THE EFFECT OF BIOMASS ACCLIMATION ON THE CO-DIGESTION OF TOXIC ORGANIC EFFLUENTS IN ANAEROBIC DIGESTERS

This Dissertation is submitted in fulfillment of academic requirements
for the degree

Master of Technology in Engineering

At

Durban University of Technology
Water Technology Research Group
Department of Chemical Engineering

By

Ziphathele Chamane
(BTech.ChemEng, VUT)

Date: June 2008
Supervisor: Prof. Lingam Pillay
Co-supervisor (s): Prof Chris Buckley, Dr Enrico Remigi

DECLARATION

The Registrar (Academic)
Durban University of Technology

I Ziphathele Chamane, student number (20519046), hereby declare that unless indicated, this dissertation/thesis titled *The Effect of Biomass Acclimation on the Codigestion of Toxic Organic Effluents in Anaerobic Digesters* is the result of my own investigation and has not been submitted in part or in full for any other degree at another University or Institution.

……. ……..
(Signature) (Date)

Declaration by supervisor

I Prof Lingam Pillay, as the candidate supervisor has recommended/not recommended for the thesis titled *The Effect of Biomass Acclimation on the Codigestion of Toxic Organic Effluents in Anaerobic Digesters* to be submitted for examination.

……. …….
(Signature) (Date)

ABSTRACT

Currently KwaZulu-Natal (KZN) province is populated with textile industry, which produces wastewater, some of which is not biodegradable. Due to the stringent environmental regulations the wastewater cannot be discharged into the rivers or public owned treatment systems. The alternative solution is to co-dispose this wastewater with easily biodegradable waste (labile effluent). The aim of this investigation was to develop a process protocol for the codigestion of high strength and toxic organic effluents under mesophilic conditions (35°C ± 2°C), with emphasis on the effect of biomass acclimation. A total of four effluents were chosen for this study, two labile (distillery and size) and two recalcitrant (scour dye and reactive dye).

Two anaerobic batch experiments and two pilot scale trials were performed. The first batch anaerobic experiment investigated the influence of biomass source in anaerobic treatability. The second batch test investigated, whether biomass acclimation enhanced the biodegradability of pollutants. The pilot scale trials were the scale up version of the biomass acclimation test.

The results showed sludge from Umbilo Wastewater Treatment Works was a superior biomass source, producing more gas and methane compared to Mpumalanga waste. For the high strength organic waste, the acclimated size and distillery samples produced 50% more biogas and methane compared to non-acclimated samples. This confirms that the biomass acclimation enhances the biodegradability. The biomass acclimation did not enhance the biodegradability of the recalcitrant effluent (scour dye). The pilot scale trials did not yield meaningful data; therefore it could not be proven if acclimation works on a larger scale.

ACKNOWLEGDEMENTS

I would like to express my deepest gratitude to the following people for their help and contribution:

Prof Lingam Pillay and Prof Chris Buckley (supervisor and co-supervisor) for giving me the opportunity to undertake this work.

Water Research Commission for financial support.

Dr Enrico Remigi (project leader) Pollution Research Group, School of Chemical Engineering, UKZN for guidance and support through out the project.

Mr. Chris Brouckert (Senior Research Fellow, PRG UKZN) for his assistance with computer problems

Staff and postgraduate students at UKZN Howard College Campus, where this investigation was undertaken.

Staff and postgraduate students at DUT for making my studies a memorable and enjoyable one.

All those who are not mentioned by name, but who supported me through out this demanding time are greatly acknowledged.

DEDICATION

This work is dedicated to the memory of my late mother (**Nonsikelelo Chamane**) who passed away before the commencement of this project. I would also like to dedicate this work to my family for their outstanding moral support during this work and my little daughter Ntokozo, whose birth gave me the full meaning of life.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

GLOSARRY

Acclimation ; the adaptation of the microbial community to degrade inherently recalcitrant compound and through prior exposure to that compound.

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

Activity: inherent ability of a microbial population to undertake the degradation of a test compound.

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

Biogas; the gas produced (primarily consists of methane and carbon dioxide), by the action of anaerobic micro-organisms on organic compounds.

Biodegradability: an intrinsic property of a test compound, primarily dependent on the molecular structure that refers to its susceptibility to undergo a biologically mediated degradation.

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

Digester: a closed vessel where microbial degradation takes place

Effluent: a stream flowing from an industrial process.

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

Inhibition: impairment (generally, irreversible) of the bacterial function

Methanogenes: bacteria which utilize volatile organic acids as substrate and produce methane and carbon dioxide.

Recalcitrant: a compound resistant to microbial degradation.

Supernatant: a liquid fraction that remains after centrifuging wastewater

ABBREVIATIONS

AAT	Anaerobic Activity Tests
ABR	Anaerobic bioreactor
APC	Advanced Process Control
APHA	American Public Health Association
ATA	Anaerobic Toxicity Assay
AWT	Anaerobic wastewater treatment
COD	Chemical oxygen demand
CSTR	Continuous Stirred Tank Reactor
DOC	Dissolved Organic Carbon
DUT	Durban University of Technology
DWAF	Department of Water Affairs and Forestry
GC	Gas Chromatography
GMS	Gas Measuring System
HRT	Hydraulic Retention Time
IWA	International Water Association
MP1	Mpumalanga Digester 1 sludge
MP2	Mpumalanga Digester 2 sludge
MTD	Month –to- Date
OFN	Oxygen Free Nitrogen gas
OLR	Organic Loading Rate
PI	Process Information
PS	Production Schedule
RCA	Root Cause Analyses
SA	South Africa
SRT	Sludge Retention Time
TCD	Thermal Conductivity Detector
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids

UASB	Upflow Anaerobic Sludge Blanket
UKZN	University of KwaZulu-Natal
VFA	Volatile Fatty Acids
VS	Volatile Solids
VSS	Volatile Suspended Solids
WW	Waste Water

CHAPTER 1

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

1.1 WATER LEGISLATION

South Africa (SA) is a water scarce country that is poorly served with natural water systems. The Department of Water Affairs and Forestry (DWAF) is mandated by the National Water Act (NO 36 of 1998) to ensure that SA water resources are protected, used, developed, conserved, managed and controlled in a sustainable and equitable manner, for the benefit of the country as a whole.

New environmental regulations have imposed limitations on a wide variety of organic and inorganic pollutants in industrial wastewaters discharged into rivers. The conventional wastewater treatment plant is depicted in **Fig 1.1**. Wastewater treatment plants are designed to treat normal effluents as far as the flow rate and organic carbon content is concerned.

The textile wastewater contains a high percentage of organics. Some of the wastewater is xenobotic, not readily biodegradable and suspected to be toxic. This wastewater, if discharged to public owned water treatment works, may inhibit the activated sludge process.

.

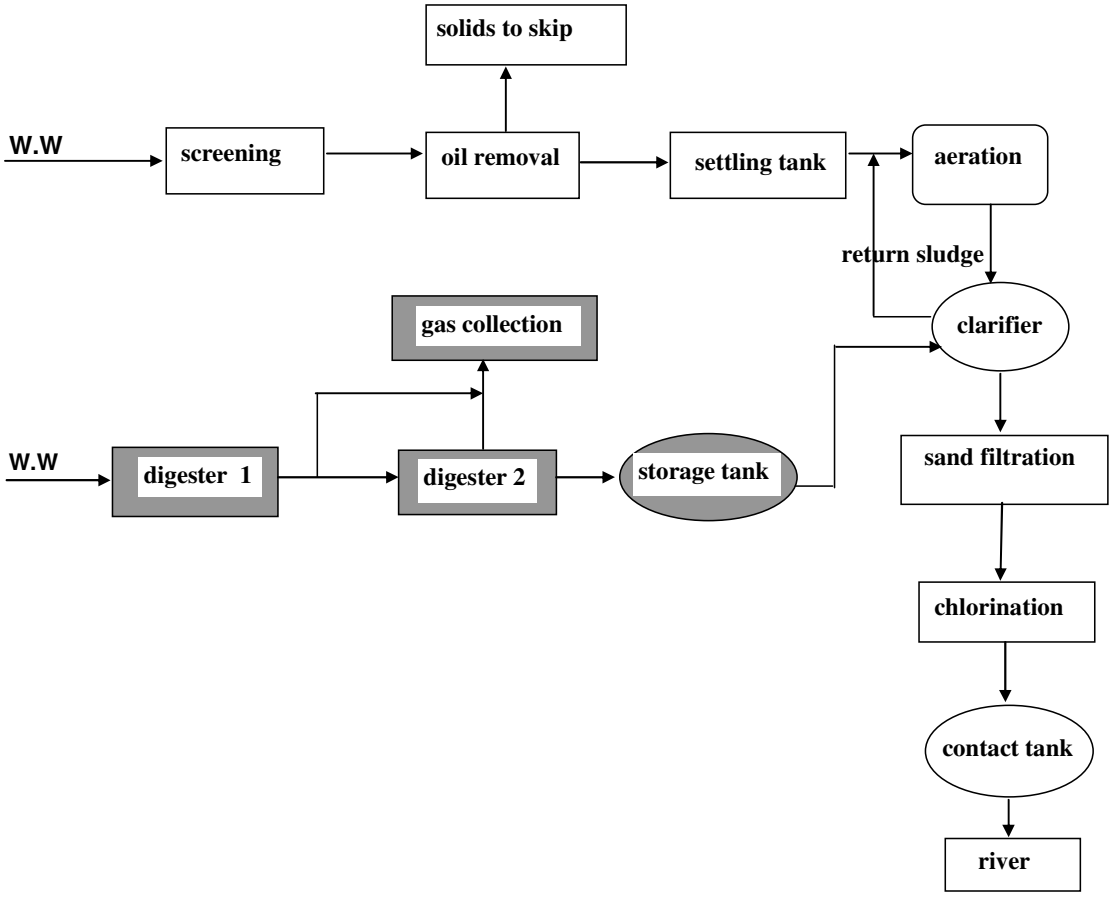


Figure 1.1: The layout of the activated sludge process (with shaded activities being a possible solution for toxic effluent treatment)

1.2 CURRENT DISPOSAL PRACTICES

Currently, industrial effluents with a high organic load or toxic organic compounds (including organics that are toxic to aquatic life) are sent to landfills or to marine outfall (Bell, 1997). In regions where there is a net surplus of rainfall, landfill sites have the potential to pollute groundwater due to saturated soil conditions. Therefore, many landfill sites should not receive liquid effluents. If the liquid wastes are disposed onto landfills, then an alternative sink is required for the treatment of high volumes of leachate that is generated. These concentrated effluents can then be treated by biological, chemical or physical methods to reduce the pollution load on rivers.

1.3 ANAEROBIC BIOTECHNOLOGY FOR INDUSTRIAL WASTE WATERS

Anaerobic digestion processes have been used for the treatment of concentrated municipal and industrial wastewaters for over a century (McCarty, 1971). Anaerobic Wastewater Treatment (AWT) is a biological unit operation that results in the conversion of organic waste to methane and carbon dioxide in the absence of molecular oxygen (Switzenbaum, 1986). The gas produced can be collected and utilised as an energy source. The residual sludge is stable and is a good soil fertilizer. It has been proven that AWT is a suitable technology for the treatment of concentrated wastewaters (Fernandez et al, 1995).

1.4 SURVEY IN KWAZULU-NATAL (KZN) PROVINCE

Several of the wastewater treatment plants in the KZN region have anaerobic digestion facilities which are under utilized, a digester which has spare hydraulic capacity and/or organic load. A digester with spare hydraulic capacity is one that is receiving a smaller volumetric load (m^3/d) than its design specification, while spare organic loading capacity implies that a digester can accept a greater organic load (kg/VS/m^3d) without experiencing an overload or shock loading (Bell,1997). A survey conducted by Bell (1997) identified wastewater plants with several digesters which were either idle or that were operating below design capacity. These digesters could receive industrial wastewater from surrounding industries without causing a hydraulic and / or organic overload. The survey showed that several wastewater treatment plants in the KZN region had available anaerobic digester capacity, which could be used to treat high strength or toxic industrial wastewater.

The availability of these digestion facilities, according to the survey by Bell (1997), can be used as a sink for the disposal of concentrated and toxic waste. The concentrated and toxic waste can then be diverted to the anaerobic digestion facilities instead of the activated sludge process. In the anaerobic digesters, municipal sludge or generally labile substrate can be treated together with industrial effluents, which are characterized by a high organic matter content (thus termed: high strength effluents) which contain substances that can be toxic for the microbial consortia. This treatment option, in which different types of wastes are treated together, is termed co-digestion (Ahring et al., 1992). It allows the disposal of

an otherwise difficult effluent, without compromising the performance of the digestion process to an un-acceptable extent.

1.5 ANAEROBIC CODIGESTION

Codigestion is a waste treatment method where different types of wastes are treated together (Ahring et al; 1992). Application of codigestion as an intelligent raw material management offers many advantages.

The main advantages of codigestion outlined by Angelidaki and Ahring (1997) are:

- Codigestion costs less per unit volume than the separate treatment systems.
- Improved digestibility of highly concentrated effluent by dilution with other wastes.
- Improved balance of nutrients, as well as increased load of biodegradable matter with a better biogas yield.
- Codigestion favours the rise of co-metabolism, which is often the only way to achieve detoxification of specific organic compounds.

The major biotechnical advantage of the codigestion is overcoming a problem of maintaining a stable pH within the methanogenesis range (Brummeler and Koster, 1990), poor buffering capacity and the possibility in of high VFA accumulation during digestion (Banks and Humphreys, 1998 and Compos et.al., 1999).

1.6 STUDY OBJECTIVES

This study was aimed at the process development of a codigestion protocol for high strength and toxic organic waste with respect to the following:

- The effect of a biomass source used as inoculum.
- The effect of biomass acclimation.
- Scale up investigation of the biomass acclimation.
- Establishing the guidelines for full scale implementation of the codigestion process.

Four potential inhibitors (effluents), two labile and two recalcitrant were used in this investigation as test compounds. The two labile effluents were distillery waste (collected from Illovo Merebank) and size effluent (collected from Frametex Textile Mills,

Pinetown). The recalcitrant effluents were scour dye (Dyefin, New Germany) and synthetic dye (simulated in the Biochem Eng Laboratory, UKZN).

1.7 PROJECT OUTLINE

The research project was executed as depicted in **Fig 1.2**

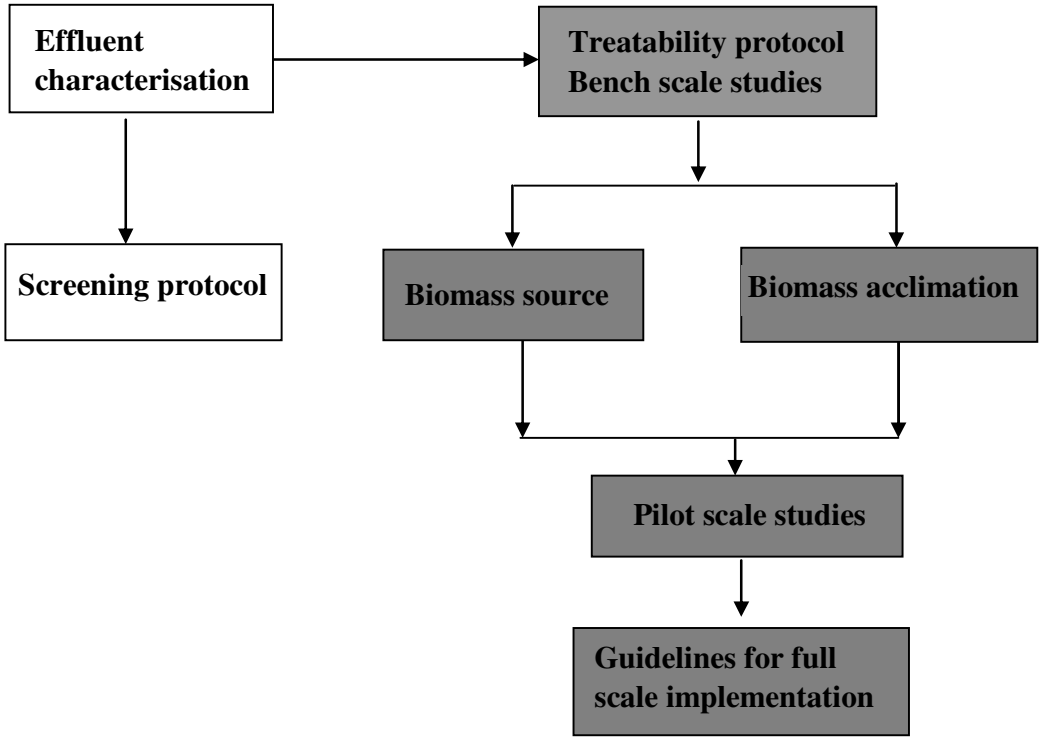


Figure 1.2: Schematic presentation of the MTechEng Project. The non-shaded activities were another MTechEng project with an input in the current project.

1.8 THESIS OUTLINE

The thesis consists of seven chapters after Chapter 1.

Chapter two: An overview of anaerobic digestion literature review, co-metabolism, as well as kinetic models. It also includes literature covering co-digestion and design/operation of bioreactors and related equipment.

Chapter three: This chapter describes the methodology of the serum bottle studies, and analytical methods used to evaluate the performance. The method for pilot plant studies is also discussed.

Chapter four: The experimental results for serum bottle studies and pilot-scale investigation are discussed at length.

Chapter five: This chapter presents the guidelines for full-scale implementation of the codigestion. It is a guide that can be followed to implement codigestion.

Chapter six: Conclusions and recommendations.

References: A full list of references cited in the text.

Appendices: A compilation of appendices (A-D)

CHAPTER 2

Anaerobic Digestion and Codigestion-Literature Review

"It is puzzling that single species of bacteria have not evolved to convert at least simple substrates such as carbohydrates, amino acids or fatty acids all the way to methane" McCarty and Smith, 1986.

This chapter introduces the reader to the general overview of the anaerobic processes **(Section 2.1)**. The concept of codigestion is discussed in **Section 2.2**. Characterization of effluents is explained in **Section 2.3**, as well as the general set-up of different reactors (**Section 2.4).**

2.1 ANAEROBIC PROCESSES

Anaerobic digestion is the process by which anaerobic organisms convert complex organic matter into biogas (mainly methane and carbon dioxide). Anaerobic conversions are amongst the oldest biological process technologies utilized by mankind, initially for food and beverage production. They have been applied and refined over many centuries, although the most dramatic advances have been achieved in the last few decades with the introduction of various forms of high rate treatment processes, particularly for industrial wastewater.

High loading rate, low sludge production and energy production in the form of methane are among the many advantages that anaerobic digestion exhibits over other biological unit operations. Energy production is the major driver for the increased application of the anaerobic process.

Anaerobic degradation of complex particulate organic materials can be described as a multistage process consisting of a series and parallel reactions (Kaspar and Wuhrmann, 1978; Bryant 1979; Zender et al.; 1982; Gujer and Zender, 1983 and Zinder 1984). It is a biological process where organic carbon is converted by subsequent oxidations and reductions to its most oxidized state (CO_2) and most reduced state (CH_4). The general

simplified flow sheet of anaerobic degradation of organics, disregarding the possible contribution of sulphide reducers, is shown in **Fig 2.1.**

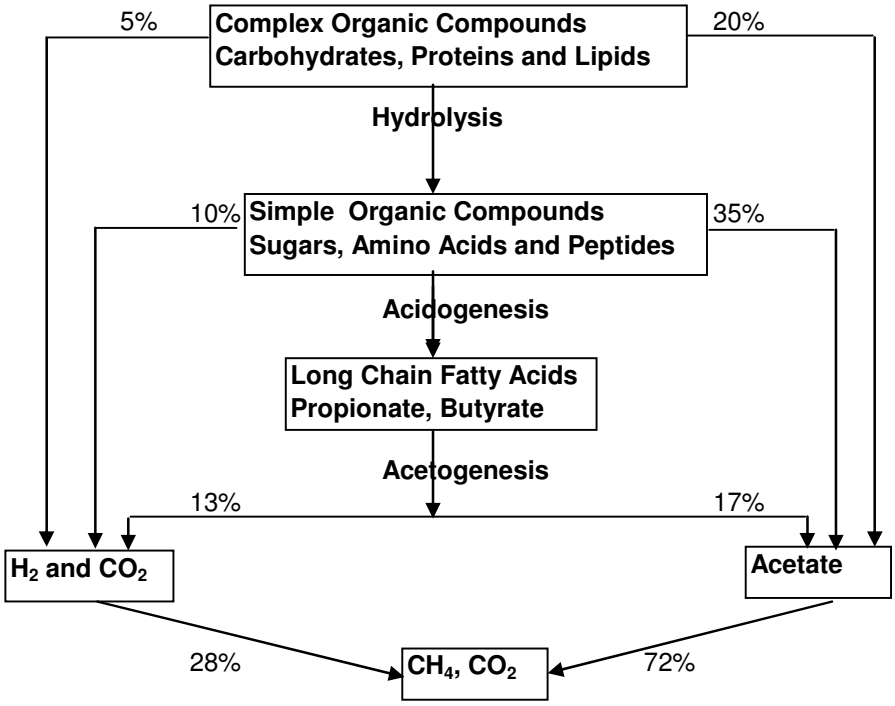


Figure 2.1: Series metabolism resulting in methanogenesis (McCarty and Smith, 1986.)

Distinguishing between available degradable (substrate) and total input Chemical Oxygen Demand (COD) is very important as a considerable fraction of the input COD may be anaerobically not biodegradable (Gossett and Belser, 1982). However, from a kinetic point of view, anaerobic treatment may generally be described as a three-step process (Remigi, 2001).

2.1.1 Stage one: Hydrolysis

Hydrolysis can be defined as the breakdown of organic substrate into smaller products that can subsequently be taken up and degraded by bacteria. Two types can be distinguished:

Primary hydrolysis: Hydrolysis of primary substrates where organic substrates present in the original wastewater is broken down.

Secondary hydrolysis: hydrolysis of secondary substrates that refers to the breakdown of substrates that have been produced by bacteria (hydrolysis of internal storage products, decay products etc).

2.1.2 Stage two: Acidogenesis

Acidogenesis (fermentation) is generally defined as an anaerobic acid-producing microbial process without an additional electron acceptor or donor (Gujer and Zehnder, 1983). In the second stage (acidogenesis), the products of the first stage (hydrolysis) are converted into acetic acid, propionic acid, hydrogen, carbon dioxide, and other low molecular weight organic acids by facultative anaerobic bacteria (acid formers).

2.1.3 Stage three: Acetogenesis

The main products of the anaerobic oxidation of short-chain fatty acids are acetate and hydrogen gas (Dolfing, 1988). These reactions are usually termed acetogenesis since acetate is the major carbon product. A number of bacteria capable of degrading butyrate and higher fatty acids have been identified; however only one acetogenic species capable of degrading propionate has been identified (McCarty and Mosey, 1991). Hence for the purpose of modeling acetogenesis, the two groups should be kept separate.

Propionate

The anaerobic oxidation reaction of propionate provided by McCarty and Mosey (1991) is:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (2.1)$$

Butyrate and High Fatty Acids

Butyrate oxidation is represented by:

$$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (2.2)$$

Homoacetogenesis

This refers to the production of acetic acid from CO_2 and H_2. The reaction provided by McCarty and Mosey (1991) is:

$$4H_2 + 2CO_2 \rightarrow 1CH_3COOH + 2H_2O \quad (2.3)$$

2.1.4 Stage Four : Methanogenesis

In the third stage, two groups of methanogenic bacteria are involved. One group converts hydrogen and carbon dioxide to methane. The other group converts acetate to methane and bicarbonate. About (72 %) of the methane produced in the anaerobic process results from the degradation of acetic acid according to the following reaction:

$$CH_3COOH^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (2.4)$$

Hydrogenotrophic Methanogeneis

Methanogenesis utilizing hydrogen and carbon dioxide can be described by the reaction:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2.5)$$

Acetoclastic Methanogenesis

In the major methanogenic step, acetate is cleaved to form methane and carbon dioxide according to the following reaction:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.6)$$

2.2 ANAEROBIC CODIGESTION (REVIEW)

The concept of co-digestion involves the use of two separate effluents as an organic substrate. One effluent is usually recalcitrant, whilst the other is labile.

Several researchers investigated the concept of codigestion. It has been shown that co-digestion of pig manure with ferrous containing waste resulted in the decrease of H_2S formation (Ahring et al., 1992). Furthermore detoxification of toxic compounds can be achieved by co-metabolism which would be favored by co-digestion. A co-metabolic process is defined as a microbial transformation of a compound by an organism which is unable to use it as an energy or carbon source (Schink, 1998). It has been previously shown that full dechlorination of pentachlorophenols could only be achieved by codigestion with glucose. (Hendriksen et al., 1992).

2.3 BIODEGRADABILTY, ACTIVITY AND INHIBITION

Numerous methods have been developed over the past 30 years since Van den Berg et al., (1974) measured the methanogenic activity by using a manometric device equipped with a photoelectric sensor to quantify the gas production. Most of the methods in literature monitor the production of biogas. These methods can be termed volumetric or manometric methods. However, this same concept can be employed to assess the activity or inhibition of individual metabolic steps preceding the methanogenic one, provided that they are rate limiting for the whole process.

The reliability of activity assessment through gas measurement is strongly dependent on the equilibrium between liquid and gas phase in a closed vessel. This can be influenced by many factors, e.g. the amount and characteristics of the test substrate, the concentration of biomass, the gas-to-liquid ratio: all these aspects need to be addressed in the standard procedures.

A working group of the International Water Association (IWA) has established a common accepted terminology that may be incorporated in reference and standard procedure. In the review the terms *biodegradability, activity* and *inhibition* are assumed to be consistent with the definitions that follow.

2.3.1 Biodegradability

Biodegradability indicates an intrinsic property of a *test substance that* refers to its susceptibility to undergo a biologically mediated degradation. The extent to which the degradation is achieved allows to further specifying biodegradability (Batters by, 2000) as:

-**ultimate**, if the organic test substance is converted into organic compounds and other products (associated with 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 substance can also be referred to as inherently biodegradable when it is potentially biodegradable only if specific steps are taken, such as pre-exposure of the inoculum to substrate, increased test duration and/or higher food-to-microorganisms 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 stochiometrically converted, i.e. based on either the elemental analysis of the test compound or its measured COD.

Under anaerobic conditions, the biodegradation of an organic substrate can be described as follows:

$$COD_0 + X_0 \rightarrow COD_{res} + X_f + Gas \qquad (2.7)$$

COD_0 and COD_{res} are the initial and residual concentration respectively of the test substrate.

X_0 and X_f are biomass concentration at the beginning and the end of the process (X_f may be equal to X_0). Gas is the net biogas and consists mainly of (CO_2 and CH_4).

Two methods are used to measure biodegradability:

Respirometric methods measure the amount of biogas produced by the activity of the biomass growing on the test substance as the sole substrate. These can be further subdivided into **manometric** and **volumetric**: the former keeps the volume constant and measure the pressure build-up due to gas production, the latter keeps the pressure constant and measures the volume of the biogas evolved.

Analytical methods: measure the extent of substrate consumption or product formation either as lumped parameters, such as dissolved organic carbon (DOC), total organic carbon (TOC) or COD (Field and Sierra, 1989, Birch et al., 1989) or by means of gas chromatography (GC).

The growth of bacteria can be described by a growth curve (**Fig 2.2)** with three distinct phases: the lag phase, exponential growth phase, and the plateau:

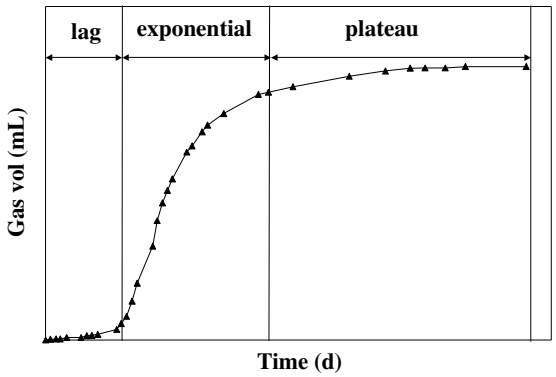


Figure 2.2: Bacterial growth curve

Lag phase: It is the period needed by micro-organisms to adapt to the test substrate i.e. the time needed to reach 10% of the maximum expected gas production.

Exponential/ biodegradation: It is the period between the end of the lag phase and 90% of the maximum expected gas production.

Plateau: It is the period after biodegradation, in which no significant gas is produced. The amount of methane expected after the complete conversion of an organic compound is referred to as theoretical methane ($ThCH_4$) and the expected biogas is ThGP. If the chemical formula of the test material is known, Buswell (1939) proposed the following equation:

$$C_cH_nO_o + (C\text{-}h/4\text{-}O/2).H_2O \rightarrow (C/2\text{-}h/8\text{-}O/4)\text{-}CH_4 + (C/2\text{-}h/8+O/4).CO_2$$

The CH_4 and CO_2 fractions of the biogas can be calculated as follows:

$$ThCH_4 = \frac{nCH_4}{C.12 + h + O.16}, \qquad ThCO_2 = \frac{nCO_2}{C.12 + h + O.16} \qquad (2.8)$$

And the biogas production is;

$$Thbiogas = (ThCH_4 + ThCO_2).m.22.4\,\text{L} \qquad \text{(L at STP)} \qquad (2.9)$$

where m is the amount of test material used.

L is the volume at STP conditions.

If neither the chemical formula nor the carbon content of the test material is available, then the theoretical biogas production can be calculated by measuring the COD.

$$ThCH_4 = 0.305mCOD, \tag{2.10}$$

Where m is the amount of test material used.

COD is the COD content.

For a volumetric system, it is convenient to express the volume of biogas measured under experimental conditions into STP conditions, also taking into account the water vapor pressure.

$$V_{STP} = \frac{273.15V(t)(p - p_W)}{101.325T} \tag{2.11}$$

Where:

p, p_w indicate the atmospheric and the water vapor pressure respectively in mbar.

$V(t)$ is the measured volume

T is the temperature in K

The percentage of biodegradation at time t can be calculated as follows:

$$\%D(t) = \frac{V_{STP}(t) - V_{STP}^{*}(t)}{Thbiogas} \tag{2.12}$$

where V_{STP}^{*} is the volume of biogas at time t and corrected for STP, which is measured in control reactors. If the gas chromatographic analysis is available, the percentage of methane can be expressed as;

$$\%DCH_4 = \frac{\%CH_4(t).V_{head}(t)}{ThCH_4} \tag{2.13}$$

where V_{head} is the volume of the headspace and $\%CH_4(t)$ is the detected methane fraction in the gas phase, at time t.

2.3.2 Activity and inhibition

Activity indicates the inherent ability of a microbial population to undertake degradation of the test material. It is generally measured as the specific rate of substrate consumption, referred to as either the total biomass (e.g. volatile suspended solids) 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 the liquid displaced by gas production and/or determine the composition of the gaseous phase by a gas chromatographic technique.

Activity can also be assessed under non-limiting or limiting substrate concentrations. The two approaches are equivalent if anabolism and catabolism (i.e. growth and energy generation respectively) are assumed to be coupled by a proportionality factor, as occurs in steady state conditions.

Inhibition indicates a detrimental effect that a *test substance* causes on the activity of a microbial population. Speece (1996) distinguishes between inhibition and toxicity: the former denotes an impairment of a particular bacterial function; the latter adversely affects bacterial metabolism as a whole.

Inhibition is assessed by comparison to a reference base activity, measured under optimal conditions, e.g. non-limiting substrate concentration. It is therefore generally referred to as percentage reduction in the specific activity. The concentration of an inhibitory agent that results in a 50% reduction in specific activity (EC_{50}) is often reported.

The inhibitory effect of the test material is generally referred to the methanogenic step, since this is generally the most vulnerable step of the entire process. Therefore, the methanogenic activity measured in the presence of the test material is compared to that measured in an optimal methanogenic condition, i.e. in presence of excess acetate, as follows:

$$I = \frac{A(b) - A(u)}{A(b)} \tag{2.14}$$

Where *I* is the inhibition coefficient, *A(u)* and *A(b)* indicate the activity measured in the test unit and the baseline methanogenic activity (control activity)

The activity A is calculated as:

$$A = \frac{1}{X}\frac{d}{dt}V_{CH} \tag{2.15}$$

Where *X* indicates the amount of biomass present in the system (in grams of volatile (gVS) or volatile suspended solids (, gVSS) and V_{CH} is the amount of methane produced. Generally, the initial biomass is used, unless significant growth has occurred and a more accurate value can be estimated. For a short-term test, the average of the initial and final values may be used.

Two distinct patterns of toxicity are mostly reported (Young and Tabak 1993): inhibition increases as the dose of the test substance increases, but the relative activity remains constant at given times throughout the test period. A situation occurs where the biomass does not recover from the toxic effect, i.e. no acclimation occurs. Inhibition occurs in early stages of the test (termed lag-phase). As the time lapse, the biomass adapts to the toxic compound by recovering its base activity.

2.3.3 FACTORS WHICH MAY AFFECT EXPERIMENTAL RESULTS

pH

Methanogens prefer nearly neutral pH conditions with a generally accepted optimum range of approximately 6.5 to 8.2. Conditions above or below this range significantly decreases the rate of methane production. The pH in an anaerobic process must be around neutral; any drop in pH will lead to the souring of the reactor, thus inhibiting the process. The only drawback of the activity and inhibition tests carried in closed vials is that the pH cannot be controlled.

Temperature

Biological activity is strongly dependent on temperature. Activity and inhibition tests are performed at constant temperature controlled room (35°C). The main outputs of a serum

bottle assay are the biodegradability of the test material and/or the inhibitory effect of the test material on the methanogenic biomass.

