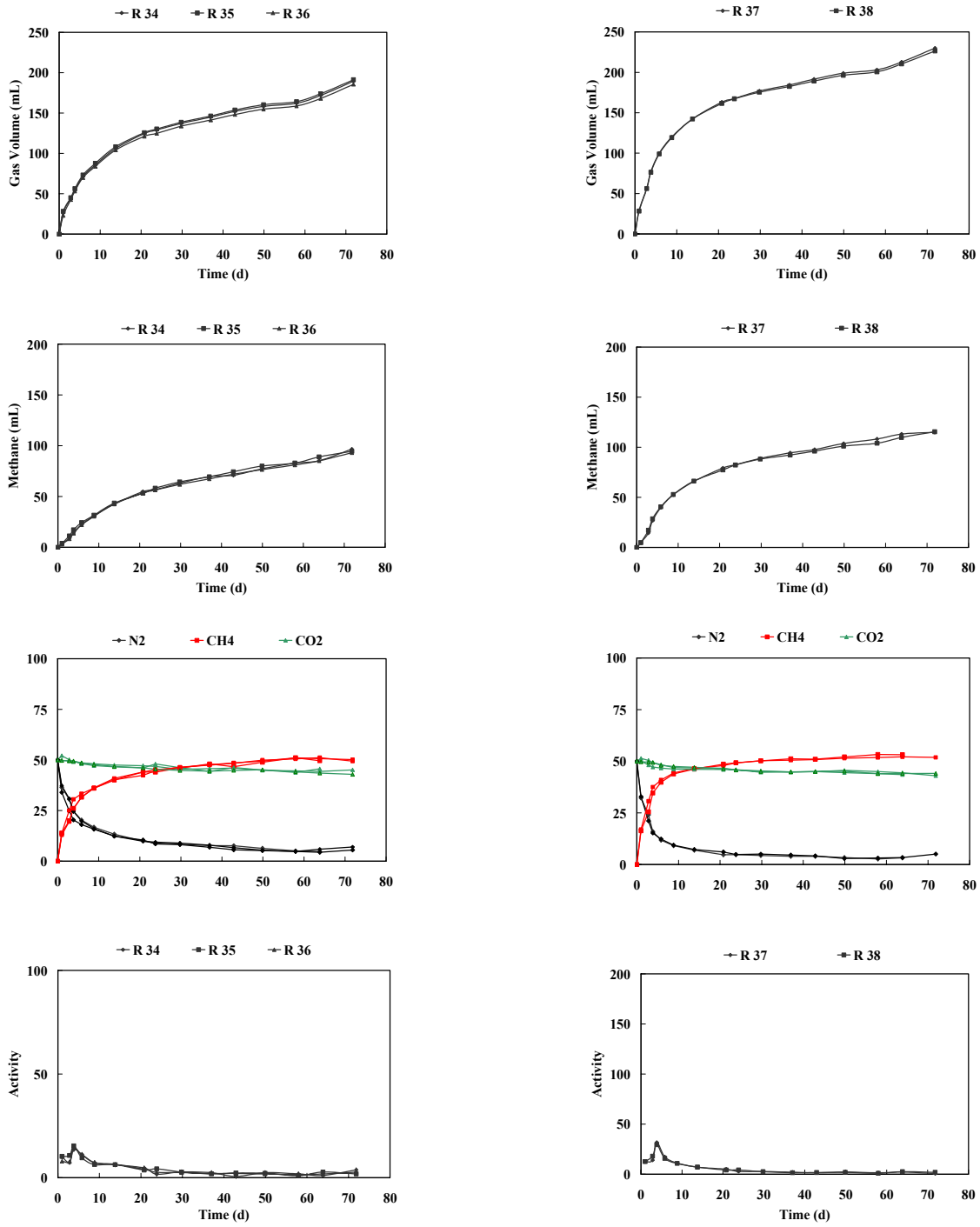


Figure: D 25 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for distillery units.

Test Compound :synthetic dye	
Low	Medium

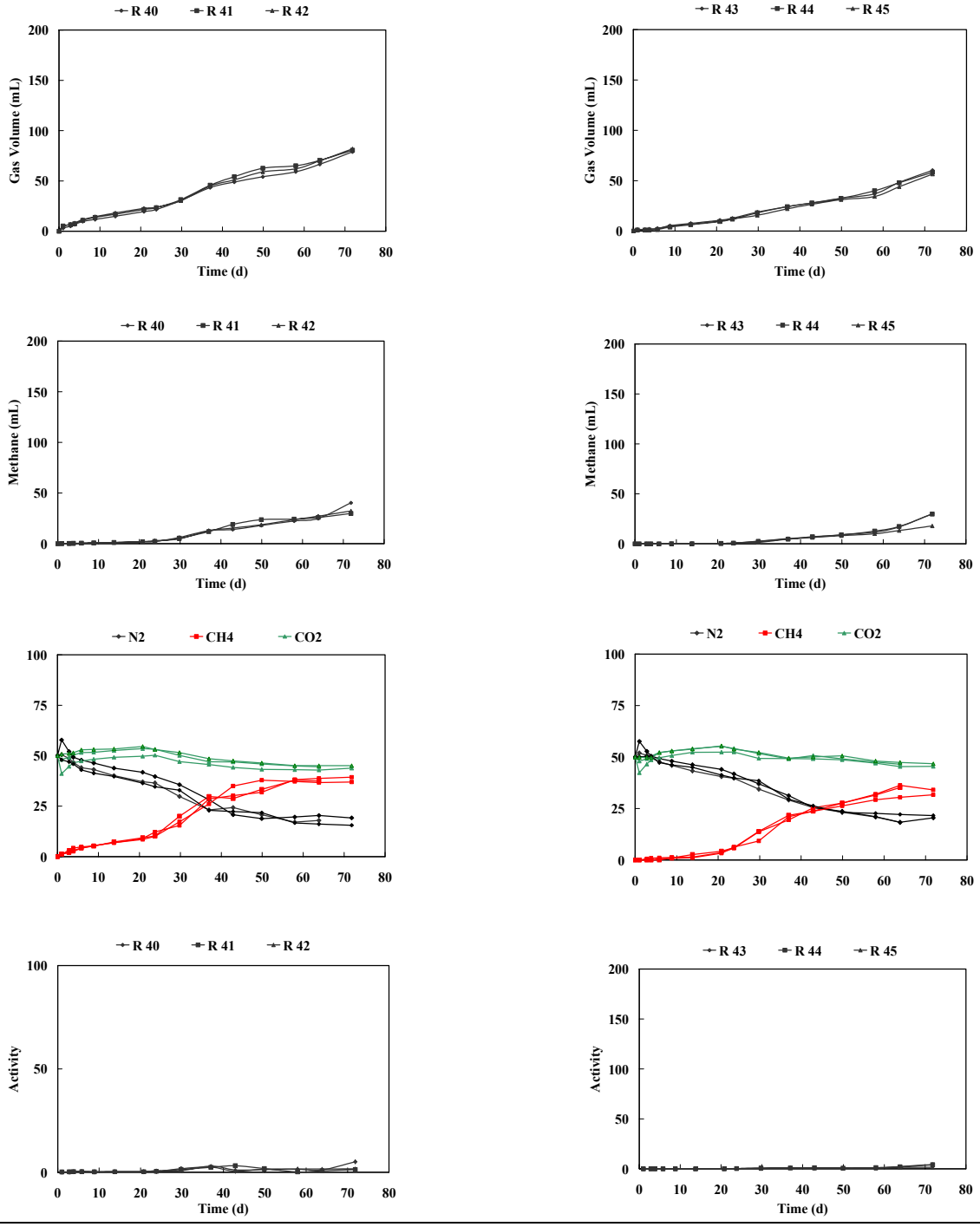


Figure: D 26 - (a) Biogas production, (b) Methane production (c) Gas composition and (d) Sludge methanogenic activity for synthetic dye units.