Key aspects

Some of the factors that are relevant to the assessment of biomass activity, and that can influence the outcome of the assay are reported in **Table 2.1**

Table 2.1: Factors that may influence microbial activity and inhibition assessment (Rozzi & Remigi 2004)

Factor	Description
Equipment	Type of bioassay; shape of the vessel
Operating conditions	Configuration (e.g. gas-to-liquid volume ratio); temperature, pH, redox potential; others (e.g. sampling frequency)
Methods of analysis	Detection principle; measuring devices; variables monitored. Inhibition of specific enzymatic pathways
Test compound	Physico-chemical properties (e.g. solubility). Characterization of the effluent (e.g. carbon content)
Inoculum	Source (e.g. municipal or industrial); physical structure (e.g. flocs or granules). Characterization (e.g. solid and carbon content)
Nutrient solution	Nutrients, minerals and trace elements; co-substrate

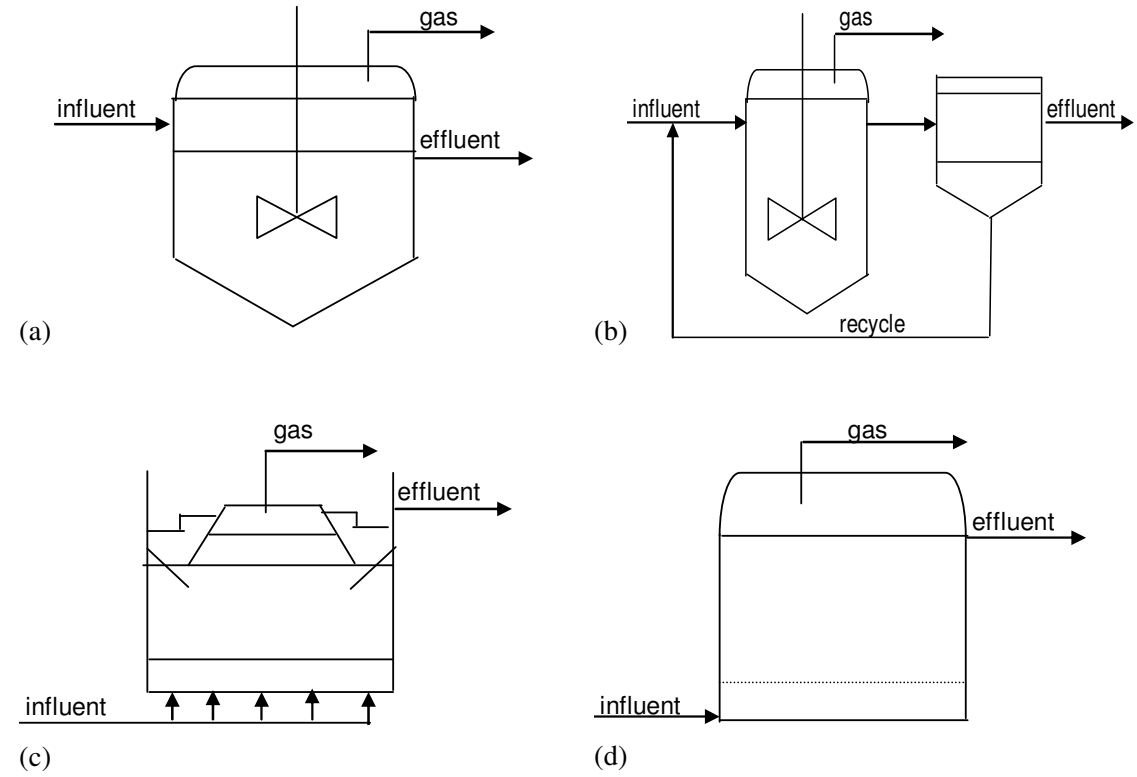
2.4 WASTE WATER TREATMENT SYSTEMS

The applicability of anaerobic processes for the treatment of industrial wastewaters and domestic sludge has been recognized for many years. However, there has been some skepticism due to the lack of quantitative information on the capability of such processes to handle potentially toxic or high strength waste

The microbial biomass responsible for anaerobic digestion can be “packaged” in a variety of process configurations. Each configuration has different implications for the ratio of Solid Retention Time/ Hydraulic Retention Time (SRT/HRT). Choosing the most appropriate configuration is critical to successful operation.

2.4.1 Continuous Stirred Tank Reactor (CSTR)

The CSTR **(Fig 2.3 a)** contains a mechanical agitation system consisting of a vertical shaft with a number of impellers and baffles around the vessel. Mixing of the vessel contents in a CSTR process is generally achieved by a paddle or screw systems or by gas diffusers (draught tube) situated near the base of the assembly, agitation tends to be intermittent in these reactors and can be maintained by a periodic recirculation of the biogas produced (Tapp, 1981).



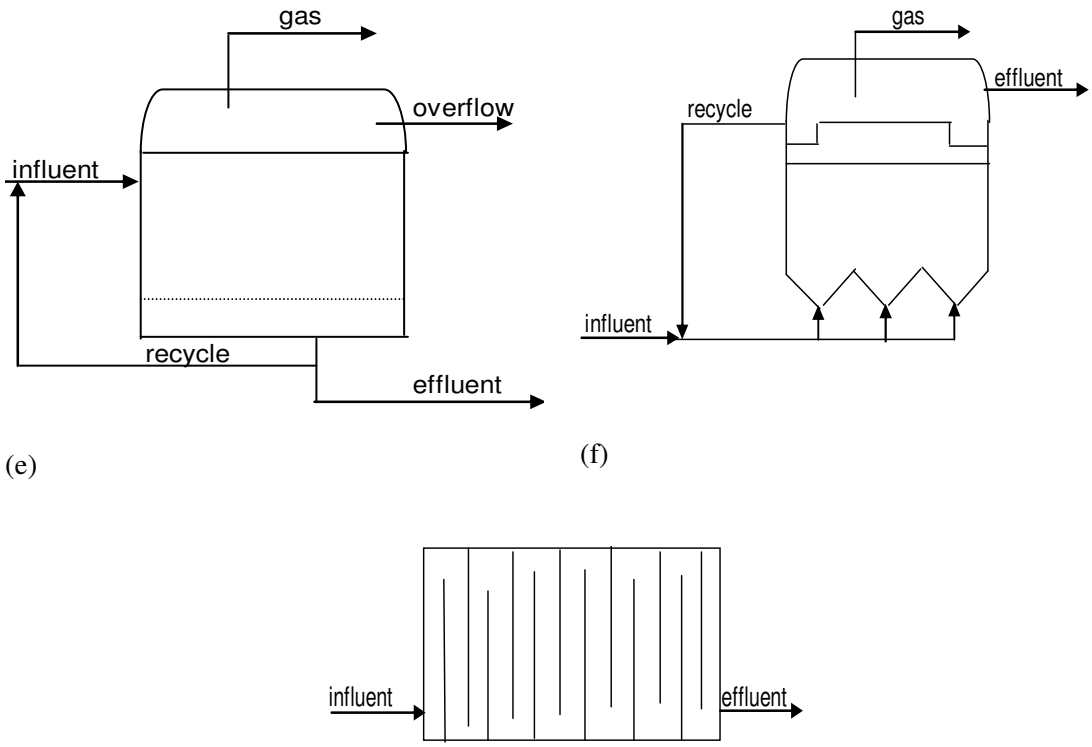


Figure 2.3: Design configuration of anaerobic reactors (a) CSTR, (b) anaerobic contact process, (c) UASB, (d) anaerobic filter, upflow configuration, (e) downflow configuration, (f) expanded/ fluidized bed reactor and (g) anaerobic baffle reactor, ABR

Mixing in anaerobic digesters provides good contact between the active biomass and the sludge. The major disadvantage of complete mixing in digesters, in addition to the cost of mixing, is the need for a facility that will enhance the separation of the digested solids from the liquid phase.

The heating of anaerobic CSTR unit can be effected by the utilization of an external heat exchanger. The vessel contents are pumped through the exchanger as necessary upon a signal received from a digester thermostat controller. The CSTR systems are susceptible to malfunction upon shock loading or subsequent to the introduction of a variety of toxic substances.

2.4.2 Anaerobic Contact Process

The contact or recycled flocs process **(Fig 2.3 b)** comprises a continuously fed, completely mixed reactor stage followed by solids/liquid separation. A degasification step is frequently included. The effluent is discharged from the settling device and the settled biomass is returned to the digester vessel, where it is mixed with the incoming feed.

The bacteria in a contact reactor appear as suspended flocs and the system is maintained in suspension by mechanical stirring or recycles. A separation of flocs and treated wastewater occurs in a separator assembly such as the sedimentation tank, from where the suspended settled flocs are recycled to the reactor at moderate rates to prevent shear forces from disturbing the floc structure. This separation of solids and liquids is crucial in the contact digester.

2.4.3 Upflow fixed/packed bed reactors

In the upflow fixed bed reactor **(Fig 2.3 c)**, the wastewater is passed through the medium particles resulting in a large proportion of the retained biomass not being attached to the packing column. This non-attached material is returned in the interstices between the medium particles partly by settling and partly through the influence of physical contact. A disadvantage of this system is blockage due to excess biomass accumulation, which ultimately leads to decreased retention capacity of the bed.

2.4.4 Down flow fixed bed reactors

With down flow fixed bed reactor **(Fig 2.3 d)**, suspended solids and biofilm solids will be carried down with the liquid flow and out of the reactor. These systems are able to withstand severe hydraulic overloading conditions with only a slight reduction in treatment efficiency.

2.4.5 Expanded and fluidized bed systems

With expanded and fluidized bed process **(Fig 2.3 f)**; attempts have been made to improve anaerobic reactor transfer characteristics by the utilization of smaller medium particles with very high surface to volume ratios. By applying high liquid up-flow velocities, the medium

can be expanded to produce a substantial increase in bed porosity (van Handel and Lettinga, 1994).

In both processes, an anaerobic biofilm is developed on the surface of the medium particles by a process of immobilization (Lettinga, 1995). The major disadvantage of these systems is the energy costs required for effluent recycle.

2.4.6 Upflow Anaerobic Sludge Blanket (UASB)

A UASB digester **(Fig 2.3 c)** is designed to treat low and medium strength wastewaters at high volumetric loading rates, and therefore, a short hydraulic retention time. 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 a lower digestion zone and an upper settling zone (van Handel and Lettinga, 1994). No support medium is added to the reactor since the process is based on the immobilization of the biomass in the form of sludge granules.

The wastewater is introduced uniformly through the bottom of the reactor. It then passes the sludge bed and the settling zone via the aperture between the phase separators. The presence of the settler on top of the digestion zone enables the system to maintain a large sludge mass in the UASB. The effluent is discharged relatively free of suspended solids.

2.4.7 Anaerobic Baffled Reactor (ABR)

The ABR **(Fig 2.3 g)** is a simple rectangular tank, which is divided into a number of equal volume compartments by means of partition from the roof and the bottom of the tank. The liquid flow is alternated upwards and downwards between the partitions. Due to its physical configuration, this type of reactor is able to treat waste with high solids content. The sludge in each compartment will differ depending on the specific prevailing environmental conditions and remaining compounds or intermediates to be degraded.

2.4.8 Anaerobic Filter

Young and Tabak (1991) introduced the anaerobic filter **(Fig 2.3 e)** which is mainly used for industrial wastewaters. An inert media is used to entrap and accumulates

microorganism in the reactor. The anaerobic filter is usually operated in the upflow mode, but it can be operated in the downflow mode (Kennedy and Berg, 1982).

CHAPTER 3

EXPERIMENTAL METHODS

3.1 INTRODUCTION

This chapter presents the methodology used in performing experimental work. It is structured into three main sections: **Section 3.1** illustrates the background scenarios used for culturing the anaerobic sludge and assessing its performance under various conditions. Also in this section, the experimental procedure for various tests that were performed is shown in detail. **Section 3.2** gives an overview of the analytical procedures used to measure the various parameters. **Section 3.3** gives a background procedure for pilot plant trials, and the set up used to perform pilot plant experiments.

3.1.1 Equipment

Two types of reactor units were used for the experiments described in this report:

Small-scale units, 125 mℓ serum glass vials, were used to conduct Anaerobic Activity Tests (AAT) in batch conditions.

Pilot-scale units, consisting of 2 to 5 ℓ continuously stirred glass vessels, were used to conduct digestion experiments. The advantages and disadvantages of these set-ups are reported in **Table 3.1** (WRC report K5/1074).

Table 3.1: Advantages and disadvantages of experimental set ups (WRC Report No K5/1074)

Set-up	Advantages	Disadvantages
Serum bottle	a large number of replicates can be carried out simultaneously;	Generally limited to batch operation and steady conditions;
	different operating conditions can be simulated, by using similar units in parallel;	Limiting possibility of monitoring the process (especially sampling), due to the large number of vials
	Possibility of simulating extreme conditions, even leading to failure, without risking the same consequences on a larger unit.	Limited to monitoring reactions which directly results in the production of biogas
Pilot-scale	It is generally the final step before the real implementation: it allows the tuning of the operational conditions and ultimately to a correctly design full-scale plant;	It requires considerable knowledge of the process, gained through extensive experimentation at a small scale;
	Industry is mostly interested in the outcome of this stage	Large equipments is needed The possibility of carrying out replicates is generally limited.

3.2 SERUM BOTTLE STUDIES

Assessment of effluent for their toxicity is important to prevent digester failure. Activity measurement of biomass is used for the proper monitoring of the loading rates in the digester. Serum bottle studies were used to assess the toxicity, as well as biomass activity.

A serum bottle anaerobic toxicity assay (ATA) and anaerobic activity test (AAT) comprises a control set which allows for the quantification of background conditions, and a number of tests units in which the anaerobic sludge is exposed to a variety of conditions. All the units are generally carried out in triplicate, to ensure the reproducibility of the results. The composition of each set varies depending on the type of test to be conducted. Activity is assessed at varying concentrations of the substrate (for a biodegradability test) or of the test compound (for a toxicity test). The general procedure for conducting a serum bottle assay was developed by Owen et al., (1979).

3.2.1 Biomass preparation and storage

The biomass for serum bottle studies was collected from the Umbilo Waste Water Works, Durban, South Africa, and Harmmarsdale Waste Water Works, Hammarsdale South Africa. It was assayed for total solids (TS) and volatile solids (VS) as per Standard Methods (APHA, 1985). The biomass for sludge activity tests was withdrawn from pilot digesters. The biomass was stored in the temperature controlled room (4°C) until use. It was taken out of the cold room 48 hours before use and placed in the temperature controlled room (35°C) to activate the micro-organisms.

3.2.2. Preparation of assay bottles

The serum bottle assays was conducted in 125 mℓ serum bottles with a working volume of 100 mℓ. The assay bottles were over-gassed with oxygen free nitrogen (OFN) gas at a flow rate of 0.5 mℓ/min for 15 min according to Owen et al., (1979). The assay concentrations were selected from non-inhibitory to severe toxic. The bottles were equilibrated at incubation temperature (35ºC). All the components (biomass, nutrients and test compounds) were added into the vials at predetermined ratios, ideally handling the sludge under strict anaerobic conditions. As a further safety precaution, each vial was flushed

with oxygen-free gas, i.e. nitrogen mixed with carbon dioxide (51%N_2, 49% CO_2). Acetate (0.35 g) substrate was added in powder form. The bottles were capped with butyl rubber septa and crimp sealed with aluminum prior to incubation at constant temperature controlled room (35°C). After equilibration for 1 h the gas volumes were zeroed to ambient pressure, with a graduated glass syringe.

3.2.3 Defined media

Concentrated stock solutions **(Table 3.2)** with long shelf life were used to prepare the defined media and were stored at 4°C. A defined solution containing trace elements, minerals and vitamins was prepared according to Owen et al., (1979) with some minor modifications. The recipe for preparing 1.8 ℓ of defined media is given in **Table 3.3**.

Table 3.2: Stock solutions for preparation of mineral salt solution

Stock solution	Composition	Concentration (g/ℓ)
S2	Resazurin	1
S3	$(NH_4)_2.HPO_4$	26.7
S4	$CaCl_2.2H_2O$	16.7
	NH_4Cl	26.6
	$MgCl_2.6H_2O$	120
	KCl	86.7
	$MnCl_2.4H_2O$	1.33
	$CoCl_2.6H_2O$	2
	H_3BO_3	0.38
	$CuCl_2.2H_2O$	0.18
	$Na_2MO_4.2H_2O$	0.17
	$ZnCl_2$	0.14
S5	$FeCl_2.4H_2O$	370
S6	$Na_2S.4H_2O$	500
S7	Biofin	0.002
	Folic acid	0
	Pyridoxine hydrochloride	0.002
	Riboflavin	0.01
	Thiamine	0.005
	Nicotinic acid	0.005
	Panthothenic acid	0.005
	p-amino benzoic acid	0.005
	Thiocitic acid	0.005

Due to unavailability of the two vitamins, p-aminobenzoic acid and thiocitic were omitted from the stock solution S7. P-aminobenzoic acid is a member of the vitamin B group.

Table 3.3: Preparation of the defined mineral salts solution

Step	Method	Volume (mℓ)	Mass (g)
1	1 ℓ of distilled water was added to a 2 ℓ Pyrex flask.		
2	The following were added		
	Stock solution S2.	1.8	
	Stock solution S3	5.4	
	Stock solution S4	27.0	
3	Distilled water was added up to 1.8 ℓ mark		
4	Boiled for 15 minutes whilst flushing with OFN gas at a rate of 1 ℓ/min		
5	Cooled to room temperature		
6	The following stock solution were added		
	Stock solution S5	18	
	Stock solution S6	1.8	
	Stock solution S7	1.8	
7	$NaHCO_3$ was added as powder		8.4
8	Flushed with OFN until pH stabilized at 7.1		
9	Autoclaved for 30 minutes at 121ºC		
10	Stored at 4ºC until use		

All the components were poured into the vials, ideally handling the sludge under strict anaerobic conditions. If this was not feasible, as a further safety precaution in all cases, each vial was flushed with oxygen-free gas (51%CO_2 and 49%N_2).

- The vials were then capped with rubber septa and crimped sealed with aluminum, and incubated at the temperature controlled room (35°C).
- After sealing, the pressure inside the vials is likely to increase due to physico-chemical adjustment; therefore, it must be re-equilibrated to the atmosphere prior to starting the test. This was done by puncturing the serum cap septa after 1 hour of incubation.
- Thereafter, the excess pressure generated by microbial activity was periodically re-equilibrated and the gas volume produced was measured using a glass syringe, gas composition was determined by gas chromatographic analysis. The following measurements were performed prior to starting a test, to optimally tune the units:

- solids concentration: volatile solids/volatile suspended solids (gVS/gVSS) are generally used to report microbial activity;
- organic content (COD) of the seed inoculum: ideally, the residual substrate in the inoculum should be negligible compared to the substrate added, to ensure a limited contribution to the biogas production.

3.3 ANAEROBIC ACTIVITY TESTS

The type of sludge (granular or flocular) and the sludge source (municipal or industrial) used as inoculum has a great effect on the treatabilty of organic waste. The source of the sludge can influence its ability to utilize the test material: for instance, a municipal sludge as compared to an industrial sludge or to an adapted culture may show a low affinity for an effluent of industrial origin. On the other hand, the reproducibility of the assessment may be improved when a non-specialized sludge is used, i.e. if taken from municipal digesters or digesters maintained on defined carbon sources.

3.3.1 Test no 1 (Biomass source)

The objective of was to establish whether the inoculum sampled from different sources will affect the degradation of the organic waste. If it does, this will form an important part of the process development protocol.

Materials and methods

The seed inoculum for AAT assays was collected from three different sources, viz the Mpumalanga digester 1, Mpumalanga digester 2 and Umbilo Wastewater Works, in Durban, South Africa. It was assayed for total solids (TS) and volatile solids (VS) according to **(Section 3.2.1)**. The biomass was stored in a cold room (4ºC) until use. It was taken out of the cold room (4ºC) 24-48 hours before use and placed in a temperature controlled room (35°C) to activate the micro-organisms and for temperature adjustments. Assay bottles were prepared as according to **Section 3.2.2**.

The AAT assay was conducted in triplicate (for reproducibility) using 125 mℓ serum bottles with a working volume of 100 mℓ. A test compound (reactive dye, from

Mediterranean Textile Mills, Mpumalanga KZN) was added at increasing concentration ranging from 1%, 5% and 10%. The set-up of the test is reported in **Table 3.4** details are listed in **(Appendix 1): Table D.1**. The test was conducted for 50 days. Gas production measurement and composition was performed as according to Owen et al., (1979) daily for the first 10 days and periodically thereafter.

Table 3.4: Biomass source experimental set-up

Reactor ID	**Mpumalanga digester 1**				**Conc. (%)**
	Sludge (mℓ)	**Nutrients (mℓ)**	**Acetate (g)**	**Reactive dye (mℓ)**	
R7-R9	40	10	0.35	0	0
R10-R12	40	10	0.35	0.5	1
R13-R14	40	10	0.35	2.5	5
R16-R18	40	10	0.35	5	10
Reactor ID	**Mpumalanga digester 2**				**Conc. (%)**
	Sludge (mℓ)	**Nutrients (mℓ)**	**Acetate (g)**	**Reactive dye (mℓ)**	
Z7-Z9	40	10	0.35	0	0
Z10-Z12	40	10	0.35	0.5	1
Z13-Z14	40	10	0.35	2.5	5
Z16-Z18	40	10	0.35	5	10
Reactor ID	**Umbilo digester**				**Conc. (%)**
	Sludge (mℓ)	**Nutrients (mℓ)**	**Acetate (g)**	**Reactive dye (mℓ)**	
U7-U9	40	10	0.35	0	0
U10-U12	40	10	0.35	0.5	1
U13-U14	40	10	0.35	2.5	5
U16-U18	40	10	0.35	5	10

3.3.2 Test no 2 (Biomass acclimation)

Biomass acclimation is believed to enhance biodegradability of inhibitory pollutants. Pre-exposure of the biomass to the potential pollutant can make the biomass to adapt to the particular waste and hence be able to degrade the organic waste. The objective of test no 2 was to determine the effect of biomass acclimation on the degradation of industrial effluents namely size, distillery waste, and scour dye.

Material and methods

The biomass used for the serum bottle assays was collected from Umbilo Waste Water Treatment Works, Durban (South Africa) and was subjected to the industrial pollutants in the first level of toxicity assessment which lasted for 100 days. The contents of the serum bottles from the previous test (level 1 toxicity assessment) were centrifuged, supernatant discarded and the biomass collected for reuse. The biomass was characterized for total solids (TS) and volatile solids (VS) according to Standard Methods (APHA, 1995) **Section 3.2.1**. The assay bottles were prepared according to **Section 3.2.2**. Test compounds (scour dye, size and distillery) were added at increasing concentrations ranging from 1%, 5% and 10% and at 9%, 23% and 41%. The set-up of the test is reported in **Table 3.5** with further details in **(Appendix 1): Table D.2**. The duration of the test was 90 days per effluent. Gas production measurement and composition was performed (as per Owen et al., 1979) daily for the first 10 days and periodically thereafter as required.

Table 3:5.a Biomass acclimation test set-up

Reactor ID	Size effluent				Conc. (%)
	Sludge (mℓ)	Nutrients (mℓ)	Acetate (g)	Size (mℓ)	
D7-D9	40	10	0.35	0	0
C1-C3	40	10	0.35	0.5	1
C4-C6	40	10	0.35	2.5	5
C7-C9	40	10	0.35	5	10
D10-D12	40	10	0.35	5	9
D13-D15	40	10	035	15	23
D16-D18	40	10	0.35	35	41

Operating conditions

Parameters	Units						
Concentration	(%-v/v)	1	5	9	10	23	41
Total liquid vol	mℓ	50.5	52.5	55	55	65	85
Gas-to-Liquid ratio		1.5	1.4	1.3	1.3	0.9	0.5
S-to-X ratio		0.41	0.61	0.9	0.9	1.9	3.9
Total COD	gCOD/g	6.12	8.9	11	12	22	35
Theoretical CH_4	mℓ	120	180	255	255	556	1158

Table 3:5.b Biomass acclimation test set-up

Reactor ID	Distillery effluent				Conc. (%)
	Sludge (mℓ)	Nutrients (mℓ)	Acetate (g)	Size (mℓ)	
D7-D9	40	10	0.35	0	0
C1-C3	40	10	0.35	0.5	1
C4-C6	40	10	0.35	2.5	5
C7-C9	40	10	0.35	5	10
D10-D12	40	10	0.35	5	9
D13-D15	40	10	035	15	23
D16-D18	40	10	0.35	35	41

Operating conditions

Parameters	Units						
Concentration	(%-v/v)	1	5	9	10	23	41
Total liquid vol	mℓ	50.5	52.5	55	55	65	85
Gas-to-Liquid ratio		1.5	1.4	1.3	1.3	0.9	0.5
S-to-X ratio		0.41	0.61	0.9	0.9	1.9	3.9
Total COD	gCOD/g	6.12	8.9	11	12	22	35
Theoretical CH_4	m	120	180	255	255	556	1158

Table 3:5.c Biomass acclimation test set-up

Reactor ID	Scour effluent				Conc. (%)
	Sludge (mℓ)	Nutrients (mℓ)	Acetate (g)	Size (mℓ)	
D7-D9	40	10	0.35	0	0
C1-C3	40	10	0.35	0.5	1
C4-C6	40	10	0.35	2.5	5
C7-C9	40	10	0.35	5	10
D10-D12	40	10	0.35	5	9
D13-D15	40	10	035	15	23
D16-D18	40	10	0.35	35	41

Operating conditions

Parameters	Units						
Concentration	(%-v/v)	1	5	9	10	23	41
Total liquid vol	mℓ	50.5	52.5	55	55	65	85
Gas-to-Liquid ratio		1.5	1.4	1.3	1.3	0.9	0.5
S-to-X ratio		0.41	0.61	0.9	0.9	1.9	3.9
Total COD	gCOD/g	6.12	8.9	11	12	22	35
Theoretical CH_4	mℓ	120	180	255	255	556	1158

3.4 ANALYTICAL METHODS

3.4.1 Determination of solids

A Standard Method (APHA, 1995) was used for determination of solids in anaerobically digested sewage sludge. Total solids (TS) and volatile solids (VS) were determined on a raw sample of sludge. Total suspended solids (TSS) and volatile suspended solids (VSS) required that the sludge is filtered, using a glass-fiber filter (0.45 µm) and the liquid fraction discarded. The solid content is generally reported as mass of solids per unit volume of sludge .i.e. gVS/ℓ

3.4.1.1 Total solids

A crucible was placed in a muffle furnace at 550°C for approximately 60 min. The crucible was then cooled in a desiccator, weighed and stored until used. A well

homogenized sample was transferred to the weighed dish and evaporated in a drying oven (105°C) for approximately 18 hours. The crucible was then cooled and re-weighed. The difference in weight represented the total residue. (**equation 3.1**).

$$TS = \frac{W_{105} - W_0}{M} \tag{3.1}$$

3.4.1.2 Volatile solids

The residue was ignited in a pre-heated muffle furnace (550°C) for 2 hours. The dish was partially cooled and then transferred to a desicator for final cooling, after which it was weighed again. The loss of weight on ignition was reported as the volatile solids (**equation 3.2**)

$$VS = \frac{W_{105} - W_{600}}{M} \tag{3.2}$$

3.4.1.3 Total and volatile suspended solids

A similar procedure was used for the determination of the suspended fractions of the solids, in which the role of the crucible was replaced by the glass-fiber filter paper (**equations 3.3 and 3.4**). Great care must be exercised in handling the filter, since the accuracy of the determination can be easily impaired, e.g. by humidity taken up from fingers or atmospheric particulate matter.

$$TSS = \frac{W_{*105} - W_{*0}}{M} \tag{3.3}$$

$$VSS = \frac{W_{*105} - W_{*0}}{M} \tag{3.4}$$

W denotes the weight (g) of the crucible or the filter (indicated by the subscript *)

M denotes the size of the sludge sample, as volume (mℓ)

The subscripts 105, 550 and 600 indicate the temperatures; $_0$ denotes the tare value.

3.4.2 Determination of organic carbon

The measurement of the Chemical Oxygen Demand (COD) is a simple, relatively inexpensive method, which gives an accurate indication of the electron equivalence. It is based on a closed or open reflux method (APHA, 1995), or on a calorimetric method. The COD is the amount of oxygen needed to stabilize organic components.

In general, a strong oxidant is used to determine this value. The oxidant must be of sufficient strength to oxidize any organic component that may be present in the sample. The oxidant most commonly used is dichromate ($Cr_2O_7^{2-}$). The principle is based on the observation that most organic matter is destroyed by a boiling solution of chromic and sulphuric acid. The amount of utilizable organic matter is proportional to the dichromate consumed. The procedure of preparing COD reagents is reported in **Table 3.6** and the COD method is discussed thereafter.

Table 3.6: Preparation of COD reagents

Reagent	Method	Volume (mℓ)	Mass (g)
Potassium dichromate (0.0417 M)	$K_2Cr_2O_7$ (dried at 103°C for 2 hours) was placed in a 1 ℓ volumetric flask.		12.26
Sulphuric acid reagent	It was diluted to the mark with distilled water. Ag_2SO_4 powder was added to 1 kg concentrated H_2SO_4, left to stand for 2 d to dissolve Ag_2SO_4.		5.5 98
Ferrous ammonium sulphate (0.25 M)	Fe $(NH_4)_2(SO_4)_2.6H_2O$ was dissolved in 1 ℓ distilled water in a 1 ℓ volumetric flask. Concentrated H_2SO_4 was added. Cooled to room temperature.	20	1.485
Ferroin indicator	o-phenanthroline was placed in 100 mℓ volumetric flask. $FeSO_4.7H_2O$ was added		0.695

The sample to be analyzed is appropriately diluted in a volumetric flask. The following sequence is then followed to determine the COD.

- A 10 mℓ aliquot of appropriately diluted sample is placed in a 250 mℓ volumetric refluxing flask. To this, a small amount of mercuric sulphate (0.04 g), several glass beads and 15 mℓ of sulphuric acid reagent are added.
- A 5 mℓ aliquot of $K_2Cr_2O_7$ (0.01417 M) solution is then added.
- The solution is mixed and allowed to cool. The flask is then attached to the condenser, cooling recycling water turned on and the mixture is boiled for 2 hours.
- A blank consisting of 10 mℓ distilled water, instead of a sample prepared in the same way. The samples are then cooled and diluted with 80 mℓ distilled water. Thereafter, they are titrated with ferrous ammonium sulphate (FAS) using ferroin indicator.
-

The COD value of the sample is calculated as follows:

$$COD = \frac{(V_B - V_S).C_{FAS}}{V} * \alpha \qquad (3.5)$$

where,

V_B and V_S denotes the volumes of FAS (mℓ) used to titrate the blank and the sample, respectively;

C_{FAS} denotes the concentration of the FAS (M) used.

V denotes the volume of the sample (mℓ); and

$\propto$ is the factor: $\propto = 8000$

Problems associated with COD measurements are:

The most common problems associated with COD measurements are listed below:

- halogens can be oxidized;
- aromatic carbohydrates and some aromatic heterocyclic compounds are not oxidized;
- volatile straight-chain aliphatic compounds are not oxidized to any appreciable degree;

- reduced inorganic compounds e.g. ferrous iron, are oxidized quantitatively under the species.

Nevertheless, the open reflux method for organic carbon measurement is still considered the most reliable and accurate.

3.4.3 Determination of pH

Different authors have different values for the optimum pH for methanogenesis. Lettinga and van Handel (1994) suggested the pH range for methanogenesis is between 6.3 and 7.8. Mosey (1974) stated that it occurs between 6.8 and 8.0. In general most researchers agree that the optimum pH is near neutrality. Acidogenic populations are significantly less sensitive to low and high pH, hence acid fermentation will predominate over methanogenic fermentation. This will results in the souring of the reactor as the pH decreases with corresponding decrease in gas production rate.

Measurement of pH was performed off-line, using a pH electrode (Toledo series 400), manually immersed into the sample of sludge or effluent. The effluent from the reactors was placed in a 50 mℓ beaker and the pH value then recorded. Caution was taken to ensure that the pH determination of a sample was a true measure of the pH of the system. If the sample is allowed to stand exposed to air, carbon dioxide will escape causing the pH to rise. Similarly, care should be taken not to vigorously stir the sample while measuring the pH, as this might strip off carbon dioxide and therefore alter the pH value.

3.4.4 Determination of the alkalinity (Alk) and volatile fatty acids (Vfa)

Alkalinity is a measure of the capacity of water to neutralize acids and is primarily due to the salts of weak acids. The alkalinity is a mixture of CO_2 and a strong base. 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).

As alkalinity is made up of different salts and weak acids, it is conventionally expressed as $CaCO_3$ equivalent. Anaerobic digesters operate optimally at neutral pH conditions in which bicarbonate is the primary constituent, therefore bicarbonate alkalinity is significant.

Various methods are available to determine volatile fatty acids concentration and alkalinity concentrations of the digesting sludge. Some of them are the pH 5 point titration procedure, and a two point titration procedure developed by Anderson & Yang (1992). A Ripple ratio (Vfa/Alk) is a useful digestion monitoring tool which increases rapidly with process upsets and decreases with recovery. In our study, a simple two-point titration method developed by Anderson & Yang (1992) which enables a precise determination of bicarbonate alkalinity and total volatile fatty acids concentrations was used. A modification of the method was adopted throughout this project and is discussed at length in **Appendix B.4.**

3.4.5 Determination of the gas volume and composition

Gas volume was measured manually with a glass syringe (for serum bottles assay) and automatically (for pilot plants) using a liquid displacement device. The composition of the biogas was determined by manually withdrawing a biogas sample with a gas lock 100 µℓ precision syringe after each gas measurement, and immediately injected into a GOWMAC Series 350 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD). The TCD detected nitrogen, methane and carbon dioxide. Helium was the carrier gas. The GC calibration procedures and settings are discussed in **Appendix B**.

3.4.5.1 Determination of the gas volume for serum bottle assays

A glass syringe fitted with a disposable needle was used to sample the headspace of the serum bottle. The syringe was lubricated with distilled water prior to sampling in order to allow the free movement of the plunger. It was then inserted into the rubber septum and held horizontal to allow the plunger to equilibrate between the bottle and atmospheric pressure. The reading was verified by drawing the plunger past the equilibrium point and releasing it to ensure that the plunger returned to the original volume. To continue the assay, gas was wasted as necessary to prevent leakages and overpressure effects.

3.4.5.2 Determination of the gas volume for the pilot-scale digester

A liquid displacement (also termed: volumetric) device was designed and constructed in our research laboratory. The measuring unit is shown in **Figure 3.1**.