Test Compound :(1distillery+2 synthetic dye) mixture units	
Low	Medium

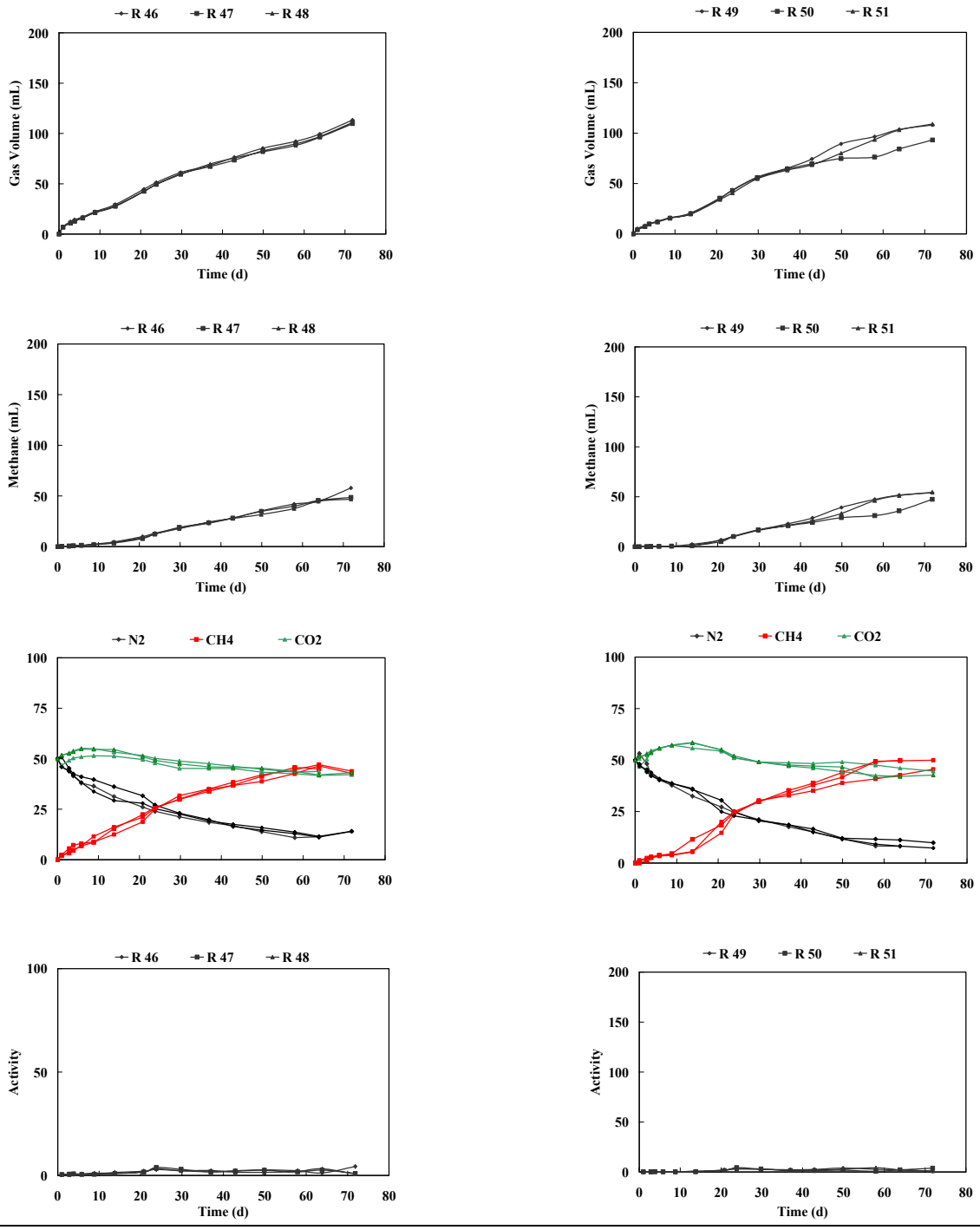


Figure:D 27 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for (1distillery+2 synthetic dye) mixture units..

Test Compound :(2 distillery+1 synthetic dye) mixture units	
Low	Medium

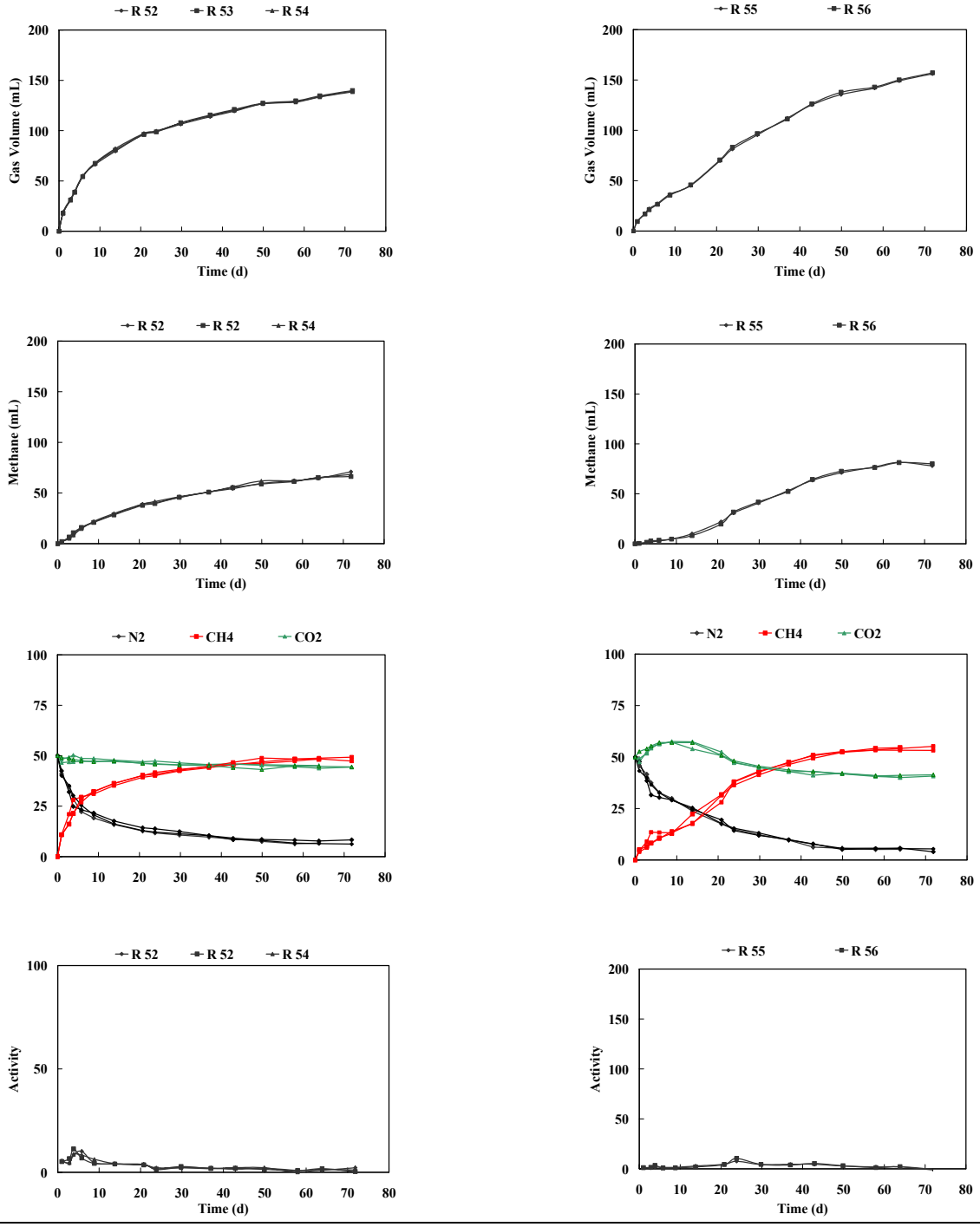


Figure: D 28 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for (2distillery+1 synthetic dye) mixture units..