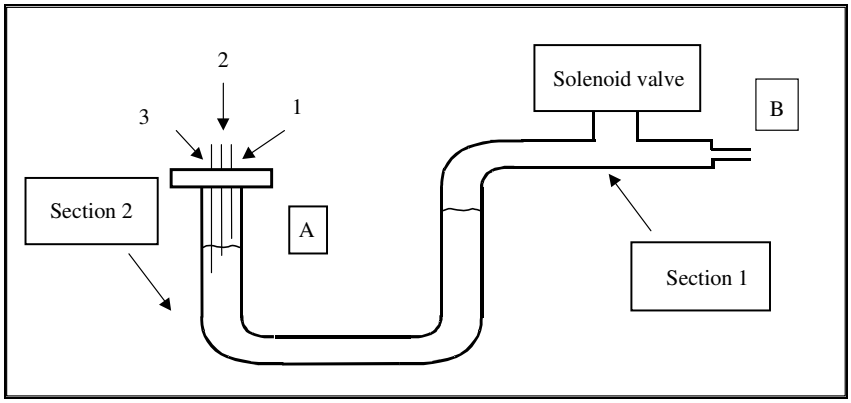


Figure 3.1: Gas measurement system

It was attached by polyflow tubing which emerged from the top of the reactor. The biogas resulting from digestion flowed into gas measuring system (GMS) through the tubing. The pressure exerted by the gas displaced the level of acidified water in the U-tube. This interference was observed as a rise in liquid level in section 2. Three level probes were inserted into the tube end A. Probes 1 and 2 are the high and low level probe, a solenoid valve B, which is normal closed, opened and vented the gas in section 1 of the tube. Venting caused the liquid level to return to its original state at low level. The opening of the solenoid valve at high-level resulted in a gas count being registered on an analogue

counter. Each count corresponded to a volume of biogas produced in the reactor. The volume was then quantified as the height difference between the high and low level probes and the inside of the tube.

The measuring unit consists of a U-tube **(A)** filled with an appropriate barrier solution (1% H_2SO_4), a solenoid three-way electro valve **(B)** and a three-level electrical sensor (1, 2 & 3). The sensor consists of an earth probe, minimum and maximum level sensors which triggers the valve to release the excess pressure.

Each count registered in an analogue was converted to the volume of biogas using the following relation:

$$V = \frac{\pi \phi^2}{4} L \qquad (3.7)$$

where:

ϕ is the inside diameter of the tube (m)

L is the height difference between the level probes

3.5 PILOT SCALE INVESTIGATIONS

The main objective of the pilot plant studies was to develop the design and operating criteria for a full-scale anaerobic reactor. The anaerobic reactors must be operated for a sufficient duration under steady state conditions before the results become representative of a prototype installation. Operation of at least 15 days to 30 days under a steady loading condition should be provided to collect reliable data (Speece, 1996).

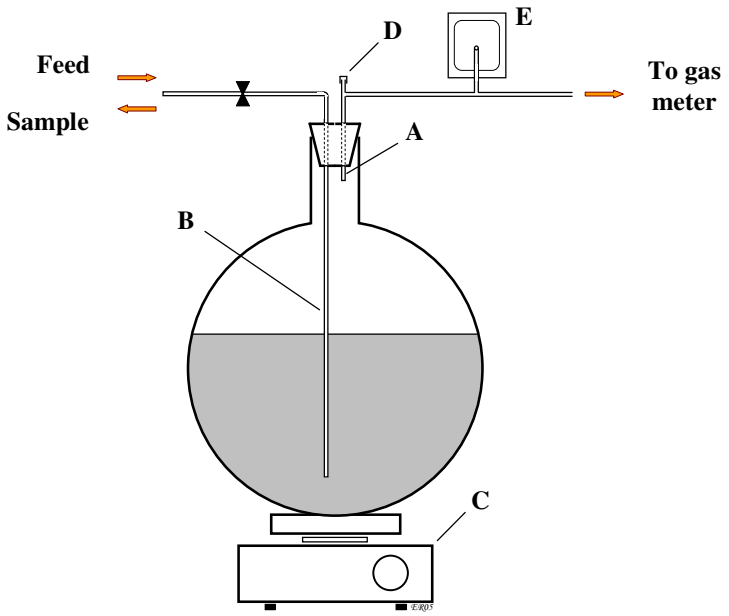


Figure 3.2 – Schematic of the pilot-scale batch or fed-batch completely stirred reactor. A, gas outlet; B, feeding tube and mixed liquor sampling port; C, magnetic stirrer; D, gas sampling port; E, compensation volume (Tedlar bag). (WRC Report, K1074/2005)

The 5 ℓ (working volume 3.5 ℓ) and 3 ℓ (working volume 1.5 ℓ) pilot-scale set-up for fed-batch and semi-continuous experiments is depicted in **Fig 3.2**. The glass flask was sealed with a rubber bung in which two holes held the gas outlet (**A**) and a 5 mm compression fitting holding a Polyurethane tubing PHHU (OD 6 mm x ID 4 mm; Parker) (**B**) which was used to feed the reactor and to sample the mixed liquor. Continuous mixing was provided by a magnetic stirrer (**C**). The gas outlet was connected to a gas sampling port, consisting of a butyl septum screwed onto a modified compression fitting (**D**), a compensation volume of 1 ℓ (**E**) and terminated at the gas measuring device. The gas sampling port (**D**) consisted of a T-compression fitting, which housed a 9.5 mm butyl septum (Supelco); a 100 µℓ gas-lock syringe (SGE syringe, Supelco) was used to puncture the septum and to withdraw 40-80 µℓ of gas for GC analysis (a gas-lock syringe was used at times).

The compensation volume consisted of a 1 ℓ Tedlar bag (Supelco). This is normally used on larger scale equipment to collect gas samples and transport them to the laboratory. It is equipped with a special fitting and a butyl septum. The former can be locked when pulling

the bag off the gas line, thus sealing the gas sample inside the bag. The septum allows the extraction of a sample of the gas. The Tedlar bag was used to provide a variable volume headspace which enabled a constant pressure to be maintained inside the reaction vessel when the liquid volume changed, during the liquid sampling and feeding operations: When a liquid sample is withdrawn, gas flows into the reactor and the bag deflates; and also, when the feed is pumped into the reactor, the gas flows back into the bag which inflates. Both operations leave the gas meter unaffected. The gas measuring device consisted of a plastic U-shaped tube (45 mm-diameter), filled with acidified water (1%H_2SO_4) to the level of the minimum level probe **(Fig 3.1)**.

The reactors were seeded with digested sludge from Umbilo Waste Water Treatment Works (UWWTW) coming from the secondary digester. The reactors were initially batch fed and after some period were continuously fed.

Routine analysis or performance parameters were evaluated to ensure the stability of the process. The routine operational procedures that were carried are summarized as follows:

- Record the gas counter reading for gas volume measurement.
- Extract a gas sample with a gas lock syringe for gas composition analysis.
- Stop the stirrer and withdraw the sludge sample from the sampling ports.
- After sampling, feed the reactor through the feeding tube with amount equal to that withdrawn, so as to maintain constant volume.

3.5.1 Test no 1: Distillery digestion

Objectives

The aim of the experiment was to commission and maintain a stable anaerobic digester. It was also to assess the biodegradability of the distillery effluent for its suitability as the codigestion candidate, using an acclimated biomass that has been pre-exposed to the distillery effluent for 150 days. The biomass was originally taken from Umbilo Waste Water Works. The process was closely controlled by monitoring the *process indicators* which are reported in **Table 3.7**. The operating conditions (HRT, OLR) which ensured sufficient (if not optimal) performance were compared to that of the unacclimated biomass.

Table 3.7: List of performance indicators in an anaerobic digester (WRC Report No K5/1074)

Parameter	Units	Indicator	Warning (*)
Gas flow (CH_4) rate	ℓ/d	-	
Gas composition	%	√	-
SMA	gCOD/gVS/d	-	-
COD concentration	gCOD/ℓ	-	√
Solids	VS	√	√
Alkalinity/VFAs	meq/ℓ	-	-
pH	-		-

Materials and Methods

An amount of 3 ℓ of acclimated sludge (to distillery) was transferred to the 5 ℓ and immediately closed with a rubber cap. The reactor was placed in a temperature controlled room (35ºC ±2ºC), where it was connected to the gas measuring system **(Fig 3.1).**

Gas production was measured automatically by a volumetric gas measurement system **(Fig 3.1)**. Measurement of the gas volume production and gas sampling for the gas composition analysis were done on average at least once daily. Feeding and sample extraction was done manually using a 50 mℓ plastic syringe. COD, solids analysis, alkalinity, VFA's concentration and pH were determined off-line daily, except for solids which were analyzed weekly.

The experiment was subdivided into two stages. In the first stage the digester was maintained in the batch fed mode, acetic acid was spiked daily to stimulate methanogenic activity and during the last four days distillery effluent was introduced. The second stage of the experiment was a semi-continuous phase where the distillery effluent was fed at increasing volumes. The summary of the experimental procedure is summarized in **Table 3.8** (batch fed mode) and **Table 3.9** (semi-continuous phase)

Table 3.8: Overview of the batch fed mode stage

Day-No (d)	Acetic acid (mℓ)	Distillery (mℓ)	Organic load (gCOD/d) *	NMS (mℓ)
1-5	5	0	5.5	0
6	7.5	0	8.3	100
7	0	20	2.4(100)	0
8	5	40	10.3(46)	0
9	5	80	15.1(63)	0
10-11	10	160	30.3(63)	0

*The fraction of the distillery effluent on the total organic load in indicated brackets (%)

Table 3.9: Summary of the semi continuous phase

Stage	Period (d)	Distillery (mℓ)	NMS *(mℓ)	HRT (d)	Organic load(gCOD/d)
I	12-16	100	0	34	3.4
II	17-41	100	100	18	3.4
III	42-69	25	75	35	1.0
IV	69-75	50	50	35	1.0
V	75-84	50	50	35	2.5

*Concentrated NMS and stock solution (10x)

3.5.2 Test no 2: Size digestion

The aim of this experiment was to commission and maintain a stable anaerobic digester, to assess the biodegradability of the size effluent for its suitability as the codigestion candidate. This was done by using an acclimated biomass that has been pre-exposed to the size effluent for 150 days. The biomass was originally taken from Umbilo Waste Water Works. The process was closely controlled by monitoring the *process indicators* which are

reported in **Table 3.9**. The operating conditions (HRT, OLR) which ensured sufficient (if not optimal) performance will be compared to that of the unacclimated biomass.

Materials and Methods

An amount of 1.5 ℓ of acclimated sludge (to size) was transferred to the 3 ℓ conical flask and immediately closed with a rubber cap. The reactor was placed in a temperature controlled room (35ºC±2ºC), where it was connected to the gas measuring system. Stirring was achieved by means of magnetic stirrer.

Gas volume produced was measured automatically by a volumetric gas measurement system **(Fig 3.1)**. Recording of the gas volume production and gas sampling for the gas composition analysis was done at least once daily. Feeding and sample extractions were done manually using a 50 mℓ plastic syringe. COD, solids analysis, alkalinity, VFAs concentration and pH determinations were performed off-line daily, except for solids analysis which was done once weekly.

The experiment was carried out in a semi-continuous phase where the size effluent was fed at increasing volumes. The summary of the experimental procedure is summarized in **Table 3.10** (semi-continuous phase).

Table 3.10: Summary of the experimental campaign (semi continuous phase)

Stage	Period (d)	Size (mℓ)	NMS *(mℓ)	HRT (d)	Organic load (gCOD/d)
I	0-29	20	20	34	3.4
II	30-54	40	40	18	3.4
III	55-68	80	80	35	1.0
IV	69-84	40	40	35	1.0

*Concentrated NMS and stock solution (10x)

Chapter 4

RESULTS AND DISCUSSION

This chapter briefly reports the experimental results and is organized into two main sections. The first section **(Section 4.1)** presents the application of the serum bottle method to the objective of biomass source and biomass acclimation. The second section **(Section 4.2)** presents the results of the pilot plant investigations. Each section is organized as an independent unit, consisting of **Results** and **Discussion** and **Conclusions**. For clarity and simplicity, only the main outcomes are discussed and the detailed and reproducibility plots are presented in the corresponding Appendices.

4.1 Test no 1 (Biomass source)

Results and discussion

4.1.1 Discussion on Controls-(0%) set

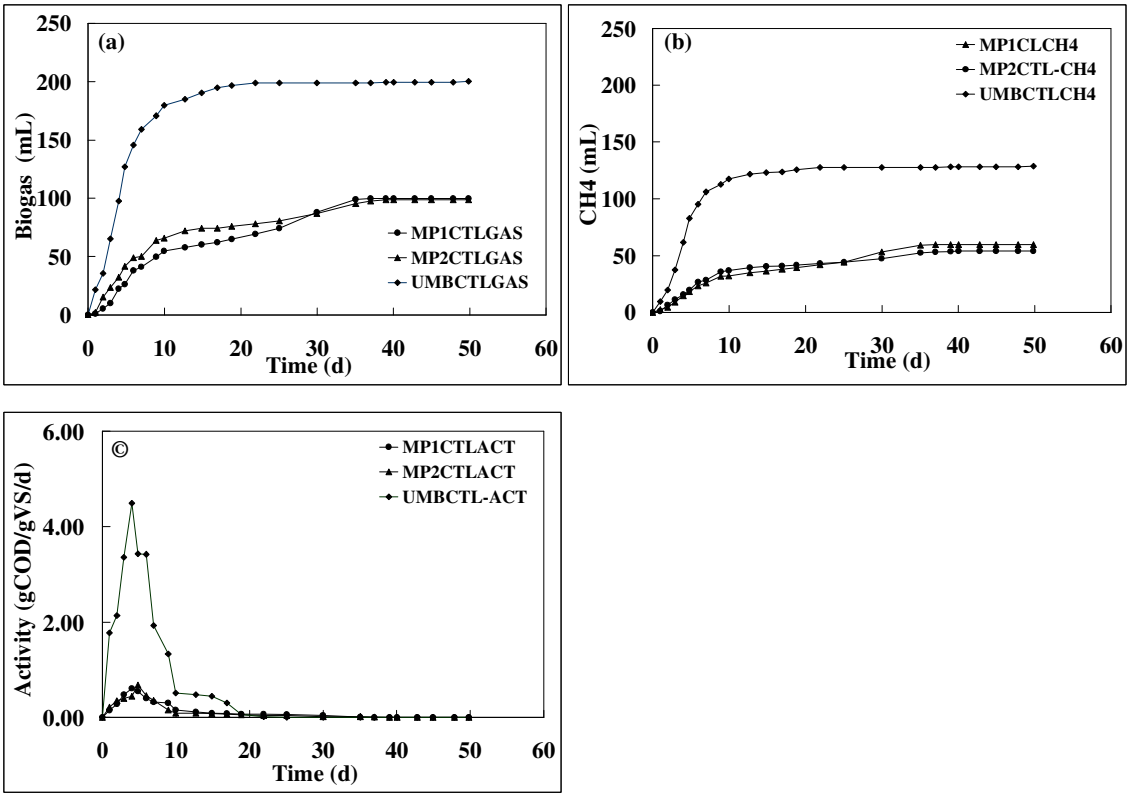


Figure 4.1: Summary plots of three control sets (a) biogas, (b) methane and (c) activity

4.1.2 Discussion on 1% concentration

The reactors seeded with Umbilo sludge produced more biogas (180 mℓ, day 20), but below the controls due to the addition of the inhibitor.

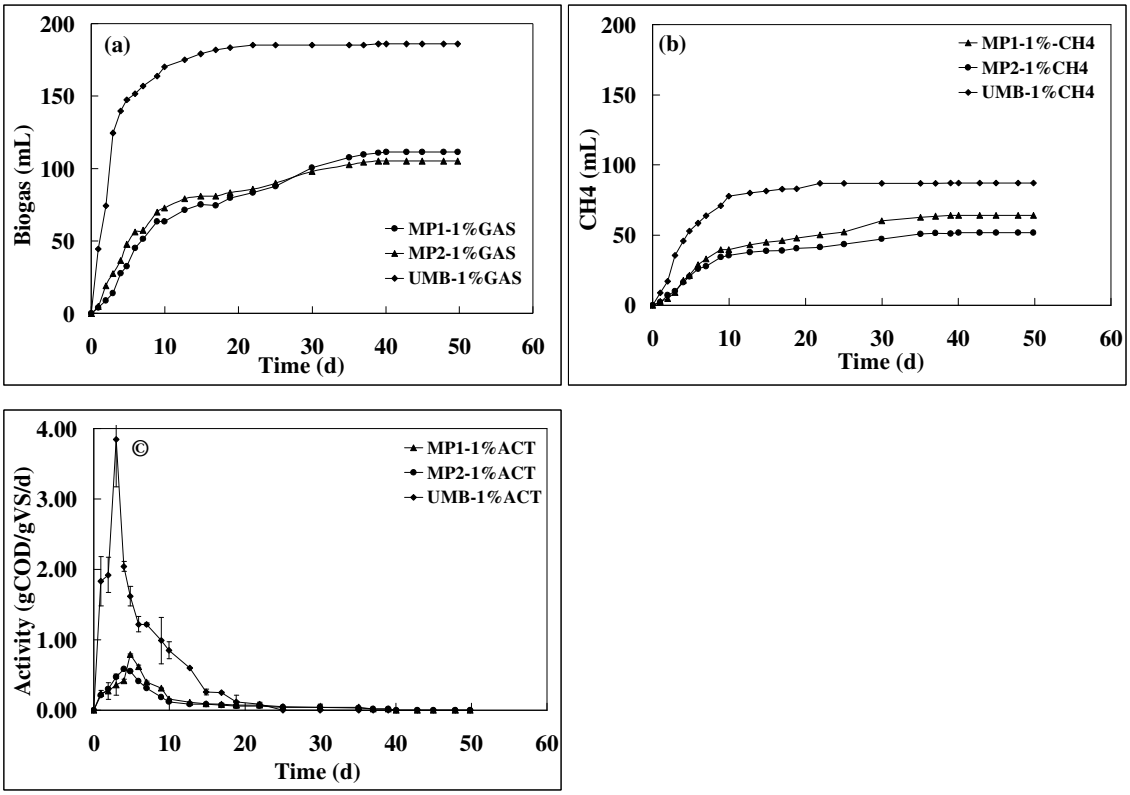


Figure 4.2: Summary plots of reactors containing 1% reactive dye (a) biogas, (b) methane and (c) activity

The reactors seeded with Mpumalanga sludge produced less biogas compared to reactors seeded with Umbilo sludge. The gas production curve for the Mpumalanga samples had two distinct growth patterns, firstly stabilizing at 80 mℓ (day 15-20) and finally stabilizing at 110 mℓ (day 40). The methane curves followed a similar pattern as gas production curves.

4.1.3 Discussion on 5% concentration

The Umbilo sludge had superior activity, with a steep gradient between day 0 and day 10 and gradually inclined before leveling off at 190 mℓ (day 20) and remained constant thereafter for the duration of the test.

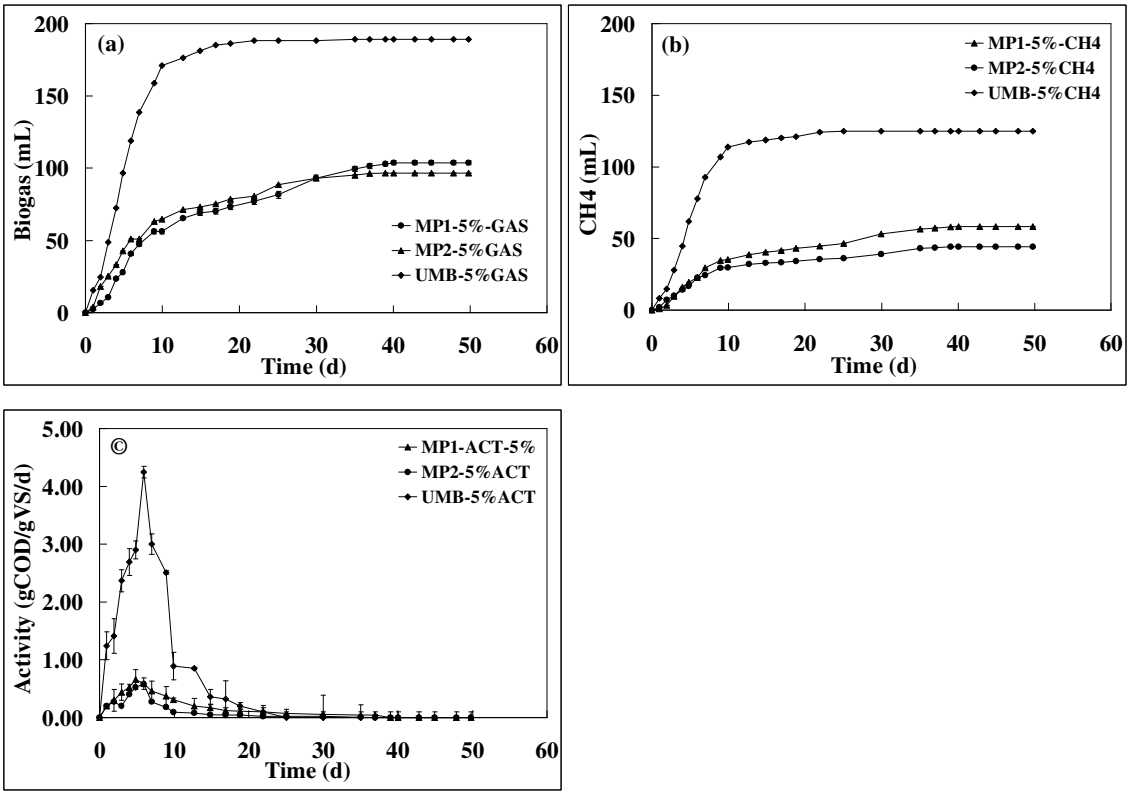


Figure 4.3: Summary plots of 5% reactors (a) biogas, (b) methane and (c) activity

The Mpumalanga sludge gas production curves had two distinct growth phases, first stabilizing on day 10 and secondly on day 40. The CH_4 produced by Umbilo sludge inoculated reactors, was twofold compared to the Mpumalanga sludge inoculated reactors. The activity of Umbilo sludge climaxed at 4.2 gCOD/gVS/d (day 8), fourfold compared to Mpumalanga sludge.

4.1.4 Discussion on 10% concentration

At 10 % concentration, Umbilo inoculated reactors produced 175 mℓ (day 20) biogas, which was twofold compared to Mpumalanga reactors (88 mℓ).

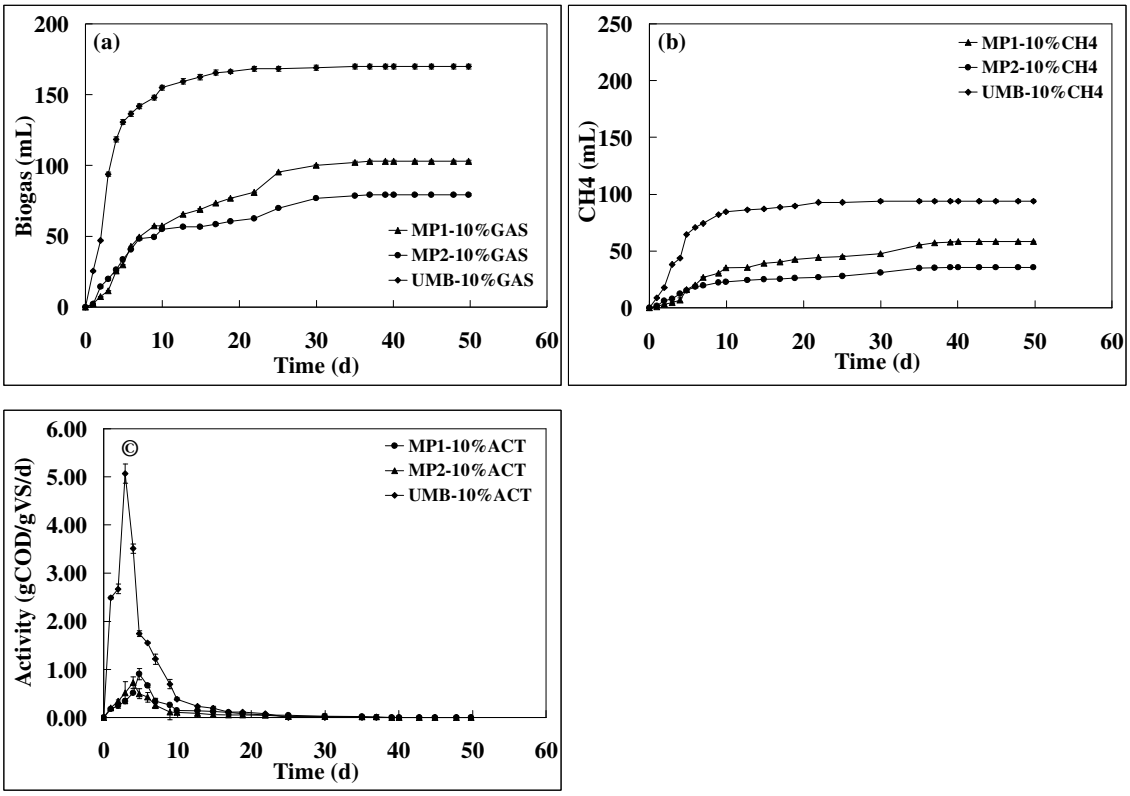


Figure 4.4: Summary plots of 10% reactors (a) biogas, (b) methane and (c) activity

The shape of the Mpumalanga reactors showed a steady incline till day 10, after which MP2 became constant between day 10 and day 20 and further incline till day 30. The MP1 inoculated reactors behaved differently with a steady incline before leveling off at 100 mℓ (day 30). The Umbilo inoculated reactors achieved the highest activity of 5.2 gCOD/gVS/d (day 7), which was fivefold compared to Mpumalanga inoculated reactors.

The summary of the results is reported in **Table 4.1.**

Table 4.1: Summary of results for test no 1

	Parameter	Units	MP1	MP2	Umbilo
Controls	Ultimate gas vol	mℓ	98 ± 3.25	99 ± 0.7	200 ± 0.28
	Ultimate CH4 vol	mℓ	60 ± 0.33	54 ± 0.2	129 ± 0.25
	Ultimate CH4 fraction	%	43	46	60
	COD-to-CH4	%	ND	ND	ND
	Max SMA	gCOD/gVS/d	0.61 ± 0	0.68 ± 0	4.49 ± 0.66
	Lag phase	d	N/A	N/A	N/A
	Parameters	**Units**	**1%**	**5%**	**10%**
Mpumalanga 1	Ultimate gas vol	mℓ	111 ± 1.3	103 ± 1.4	103 ± 0.83
	Ultimate CH4 vol	mℓ	64 ± 1.74	58 ± 0.26	58 ± 0.4
	Ultimate CH4 fraction	%	41	40	41
	COD-to-CH4	%	ND	ND	ND
	Max SMA	gCOD/gVS/d	0.79 ± 0.02	0.66 ± 0.01	0.9 ± 0.11
	Lag phase	d	N/A	N/A	N/A
Mpumalanga 2	Ultimate gas vol	mℓ	105 ± 1.4	97 ± 0.31	79 ± 0.7
	Ultimate CH4 vol	mℓ	52 ± 1.17	44 ± 0.14	36 ± 0.3
	Ultimate CH4 fraction	%	45	40	36
	COD-to-CH4	%	ND	ND	ND
	Max SMA	gCOD/gVS/d	0.58 ± 0.04	0.58 ± 0.03	0.72 ± 0.1
	Lag phase	d	N/A	N/A	N/A
Umbilo	Ultimate gas vol	mℓ	186 ± 2.58	189 ± 0.99	170 ± 1.76
	Ultimate CH4 vol	mℓ	87 ± 0.8	125 ± 0.54	94 ± 1.25
	Ultimate CH4 fraction	%	65	64	62
	COD-to-CH4	%	ND	ND	ND
	Max SMA	gCOD/gVS/d	3.85 ± 0.68	4.25 ± 0.44	5.07 ± 1.38
	Lag phase	d	N/A	N/A	N/A

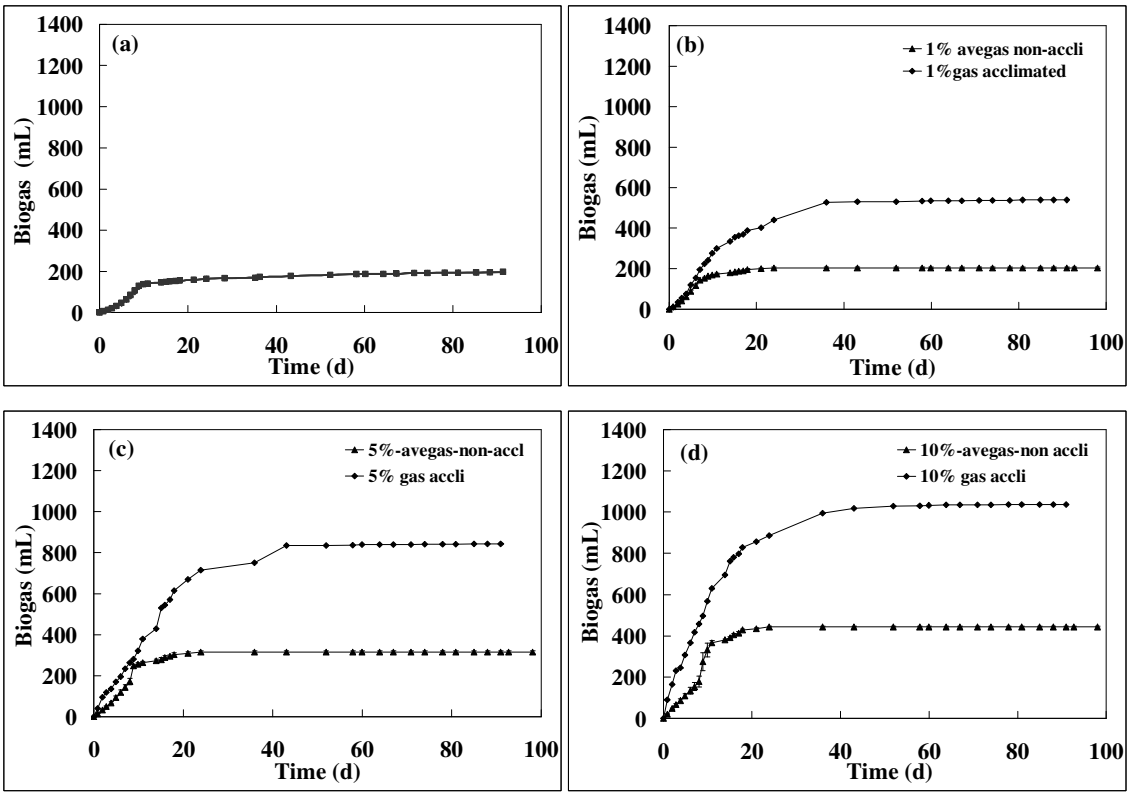
NB: For controls MP1 refers to Mpumalanga 1, MP2 refers to Mpumalanga 2 and Umbilo refers to Umbilo sludge

The reproducibility plots of this experiment are given in **Appendix D (Fig D1-D16)**.

4.2 Test no 2 (Biomass acclimation)

4.2.1 Biogas production from biomass exposed to distillery effluent

The average cumulative gas production profiles for distillery samples are shown in **Fig 4.5**. The biogas production for controls was the same for both acclimated and non-acclimated samples. The acclimated 1% distillery samples produced three fold biogas compared to the non-acclimated samples. Unexpectedly though, acclimated samples reached a plateau on day 36 (528 mℓ) compared to the non-acclimated samples which stabilized on day 24 (205 mℓ).



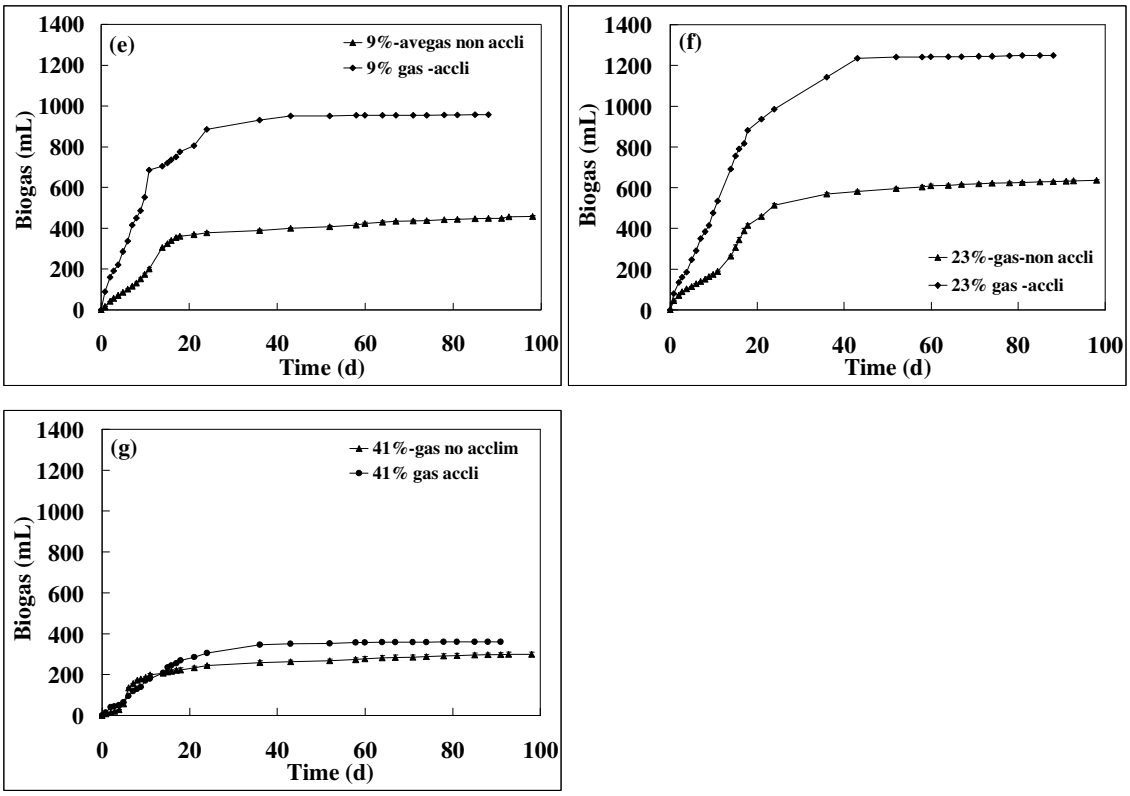


Figure 4.5: Biogas production plots for distillery samples (a) controls (0%v/v), (b) 1% v/v, (c) 5% v/v, (d) 10% v/v, (e) 9% v/v, (f) 23% v/v and (g) 41% v/v

At 5% concentration level the acclimated distillery samples were rapidly metabolized between day 1 and day 24, climaxing on day 43 (835 mℓ) with no significant gas production for the remainder of the test. The non-acclimated samples attained a maximum gas production of 315 mℓ (day 25).