Test Compound :(1 distillery+1 synthetic dye) mixture units	
Low	Medium

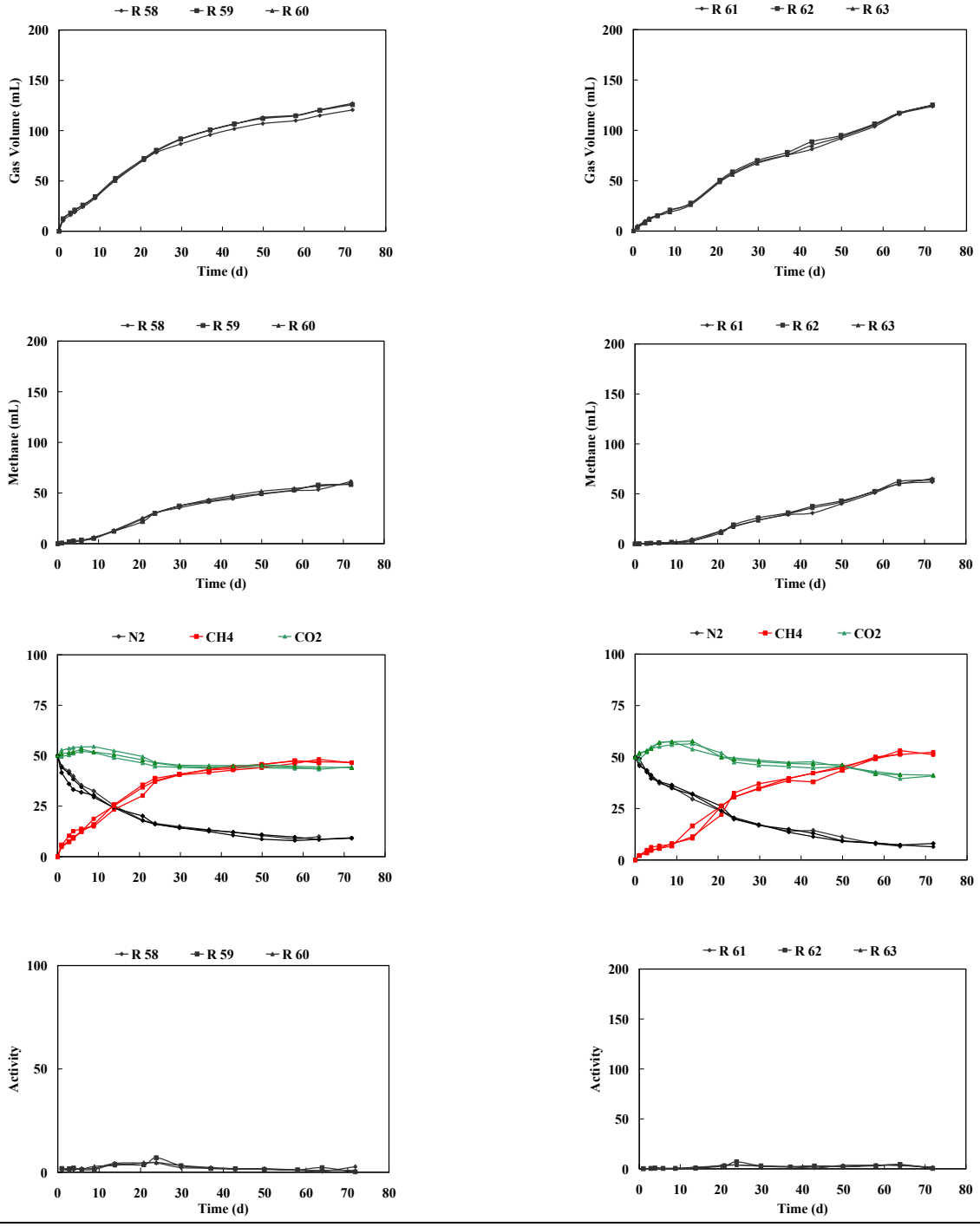


Figure: D 29 - (a) Biogas production, (b) Methane production (c) Gas composition and (d)Sludge methanogenic activity for (1distillery+1 synthetic dye) mixture units..

Annexure E: Laboratory scale results

E.1 LABORATORY SCALE A: DISTILLERY EFFLUENT

N.D. Time (d)	Comm. Gas Volume (L)	COD gCOD/L	CH4 (%)	CO2 (%)	Alkalinity (meq/L)	t-VFAs (meq/L)	pH	Solids gVSS/L
7.00	1.29		63.00	34.00				
8.00	7.34		63.00	35.00				
9.00	18.68		51.00	47.00				
10.00	37.73		61.00	36.00				
12.00	44.64	3.70	64.76	31.25	118.10	26.30		
13.00	50.07	5.32	59.71	37.92	112.90	32.15		
14.00	54.74	7.20	58.03	39.17	107.70	38.00	7.36	16.14
15.00	58.31	8.56	54.82	42.26	102.60	39.20	7.23	
16.00	59.33	8.90	54.78	42.79	97.50	40.40	7.22	
17.00	61.07	8.84	55.08	42.24	104.20	46.10	7.25	
18.00	64.28	8.80	57.12	40.93	110.90	51.80	7.34	
19.00	69.78	10.22	57.41	40.14	127.90	43.70	7.38	
20.00	77.06	10.54	56.82	40.63	137.33	51.65	7.40	
21.00	84.06	13.08	56.34	40.98	142.95	53.96	7.45	14.15
22.00	90.93	13.10	54.87	42.06	153.76	74.11	7.48	
23.00	97.58	14.76	55.24	42.75	159.66	62.39	7.46	
24.00	102.60	16.00	53.82	43.80	159.88	71.57	7.55	
25.00	107.00	17.04	49.53	48.27	160.10	80.75	7.45	
26.00	110.90	17.04	48.87	49.51	177.40	77.25	7.46	
27.00	114.65	19.24	45.22	52.73	169.13	102.39	7.45	
28.00	119.57	22.18	42.40	55.55	176.69	108.07	7.37	11.23
29.00	122.49	25.00	33.27	65.04	150.80	116.67	7.25	
30.00	123.92	25.67	27.20	70.90	136.06	162.22	7.11	
31.00	124.29	24.22	32.39	65.99	142.05	159.80	7.15	
32.00	124.47	22.90	38.43	59.40	148.03	157.37	7.22	
33.00	125.21	21.55	47.10	50.26	162.36	152.16	7.36	
34.00	126.02	26.39	38.55	59.26	155.55	159.50	7.26	
35.00	126.92	27.08	34.70	63.33	155.55	171.86	7.19	10.45
36.00	127.91	28.96	30.14	66.23	148.77	160.89	7.25	
37.00	128.94	29.55	29.65	67.55	153.82	185.05	7.21	
38.00	129.98	30.94	29.37	68.24	128.67	203.45	6.92	
39.00	130.91	32.30	29.82	67.14	116.09	212.65	6.89	
40.00	131.28	26.80	30.60	65.80	103.51	221.85	6.93	
41.00	131.45	27.78	31.53	66.34	91.38	261.14	6.90	
42.00	131.60	28.76	33.16	64.67	99.72	229.33	6.76	11.91
43.00	131.60	29.41	37.76	59.67	102.00	238.57	6.89	
44.00	131.60	31.26	43.02	53.45	122.33	219.57	7.01	
45.00	131.60	33.17	48.35	49.56	142.66	210.07	7.18	
46.00	131.60	29.48	52.12	45.11	159.59	200.57	7.28	
47.00	131.67	31.49	48.37	48.75	177.54	173.21	7.31	