At 10% concentration level there was a sharp incline in gas production for the acclimated samples between day 0 and day 18, followed by a steady incline and finally stabilizing on day 43 at 1029 mℓ. The non-acclimated samples attained a maximum gas production of 442 mℓ (day 25) with no significant gas production for the remainder of the test.

In the second set of samples (9, 23 and 41%), the same trend continued, as samples inoculated with acclimated biomass produced more biogas compared to samples with non-acclimated biomass. The 9% acclimated sample attained maximum gas production of

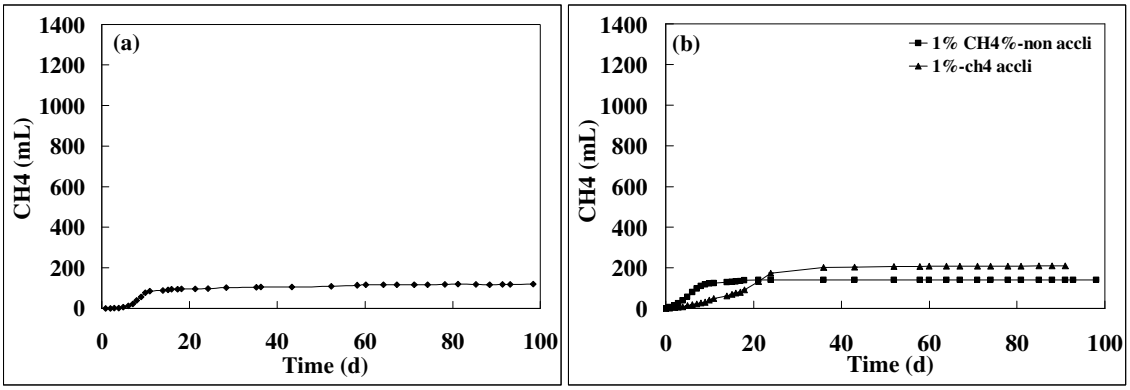
954 mℓ, reaching a plateau on day 40. The non-acclimated sample climaxed at 435 mℓ (day 21).

At 23% concentration level, the acclimated samples achieved a maximum gas production of 1249 mℓ (day 43), after a sharp increase in gas production between day 1 and day 18, and a steady incline till day 43. The non acclimated samples climaxed at 626 mℓ (day 36), with a steep incline between day 0 and day 11, followed by another sharp incline till day 24, and stabilizing on day 36.

In a 41% concentration level the biomass activity was reduced dramatically with a maximum biogas production of 361 mℓ (day 40) for acclimated samples, compared to 300 mℓ (day 21) for non-acclimated samples.

4.2.2. Methane production from biomass exposed to distillery effluent.

The cumulative methane production curves are shown in **Fig 4.6**. The 1% acclimated samples had a lag period of 6 days, climaxing at 201 mℓ on day 36, with no further CH_4 production for the remainder of the test. The non-acclimated distillery samples had a lag period of 2 d, leveling off at 137 mℓ (day 18), with insignificant CH_4 production thereafter.



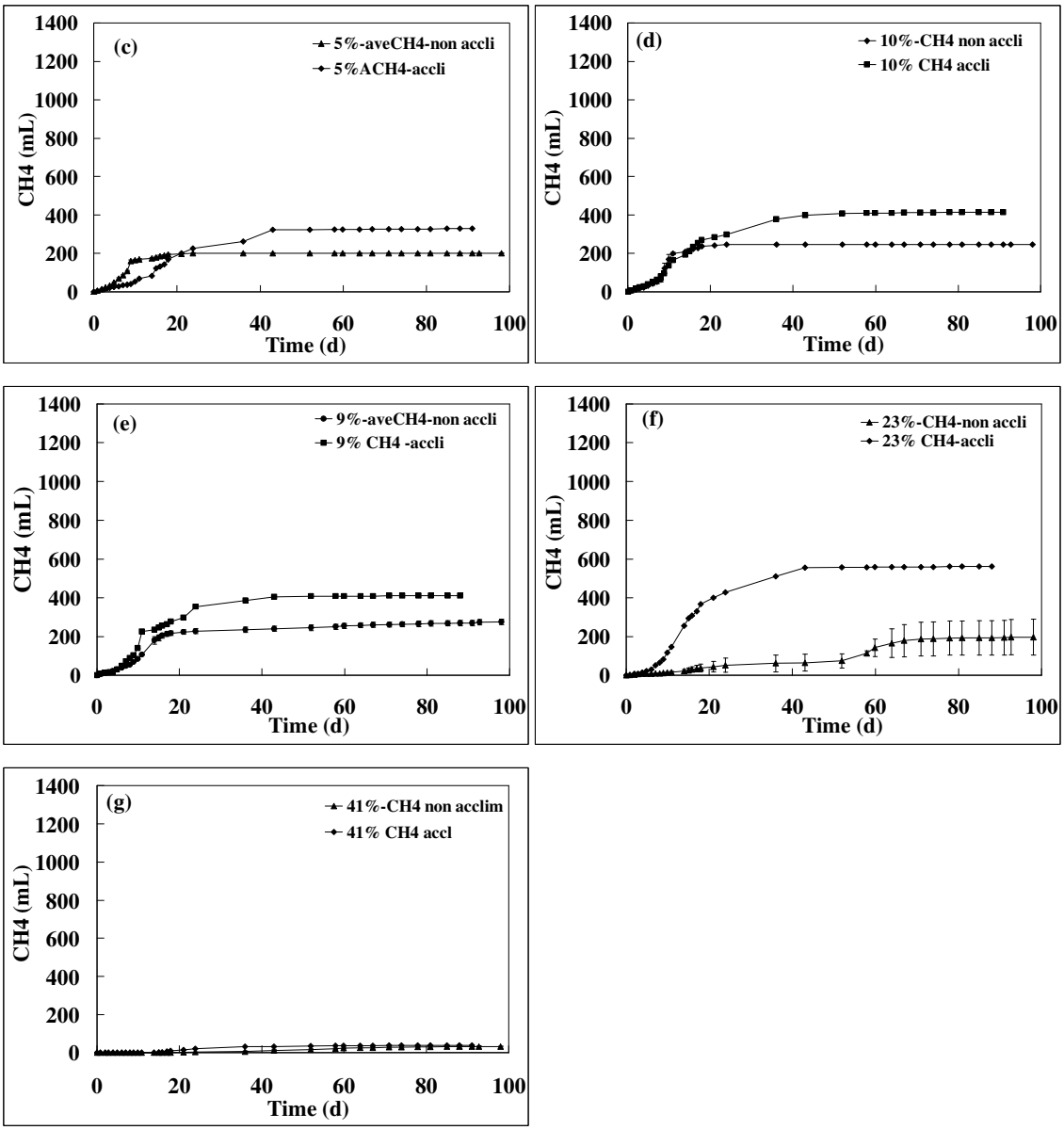


Figure 4.6: Methane production plots for distillery samples (a) controls (0%), (b) 1% v/v, (c) 5% v/v, (d) 10% v/v, (e) 9% v/v, (f) 23% v/v and (g) 41% v/v

The gas composition profile during this period showed a larger percentage of CO_2 at the beginning of the test and a gradual increase in CH_4 content at a later stage.

At 5% (v/v) level, the acclimated samples had a lag period of 7 days, with a sharp incline in CH_4 production and stabilizing at 323 mℓ (day 43). The non-acclimated samples had a lag period of 4 days, followed by a sharp incline in CH_4 production till day 10, with a second

lag period (day 10-15). The CH_4 production reached a plateau on day 18 (201 mℓ). The gas composition profile showed a greater CO_2 content at the beginning of the test and a gradual increase in CH_4 content with an ultimate CH_4 fraction of 65%.

The acclimated 10% distillery samples had a lag period of 5 days, and climaxed at 410 mℓ (day 43). The non-acclimated distillery samples had a lag period of 3 days, and reached a plateau of 254 mℓ (day 21).

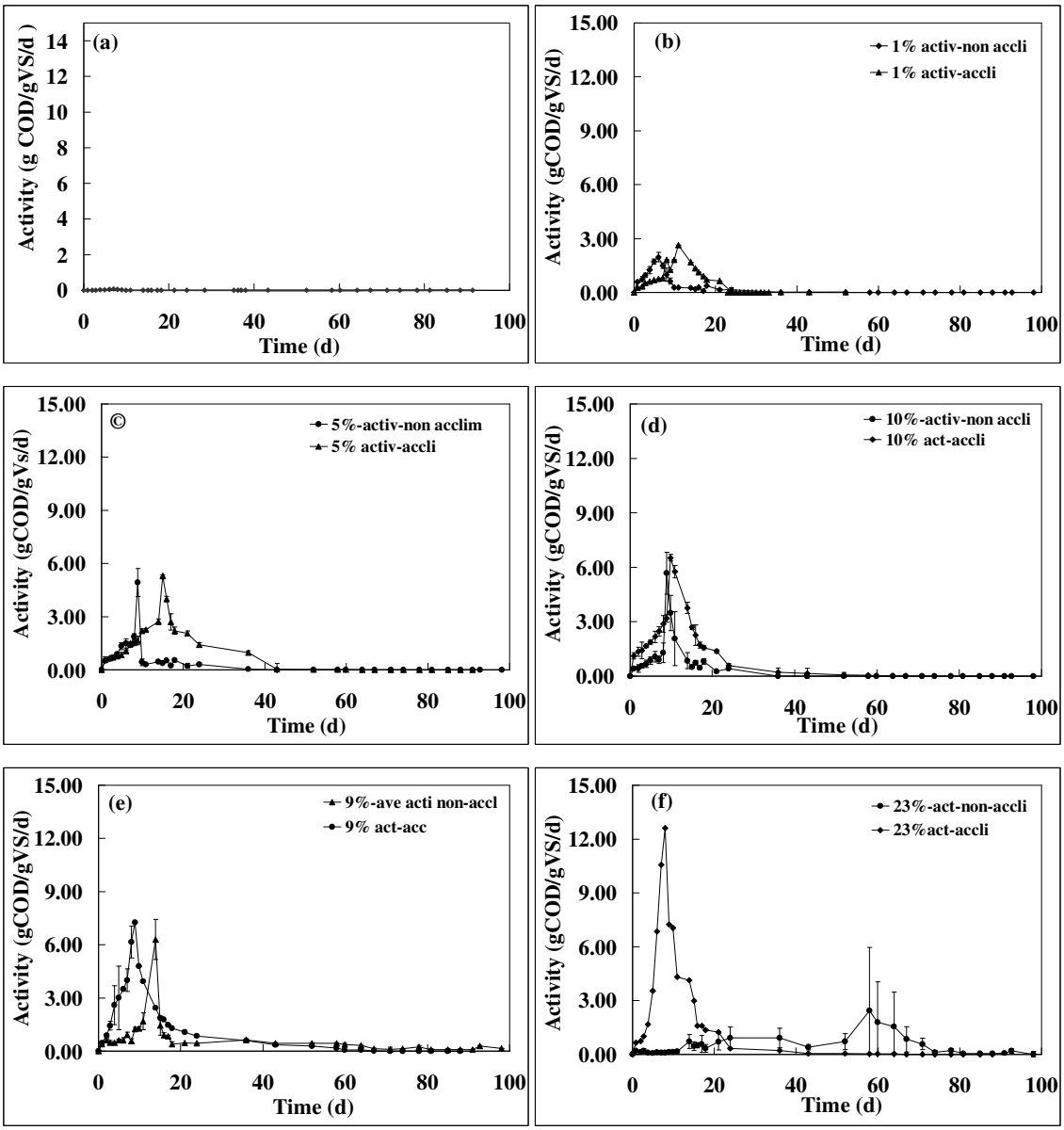
The 9% concentration level acclimated samples had a lag period of 6 days, with a sharp increase in CH_4 production between day 6 and day 11, with a second lag period (day 11-14), and climaxed at 410 mℓ (day 43). The non-acclimated distillery samples had a lag period of 5 days, with the sharp incline till day 18 and finally leveling off at 275 mℓ (day 24).

The lag period for the acclimated samples at 23% concentration level was 7 days, followed by a sharp incline in CH_4 production till day 18, and a steady incline climaxing at 561 mℓ on day 43. The non-acclimated samples had a lag period of 14 days, followed by the steady incline till day 24, with a second lag period between day 24 and day 52, and ultimately climaxing at 197 mℓ on day 67.

At 41% concentration level, CH_4 production reached a maximum of 39 mℓ (lag period of 16 d) for the acclimated samples. The non-acclimated samples had a lag period of 24 days, and leveled off at 33 mℓ (day 64). There was great biomass inhibition at this concentration level, hence reduced rates of biogas and methane production.

4.2.3 Activity of biomass exposed to distillery effluent

The Specific Methanogenic Activity (SMA) plots are shown in **Fig 4.7**. At 1% concentration level the acclimated samples had a maximum activity of 2.65 gCOD/gVS/d with a corresponding value of 1.97 gCOD/gVS/d for non-acclimated biomass samples. At 5% concentration level the acclimated samples attained a maximum activity of 5.3 gCOD/gVS/d compared to 4.92 gCOD/gVS/d for the non-acclimated samples.


(a)
Activity (g COD/gVS/d)
Time (d)
(b)
1% activ-non accli
1% activ-accli
Activity (gCOD/gVS/d)
Time (d)
©
5%-activ-non acclim
5% activ-accli
Activity (gCOD/gVs/d)
Time (d)
(d)
10%-activ-non accli
10% act-accli
Activity (gCOD/gVS/d)
Time (d)
(e)
9%-ave acti non-accl
9% act-acc
Activity (gCOD/gVS/d)
Time (d)
(f)
23%-act-non-accli
23%act-accli
Activity (gCOD/gVS/d)
Time (d)

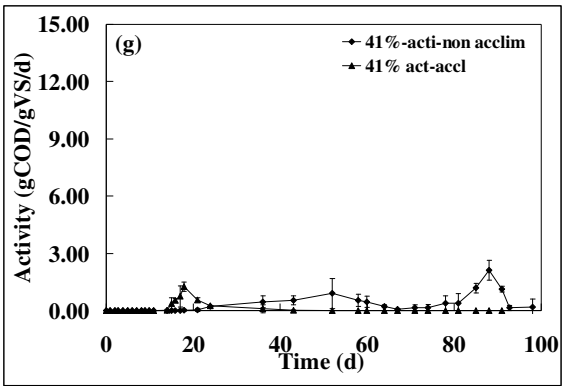
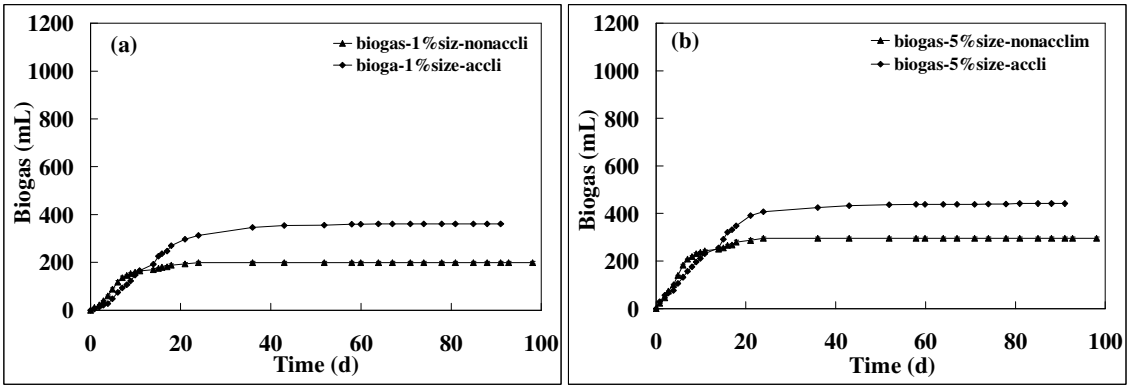


Figure 4.7: Methanogenic activity plots for distillery samples (a) controls (0%v/v), (b) 1% v/v, (c) 5% v/v, (d) 10% v/v, (e) 9% v/v, (f) 23% v/v and (g) 41% v/v

At 10% concentration level the maximum activity for the acclimated was samples 6.52 gCOD/gVS/d compared to 5.68 gCOD/gVS/d attained by the non-acclimated samples.

4.2.4. Biogas production of biomass exposed to Size effluent

The average cumulative gas production plots for size samples are shown in **Fig 4.8**. The 1% acclimated size samples had a lag period of 4 days, and a maximum gas production of 360 mℓ (day 36). The non-acclimated samples had a lag period of 3 days, with the maximum gas production of 199 mℓ (day 24) and remained constant for the duration of the test.



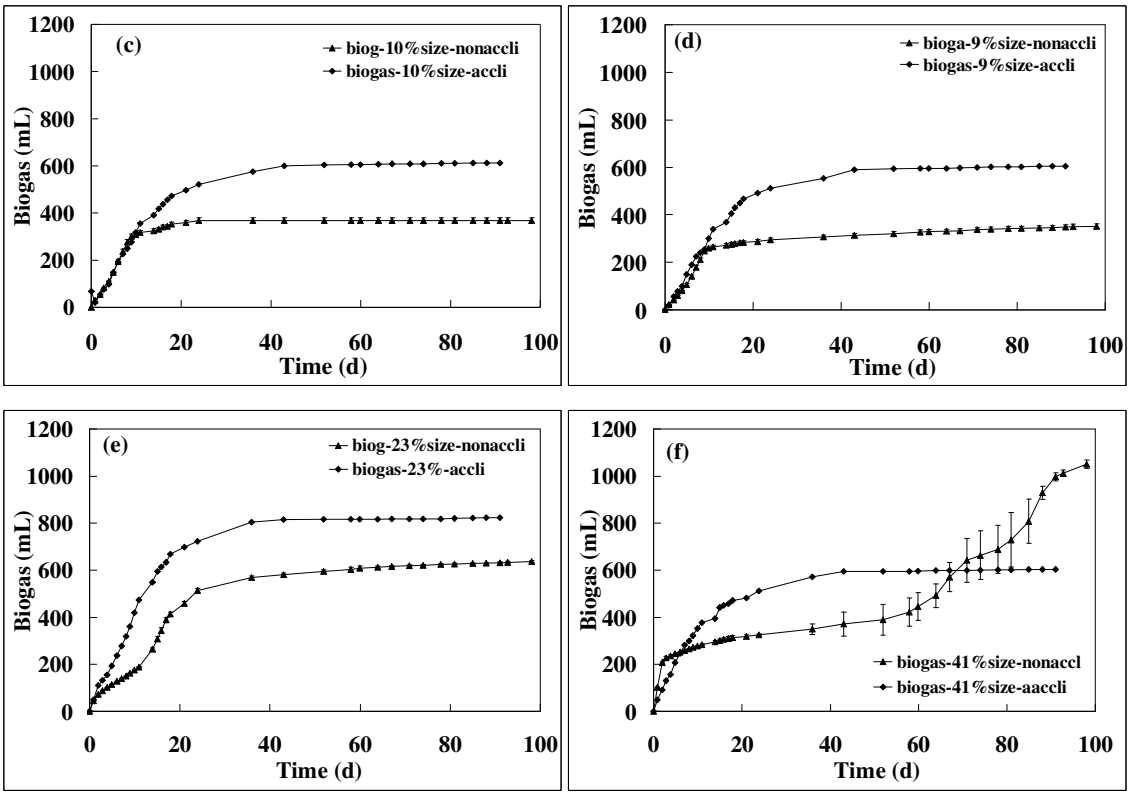


Figure 4.8: Biogas production plots for size samples (a) 1% v/v, (b) 5% v/v, (c) 10% v/v, (d) 9% v/v, (e) 23% v/v and (f) 41% v/v

At 5% concentration level the acclimated size samples produced 438 mℓ (day 36), compared to 296 mℓ (day 24) produced by the non-acclimated samples. The biogas production curve shows that size is metabolized immediately after incubation, with no second lag phase.

At 10% concentration level, the acclimated size samples were rapidly metabolized and climaxed at 600 mℓ (day 36) with no further gas production. The non-acclimated samples evolved a maximum gas production of 369 mℓ (day 23).

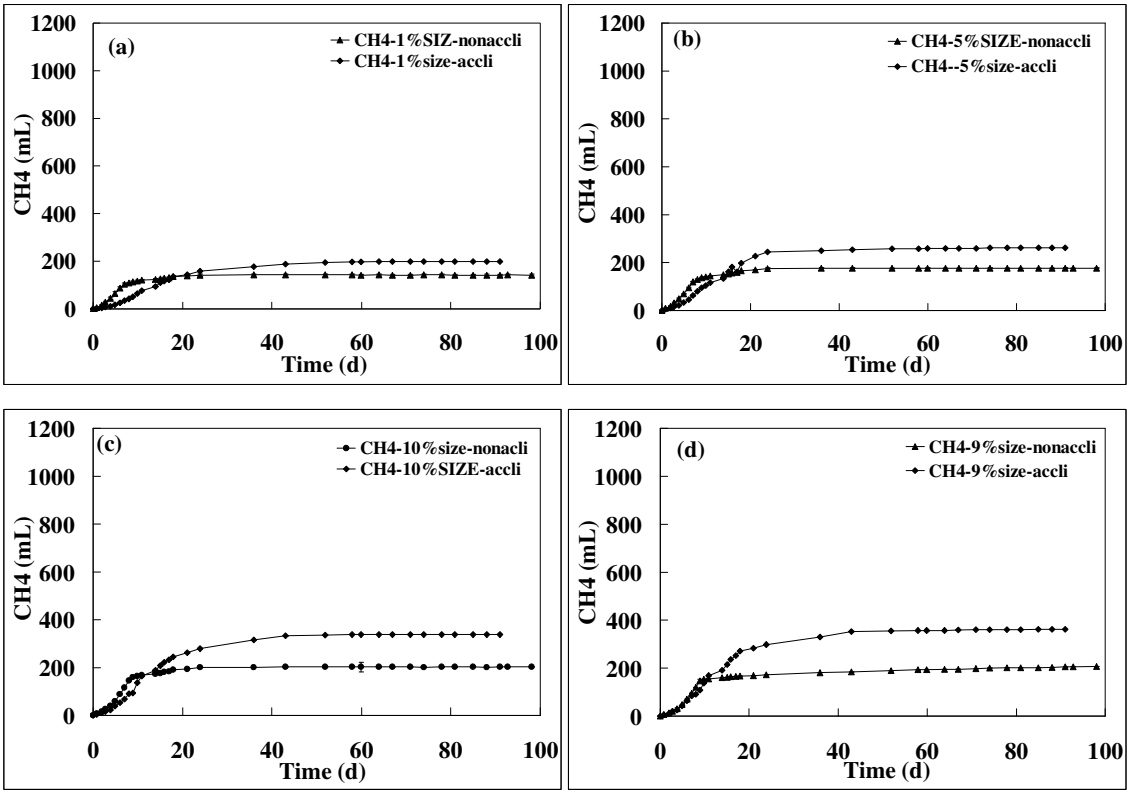
The 9% acclimated samples produced a maximum gas production of 596 mℓ (day 43). The non-acclimated sample climaxed at 327 mℓ (day 36) with negligible gas production for the remainder of the test period.

The 23% acclimated size samples had an ultimate gas production of 817 mℓ (day 43), compared to 604 mℓ (day 52) produced by the non-acclimated samples.

At the highest concentration level (41%) the acclimated size samples produced less gas compared to the non-acclimated samples. The acclimated samples climaxed at 595 mℓ (day 43), compared to 1050 mℓ produced by the non-acclimated samples. This was not the expected outcome. This shows that size wastewater is inhibitory at the highest concentration. The biomass acclimation did not improve the size biodegradability.

4.2.5. Methane production of biomass exposed to Size effluent

The cumulative methane production plots are shown in **Fig 4.9**. The 1% acclimated size samples had a lag period of 5 days, and climaxed at 195 mℓ (day 36), with no CH_4 production for the remainder of the test period. The non-acclimated size samples had a lag period of 2 days, and leveled off at 142 mℓ.



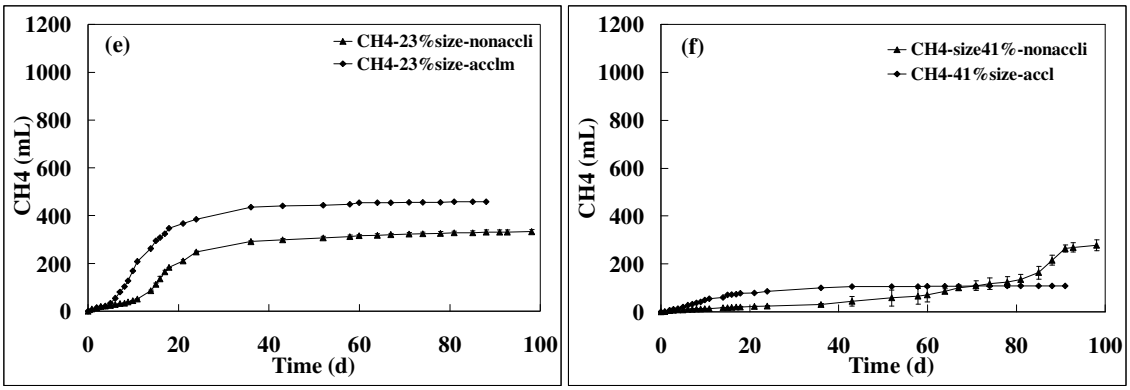


Figure 4.9: Methane production plots for size samples (a) 1% v/v, (b) 5% v/v, (c) 10% v/v, (d) 9% v/v, (e) 23% v/v and (f) 41% v/v

The acclimated 5% size samples had a lag period of 3 days, climaxed at 249 mℓ (day 23) and remained stable for the duration of the test. The non-acclimated samples had a lag period of 3 days, and climaxed at 177 mℓ (day 24).

The 10% acclimated samples had a lag of 5 days, reached a plateau of 336 mℓ (day 41). The non-acclimated samples had a lag period of 3 days, and attained a maximum CH_4 production of 202 mℓ (day 18).

The acclimated 9% size samples had a lag period of 3 days, climaxed at 335 mℓ (day 43) with no further CH_4 production for the remaining test period. The non-acclimated 9% size samples had a lag period of 3 days, achieved a maximum CH_4 production of 181 mℓ (day 18) and remained stable for the remainder of the test period.

The acclimated 23% size samples had a lag period of 5 days, climaxed at 440 mℓ (day 36). The lag period for the non-acclimated was 7 days, with a maximum CH_4 production of 298 mℓ (day 52) and remained stabilized for the remainder of the test period.

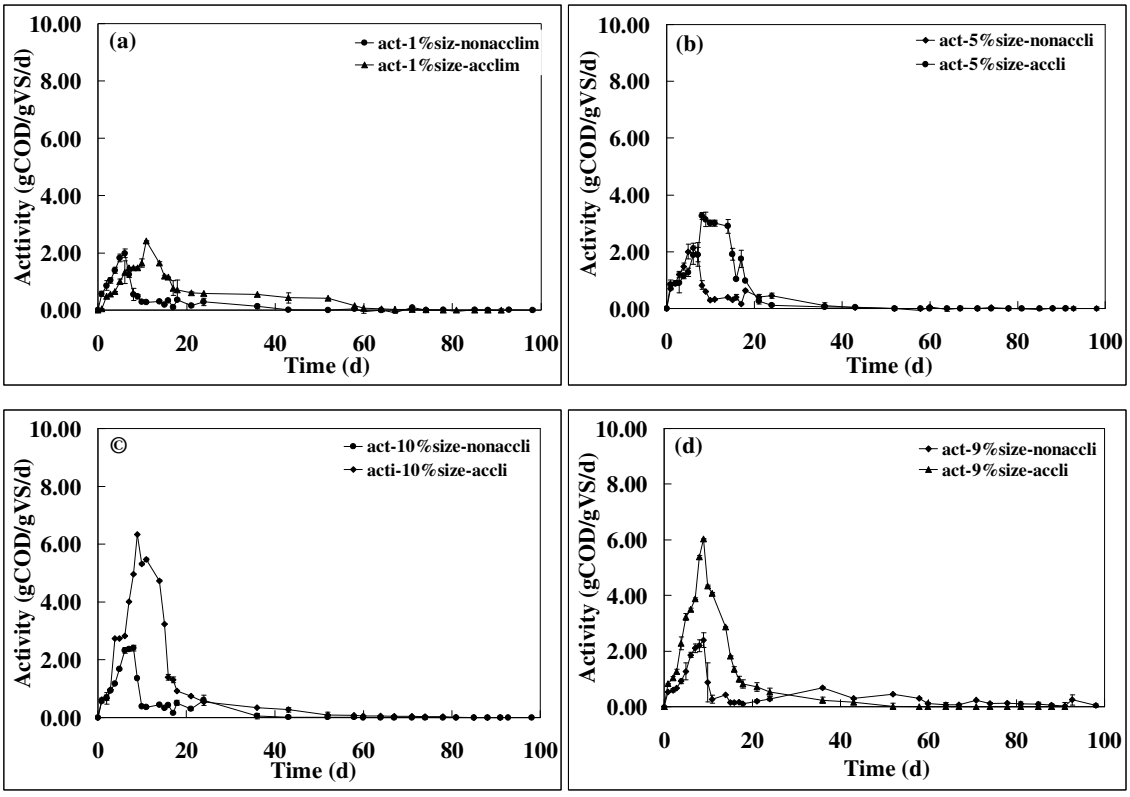
The highest concentration (41%) produced 105 mℓ for acclimated samples, and 270 mℓ for the non-acclimated biomass samples. The lag period was 3 days for acclimated samples and 25 days for non-acclimated samples. At the highest concentration size effluent is toxic at the beginning of the test.

4.2.6 Activity assessment of biomass exposed to Size effluent

The activity assessment plots are shown in **Fig 4.10**. At 1% concentration level the maximum activity for the acclimated samples was 2.42 gCOD/gVS/d compared to 1.98 gCOD/gVS/d attained by the non-acclimated samples.

At 5% concentration level the maximum activity was 3.26 gCOD/gVS/d for the acclimated samples s and 2.1 gCOD/gVS/d for the non-acclimated samples. The 10% concentration acclimated samples achieved the maximum activity of 6.33 gCOD/gVS/d compared to 2.4 gCOD/gVS/d attained by the non-acclimated samples.

In the second set, the lowest concentration (9%) acclimated samples attained a maximum activity of 9.26 gCOD/gVS/d compared to 3.5 gCOD/gVS/d attained by the non-acclimated samples.



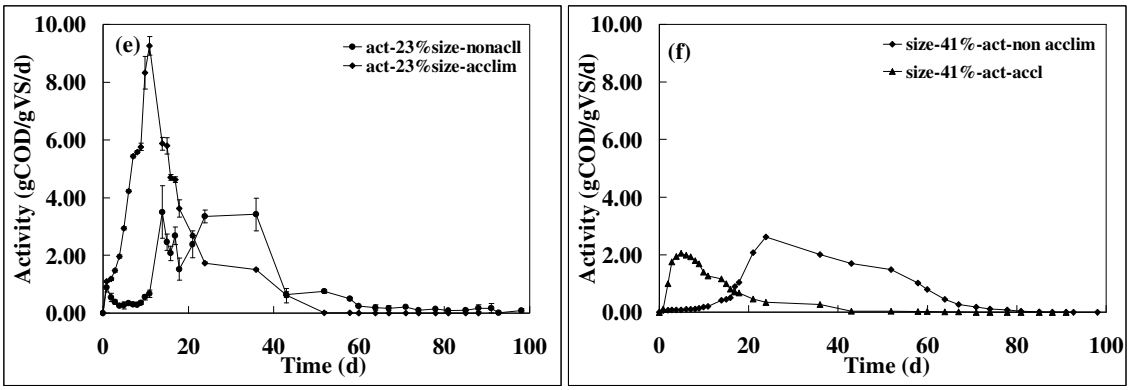


Figure 4.10: Methanogenic activity plots for size samples (a) 1% v/v, (b) 5% v/v, (c) 10% v/v, (d) 9% v/v, (e) 23% v/v and (f) 41% v/v

In the second set, the lowest concentration (9%) acclimated samples attained a maximum activity of 9.26 gCOD/gVS/d compared to 3.5 gCOD/gVS/d attained by the non-acclimated samples.

At 23% concentration level the acclimated samples attained a maximum activity of 9.6 gCOD/gVS/d compared to 3.8 gCOD/gVS/d of the non-acclimated samples. In highest concentration (41%) the acclimated samples attained a maximum activity of 2.1 gCOD/gVS/d compared to 2.6 gCOD/gVS/d achieved by the non-acclimated sample.

4.2.7 Biogas production from biomass exposed to Scour effluent

The average cumulative gas production profiles for scour samples are shown in **Fig 4.11**. The 1% acclimated scour samples had a lag period of 17 days, with steady incline in gas production and finally stabilizing at 33 mℓ on day 41. The non-acclimated 1% scour samples produced a maximum gas production of 185 mℓ (day 24).

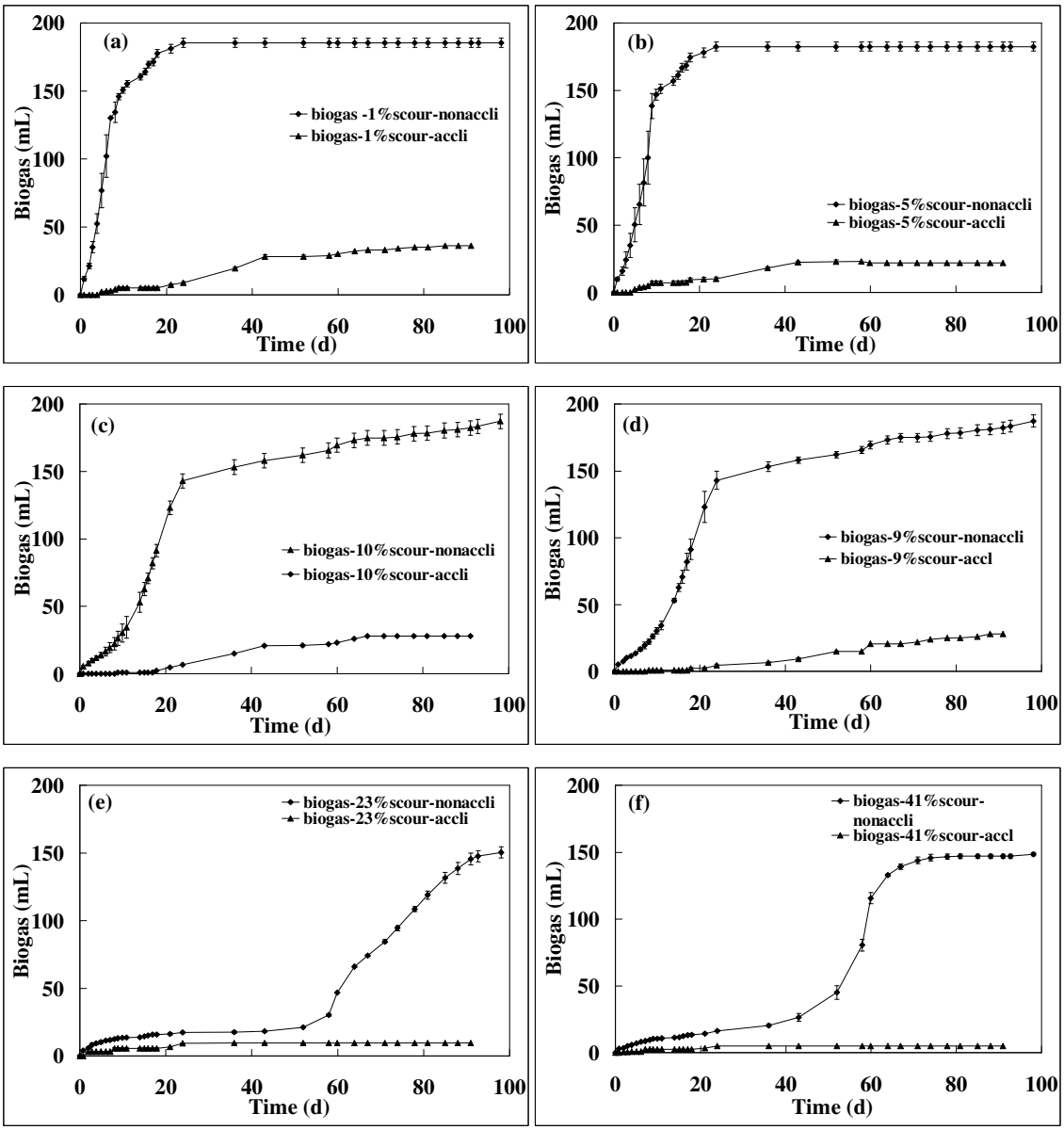


Figure 4.11: Gas production plots for scour samples (a) 1% v/v, (b) 5% v/v, (c) 10% v/v, (d) 9% v/v, (e) 23% v/v and (f) 41% v/v

At 5% concentration level, the acclimated scour samples had a lag of 10 days and climaxed at 28 mℓ. The non-acclimated samples had a lag of 5 days, with a maximum gas production of 183 mℓ (day 43) with no further gas production for the remainder of the test period.

The 10% acclimated samples had a lag period of 18 days, climaxed at 28 mℓ (day 60) with no further gas production afterwards. The non-acclimated samples had a sharp incline between day 0 and day 24, with at steady incline till day 66 and leveling of at 183 mℓ with no further gas production.

The (9%) acclimated scour sample had a lag of 21 days, with a steady incline till day 52, and climaxed at 28 mℓ. The non-acclimated scour samples had a lag of 6 days, followed by a sharp incline of gas production till day 24, and stabilized at 183 mℓ.

At 23% concentration level the lag period was 18 days for the acclimated samples, with a maximum gas production of 9 mℓ (day 23). The non-acclimated scour samples had a steady incline between day 0 and day 24, and climaxed at 151 mℓ (day 93) and remained constant for the remainder of the test period.

The acclimated 41% scour samples had a lag period of 6 days, inclined steadily till day 24 and stabilized at 5 mℓ (day 24) and remained stable for the remainder of the test period. The non-acclimated samples had a lag period of 24 days, climaxed at 146 mℓ (day 70) with no further gas production for the remainder of the test period.

4.2.8 Methane production from biomass exposed to Scour effluent

The cumulative methane production plots are shown in **Fig 4.12**. The 1% acclimated samples had a lag period of 24 days, with a maximum CH_4 production of 12 mℓ. The non-acclimated samples had a lag period of 2 days, followed by a sharp incline and climaxed at 131 mℓ (day 36).

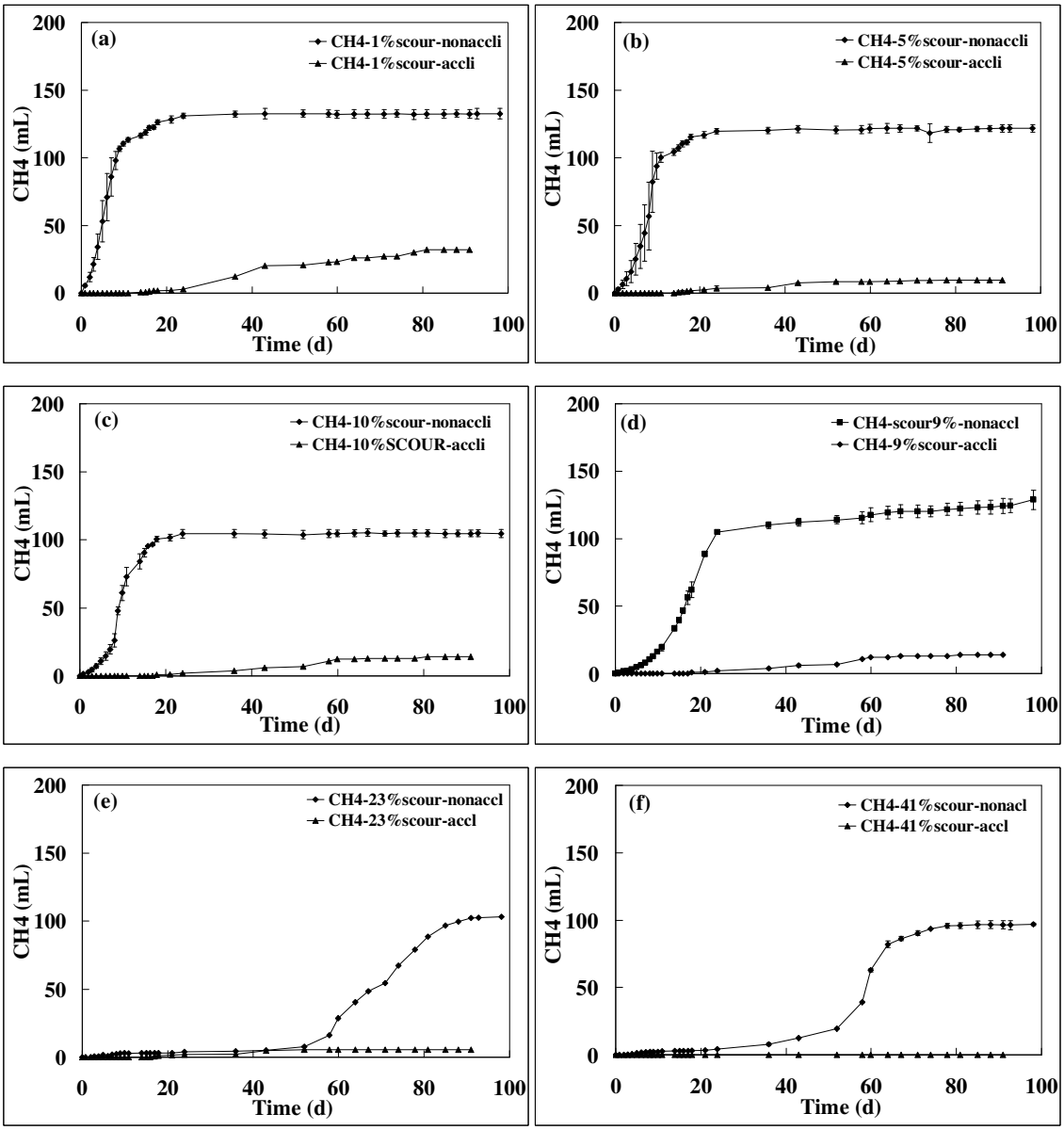


Figure 4.12: Methane production plots for scour samples (a) 1% v/v, (b) 5% v/v, (c) 10% v/v, (d) 9% v/v, (e) 23% v/v and (f) 41% v/v

The acclimated 5% scour samples had a lag period of 17 days, reached a plateau of 8 mℓ (day 43). In the non-acclimated 5% scour samples the lag period was 5 days, with maximum CH_4 production of 120 mℓ (day 24).

The 10% acclimated scour samples had a lag period of 24 days, with a maximum CH_4 production of 13 mℓ (day 60) and remained stable for the remaining test period. The non-acclimated samples had a lag period of 5 days, with a sharp incline between day 5 and day 11, and finally stabilized at 105 mℓ (day 24).

The (9%) acclimated scour samples had a lag period of 24 days, climaxed at 12 mℓ (day 60). In the non-acclimated samples, the lag period was 8 days, followed by a sharp incline till day 24, with a further steady incline and finally leveling off at 125 mℓ (day 60) with no further CH_4 production for the remainder of the test period.

The 23% acclimated samples had a lag period of 6 days, climaxed at 5 mℓ (day 43) and remained stable thereafter. The non-acclimated samples had a lag of 58 days, with a sharp incline climaxing at 103 mℓ (day 88) with no further CH_4 production.

At 41% concentration level the acclimated samples produced no methane at all. The non-acclimated samples had a lag period of 36 days, with a steady decline between day 36 and day 52, and stabilized at 96 mℓ (day 74), with no further CH_4 production.

4.2.9 Activity assessment of biomass exposed to scour effluent

The activity profiles for the scour samples are shown in **Fig 4.13**. The activity for the 1% acclimated samples was 0.1 gCOD/gVS/d compared to 1.7 gCOD/gVS/d attained by the non-acclimated samples. At 5% concentration level the acclimated samples had an activity of 0.32 gCOD/gVS/d compared to 2.5 gCOD/gVS/d of the non-acclimated biomass. At 10% concentration level, the maximum biomass activity for the acclimated samples was 0.1 gCOD/gVS/d compared to 1.82 gCOD/gVS/d attained by the non-acclimated samples.

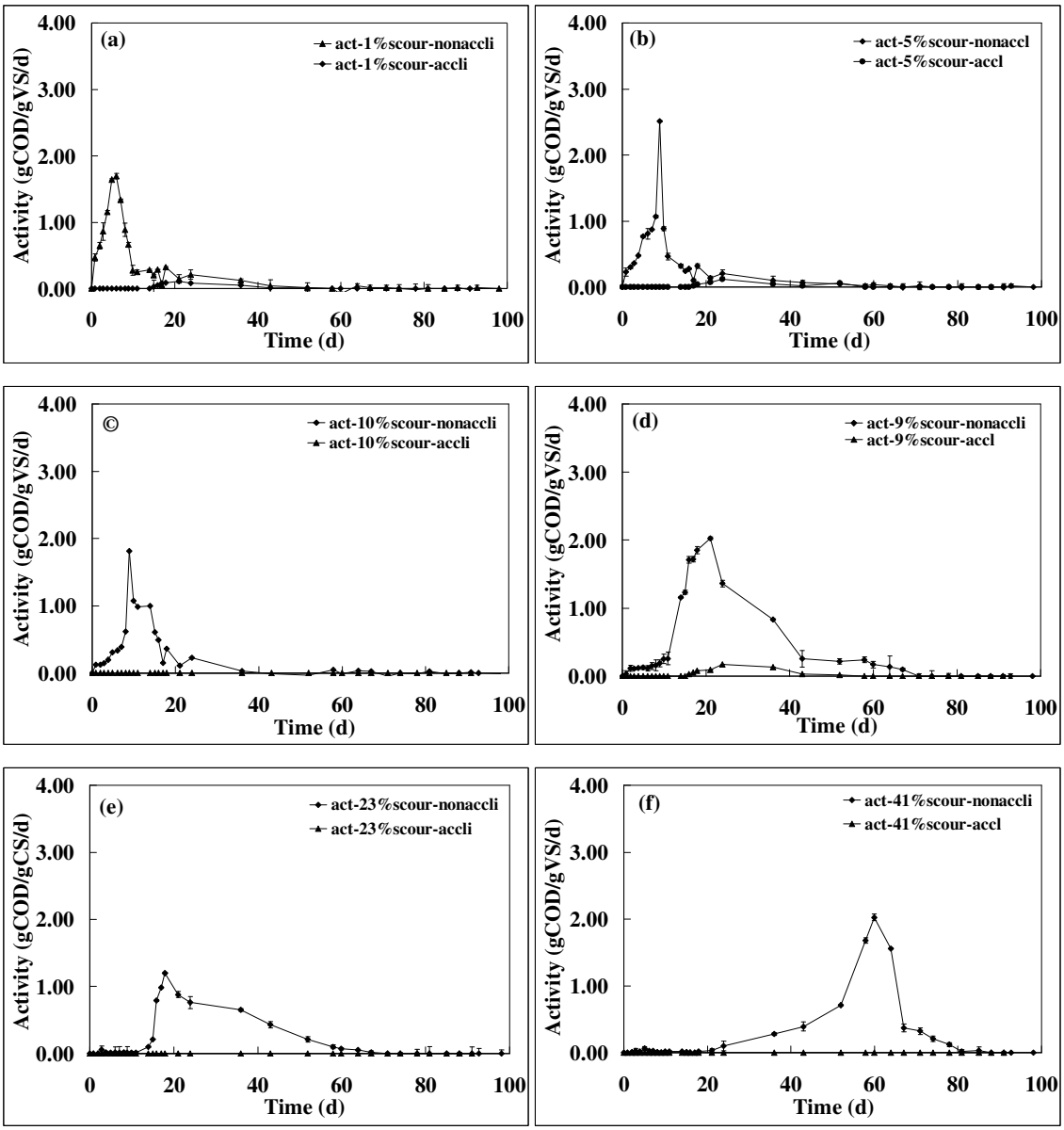


Figure 4.13: Methanogenic activity plots for scour samples (a) 1% sample, (b) 5% samples, (c) 10% samples, (d) 9% samples, (e) 23% samples and (f) 41% samples

At 9% concentration level, the acclimated samples attained the maximum biomass activity of 0.02 gCOD/gVS/d compared to 2.03 gCOD/gVS/d of the non-acclimated samples.

The 23% concentration level the maximum activity of the acclimated samples was 0.1 gCOD/gVS/d compared to 1.2 gCOD/gVS/d attained by the non-acclimated samples.

At 41% concentration level the acclimated samples attained the maximum activity of 0.1 gCOD/gVS/d, compared to 2.02 gCOD/gVS/d attained by the non-acclimated samples. The summary of the results is reported in **Table 4.2**.

Table: 4.2 Summary for section 1 experimental results

	Parameter	Units	1%		5%		10%		9%		23%		41%	
			acc	un-acc	acc	un-acc	acc	un-acc	acc	un-acc	acc	un-acc	acc	un-acc
Distillery	Ult gas vol	mℓ	528	205	835	315	1018	416	951	442	1236	604	346	266
	Ult CH_4 vol	mℓ	210	141	327	201	413	245	409	267	559	197	39	33
	Max SMA	gCOD/gVS/d	2.7	1.8	5.3	4.92	6.52	5.68	7.26	6.3	12.6	12.4	2.13	0.2
	Lag phase	d	7	2	7	3	5	4	6	5	7	13	16	24
Size	Ult gas vol	mℓ	355	219	425	296	590	369	600	321	824	634	603	105
	Ult CH_4 vol	mℓ	100	9	261	177	338	202	363	206	458	332	109	278
	Max SMA	gCOD/gVS/d	2.4	141	3.1	2.1	6.3	2.4	6.02	2.4	9.26	3.5	2.05	2.6
	Lag phase	d	6	2.0	5	2	5	31	4	2	5	8	3	3
Scour	Ult gas vol	mℓ	36	185	22	183	28	183	28	187	20	151	5	147
	Ult CH_4 vol	mℓ	32	153	10	122	14	105	14	129	5	103	0	97
	Max SMA	gCOD/gVS/d	0.1	1.7	0.1	2.51	0	1.82	0.2	2.0	0	1.2	0	2.0
	Lag phase	d	24	2	19	3	21	5	21	39	17	52	98	36

The reproducibility plots of the above experiments are shown in **Appendix D (Fig D17 to D44)**.

4.3 DISCUSSION SUMMARY OF BIOMASS EXPERIMENTS

4.3.1 Biogas production from biomass exposed to different effluents

This section discusses the observations noted in the biogas production from the biomass that was exposed to the different effluents.

4.3.1.1 .Distillery effluent (biogas production)

The biogas production for the acclimated samples at all concentration levels tested (except 41%) was two-and half fold compared to non-acclimated samples. The maximum safe concentration is 23% (v/v) beyond which the biomass activity is greatly inhibited. An unexpected observation was that the acclimated samples took twice as long to reach plateau, compared to the non-acclimated samples. One explanation for this observation is that the non-acclimated samples reached a false plateau. A second explanation is that the acclimated samples had endured the cumulative inhibition.

The concentration effect of the distillery samples on biogas production is shown in **Fig 4:14(a)** and reported in **Table 4.3 (a).**

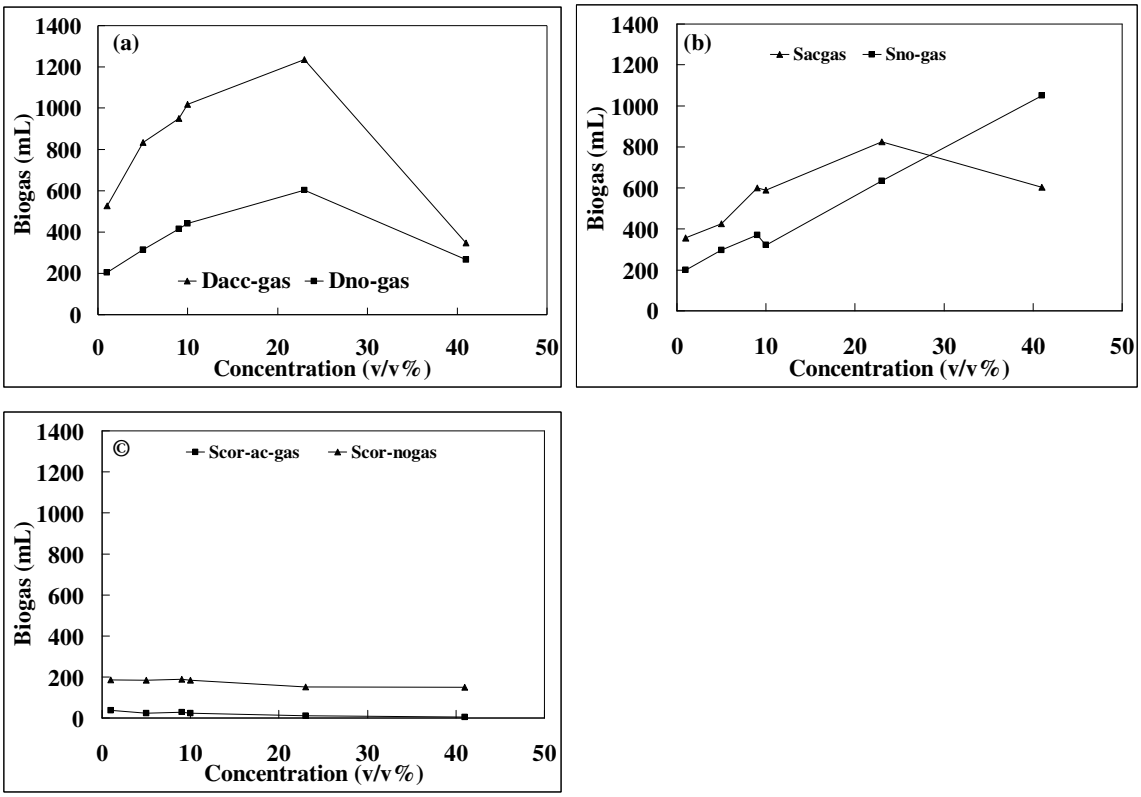


Figure 4.14: Ultimate gas production profile for acclimated and non-acclimated samples (a) distillery, (b) size and (c) scour

4.3.1.2 Size effluent (biogas effluent)

The biogas production in the acclimated size samples was twofold compared to non-acclimated samples at all concentration levels tested, except 41% concentration level. At 41% concentration level the non-acclimated samples produced twice biogas compared to the acclimated samples. This outcome was not expected. The 23% concentration level is the safe concentration to prevent biomass failure. These observations are shown in (**Fig 4.14(b)**) and reported in **Table 4.3.(b).**

4.3.1.3 Scour effluent (biogas production)

The non-acclimated scour samples produced fivefold much biogas compared to the acclimated samples at all concentration levels tested. This outcome shows that the scour dye effluent is severe toxic. It is permanent inhibitory to the biomass. Pre-exposure of the

biomass to the scour effluent resulted in cumulative inhibition (poisoning). This outcome is shown in **Figure 4.14. (c)** and reported in **Table 4.3. (c)**.

Table: 4.3 (a) Comparison of acclimated and non-acclimated gas production of distillery samples

Conc. (%)	Biogas-acclimated (mℓ)	Biogas nonacclimated (mℓ)	Ratio (Acc/non-accl)
1	528	205	2.6
5	835	315	2.7
9	951	442	2.3
10	1018	416	2.3
23	1236	604	2
45	346	266	1.3

(b): Comparison of acclimated and non-acclimated gas production of size samples

Conc. (%)	Biogas-acclimated (mℓ)	Biogas –nonacclimated (mℓ)	Ratio (Acc/non-accl)
1	355	199	1.8
5	425	296	1.4
9	590	369	1.6
10	600	321	1.8
23	824	634	1.3
45	603	1050	0.6

(c): Comparison of acclimated and non-acclimated gas production of scour samples

Conc. (%)	Biogas-acclimated (mℓ)	Biogas –nonacclimated (mℓ)	Ratio (Acc/non-accl)
1	36	185	0.2
5	22	183	0.12
9	28	183	0.15
10	28	187	0.15
23	20	151	0.07
45	5	147	0.03

4.3.2 Methane production from biomass exposed to different effluents

4.3.2.1 Distillery effluent (methane production)

The acclimated distillery samples produced more CH_4, compared to the non-acclimated samples at all concentration levels tested, with severe inhibition at the highest concentration (41%). The distillery effluent is slightly inhibitory, as at the beginning of the test, as the larger fraction of the biogas was CO_2, as the test progressed the CH_4/CO_2 ratio shifted in favor of CH_4 production. This observation is shown in **Fig 4.15(a)** and reported in **Table 4.4 (a).**

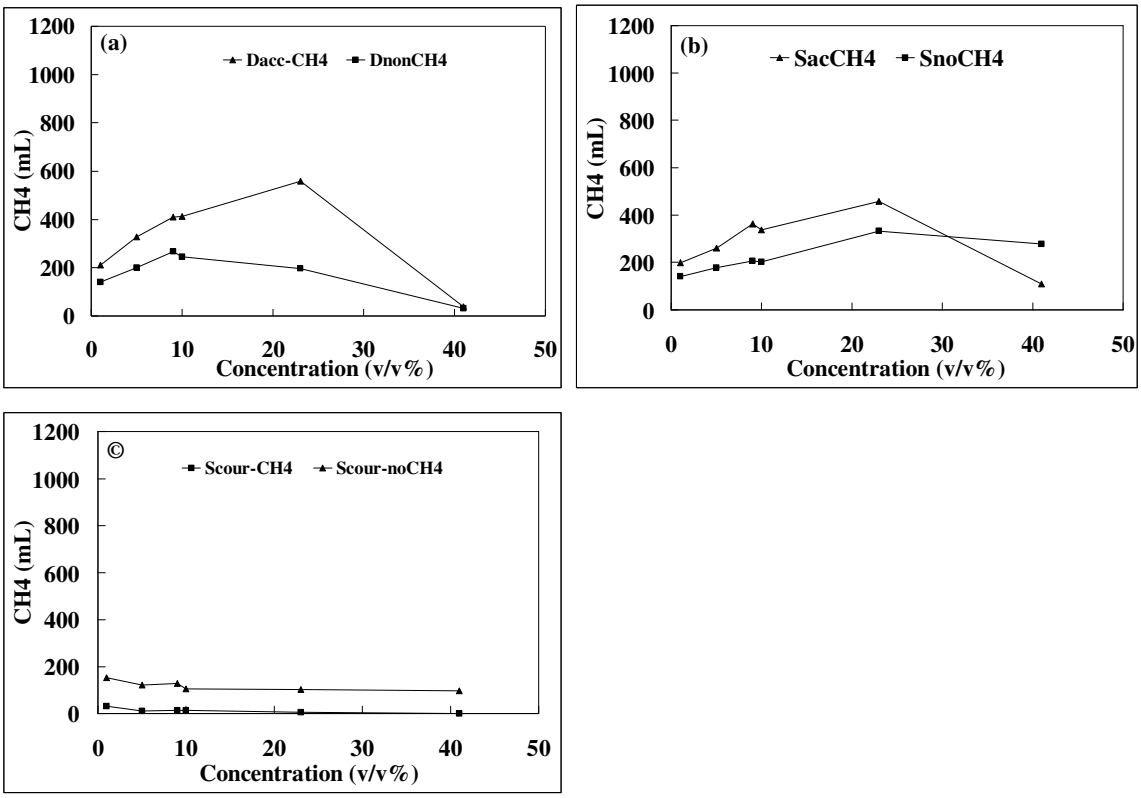


Figure 4.15: Ultimate CH_4 production profile for acclimated and non-acclimated samples (a) distillery, (b) size and (c) scour

Table 4.4(a): Comparison of acclimated and non-acclimated CH_4 production for distillery samples

Conc. (%)	CH_4-acclimated (mℓ)	CH_4-nonacclimated (mℓ)	Ratio (Acc/non-accl)
1	210	141	1.48
5	327	201	1.63
9	413	245	1.66
10	409	267	1.53
23	559	197	2.84
41	39	33	1.18

(b): Comparison of acclimated and non-acclimated CH_4 production for size samples

Conc (%)	CH_4-acclimated (mℓ)	CH_4-non-acclimated (mℓ)	Ratio (Acc/non-accl)
1	199	141	1.41
5	261	177	1.47
9	338	202	1.67
10	363	206	1.76
23	458	332	1.38
41	109	278	0.39

(c): Comparison of acclimated and non-acclimated CH_4 production for scour samples

Conc. (%)	CH_4-acclimated (mℓ)	CH_4 non-acclimated (mℓ)	Ratio (Acc/non-accl)
1	32	153	0.21
5	10	122	0.08
9	14	105	0.13
10	14	129	0.11
23	5	103	0.05
41	0	97	0

4.3.2.2 Size effluent (methane production)

The acclimated size samples produced more CH_4 compared to non-acclimated samples, with the exception of the high concentration (41%). At 41% concentration, there was more CH_4 production in the non-acclimated sample compared to acclimated samples. This comparison is reported in **Table 4.4(b)**, and shown in **Fig 4.15(b).**

4.3.2.3 Scour methane production

The non-acclimated scour samples produced more CH_4 than the acclimated samples, with reduced production as the concentration increased; this is due to the inhibitory nature of the

scour effluent. The scour effluent caused permanent inhibition to the biomass, with no CH_4 production at the highest (41%) concentration. This comparison is reported in **Table 4.4 (c)** and shown in **Fig 4.15 (c).**

4.3.3. Specific methanogenic activity of biomass exposed to different effluents

There was more activity for the acclimated samples compared to the non–acclimated samples (distillery and size). The scour activity in acclimated samples was negligent. The comparison is reported in **Table 4.5** and shown in **Fig 4.16 (a, b and c)**

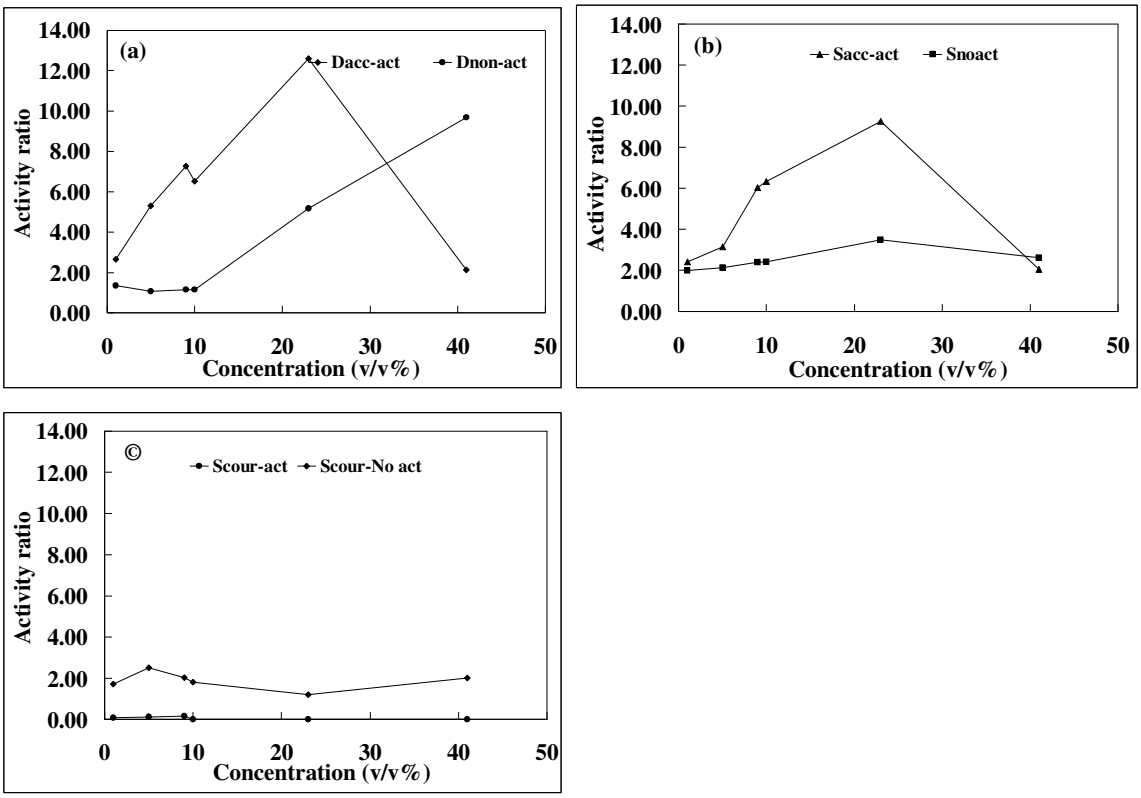


Figure 4.16: Ultimate activity profile for acclimated and non-acclimated samples (a) distillery, (b) size and (c) scour

Table 4.5(a): Comparison of acclimated and non-acclimated activities for distillery samples

Concentration (%)	Activity-acclimated (gCOD/gVS/d)	Activity-nonacclimated (gCOD/gVS/d)	Ratio (Acc/non-accl)
1	2.65	1.97	1.35
5	5.3	4.92	1.08
9	6.52	5.68	1.15
10	7.26	6.3	1.15
23	12.6	2.44	5.16
41	2.13	0.22	9.68

(b): Comparison of acclimated and non-acclimated activities for size samples

Concentration (%)	Activity-acclimated (gCOD/gVS/d)	Activity-nonacclimated (gCOD/gVS/d)	Ratio (Acc/non-accl)
1	2.42	1.98	1.22
5	3.14	2.13	1.47
9	6.33	2.41	2.63
10	6.02	2.4	2.51
23	9.26	3.5	2.65
41	2.05	2.61	0.79

(c): Comparison of acclimated and non-acclimated activities production for scour samples

Concentration (%)	Activity-acclimated (gCOD/gVS/d)	Activity nonacclimated (gCOD/gVS/d)	Ratio (Acc/non-accl)
1	0.09	1.7	0.05
5	0.12	2.51	0.05
9	0	1.82	0
10	0.17	2.03	0.08
23	0	1.2	0
41	0	2.02	0

The above results illustrated the dilemma faced by researchers in anaerobic digestion. In many instances some conflicting results have been reported. Based on the results of distillery and size, biomass showed successful acclimation to high strength organic effluents, with the exception of the highest concentration.

These results are consistent with literature. Several researchers have investigated the effect of biomass acclimation. Young et al., (1980), noted that when methanogenes were first exposed to 2.5 mg/ℓ of chloroform, the gas production almost ceased, requiring about a week for recovery. Repeated exposure to the same dosage of chloroform showed no reduction in the rate of activity, demonstrating the potential for acclimation. Bagley and Gosset (1995) demonstrated that biodegradation was taking place when chloroform was supplemented to anaerobic systems receiving a co-substrate of methanol. Bhattacharya and Parkin (1986) reported that, upon acclimation, the inhibition coefficient increased five fold for Ni toxicity.

4.4 PILOT SCALE STUDIES

4.4.1 Scale-up of batch conditions to pilot scale

The aim of performing batch studies (serum bottles) was to determine the safe set of operating conditions for anaerobic digestion, to be implemented in the scale up process. In a batch system, the biomass is initially exposed to a relatively high concentration of the effluent and has unlimited time to degrade the effluent. In a continuous system, the biomass is constantly exposed to a certain constant concentration of the substrate and the time to adapt is limited. The parallel between a serum bottle method and a continuous fed rector is illustrated in **Fig 4.17** (WRC Report no K5/1074, 2005).