48.00	133.05	32.50	45.85	50.52	167.78	196.62	7.32	
49.00	134.57	34.51	44.26	52.12	196.45	205.31	7.35	9.10
50.00	135.81	29.48	57.26	40.23	208.38	164.28	7.53	
51.00	137.15	24.13	63.73	33.34	230.76	168.29	7.66	
52.00	138.45	23.44	66.79	29.73	237.22	152.01	7.78	
53.00	139.73	22.34	69.80	27.08	263.68	147.74	7.86	
54.00	140.97	22.64	71.07	24.91	258.22	117.87	7.92	
55.00	142.13	22.90	66.72	29.14	290.00	104.82	7.90	
56.00	143.25	24.92	67.80	28.11	300.39	99.80	7.93	8.79
57.00	145.41	23.44	68.48	28.13	307.79	102.81	7.97	
60.00	145.89	19.75	66.90	27.80	340.40	70.47	8.09	
61.00	146.34	16.98	66.19	29.83	353.99	59.28	8.03	
62.00	146.73	21.24	65.90	31.50	335.01	65.45	8.03	
63.00	147.26	21.49	64.85	33.05	347.46	66.61	8.05	
64.00	147.74	21.81	64.15	34.02	356.22	62.25	8.08	8.51
65.00	148.22	23.69	64.22	34.21	365.30	43.68	8.13	
66.00	148.67	24.88	64.66	34.00	354.63	42.72	8.12	
67.00	149.06	24.50	61.22	35.66	358.76	36.23	8.16	
68.00	149.40	26.08	59.79	36.51	350.41	44.73	8.13	
69.00	149.73	21.88	60.01	37.37	350.53	31.99	8.09	
70.00	150.12	22.83	53.98	42.48	333.68	39.64	8.09	7.53
71.00	150.48	20.70	49.17	46.85	323.36	43.91	8.06	
72.00	150.92	25.33	47.66	49.62	333.75	43.42	8.09	
73.00	151.37	24.30	42.73	53.98	335.95	50.43	8.07	
74.00	151.86	23.44	40.40		329.37	53.13	8.07	
75.00	152.37	23.42	37.75		325.43	55.73	8.01	
76.00	152.96	24.86	35.08		327.21	58.99	7.98	
77.00	153.81	27.07	32.42	63.69	310.65	63.15	7.95	8.18
78.00	154.68	26.12	28.51	67.03	310.39	70.25	7.90	
79.00	155.61	25.16	28.31	70.15	309.86	79.79	7.86	
80.00	156.54	27.84	27.87	70.21	310.96	83.56	7.88	
81.00	157.46	27.17	27.64	71.44	300.80	87.55	7.86	
82.00	158.39	30.15	27.04	72.11	297.80	98.07	7.86	
83.00	159.32	30.18	26.46	72.69	284.46	103.61	7.79	
84.00	160.22	30.20	25.71	73.04	290.69	106.03	7.79	
85.00	161.18	31.88	26.90	72.53	288.02	109.71	7.79	
86.00	162.36	31.25	26.04	73.23	285.24	113.53	7.76	
88.00	163.23	31.40	28.92	70.08	279.15	122.62	7.76	6.73
89.00	164.12	31.48	27.58	71.47	279.22	119.75	7.71	
90.00	164.99	28.98	26.55	72.60	271.83	131.73	7.73	
91.00	165.80	32.84	26.09	72.81	269.37	136.93	7.66	
92.00	166.67	35.96	25.72	72.98	267.37	141.15	7.68	
93.00	167.72	37.04	26.09	72.18	266.09	139.64	7.64	
95.00	168.56	38.79	28.90	69.95	264.02	137.23	7.66	
96.00	168.73	40.52	27.33	71.78	264.62	141.17	7.66	
97.00	169.27	42.48	32.10	67.07	265.31	145.63	7.67	
98.00	169.48		29.50	69.83	266.89	146.84	7.68	
99.00	170.05		30.92	68.34	268.88	148.34	7.69	8.04
100.00	170.30		32.29	66.90	270.70	150.72	7.71	