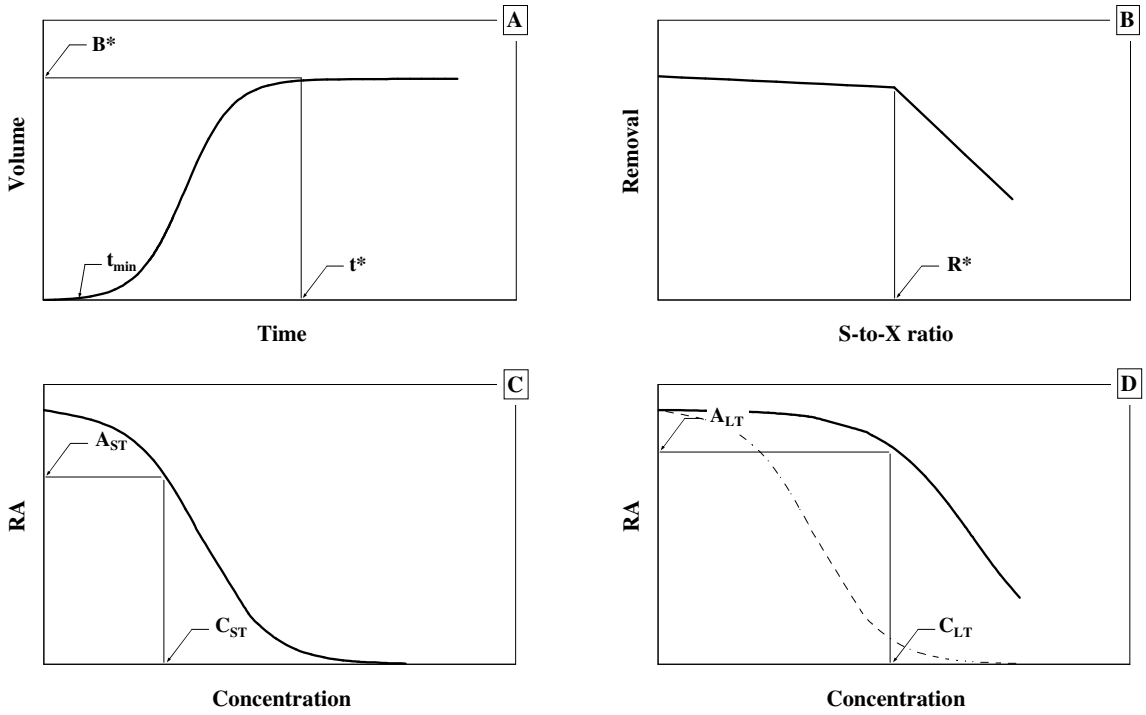


Figure 4.17: Schematic outcome of a series of serum bottle activity tests, on a generic test material and relevant parameters to be transferred to the start-up of an anaerobic digester.(WRC Report No K5/1074)

The following guidelines were used, based on **Fig 4.17** (WRC Report no K5/1074):
If t* is the period (days) needed for the biomass to degrade the test material to an extent B and t_{min} is the period needed to adapt to the test compound (e.g. to overcome initial inhibition), the HRT is ideally set to equal t*. If t* is excessively long, hence no feasible HRT, a lower retention time could be set that would result in a lower removal rate. This limit should be set at t_{min}. Any HRT below t_{min} will result in test compound leaving the reactor undegraded.

If R* is the limit of the substrate-to-biomass ratio (gCOD/gVS/d) that ensures a sufficient level of biodegradation, the Organic Loading Rate (OLR) can be set according to:

$$OLR = \frac{R}{HRT}.X \qquad (4.1)$$

where *X* is the biomass
The biodegradability and toxicity can be used to determine show both the short and long term inhibition effect of the test material.

4.4.2 Anaerobic digestion of distillery effluent

During the batch fed-mode, the reactor seemed to be stable with all the conditions that were monitored. The results corresponding to different periods are shown in **Fig 4.18**.

4.4.2.1. Period I

The pH for the acclimated biomass averaged 8.5, compared to that of the unacclimated biomass (7.2). In both samples, the pH fluctuated slightly, but within the optimal range. The pH stability was a sign of stable reactor operation.

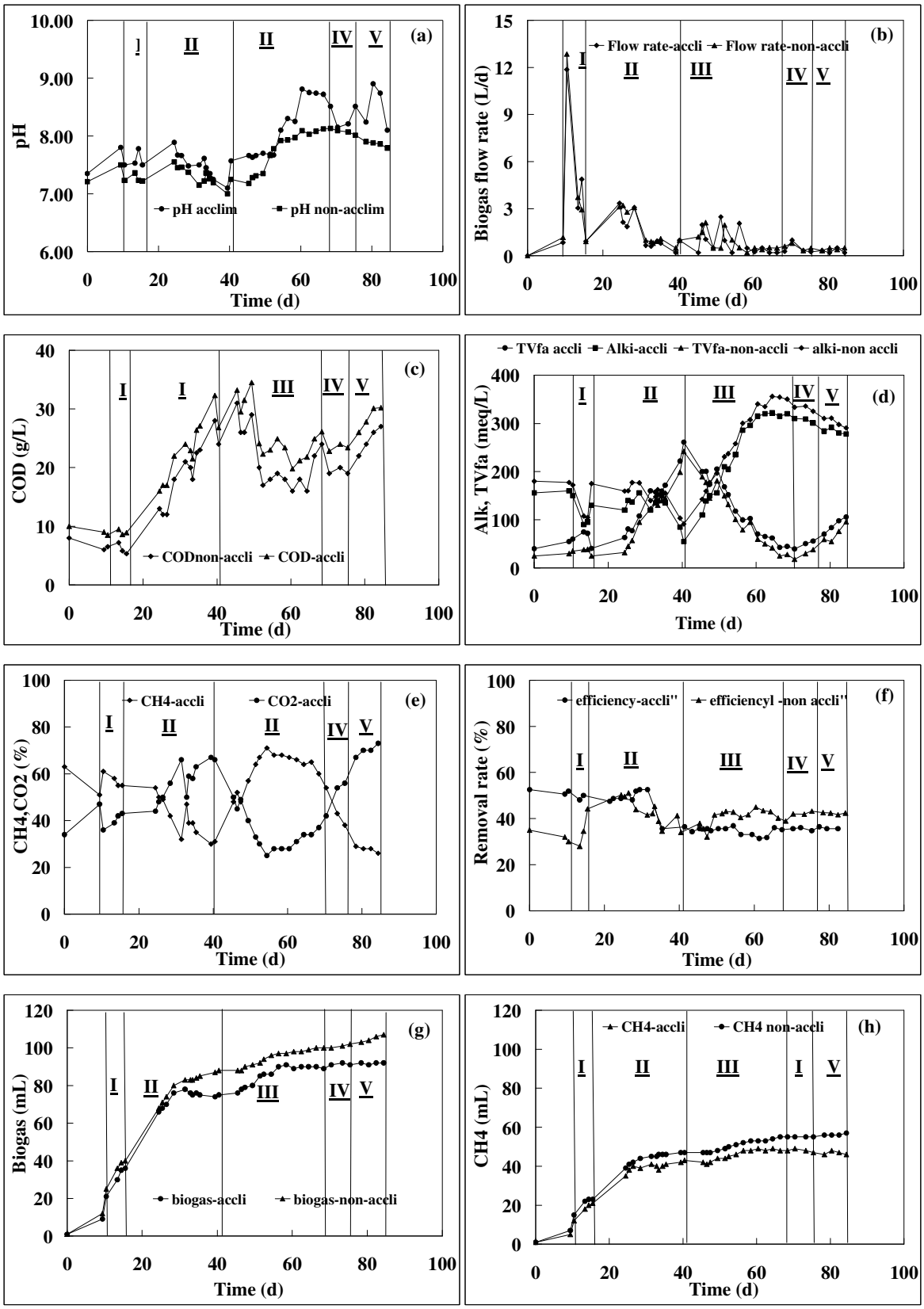


Figure 4.18: Summary of results of the distillery pilot reactor: (a) pH, (b) biogas flow rate, (c) residual COD, (d) VFA and alkalinity, (e) gas composition, (f) COD removal rate, (g) biogas production and (h) methane production.

There was a surge in biogas production for both acclimated and non acclimated samples. A major fraction of the biogas during this period was CO_2, matched by a corresponding pH drop and an increasing VFAs concentration. This degradation pattern was observed in the serum bottle studies.

The residual COD fluctuated around (10 g/ℓ), with the acclimated sample having more residual COD compared to the non-acclimated sample. The COD removal rate of the non-acclimated sample was stable (52%) while the acclimated sample showed a recovery. The extent of biogas and methane production was relatively the same, exhibiting the increasing trend.

4.4.2.2. Period II

The pH decreased on day 30 matched by the corresponding increase in VFAs concentration, with a corresponding rate of biogas flow rate. When the residual COD approached its peak, it was matched by the decreasing COD removal efficiency. The biogas and CH_4 production continued with an increasing trend with methane production approaching stability (first plateau, indication of acclimation).

4.4.2.3. Period III

The pH showed recovery at the beginning of this period, for both acclimated and non acclimated samples. The biogas flow rate stabilized with no further fluctuation. The residual COD fluctuated a lot, very unsettled. This was an indication of the system shift, establishing a new steady state. The biogas and methane production approached stability.

4.4.2.4. Period IV

There was stability in pH, and biogas flow rate. The COD removal rate stabilized as well. Biogas and methane production were stable with no further increase.

4.4.2.5. Period V

The pH showed signs of depression (decreasing trend), whist the biogas flow rate was stabilizing. The residual COD showed a shift towards a new steady state, with COD removal rate remaining constant. Judging from the reactor gas composition, the reactor performance was deteriorating, with no further sign of biogas and methane production recovery.

4.4.3 Anaerobic digestion of Size effluent

A 3 ℓ volume flask (working volume 1.5 ℓ) was used to set up the completely stirred reactor for size digestion as per **Section 3.2**. The reader is requested to refer to **Fig 4.19** for discussion.

4.4.3.1. Period I

The pH fluctuated between 6.92 and 8.05 for the non-acclimatized sample, whilst the acclimated sample had a stable pH of 8.00. The biogas flow rate was unstable peaking at 2.46 ℓ for non-acclimated sample. The behavior of the acclimated sample exhibited the similar trend similar to that of the non acclimated sample.

The residual COD for the non acclimated sample was low compared to the acclimated sample, fluctuating between 30 g/ℓ and 15 g/ℓ.

The alkalinity of both the acclimated and non acclimated sample was similar during this period. There was a slight suppression of methane production, with methane content in the reactor declining from 66% to 35%, with a corresponding increase in CO_2 indicating a transition to the acidogenic phase.

The COD removal efficiency decreased reduced to 26% on day 21 and slightly recovered again towards the end of period 1.

4.4.3.2. Period II

The pH remained stable during period 2 at optimum (8.1) for the non-acclimated sample, with biogas flow rate fluctuating during this period for both samples.

The residual COD for both samples followed similar trend, first with an increase on day 27 until day 35, displaying a downward trend until day 47, and started to accumulate again until day 56, with the acclimated sample displaying a larger COD removal efficiency. The methane content increased in favor of biogas and methane production.

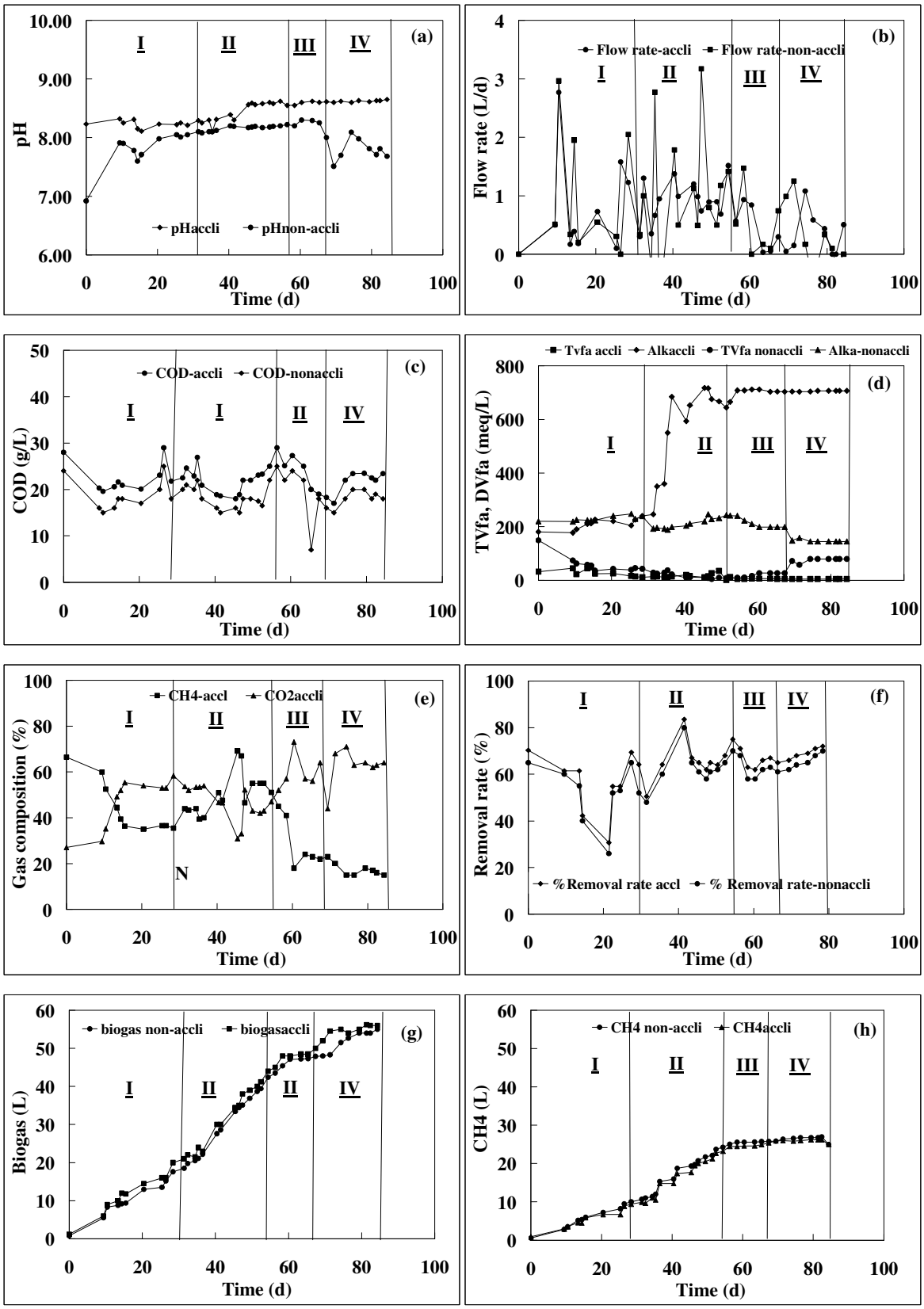


Figure 4.19: Summary of results of the size pilot reactor: (a) pH, (b) biogas flow rate, (c) residual COD, (d) VFA and alkalinity, (e) gas composition, (f) COD removal rate, (g) biogas production and (h) methane production

4.4.3.3. Period III

The pH remained stable during this period with an acclimatized sample having a higher pH of 8.6, compared to the non acclimated sample with a pH of 8.2, and decreased towards the end of this period. The biogas flow rate decreased after day 58 to almost zero, before recovering again. The residual COD displayed a downward trend, dropping from 30 g/ℓ (acclimated) to 18 g/ℓ at the end of the period III. The alkalinity and VFA during this period displayed a similar trend.

The CO_2 composition increased between day 56 and day 60, thereafter it decreased and stabilized, with a corresponding increase in biogas production and stabilization in CH_4 production.

4.4.3.4. Period IV.

During this period the pH stabilized at around 8.6 for acclimated sample, and fluctuated between 7.5 and 8 for non-acclimated sample. The biogas flow rate was unstable during this period.

The residual COD initially increased and finally stabilized at 23.5 g/ℓ (acclimated) and 18 g/ℓ for the non-acclimated. The VFA and alkalinity reached stability during this period.

The CH_4 content continued with a downward trend, as CO_2 finally stabilizing at 64%, an undesirable amount for the well functioning anaerobic process. The biogas and CH_4 production also stabilized during this period.

4.5 Conclusions

1. There was no significant difference in the digestion of acclimated and non-acclimated samples for both size and distillery effluent.

2. The pilot plant investigation highlighted some shortfall in the digester design, some of which are:

- The reliability of the gas measurement system.
- The need for phase separation between liquid and solids.
- The digester configuration type, the reactor configuration used was a simple low cost CSTR.

CHAPTER 5

GUIDELINES FOR FULL SCALE IMPLEMENTATION

5.1 INTRODUCTION

The critical step in the process development, is the scale up from laboratory and pilot scale to full scale. Some of the potential problems experienced during the research phase are highlighted. This Section is subdivided in the following main subjects:

i) how to **operate** an anaerobic digester;

ii) how to **monitor** the performance of an anaerobic digester; and

iii) how to **assess** the performance of an anaerobic digester.

iv) process management workflow (process business model)

5.2 OPERATION OF AN ANEROBIC (CSTR) DIGESTER

The set-up of the pilot-scale digester used for the investigation is discussed in **Section 3.3** and is regarded as anticipated prototype for a full-scale digester. The essential components are:

- the reaction vessel,
- the stirring device,
- the temperature control system,
- the feed inlet and product outlet ports
- gas measuring system.

The size and shape of the reaction vessel has an influence on other characteristics of the experiment and to some extent on the performance of the process itself. Mixing in the reactor can be achieved by means of an external recycle or by the use of a mechanical stirring device. Great care should be taken in the design of the mixing device not to create high turbulence as the shear forces created by aggressive stirring can damage the biomass structure.

Based on our research experience, we proposed that the anticipated prototype be housed in the temperature controlled environment/house, ideally have your digester erected under a digester house with a thermo stated controlled temperature, thus minimizing energy requirements.

The key factors to operate a digester are the sludge retention time (SRT) and the hydraulic retention time (HRT) and the organic loading rate (OLR). The sludge and the hydraulic

retention times coincide if no sludge separation is performed prior to liquid extraction. The simple set-up adopted in our research did not allow sludge separation, but it is highly recommended that in the full-scale digester there should be phase separation between liquid and solids. In general, the SRT should be decided in a way that guarantees the minimum retention time needed for the most vulnerable microbial population.

The HRT will define the feed flow rate, according to the formula:

$$Q = \frac{V}{HRT} \tag{5.1}$$

where V indicates the volume of the contents of the reactor.

HRT is the hydraulic retention time

5.3 MONITORING ANAEROBIC DIGESTION PROCESS

Monitoring the performance of a full-scale digester requires the estimation of the *state* of the system in response to the *input* and the operating conditions. The question therefore is: which variables must be measured in order to reliably assess the behaviour of the digester?. Our experience however suggests that the following variables **(Fig 5.1)** are sufficient to obtain an accurate picture on the current state of an anaerobic process.

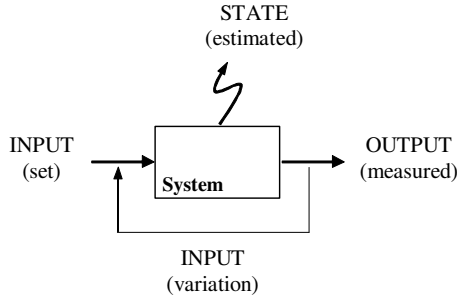


Figure 5.1: The concept of monitoring an anaerobic process: the input variables are set by the operator; the output variables are analytically or instrumentally measured; the state of the system is estimated, based on the observation of output and the knowledge of the input. This ultimately enables to manipulate the input, in order to achieve better performance. (WRC Report No K5/1074, 2005)

5.3.1 Gas production

This is the most direct output of an anaerobic process; in fact the majority of the existing methods to assess biodegradability of a substance and the sludge activity target this variable (Rozzi and Remigi, 2004). Using qualitative analysis, one can say that a high biogas flow rate is indicative of an effective digestion process. However, the knowledge of this variable alone is not sufficient and may even be misleading if not accompanied with the knowledge of

the composition of the gas: typically, a high gas flow rate with a low methane fraction may be indicative of a predominantly cacogenic process in which the methanogenic biomass may be inhibited. If these conditions are not detected on time, they may ultimately lead to the failure of the digester.

In industry a reliable gas measurement system using utility/instrument air can be used to measure the rate of biogas production. In order to ensure that the measurement of the biogas volume is accurate and consistent throughout the process, frequently planned preventative maintenance and calibration of the instrument must be performed.

5.3.2 Gas composition

In the full-scale plant the gas analyzers (installed on the product pipeline) can be installed to analyze the gas composition on-line, with a laboratory analysis (GC method) as a back up. The typical system can consist of three analyzers, where the process failure can be measured by two out of three analyzers. The high and low alarm conditions, as well as trip conditions, can be set such that the operator at the console is able to receive early warning, if the gas composition deteriorates and thus corrective action can be taken. In addition to this, routine gas sampling can be performed on shift basis to verify the analyzer reading, whereby an operator can extract gas samples and take them to the quality control laboratory for specification confirmation. A generic guide similar to **Table 5.1** can be employed as a quality control tool.

Table 5.1: A generic guide of a digester operating values

Component	Minimum (%)	Maximum (%)	Alarm	Trip
H_2	1	5	-	
CH_4	10	65	10	<10
CO_2	10	30	35	
N_2	1	5	5	
impurities	≤ 0.001	0	0	

5.3.3 pH, alkalinity, COD and VFAs concentration

The full-scale plant must be equipped with instrument analyzers, capable of doing online measurements for the above parameters. The normal trend in industry is to have three

analyzers measuring the same parameter. If two out of three analyzers show a deviation, an alarm can be activated on the console, thus enabling the operator to take corrective action.

Routine sampling must be followed as a back up, to prevent the digester failure. In case of plant upsets the special sampling with the quality control laboratory can be arranged until such time the stability has been restored on the plant.

A production management tool like Portal, a web based software, can be installed to allow managers and specialists (engineers and technicians) to have an overview of the process. Once the laboratory analysis is complete, the results are loaded on the laboratory information management system (LIMS) which is linked to the Portal system and become available on the web, except for special samples which will require the direct communication between operation and laboratory personnel.

5.4 ESTIMATING THE PERFORMANCE OF THE PROCESS

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

$$\eta_1 = \frac{COD_{out}}{COD_{in}} \tag{5.2}$$

where COD_{in} and COD_{out} denote the COD concentrations in the feed and in the effluent respectively. A mass balance on the COD is a better approach in that it compares the *cumulative* amount of organic matter fed, to the *cumulative* amount of residues, as:

$$\eta_2 = \frac{COD_{removed}}{COD_{fed}} = \frac{\sum C_{in,t}.V_{in,t} - \left[C_{out,t}.V_R + \sum\left(C_{out,t}.V_{out,t}\right)\right]}{\sum\left(C_{in,t}.V_{in,t}\right)} \tag{5.3}$$

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 and of mixed liquor respectively, at time t; and V_R is the liquid volume.

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

$$\eta_3 = \frac{COD_{toCH4}}{COD_{fed}} = \frac{\sum M_{ch,t}}{\sum (C_{in,t}.V_{in,t})} \quad (5.4)$$

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

Possible reasons for the η_2 and η_3 efficiency not been equal are:

i) the COD measured analytically is not accurate or not representative of the actual organic matter in the mixed liquor; this is possible since the COD is often measured on the filtered mixed liquor, i.e. the portion of COD that has been stored by the biomass is not taken into account;

ii) 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.

$$\Delta V_{CH} = \Delta V_{CH,out} + \Delta V_{CH,headspace} \quad (5.5)$$

Where $\Delta V_{CH,out}$ denotes the volume of methane that has left the reactor during the interval $(t-1, t)$ with the biogas; and $\Delta V_{CH,headspace}$ indicates the variation of the methane volume in the headspace in the same interval.

The two terms can be calculated as:

$$\Delta V_{CH,out} = \int f_{CH}(t).Q_{gas}(t).dt \quad (5.6.a)$$

$$\Delta V_{CH,headspace} = V_H.(f_{CH,t} - f_{CH,t-1}) \quad (5.6.b)$$

Where $f_{CH,t}$ is the methane fraction in the off-gas (generally a function of time); $Q_{gas,t}$ is the biogas flow rate (ℓ/d) (also a function of time); f_{CH},t and $f_{CH,t-1}$ denote the methane fraction, at time *t* and *t-1* respectively; and V_H is the volume of the headspace (generally constant).

Table 5.2: Operation guidelines for process monitoring of the full-scale digester

Variable	Frequency of determination	Optimal range
Gas flow rate	Continuous (*at least* twice per shift)	Function of the sludge activity and organic loading rate.
Gas composition	*At least* twice per shift	Function of the feed composition and microbial dynamics.
pH	Online determination and routine sampling	6.5 to 7.5
Alkalinity	Online determination and routine sampling	
VFA concentration	Online determination and routine sampling	
COD	Online determination and twice per shift	Should not display a clearly *increasing* trend
Solids	Weekly	Should not display a clearly *decreasing* trend
SMA (*)	As for solids	> 60 mg COD/g VSS/d

(*) The variable measured is the methanogenic activity of the *sludge*, combined with the previous knowledge of the solids concentration. The methanogenic fraction of the biomass is a derived quantity.

Note. These guidelines are useful for the monitoring during plant commissioning, as well as during steady state. Special sampling campaign must be executed during upset conditions.

5.5 CONTROLING THE DIGESTION PROCESS

Based on the experience from our research laboratory, it was realized that unless an anaerobic digestion unit was highly automated, e.g. with online data acquisition of gas production and composition, pH, automated feeding and sampling procedures, etc., the accurate monitoring of the process can be very time-consuming.

In the process industry, Advanced Process Control (APC) can be implemented to control the digestion process. The APC can be coupled with commercial software known as Process Information (PI) system. This program offers many advantages, among them such as:

- The ability to view process shows at anytime
- The ability to trend the history of process performance, thus making it easy to trace incidents and initiate Root Cause Analysis (RCA) investigations.

This system is also highly recommended for the full-scale application since you are likely to have different users, with different requirements in relation to the smooth operation and

control of the plant. Three types of users encountered in the process industry are process managers, specialists and operators.

Process manager's main interest is in the overall overview of the process. These users will access the PI system with the intention of viewing process shows and any points of interest to them. Managers cannot make any data inputs to the system. Their concerns are communicated to either specialist group or operators.

The specialist users (engineers/technologists and technicians) are interested in the overall overview of the process. Unlike the process managers, specialists have a mandate to analyze data and make recommendations to the process managers and operators. Specialists will make input in the system, modify formulae and perform calculations, create plots or trends, and view lab results (reports).

The last users (operators) are interested in viewing process shows, with the intention of monitoring the process for deviations management. They will also make input to the data base system only at the directive from specialist group in the form of operating philosophy.

From the above discussion it is clear that the process development also need to include information and data management for the smooth and effective running of the plant. The success of the full-scale implementation will be highly dependant on two major areas. The first is the process design itself, and the second one is the choice of business and process management model chosen by the organization.

CHAPTER 6

CONCLUSIONS AND RECOMENDATIONS

This chapter summarises the conclusions and recommendations drawn from this study.

6.1 CONCLUSIONS

The following observations were made from the serum bottle studies: experimental results:

- The sludge sampled from Umbilo produced 189 mℓ biogas and 100 mℓ of CH_4 compared to 100 mℓ biogas and 60 mℓ CH4 produced by the sludge sampled from Mpumalanga digesters.
- The activity of the biomass exposed to distillery and size effluents was enhanced by acclimation. The biogas and methane production of the biomass exposed to both effluents was doubled, except for the highest concentration of 41%.
- Distillery effluent is slightly inhibitory (temporary inhibition at the beginning of the incubation period due to larger fraction of CO_2 in the biogas, which results in the increase of VFA concentration), but after some exposure time has lapsed the biomass is able to recover with the exception of the highest concentration (41%) for the acclimated samples. The non-acclimated samples are inhibitory after 10% concentration.
- The scour effluent is severely toxic and causes permanent inhibition of the biomass activity. This was evidenced as reduced biogas and CH_4 production in the acclimated biomass exposed to scour

Based on the above observations the following conclusions are valid:

1. Biomass source plays a role in the treatability of the organic waste. Effluents inoculated with Umbilo sludge, were highly degraded compared to samples inoculated with a biomass from Mpumalanga source.
2. Biomass acclimation enhanced the treatabilty of organic waste.
3. The treatability of recalcitrant effluent was not improved by biomass acclimation.
4. Size and distillery are good candidates for codigestion. They are highly biodegradable and can be digested with recalcitrant effluent, provided it is adequately diluted to prevent permanent biomass inhibition.
5. Recalcitrant effluents can only be codigested at lower concentrations.

Pilot scale results

The pilot scale experiments did not produce good data. This could be attributed to the scale up problems

6.2 RECOMMENDATIONS

The following recommendations can be made based on the investigation.

- The biomass source and biomass acclimation must be included in the codigestion protocol.
- Future work on the process development must focus on the reactor design or reactor configuration.
- The reliability of the gas measuring system in the full scale process must be validated.
- The full-scale plant design should encompass the phase separation between sludge and supernatant.

List of References

Ahring, BK, Angelidaki, I and Johansen, K. 1992. *Anaerobic treatment of manure together with organic industrial waste. Wat Sci Tech* **(7)**:311-318.

Anderson, GK and Yang, G. 1992. *.Determination of bicarbonate alkalinity and volatile acid concentration in anaerobic digesters using a simple titration. Wat Environ Res* (64): 3-59.

Angelidaki, I and Ahring, BK. 1997. *Codigestion of olive oil mill wastewaters with manure, household waste or sewage sludge. Biodegradation (8)***:** 221-226.

APHA. 1995. *Standard Methods for the Examination of Water and Waste Water.* 20th Edition. American Public Health Association. Washington DC.

Bagley, DM and Gosset, J. 1995. *Chloroform degradation in Methanogenic Methanol Enrichment Cultures and by Methanosarcina Barkeri227. Appl Environ Microbiol.* (61): 3195-3201

Bhattachariya, S.K and Parkin, GF. 1986. *Toxicity of Nickel in Methane Fermentation System: Fate and Effect of Process Kinetics.* In: International Conf on Innovative Biological Treatment of Toxic Wastewaters, Arlington, VA, USA.

Banks, CJ and Humphreys, PN. 1998. *The anaerobic treatment of lingo-cellulose substrate offering little natural pH buffering capacity. Wat Sci Tech* 38(4/5):29-35.

Bell, J. 1997. *Anaerobic digestion of high strength or toxic organic effluents.* MScEng. Thesis, University of Natal (Durban), South Africa.

Brumeller, ET and Koster, IW. 1990. *Enhancement of dry anaerobic batch digestion of the organic fraction of municipal solid waste by anaerobic pretreatment step. Biol Wastes* 31(3):199-210.

Bryant, MP. 1979. Microbial *methane production "Theoretical aspects*". *Animal Sciences (48):*193-201.

Battesrby, NS. 2000). *The biodegradability and microbial toxicity testing of lubricants, some recommendations. Chemosphere* (41):1011-1027.

Birch, RR, Biver, C, Campagna, R, Gledhill, WE, Pagga, U, Steber, J, Reust, H and Boutinok, WJ. 1989. *Screening of chemicals for anaerobic biodegradability. Chemosphere* 19(10-11):1527-1550.

Buswell, A. 1939. *Anaerobic Fermentation. State Water Serv Bull. 32.*

Campos, E, Palatsi, J and Flotats, X. 1999. *Codigestion of pig slurry and organic wastes from food industry.* In: Proceedings of the second international symposium on anaerobic digestion of solid wastes, vol 2. Barcelona, Spain, 15-17 June, pp192-195.

Dolfing, J. 1988. *Acetogenesis, Biology of anaerobic microorganisms*, New York, John Wiley and Sons, Incorporated.

Field, J and Sierra, R. 1989. Biodegradability and toxicity lecture series (International Course of Anaerobic Wastewater Treatment). Dept of Water Pollution Control, Wageningen Agricultural University, Netherlands.

Fernandez, J.M, Mendez, R and Lema, J.M. 1995. *Anaerobic treatment of Eucalyptus fibreboard manufacturing waste water by a hybrid USBF lab scale reactor*. Environ. Technol. 16 (7):667-684.

Gossett, JM and Belser, RL. 1982. *Anaerobic digestion of waste activated sludge*. *ASCE J Environ Eng* (108):1101-1120.

Gujer, W and Zehnder, AJB. 1983. *Conversion processes in anaerobic digestion*. *Wat Sci Tech* (15):127-167.

Hendriksen, H.V, Larsen, S and Ahring, B.K. 1992. *Influence of supplemental carbon source on anaerobic dechlorination of pentachlorophenol in granular sludge*. *Appl Environ Microbial.* 58:36-370.

Kaspar, HF and Wuhrmann, K. 1978. *Kinetic parameters of relative turnovers of some important catabolic reactions in digester sludge*. *Appl Environ Microbiol* (36):1-7.

Kennedy, KJ and Van den Berg, L. 1982. *Anaerobic digestion of piggery wastes using stationery Film Fixed reactor*. *Agricultural Wastes* (4):151-158.

Lettinga, G. 1995. *Anaerobic digestion and wastewater treatment systems*: Antonie van Leeuwenhoek, (67):3-28.

McCarty, PL and Smith, DP. 1986. *Anaerobic wastewater treatment*. *Environ Sci Tech* (20):120-1206.

McCarty, PL. 1971. *Energetics and kinetics of anaerobic treatment: In; Anaerobic biological treatment processes*: *Advances Chem. Series*, (105):91-107.

McCarty, PL and Mosey, FE. 1991. *Modeling of anaerobic digestion process*. *Wat Sci Tech* (24):17-33.

Mosey, F.E. 1974. *Anaerobic digestion*. *Water Poll Contl Federation.* 12:24.

Owen, WF, Stucky, DC, Healy, JB, Young, LY and McCarty, PL. 1979. *Bioassay for monitoring biochemical methane potential and anaerobic toxicity.* *Wat Res* (13):485-492.

Remigi, ER. 2001. *Activity and Biodegradability assessment under anaerobic conditions, PhD Thesis*, Politecnico di Milano (Italy).

Ross, W.R.P and Norella, H. 1992. *Anaerobic digestion of Waste water sludge: Operation guide.*

Rozzi, A and Remigi, E.U. 2004. *Methods of Assessing Microbial Activity and Inhibition under Anaerobic conditions: A Literature Review*. *Environ Sci. Biotechnol*. 3(21):93-115.

Schink, B. 1988. Principles and limits of anaerobic degradation**:** *Environmental and technological aspects: In: Zehnder, AJB (Ed): Biology of anaerobic microorganisms, Chapter 14, A Wiley Interscience Publication, John Wiley & Sons.*

Speece, R.E. 1983. *Anaerobic Biotechnology for Industrial Wastewaters*. *Environ. Sci. Tech* 17(9):416A-427A.

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

Switzenbaum, M.S. 1986. *Obstacles in the implementation of anaerobic treatment technology*. *Bioresource Technol*., 53:255-262.

Tapp, M.D.J. 1981. *A commercial biogas producing plant*. In: Vogt F (Ed) *Energy conservation and use of renewable energies in the bioindustries. Pergamon, Oxford*: pp473.

Van den Berg, L, Lentz, L.C.P, Anthony, RJ and Rooke, EA. 1974. *Assessment of methanogenic biomass in anaerobic sludges*. *J Watt Poll Control Fed* (55): 1191-1195**.**

Van Handel, A.C and Lettinga, G. 1994. *Anaerobic sewage treatment*, Wiley, London UK.

Water Research Commission Report K51/1074. 2005. *Codigestion of high strength/toxic organic effluents in Anaerobic digester at Water Treatment Works.*

Yang, J, Speece, R.E, Parkin, G.F, Gosset, J and Kocher, W. 1980. *The response of methane fermentation to cyanide and chloroform. Prog Wat Tech, IAWP* ((12): 977-989.

Yang, H.W and Yang, B.S. 1989. *Design consideration for full scale Anaerobic filter*. *Journal of Wat Poll Contl Fed,* 16:1576**.**

Young, J and Tabak, H.H. 1991. *Multilevel protocol for assessing the fate and effect of toxic organic chemicals in anaerobic treatment processes*. *Wat Environ Res***,** 65(1): 34-35.

Zehnder, A.J.B, Ingroversen, K and Marti, T. 1982. *Microbiology of Methane bacteria*. *Elsevier, Biomedical Pres*, (13): 45-68.

Zinder, S.H. 1984. *Microbiology of anaerobic conversion or organic wastes to methane, recent developments.* ASM Nens, (50): 294-298.

Appendix A

A1. THEORETICAL METHODS

A.1.1 ORGAINC CARBON CONTENT

The theoretical COD of a compound with a known chemical composition can be calculated as follows:

$$Cr_2O_7^{2-} + 8H^+ \rightarrow 2Cr^{3+} + 4H_2O + 3O \quad \text{(A.1)}$$

$$C_cH_hO_oN_n + \alpha Cr_2O_7^{2-} + (8\alpha + n))* H \rightarrow cCO_2 + \frac{h + 8\alpha - 3n}{2} * (H_2O + N)* NH_4^+ + 2\alpha.Cr^{3+} \quad \text{(A.2)}$$

Where $\alpha = \frac{2}{3}c + \frac{1}{6}h - \frac{1}{3}o - \frac{1}{2}n$ (A.3)

$$COD = \frac{3}{2}\alpha molO_2 \quad \text{(A.4)}$$

Sample calculation

An empirical formula for biomass is $C_5H_7O_2N$ and substituting c=5, h=7, o=2 and n=1 in equation (A.3) and equation A.4.

$\alpha = \frac{10}{3}$ and 1 mol $C_5H_7O_2N = \frac{3}{2}\alpha$, =5mol$O_2$

Considering the formula weight of the biomass (113 g/mol), this translate to 1.416 gCOD/g biomass.

The application of this calculation is illustrated in **Table A.1**.

Table.A.1 COD estimation from chemical formula

Compound	Formula	Molecular weight (g/mol)	Equivalent COD (gCOD/g)
Biomass	$C_7H_7O_2N$	113	1.416
Ethanol	C_2H_5OH	46	2.087

The COD of the biomass and industrial effluents in this work were determined experimentally (APHA, 1999) and appropriate dilutions were performed to achieve the required concentrations.

A.2 REAGENTS PREPARATION

The 0.1MHCℓ and 0.1M NaOH titrants for Alkalinity and VFA concentrations were prepared as follows.

A.2.1 Sodium hydroxide

The molar mass of NaOH is 40 g/mol. In order to prepare 0.1 M (mol/ ℓ) in 1 ℓ the mass needed to be weighed and dissolved in 1 ℓ is:

$$m = nM_m$$

=0.1 (mol/ ℓ) *40 g/mol

=4 g/ ℓ

Hence 4 g of 98% NaOH pellets was dissolved in 1 ℓ to prepare a concentration of 0.1 M.

A.2.2 Hydrochloric acid

The 32% w/w) concentrated of 0.1 M HCℓ used as an acid titrant was prepared in the following way:

$$C_{HCL} = \rho S M_m$$

Where C_{HCL} is the acid concentration

S is the percentage strength of the acid

M_m is the molar mass

= (1.16*10^3 g reagent/ ℓ reagent)* (32 gHC ℓ)/100 g reagent *1mol/36.45 gHCℓ

=10.1838 N

$$n = CV$$

=0.1 M*1 ℓ

=0.1 mol

$$C_1V_1 = C_2V_2$$

$$V_1 = \frac{C_2V_2}{C_1}$$

= (0.1 M *1 ℓ)/10.1838 M

=0.009819517 ℓ

=9.82 mℓ HCℓ

To prepare 0.1 M HCl titrant, 9.82 mℓ HCL was diluted to 1 ℓ of distilled water.

Appendix B

B.1 CALIBRATION OF GAS CHROMATOGRAPH

The accuracy of a gas chromatography in practice is limited by the accuracy of the peak area evaluation and by the accuracy of the calibration method. In this study calibration curves were prepared by injecting increasing volumes of high purity methane (CH_4), nitrogen (N_2) and carbon dioxide (CO_2).

B2. GC METHOD

The biogas composition was analysed with a GOW MAC 350 gas chromatograph, equipped with a thermal conductivity detector (TCD) which can 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, according to the following conditions:

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 retention 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- or pressure-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. To set-up the integrator, the following procedure is to be used

- Switch the power on; then press the DIALOG key.
- Enter a FILE NAME if desired, and then press ENTER.

The batch program is ready to acquire the set of parameters which are needed to interpret the chromatogram and automatically integrate the peak areas. A matrix of (5 x 3) parameters is to be entered. The three parameters indicate the times (TT and TV) and the codes (TF) which identify each one of five distinct events. E.g. should the detector be receiving a signal

stronger than the background after 5 min (TT: 5), this would be treated as an error (TF: ER) and the detection period would be terminated (Line 5 in the sequence corresponds to the 'safety' termination).

Press ENTER after each entry, to prompt the next parameter in the sequence.

	TT	**TF**	**TV**
Line 1	0.01	AZ	1
Line 2	0.01	CS	0.5
Line 3	0.01	PM	1
Line 4	0.01	AT	1
Line 5	5	ER	5

At the next prompt, press ENTER to exit.

Press ENTER, to END DIALOG.

Press PRINT FILE to display program code

The calibration of the gas chromatograph was performed using pure gases (UHP, Afrox Scientific). Gas samples of given volumes were extracted by inserting the gas-lock syringe into a butyl rubber septum positioned on the outlet of the gas bottle. The flow rate 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.

- 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 to 35 injections, to prevent gas leaks through the injection port.
- Check the reading on the pressure gauge and ensure that there is no fluctuation.
- Record the ambient temperature and the gauge pressure.
- extract a sample of the gas ($i = N_2$, 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 in the GC and wait for integrator analysis.
- Record the retention time (RT) and Area ($A_{i,j}$) of the gas with the highest area.
- Repeat steps 5 to 7, for all the j volumes of the first gas, from the lowest to the highest volume.

- Repeat steps 5 to 7, a number of times sufficient to generate a statistically significant set of data for the first gas (five replicates should be sufficient).
- Repeat steps 5 to 9, for the other gases.

B.2.1 Precautions taken during calibration

In order to generate reproducible results during calibration, the injected volumes must be as accurate as possible during injection. Good quality syringes must be used and checked regularly for blockages. The tightness of the piston plunger seal for the gas must be checked regularly. For each calibration sample, the component used must be flushed at least three times before withdrawing the final sample to be injected on the GC. This is done to remove impurities within the needle and syringe as well as to prevent entrainment of air bubbles in the syringe.

The GC injector septa must be replaced after every 30 injections. This is done to avoid errors due to septum leakage, which could be detected by a drop in the column head pressure.

B3. GAS COMPOSITION ANALYSES

1. The biogas composition was determined from the gas chromatograms.
2. The volume of biogas was corrected to STP.
3. Biogas samples were analysed for nitrogen, methane and carbon dioxide.
4. The peak area of each component was recorded.

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

$$PV = nRT \qquad \text{(B.1)}$$

Where P 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:

$$P_1 = (P_{atm} - P_w).x_i \qquad \text{(B. 2)}$$

Where P_{atm} is the atmospheric pressure: 1.013 bar, P_w is the water vapour pressure (bar); xi is the molar fraction of each constituent (mol/mol).

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

$$Pw = 3.225 * 10^9 * e^{0.05393T} \qquad \text{(B.3)}$$

B.4 GC METHOD APPICATION

The raw data for the GC calibration on methane and on carbon dioxide are reported in **Tables B.1** and **B.2**. The respective calibration curves are plotted in **Fig B.1** and **B.2**: the data points (diamonds) are the average values of each set of replicates (column *Ave*, in the Tables); the solid lines represent the 95 % confidence intervals for the calibration curve (the slopes and intercepts of the regression lines are indicated below the Figures, as *calibration factor* and *detection limit*). As one can see, the determination coefficient of the regression is very high ($R^2 \geq 99.9\%$) and the detection limits very low ($v_0 \leq 5$ µℓ).

Table B.1 – Raw data for the calibration curve of methane.

Volume	Area					Ave	StDev	VC
(µℓ)	1st	2nd	3rd	4th	5th			(%)
10	4 183	4 150	4 495	4 269	4 343	4 288	138	3
20	10 557	11 244	11 047	11 380	11 320	11 110	333	3
30	15 299	16 226	15 939	17 284	17 316	16 413	877	5
40	22 471	22 565	22 609	23 659	23 800	23 021	651	3
50	29 938	29 481	29 579	30 229	30 209	29 887	347	1
60	36 069	35 810	36 004	36 624	36 724	36 246	404	1

Table B.2 – Raw data for the calibration curve of carbon dioxide.

Volume	Area					Ave	StDev	VC
(µℓ)	1st	2nd	3rd	4th	5th			(%)
10	6 196	5 755	5 763	5 269	5 421	5 681	359	6
20	15 015	14 192	13 920	13 866	14 088	14 216	465	3
30	21 677	23 511	22 056	21 035	21 687	21 993	925	4
40	29 753	31 234	31 949	29 617	29 354	30 381	1 142	4
50	41 226	40 265	38 024	37 575	37 632	38 944	1 688	4
60	47 785	45 063	n.p.	n.p.	n.p.	46 424	1 925	4

n.p., not performed; Ave, average; StDev, standard deviation; VC, variation coefficient

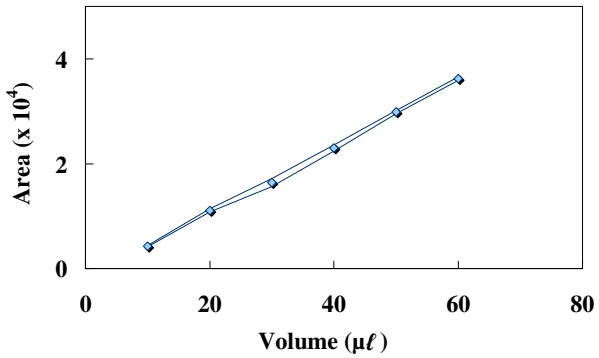


Figure B.1 – Calibration curve for methane.
Calibration factor: 636 Area/µℓ (R^2 99.90 %)
Detection limit: 3 µℓ

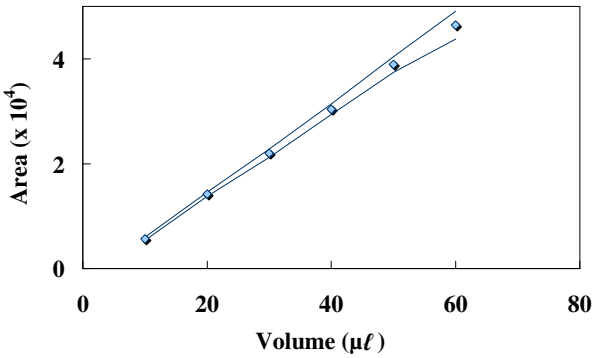


Figure B.2 – Calibration curve for carbon dioxide.
Calibration factor: 818 Area/µℓ (R^2 99.97 %)
Detection limit: 3 µℓ

Appendix C

ANALYTICAL PROCEDURES

C.1. COD MEASUREMENTS

The method used was standard open reflux (American Public Health Association, 1985). The COD reagents were prepared as described in **Table 3.1.** This method is based on the fact that most organic matter is oxidized by a boiling mixture of chromic and sulphuric acids. A sample is refluxed in strongly acidic solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the remaining unreduced dichromate is titrated with ferrous ammonium sulphate (FAS) to determine the amount of $K_2Cr_2O_7$ consumed and the oxidisable organic matter is calculated in terms of oxygen equivalent.

C.1.1 Procedure

- Place 50 mℓ of appropriate diluted sample in a 500 mℓ refluxing flask. Add 1 g $HgSO_4$, several glass beads and slowly add 5 mℓ sulphuric acid reagent, with mixing to dissolve $HgSO_4$.
- Add remaining sulphuric acid reagent (70 mℓ) through the cooling coils of the open end of condenser.
- Cover open end of the condenser with a small beaker to prevent foreign material from entering refluxing mixture and reflux for 2 h.
- Cool to room temperature and titrate excess (unreduced) $K_2Cr_2O_7$ with FAS, using 2 to 3 drops ferroin indicator.
- The end point of the titration is the first sharp colour change, from blue-green to reddish brown.
- Repeat the same procedure for your blank reagent. The recipe to prepare the COD reagents is reported in **Table 3.2**

C.2 COD CALCULATION

C.2.1. Standardisation of $K_2Cr_2O_7$

Dilute 10 mℓ standard $K_2Cr_2O_7$ to about 100 mℓ. Add 30 mℓ concentrated H_2SO_4 and cool. Titrate with FAS titrant using 2 to 3 drops ferroin indicator. Molarity of the FAS solution is determined as follows:

Molarity is the volume of 0.0471 M $K_2C_{r2}0_7$ solution titrated

$$COD(mgO_2/l) = \frac{(A-B)*M*8000}{mlsample}$$

Where:

A = $m\ell$ FAS used for blanks

B = $m\ell$ FAS used for sample

M = molarity of FAS

C3 TWO POINT TITRATION METHOD

A spreadsheet based on the two-point titration method developed by Anderson & Yang (1992) to determine the alkalinity and VFA concentration was implemented **(Table C.1)** to analyse titration data and is described hereafter

Table C.1. Two point titration method for VFA and alkalinity

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:

$; H_o = 10^{-pH}$ $H_1 = 10^{-5.1}$; $H_2 = 10^{-3.5}$

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

Calculate the following:

$$; A_1 = \frac{H_1 - H_0}{K_2 + H_1} \qquad ; B_1 = \frac{H_1 - H_0}{K_1 + H_1} \qquad c_1 = \frac{V_1 c}{V}.\left(1^0 = 1,2\right) \quad \text{(i = 1, 2)}$$

Table C: 2 Outputs of the two point titration method

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

Analytical procedure

- Withdraw a liquid sample from the reactor (e.g. 50 mℓ) and place it in the beaker.
- Measure the pH of a sample, using an accurate (recently calibrated pH meter)
- Note the starting pH value
- Use a strong acid titrant. Counter titrate it to determine its concentration
- Fill up the burette with the titrant, place the beaker containing a sample under on a magnetic stirrer to keep it homogenized during the procedure.
- Use a strong acid titrant. Counter titrate it to determine its concentration.
- Insert the pH electrode into the sample, to continuously monitor the pH during titration.
- Start titrating, until you reach the first titration point (pH5.1) and record the titrated volume.
- Continue titrating until you reach the second titration point (pH3.5) and record the volume.

The bicarbonate alkalinity and VFAs are then calculated as per **Table C.2**.

Appendix D

This section contains the results of the experiments that are discussed in **Chapter 4**. Anaerobic activity tests (AAT) on Mpumalanga reactive dye, using biomass from different sources are presented in **Section D.1.1**.
Anaerobic activity tests (AAT) on industrial effluents namely size, distillery, scour dye and synthetic dye aimed at assessing the effect of biomass acclimation are reported in **(Section D.1.2 to D.1.4)**. The results are particularly interesting in that they clearly indicate that the biomass was eventually able to adapt to the initially inhibiting substances present in the effluent.

Anaerobic activity tests (AAT) which were aimed at assessing the sludge methanogenic activity (SMA) are reported in **Section D.1.5 to D.1.6**. Lastly the method of the accurate calculation of the mass balances in serum bottle activity tests is discussed in **Section D.1.7**.

A consistent format has been used to report the outcome of the assays. Firstly the composition of the serum bottles, for each test is reported in the form of a table, by listing all constituents and a few parameters of interest that characterize each group of replicates. Each column in theses tables indicates a group of replicates (for reproducibility)

D.1 SERUM BOTTLES (BIOMASS SOURCE)

This Section presents the detailed results of the first test conducted on the reactive dye effluent. **Table D.1** reports the quantities of each component that were added to the vials, in the section *constituents*; and some *parameters* of interest which characterise each group of replicates. Each column in the **Table D.1** indicates a group of three replicates. In the following pages, the outcomes of the 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. As discussed in **Chapter 4**, the AAT was initially designed to evaluate the inhibitory effect of the test materials on the aceticlastic biomass. Therefore, two 'functionally' different types of organic substrate are present in each unit, i.e. acetate as reference and the respective test materials. The test compound was Mpumalanga reactive dye supplemented on serum bottles

inoculated with the biomass from Mpumalanga digester 1, Mpumalanga digester 2 and Umbilo secondary digester.

Table D.1 – Composition of the serum bottles for the activity test on Mpumalanga dyes

Constituents		**Controls**			**Mpumalanga 1**			**Mpumalanga 2**			**Umbilo**		
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	0	0	0.5	2.5	5	0.5	2.5	5	0.5	2.5	5
Concentration	%	0	0	0	1	5	10	1	5	10	1	5	10
Parameters													
Total volume	mℓ	50	50	50	50.5	52.5	55	50.5	52.5	55	50.5	52.5	55
Organic m. [(1)]	gℓ	5.46	5.46	5.46	5.42	5.28	5.12	5.43	5.28	5.12	5.42	5.28	5.12
Solids [(2)]	gℓ	34.05	34	20.1	34.05	34.05	34.05	34	34	34	20.1	20.1	20.1
S-to-X	-	0.2	0.2	0.34	0.2	0.2	0.21	0.2	0.2	0.21	0.34	0.35	0.35
G-to-L	%	1.5	1.5	1.5	1.48	1.38	1.27	1.45	1.38	1.27	1.48	1.38	1.27
Theo CH_4	mL	105.1	105.1	105.1	105.4	106.8	109	105.4	107	108	105.4	107	109

[(1)] in COD units;
[(2)] expressed as Volatile Solids (VS).

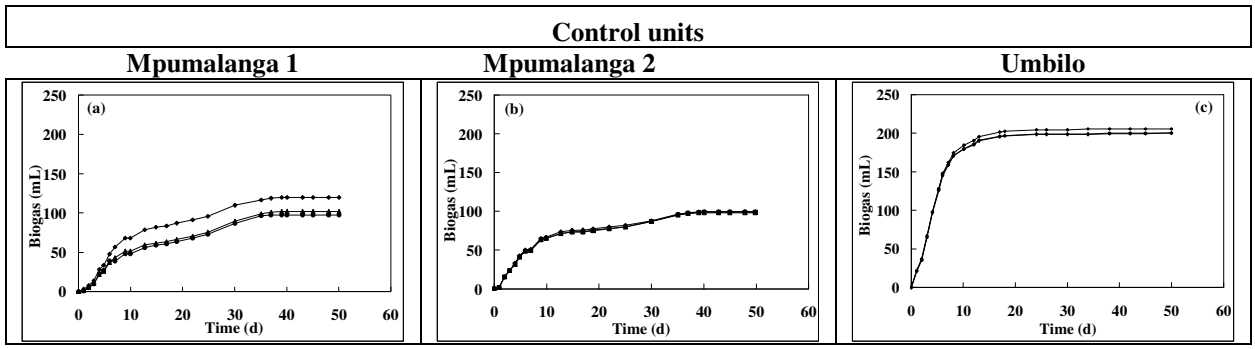


Figure D.1 – Biogas production.

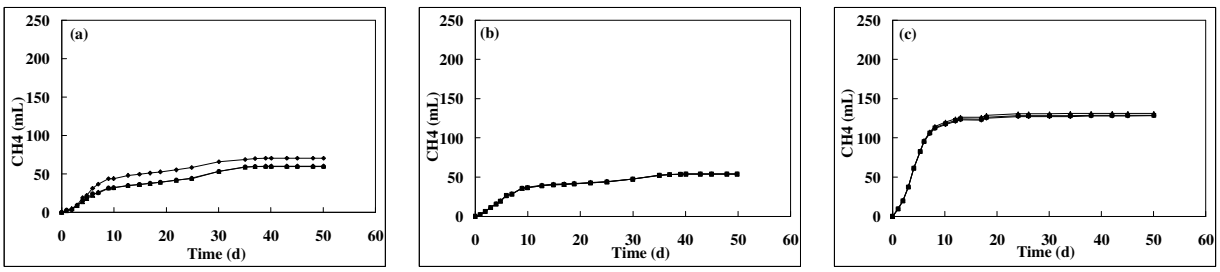


Figure D.2 – Methane production.

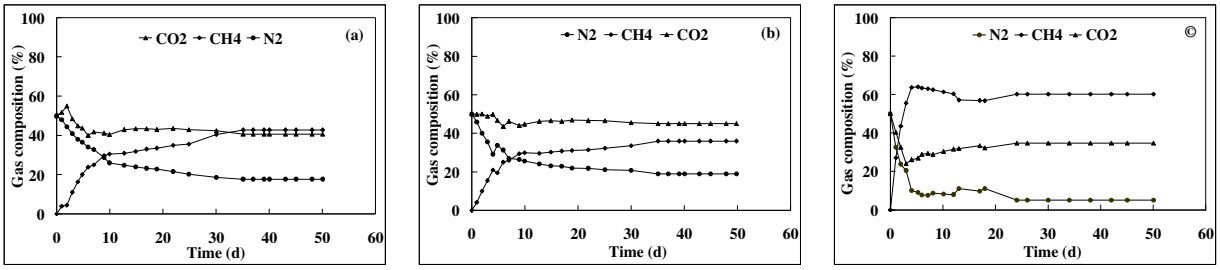


Figure D.3 – Gas composition.

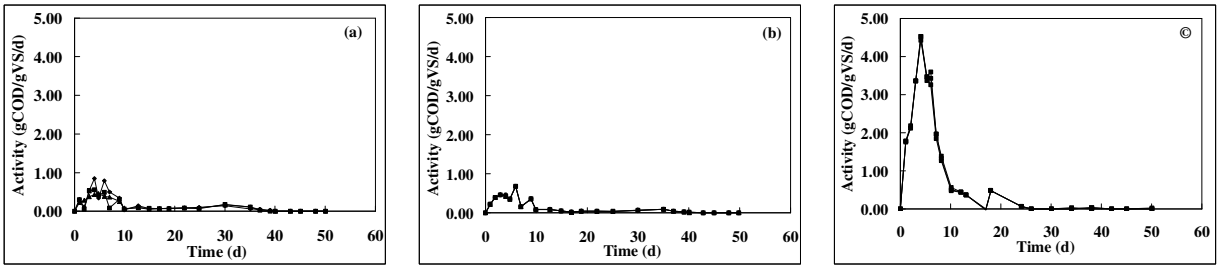


Figure D.4 – Specific methanogenic activity.

Test compound: Reactive dye (Mpumalanga 1)		
1% (Low)	**5% (Medium)**	**10% (High)**

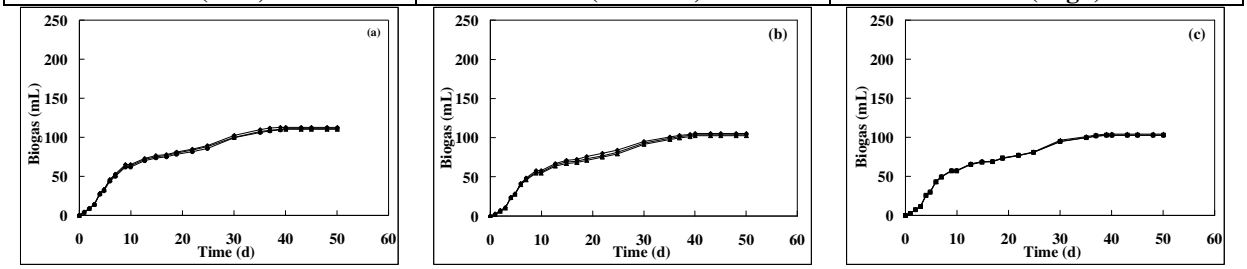


Figure D.5 – Biogas production.

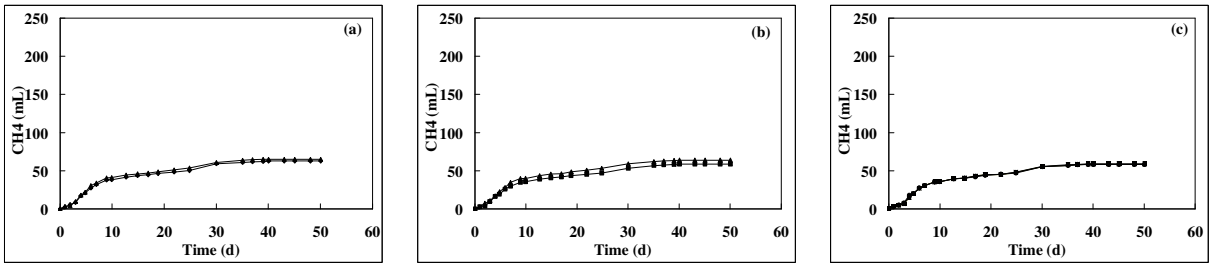


Figure D.6 – Methane production.

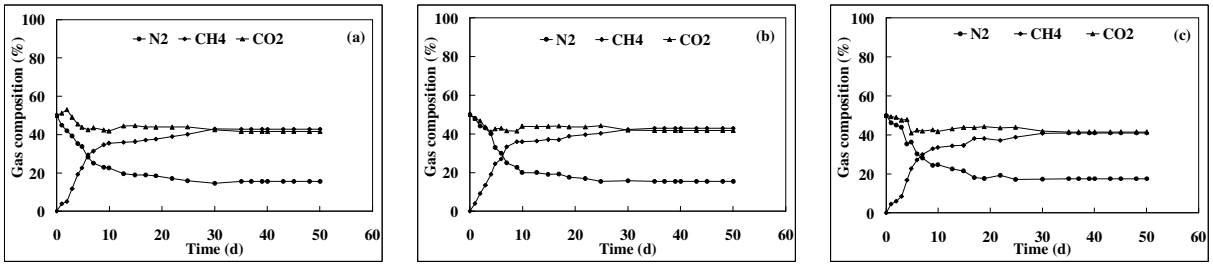


Figure D.7 – Gas composition.

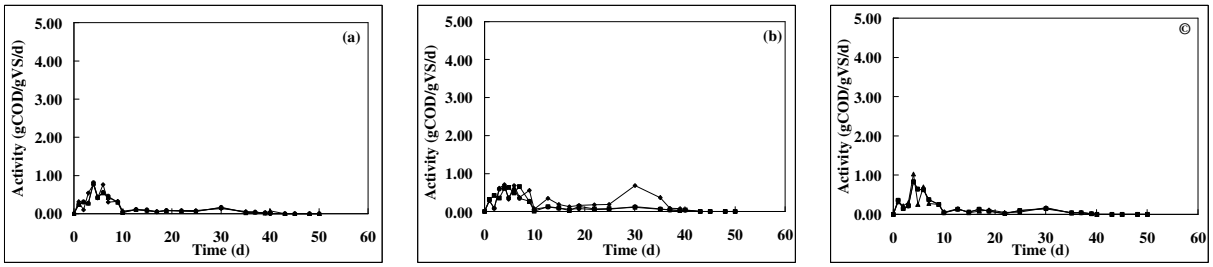


Figure D.8 – Specific methanogenic activity.

Test compound: Reactive dye (Mpumalanga 2)		
1% (Low)	**5% (Medium)**	**10% (High)**

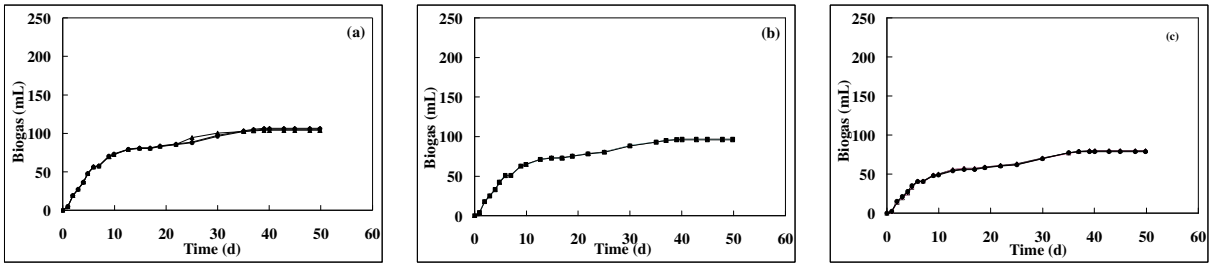


Figure D.9 – Biogas production.

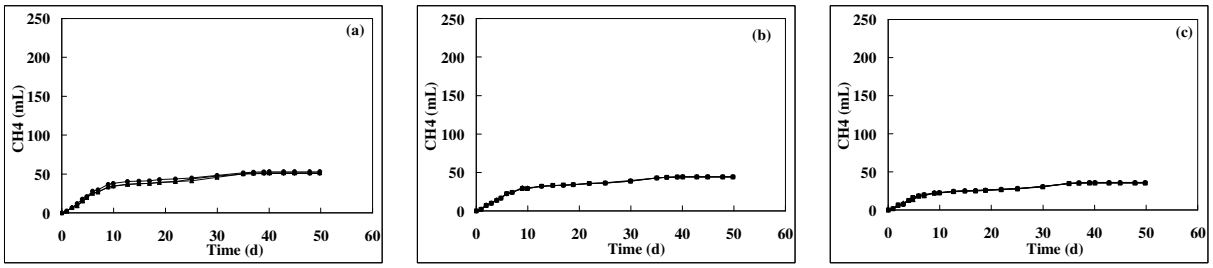


Figure D.10 – Methane production.

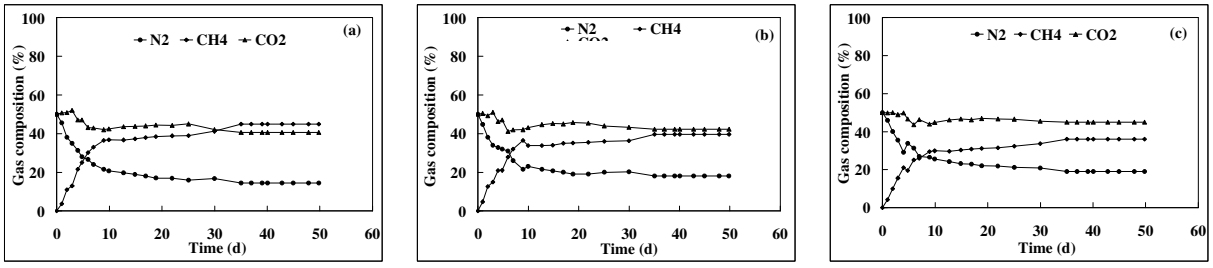


Figure D.11 – Gas composition.

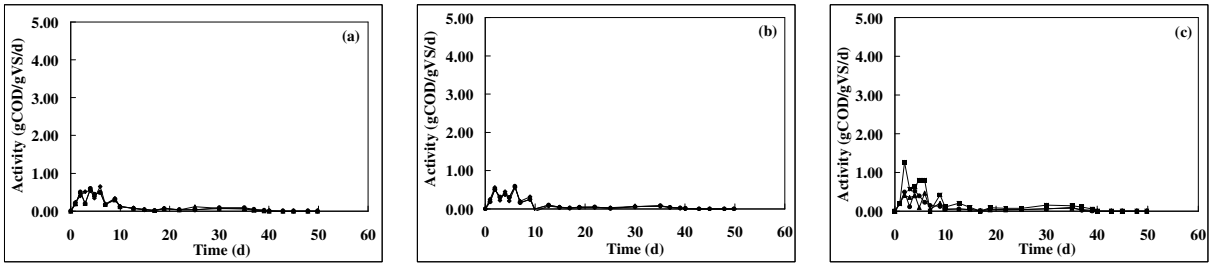


Figure D.12 – Specific methanogenic activity.

Test compound: Reactive dye (Umbilo)		
1% (Low)	**5% (Medium)**	**10% (High)**

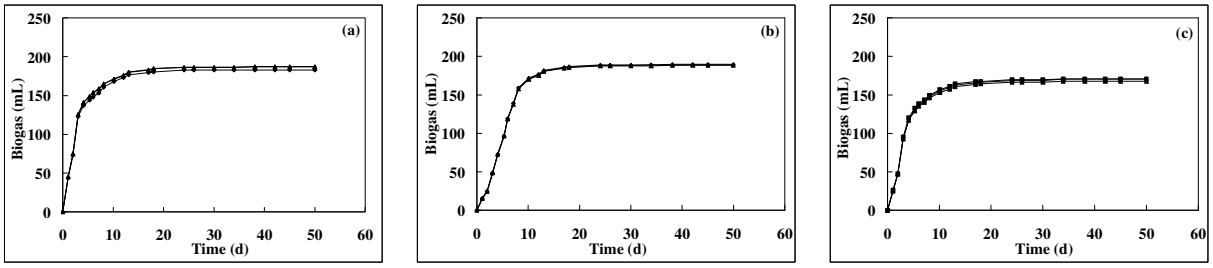


Figure D.13 – Biogas production.

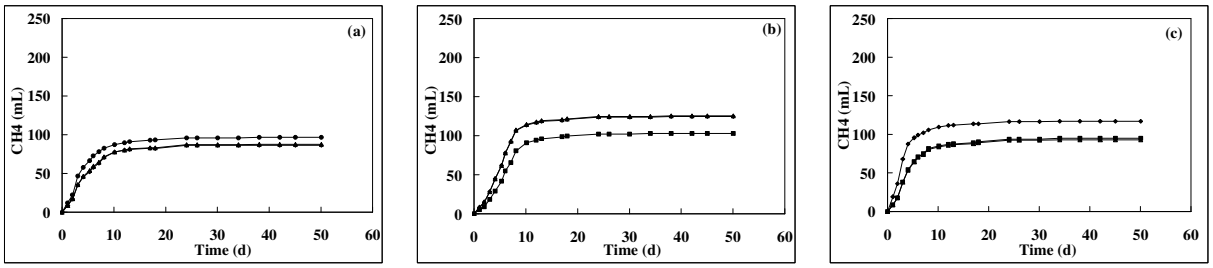


Figure D.14 – Methane production.