101.00	170.56		36.78	62.36	272.29	152.79	7.74	
102.00	171.10	43.88	41.79	57.28	274.06	155.11	7.76	
103.00	171.39		38.20	60.82	272.16	154.75	7.78	
104.00	171.91		44.26	55.03	270.25	154.40	7.79	
105.00			38.66	60.25				
106.00								
107.00								
108.00								
109.00								
110.00								
111.00								
112.00								
113.00								
114.00	174.44							
115.00	174.80							
116.00	175.16	33.08	78.82	18.86	328.34	97.77	8.47	
117.00	175.60	34.15	71.36	26.14	337.42	88.70	8.47	
118.00	176.05	34.68	63.90	33.43	341.96	84.17	8.46	
119.00	176.53	35.21	61.60	36.64	346.50	79.64	8.46	
120.00	177.15		59.47	39.62				
121.00	177.95		61.61	37.36				
122.00	178.35		65.20	33.58				
123.00	178.76	29.85	70.71	27.78	377.77	62.20	8.45	8.86
124.00	179.31	30.06	68.52	29.99	374.03	61.26	8.45	
125.00	179.92	30.27	65.44	33.08	370.28	60.31	8.44	
126.00	180.52		56.99	40.68	369.70	54.15	8.36	
127.00	181.02		64.03	35.34	369.11	47.98	8.28	
128.00	181.79		64.85	34.15	371.64	48.53	8.39	
129.00	182.56		65.65	33.00	372.91	48.80	8.45	
130.00	183.05	32.59	66.48	31.83	374.17	49.07	8.50	
131.00	183.34		55.90	42.69	385.75	38.29	8.47	
132.00	183.63		59.76	38.77	391.53	32.90	8.45	
133.00	184.04		64.22	34.26	397.32	27.51	8.43	8.51
134.00	184.41		51.85	47.50				
135.00	184.60		55.17	43.90				
136.00	184.79		58.49	40.31				
137.00	185.21	30.98	61.88	36.64	385.06	43.99	8.44	
138.00	185.45	30.58	51.98	47.03	383.11	46.89	8.41	
139.00	185.86	30.18	56.81	41.81	381.15	49.80	8.38	
140.00	186.13		50.58	47.44	388.83	45.91	8.39	
141.00	131.68		58.29	40.47	396.51	42.01	8.39	

E.1 LABORATORY SCALE B: SIZE EFFLUENT

ND-Time	Gas Volume	COD	CH4	CO2	Alkalinity	VFA	pH	Solids
(d)	(L)	(g COD/L)	(%)	(%)				gVSS/L
0.00								
1.00		28.80	49.00	49.00			6.92	
2.00			51.00	46.00				
3.00		29.50	53.00	45.00			7.13	
4.00		25.50	62.00	36.00			7.28	
5.00		26.80	66.00	30.00	124.99	148.91	7.67	
6.00	0.84	25.10	70.00	28.00	147.40	129.35	7.67	
7.00	2.42	22.80	74.00	24.00	196.45	205.31	7.81	
8.00	4.28	24.10	75.00	22.00	208.38	164.28	7.97	
9.00	5.54	20.30	72.00	26.00	218.84	73.05	7.91	
10.00	6.40	16.70	65.00	31.00	225.51	24.62	7.90	
11.00	6.96	19.60	60.00	38.00	239.52	27.37	7.78	
12.40	7.60	19.60	55.00	45.00	232.52	44.69	7.78	
13.31	8.26	19.60	54.00	42.00	225.52	62.01	7.78	11.00
14.40	8.78	20.60	53.00	42.00	224.59	58.90	7.75	
15.30	9.16	21.60	53.00	41.00	224.12	57.35	7.73	
16.02	9.42	21.05	49.00	43.00	223.65	55.79	7.71	
17.02	9.92	20.78	41.00	55.00	226.68	63.30	7.90	
18.09	10.22	20.50	41.00	52.00	227.93	65.26	7.79	
19.50	10.60	20.90	41.00	52.00	229.18	67.21	7.68	
20.36	11.02	21.30	40.00	56.00	226.26	60.18	7.78	
21.34	11.50	21.50	43.00	53.00	220.42	57.11	7.76	
22.54	12.00	21.70	45.00	52.00	229.14	57.08	7.84	9.00
23.46	12.50	21.70	46.00	48.00	238.15	47.52	7.98	
24.38	13.00	20.90	50.00	49.00	240.23	42.32	7.98	
25.46	13.50	20.10	59.00	38.00	247.68	38.58	8.05	
26.34	13.98	23.10	53.00	45.00	229.15	45.57	8.01	
27.46	14.62	22.00	55.00	41.00	229.37	58.28	8.03	
28.31	15.16	29.00	56.00	40.00	239.28	42.57	8.05	
29.46	15.80	20.90	50.00	47.00	201.66	32.03	8.05	6.00
30.31	16.48	21.80	55.00	43.00	199.71	28.39	8.07	
31.48	17.62	23.20	50.00	47.00	196.17	28.25	8.10	
32.34	18.48	24.60	54.00	42.00	192.63	28.10	8.08	
33.45	19.78	22.90	56.00	39.50	195.61	24.90	8.10	
34.54	21.14	26.90	58.00	37.00	188.63	37.41	8.10	
35.53	22.24	20.90	58.00	40.00	198.68	21.60	8.12	8.00
36.44	23.50	22.20	56.00	40.00	203.60	22.06	8.28	
37.34	24.74	18.00	59.00	39.00	191.71	23.35	8.28	
38.36	26.24	18.30	63.00	35.00	197.54	16.37	8.27	
39.44	27.56	18.45	65.00	33.00	203.37	9.38	8.20	
40.37	28.60	18.60	59.00	40.00	211.53	11.39	8.19	
41.40	29.58	17.30	57.00	42.00	195.71	26.94	8.15	
42.46	30.50	16.40	56.00	43.00	205.08	21.90	8.19	8.00

43.46	31.42	19.90	56.00	43.00	166.85	67.25	8.16	
44.48	32.38	19.90	57.00	42.00	212.92	24.96	8.20	
45.40	33.40	19.40	57.00	42.00	229.58	16.75	8.19	
46.48	34.40	18.90	61.00	37.00	246.24	8.54	8.18	
47.40	35.14	22.00	57.00	42.00	228.52	4.62	8.19	
48.44	36.10	18.20	58.00	41.00	242.65	3.08	8.24	
49.38	36.90	42.00	55.00	43.00	232.37	9.09	8.20	
50.50	37.84	39.50	56.00	42.00	242.99	0.00	8.21	
51.50	38.66	11.65	55.00	42.00	239.13	0.48	8.22	
52.43	39.38	17.48	55.00	43.00	237.19	0.72	8.20	
53.52	40.36	23.30	56.00	40.00	235.26	0.96	8.19	5.00
54.56	42.36	26.30	51.00	47.00	240.28	9.21	8.30	
55.34	42.91	29.30	43.00	55.00	220.85	3.53	8.19	
56.48	43.46	31.70	45.00	52.00	222.67	10.18	8.22	
57.38	44.48	34.10	39.00	60.00	221.67	11.26	8.20	
58.34	45.40	25.10	41.00	57.00	210.16	16.42	8.15	
59.36	46.66	26.20	21.00	77.00	204.42	21.53	8.23	
60.65	47.12	27.30	18.00	73.00	198.67	26.63	8.30	
61.36	47.18	26.10	18.00	64.00				
62.36	47.18	25.20	24.00	57.00				5.00
63.36	47.18	24.00	23.00	56.00				
63.98	47.30	23.60	22.50	60.00				
65.61	47.30	22.70	22.00	64.00				
65.98	47.86	21.60	22.50					
67.36	48.04	20.40	23.00	44.00				
68.40	48.04	18.30	17.00		148.00	71.83	7.51	
69.36	48.04	17.65	11.00	46.00	153.12	64.71		
70.48	48.30	17.00	12.00	46.00	158.24	57.59	7.70	
71.44	49.62	14.80	12.00	46.00	165.17	57.59	7.98	
72.48	50.34	14.70	14.00	49.00	165.21	57.55	7.95	
73.48	51.48	19.05	15.00	71.00	144.76	79.50	8.09	4.00
74.50	51.96	23.40	16.00	68.00	165.41	64.49	8.09	
75.56	52.70	23.00	17.00	63.00	165.00	53.00	8.10	
76.48	53.30	23.00	17.00	64.00	165.20	54.00	8.04	
77.48	53.78	23.40	17.00	64.00	164.60	59.00	8.06	
78.31	54.04	24.00	17.00	64.00	164.60	56.00	8.00	