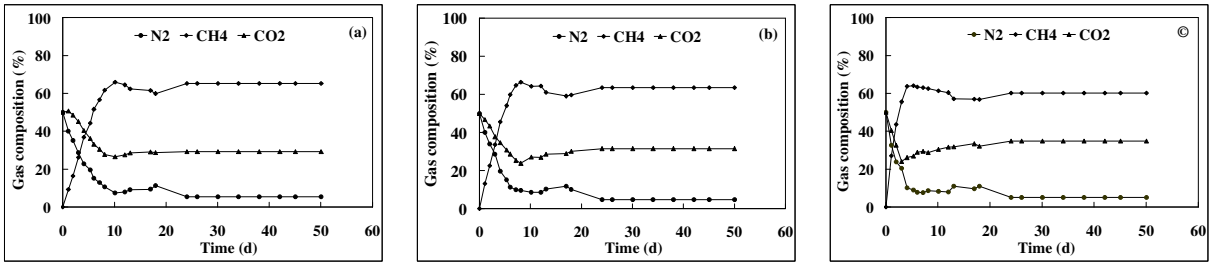


Figure D.15 – Gas composition.

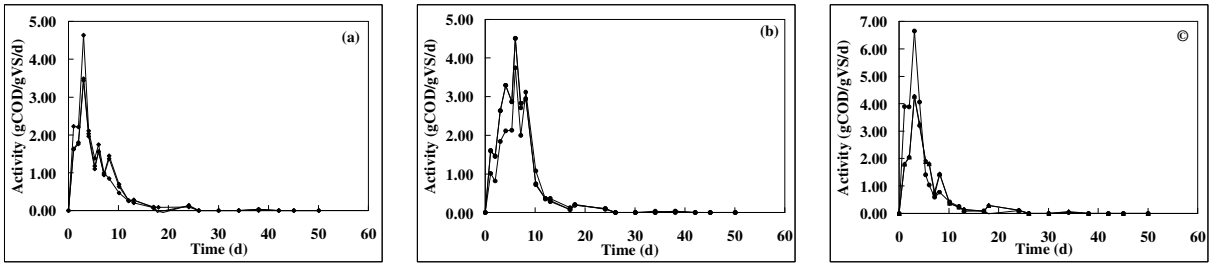


Figure D.16 – Specific methanogenic activity.

D.2 SERUM BOTTLES STUDIES (BIOMASS ACCLIMATION)

This Section presents the detailed results of the activity tests conducted on the industrial effluent namely distillery, size and scour dye effluent. **Table D.2** and **Table D.3** reports the quantities of each components that were added to the vials, in the section *constituents*; and some *parameters* of interest which characterise each group of replicates. Each column in the **Table D.2** and **Table D.3** indicates a group of three replicates. In the subsequent pages, the outcomes of groups, 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. The activity tests presented here were aimed at assessing the effect of biomass acclimation on inhibition and biodegradability. Therefore, two 'functionally' different types of organic substrate are present in each unit, i.e. acetate as reference and the respective test materials.

Table D.2 – Composition of the serum bottles for the activity tests on industrial effluents

Constituents		**Controls**			**Distillery**			**Size**			**Scour dye**		
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	0	0	0.5	2.5	5	0.5	2.5	5	0.5	2.5	5
Concentration	%	0	0	0	1	5	10	1	5	10	1	5	10
Parameters													
Total volume	mℓ	50	50	50	50.5	52.5	55	50.5	52.5	55	50.5	52.5	55
Organic m. [(1)]	gℓ	5.46	5.46	5.46	6.58	10.86	15.76	6.18	8.92	12.10	5.45	5.4	5.34
Solids [(2)]	gℓ	34.05	34	20.1	19.32	19.32	19.32	19.08	19.08	19.08	17.26	17.26	17.26
S-to-X	-	0.2	0.2	0.34	0.43	0.74	1.12	0.41	0.61	0.87	0.4	0.41	0.43
G-to-L	%	1.5	1.5	1.5	1.47	1.38	1.27	1.48	1.38	1.27	1.48	1.38	1.27
Theo CH_4	mL	105.1	105.1	105.1	128	220	334	120	180	255	106	109	113

[(1)] in COD units;
[(2)] expressed as Volatile Solids (VS).

Table D.3 – Composition of the serum bottles for the activity tests on industrial effluents

Constituents		Controls			Distillery			Size			Scour dye		
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	0	0	5	15	35	5	15	35	5	15	35
Concentration	%	0	0	0	9	23	41	9	23	41	9	23	41
Parameters													
Total volume	mℓ	50	50	50	55	65	85	55	65	85	55	65	85
Organic m. [(1)]	g/ℓ	5.46	5.46	5.46	15.76	31.62	52.13	12.1	22.22	35.37	5.34	5.15	4.9
Solids [(2)]	g/ℓ	34.05	34	20.1	19.32	19.32	19.32	19.08	19.08	19.08	17.26	17.26	17.26
S-to-X	-	0.2	0.2	0.34	1.12	2.66	5.73	0.87	1.89	3.94	0.53	0.61	0.76
G-to-L	%	1.5	1.5	1.5	1.27	0.92	0.47	1.27	0.92	0.47	1.27	0.92	0.47
Theo CH_4	mL	105.1	105.1	105.1	334	791	1706	255	556	1158	113	129	160

[(1)] in COD units;
[(2)] expressed as Volatile Solids (VS)

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

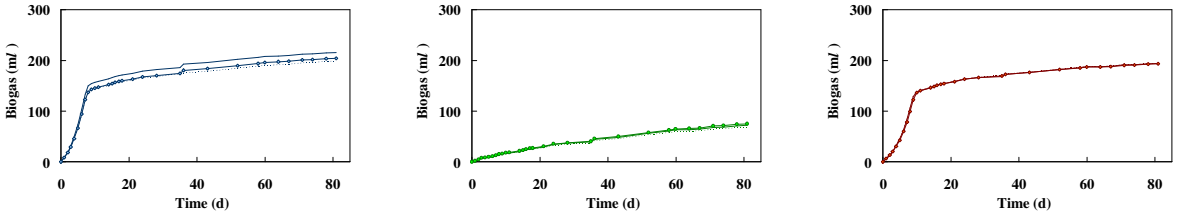


Figure D.17 – Biogas production.

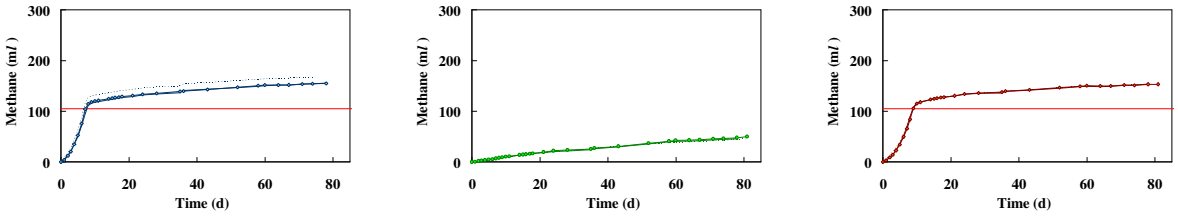


Figure D.18 – Methane production.

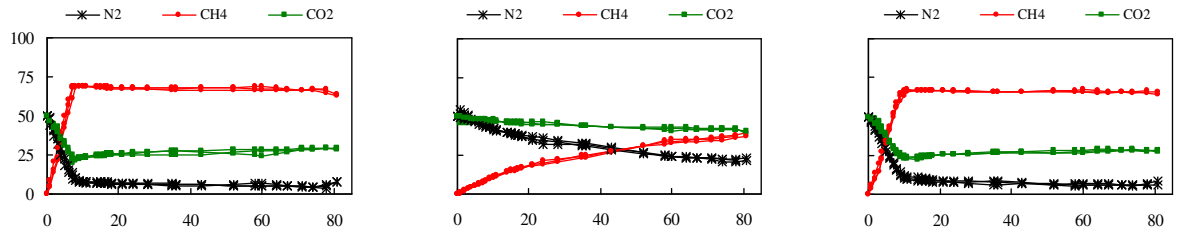


Figure D.19 – Gas composition.

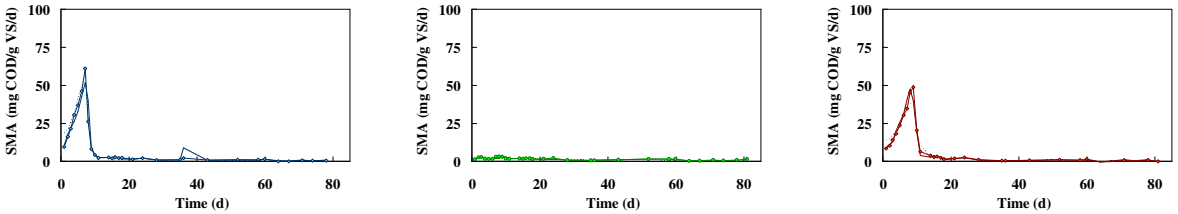


Figure D.20 – Specific methanogenic activity.

Test compound: Distillery effluent		
1% (Low)	**5% (Medium)**	**10% (High)**

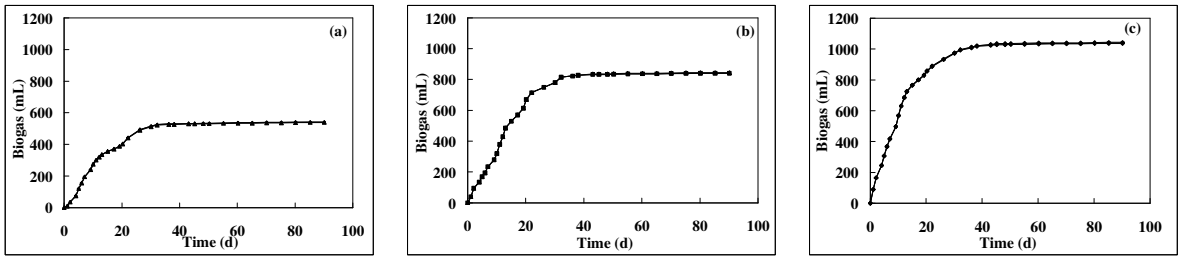


Figure D.21 – Biogas production.

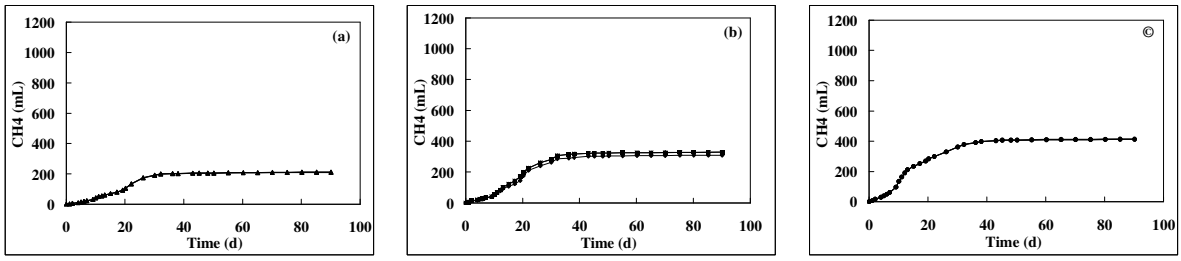


Figure D.22 – Methane production.

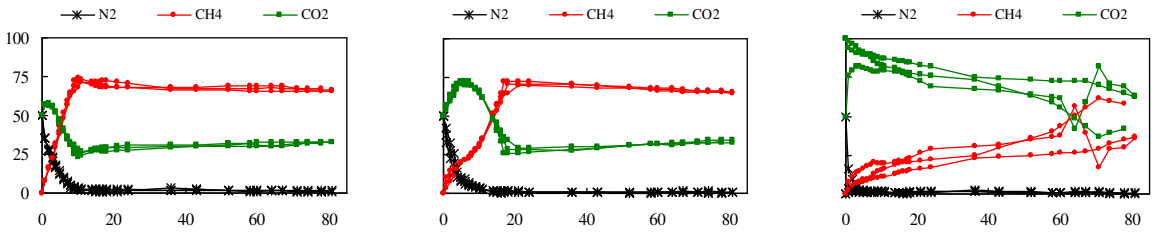


Figure D.23 – Gas composition.

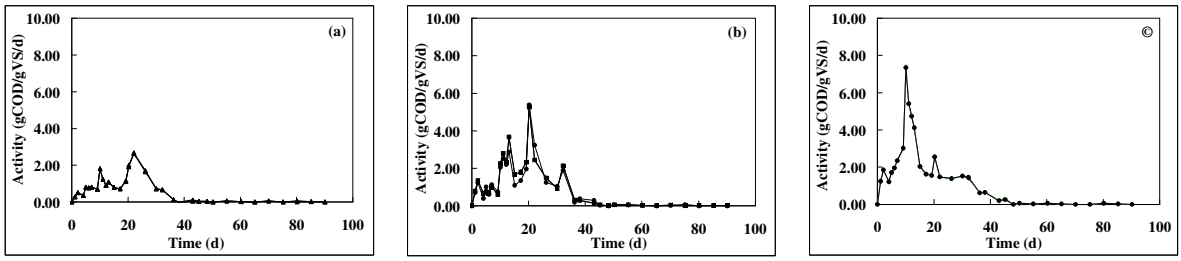


Figure D.24 – Specific methanogenic activity.

Test compound: Size effluent		
1% (Low)	5% (Medium)	10% (High)

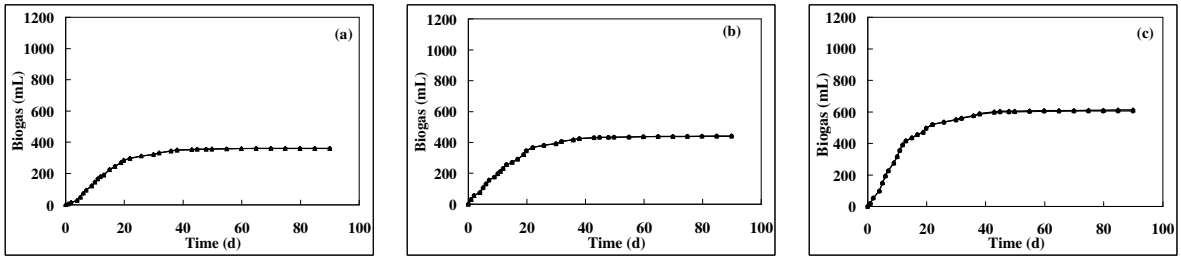


Figure D.25 – Biogas production.

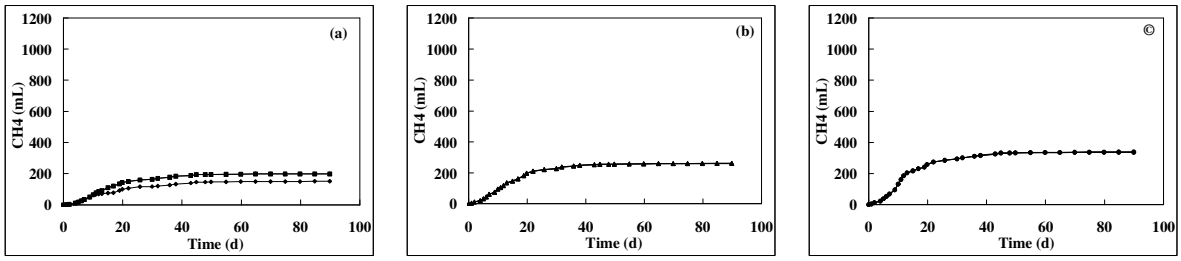


Figure D.26 – Methane production.

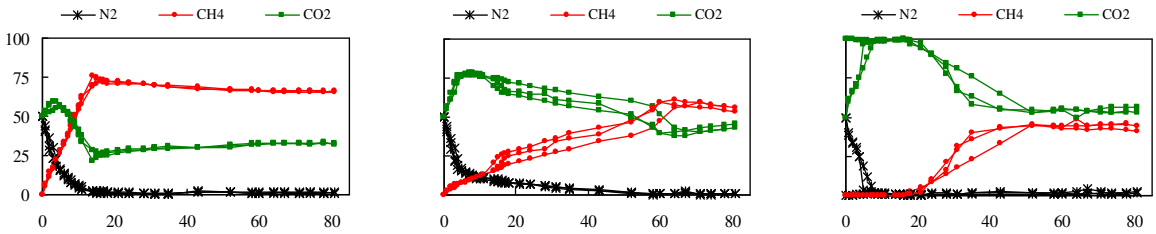


Figure D.27 – Gas composition.

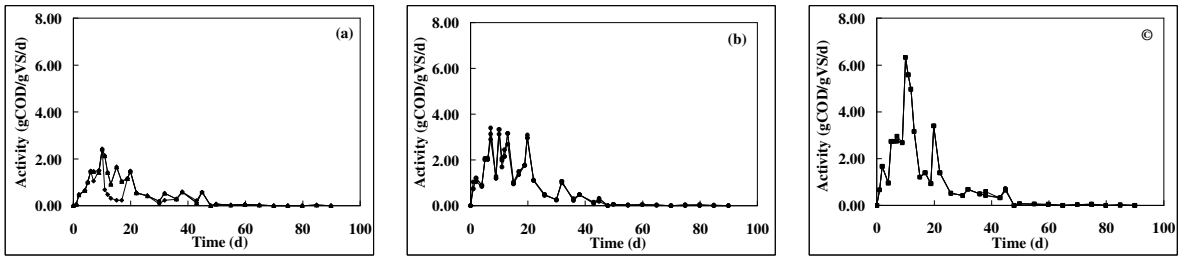


Figure D.28 – Specific methanogenic activity.

Test compound: Scour effluent		
1% (Low)	**5% (Medium)**	**10% (High)**

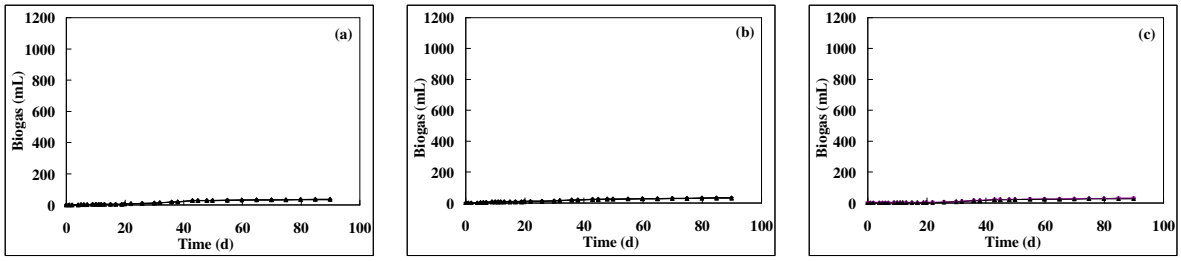


Figure D.29 – Biogas production.

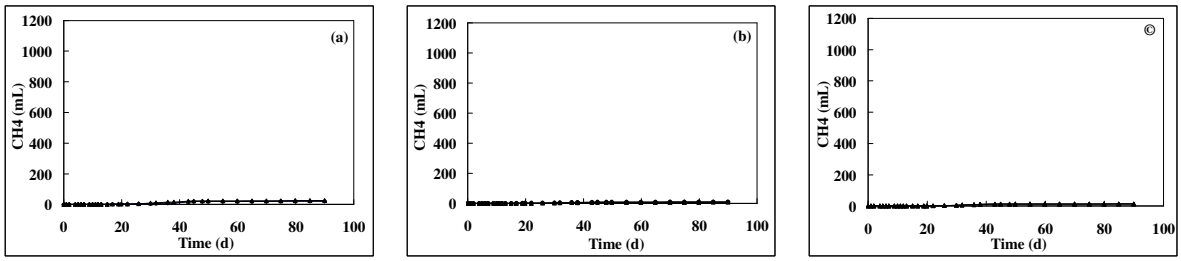


Figure D.30 – Methane production.

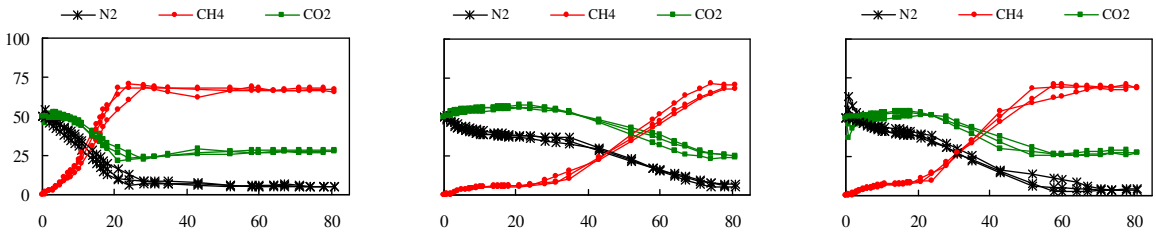


Figure D.31 – Gas composition.

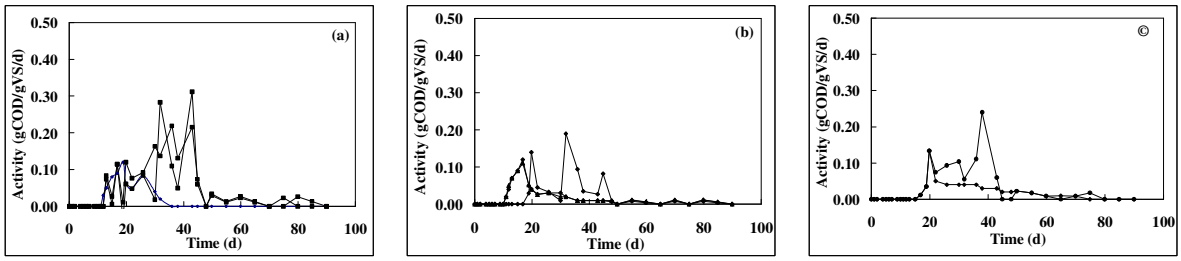


Figure D.32 – Specific methanogenic activity.

Test compound: Distillery effluent		
9% (Low)	**23% (Medium)**	**41% (High)**

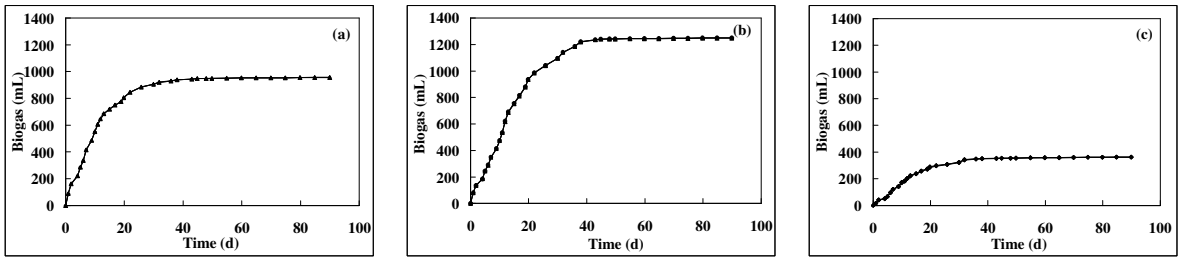


Figure D.33 – Biogas production.

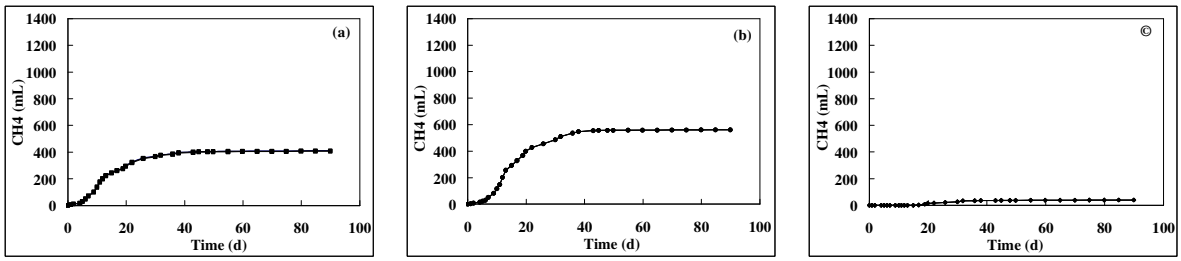


Figure D.34 – Methane production.

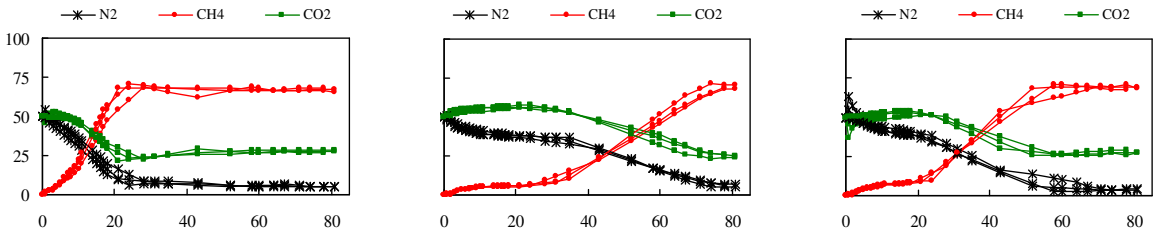


Figure D.35 – Gas composition.

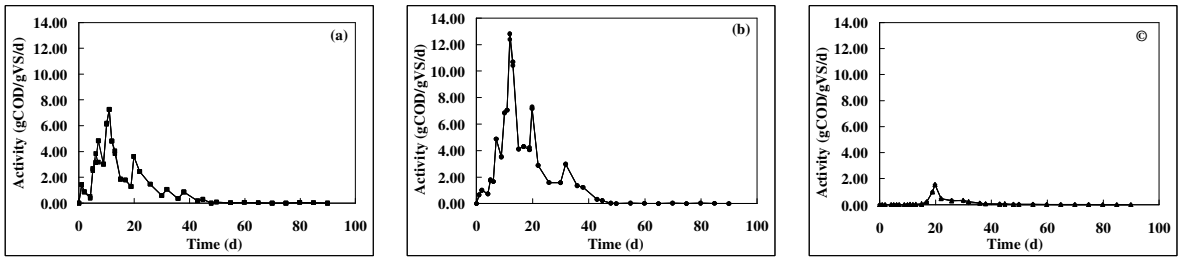


Figure D.36 – Specific methanogenic activity.

Test compound: Size effluent		
9% (Low)	**23% (Medium)**	**41% (High)**

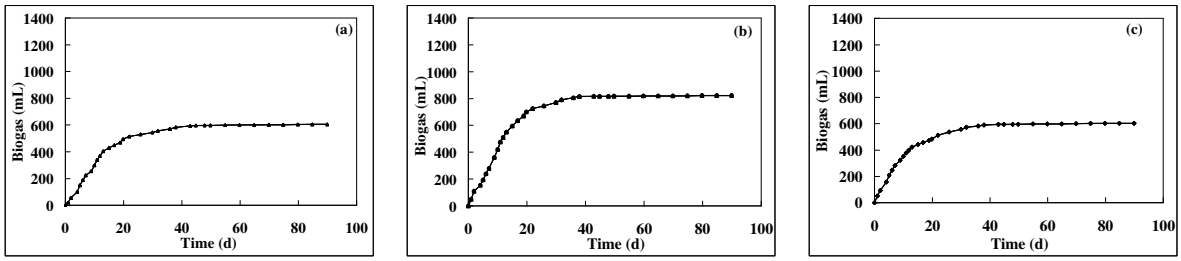


Figure D.37 – Biogas production.

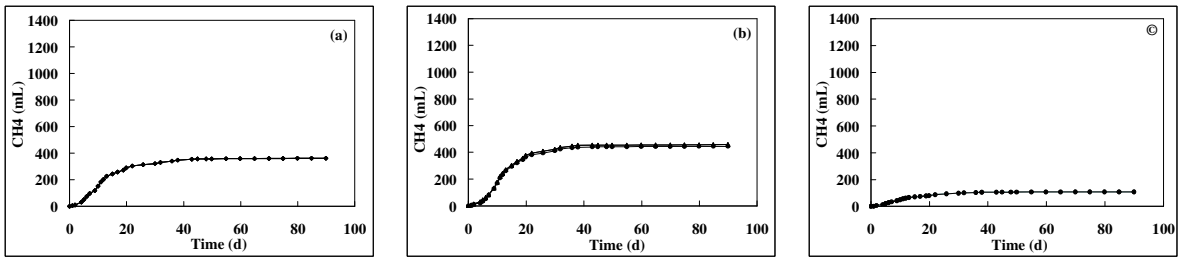


Figure D.38 – Methane production.

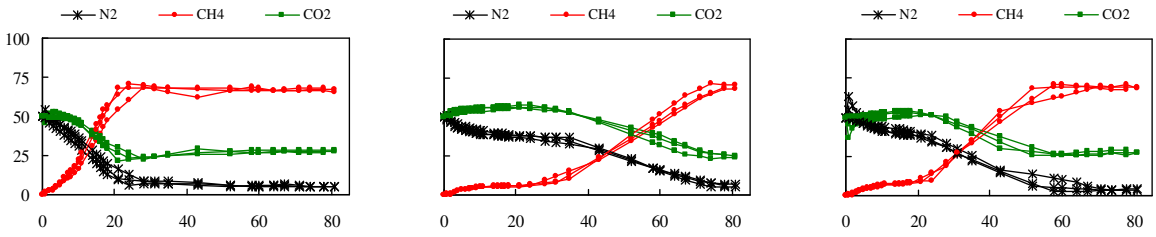


Figure D.39 – Gas composition.

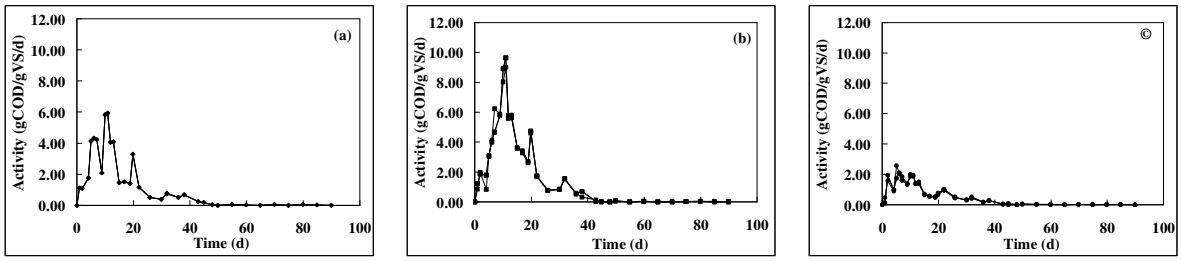


Figure D.40 – Specific methanogenic activity.

Test compound: Scour effluent		
9% (Low)	**23% (Medium)**	**41% (High)**

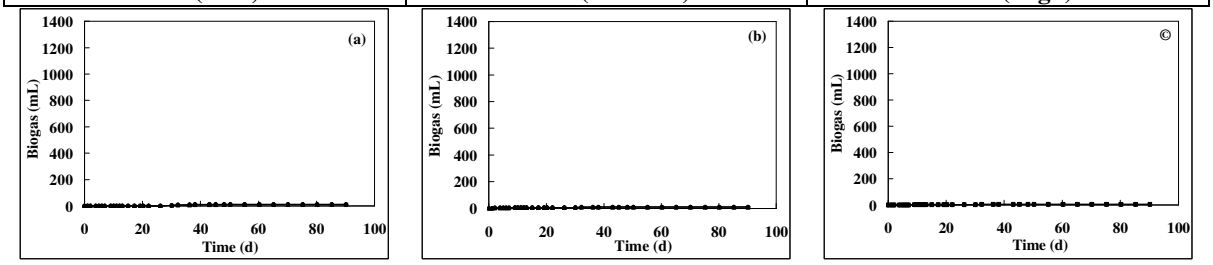


Figure D.41 – Biogas production.

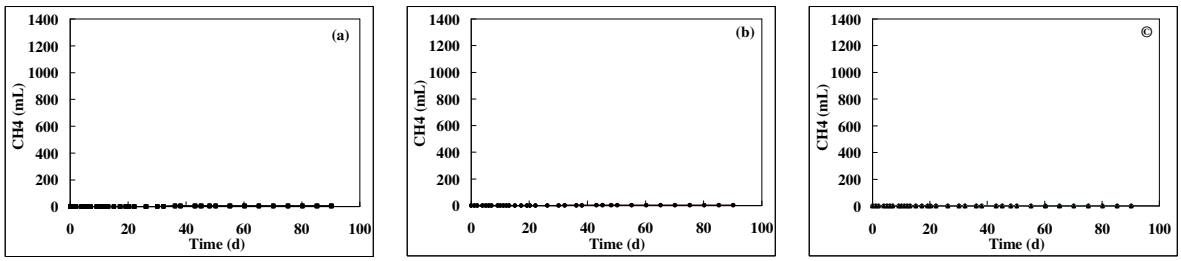


Figure D.42 – Methane production.

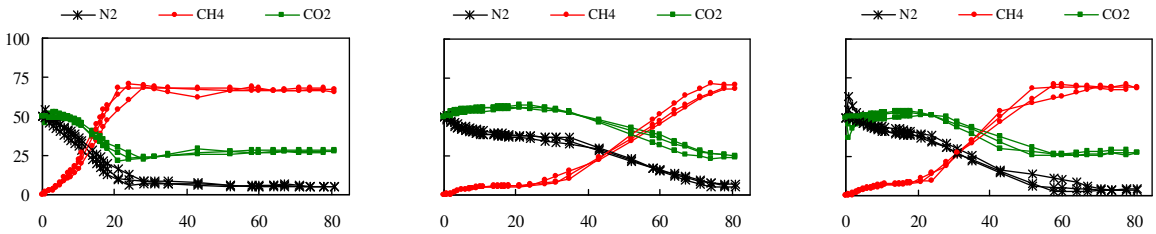


Figure D.43 – Gas composition.

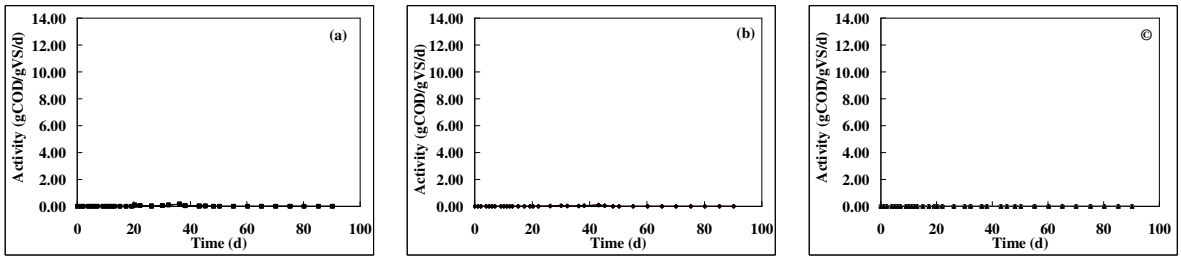


Figure D.44 – Specific Methanogenic activity.