Microbial Community Analysis of a UASB Reactor and Application of an Evolutionary Algorithm to Enhance Wastewater Treatment and Biogas Production

This work is submitted in complete fulfillment for the degree of Doctor of Philosophy (Biotechnology) in the Department of Biotechnology and Food Technology, Faculty of Applied Sciences at the Durban University of Technology, Durban, South Africa

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ABSTRACT

Anaerobic digestion, a proven and highly efficient biological process for treating industrial wastewater and biogas generation is an underutilized technology in South Africa. Some of the industries that have on-site anaerobic reactors tend to face problems in operating these reactors due to poor understanding of the process and implementation of the technology. This has resulted in high pollutant loads in their final effluents and low energy recovery. In this study, an on-site full–scale upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater was extensively monitored in order to evaluate the efficiency in terms of effluent quality, biogas production and microbial structure. Furthermore, developed and adopted kinetic models were used to optimize the performance of the full–scale UASB reactor using a combined Pareto differential evolution (CPMDE) algorithm.

A preliminary analysis of the raw wastewater has shown that the wastewater produced from the brewery industry was high in organic matter with a total chemical oxygen demand (COD) between 1096.41 to 8926.08 mg/L. The average removal efficiency of COD from the UASB reactor after treatment was 79% with a methane (CH_4) production of 60-69% at temperature ranges of 28-32˚C and hydraulic retention time (HRT) of 12 h within the optimal pH range for anaerobic bacteria (6.6 and 7.3) under various organic loading rates. However, the results also showed an increase in total suspended solids (TSS), nitrogen (N_2), ammonia (NH_3) and orthophosphate concentrations when comparing the influent to the effluent, which indicated the necessity for further optimization of the reactor condition in order to reduce these effluent parameters to acceptable standards and to increase CH_4 production.

In order to optimize the process, a thorough understanding of microbial interaction was essential. A combination of different molecular techniques viz., fluorescence *in–situ* hybridization (FISH), polymerase chain reaction (PCR) and quantitative real-time PCR (QPCR) were employed to understand the microbial community structure of the granular sludge samples using species specific primers and probes. The results revealed that the dominance of diverse groups of eubacteria belonging to phyla *Proteobacteria, Firmicutes* and *Chloroflexi* and an uncultured candidate division WS6 with four different orders of methanogenic Archaea viz., *Methanomicrobiales,*

Methanococcales, *Methanobacteriales* and *Methanosarcinales* belonging to hydrogenotrophic and aceticlastic methanogens were within the reactor samples. Quantification of the 16S rDNA copies of eubacteria and methanogenic Archaea using species-specific primers further confirmed the spatial distribution of these microorganisms within the different compartments of the reactor where, the upper compartments were dominated by eubacteria and the lower compartments by methanogenic Archaea. The concentration of Archaea per nanogram of DNA was much higher (96.28%) than eubacteria (3.78%) in lower compartments, while, the eubacteria concentration increased to 98.34% in upper compartments with a decrease in Archaea quantity (1.66%).

A modified kinetic methane generation model (MMGM) was developed on the basis of mass balance principles with respect to substrate (COD) degradation and the endogenous decay rate to predict CH_4 production efficiency of the reactor. Furthermore, a Stover–Kincannon kinetic model was adopted with the aim of predicting the final effluent quality in terms of COD concentration and model coefficients were determined using the data collected from the full–scale reactor. Thereafter, a model-based multi-objective optimization was carried out using the CPMDE algorithm with three–objective functions namely; maximization of volumetric CH_4 production rate; minimization of effluent substrate concentration and minimization of biomass washout, in order to increase the overall efficiency of the UASB reactor. Important decision variables and constraints related to the process were set for the optimization. A set of non-dominated solutions with high CH_4 production rates of between 2.78 and 5.06 L CH_4/g COD/day at low biomass washout concentrations were obtained at almost constant solution for the effluent COD concentration. A high COD removal efficiency (85-87%) at ~30-31˚C and 8-9 h HRT was obtained for the multi-objective optimization problem formulated.

This study could significantly contribute towards optimization of a full–scale UASB reactor treating brewery wastewater for better effluent quality and biogas production. Knowledge on the activity and performance of microbial community present in the granular sludge taken from the full–scale UASB reactor would contribute significantly to future optimization strategies of the reactor. In addition, optimization using an evolutionary algorithm under different operational conditions would help to save both time and resources wasted in operating anaerobic bioreactors.

DECLARATION

"I declare that the thesis herewith submitted for the degree Doctor of Philosophy: Biotechnology at the Durban University of Technology is my original work and has not been previously submitted for a degree at any other institution of higher education, and that its only prior publication was in the form of conference papers, book chapter and/or journal articles. I further declare that all the sources cited or quoted are acknowledged and indicated by means of a comprehensive list of references".

A.M. Enitan

I hereby approve the final submission of the following thesis.

Dr F. M. Swalaha	**Prof. F. Bux**	**Prof. J. Adeyemo**	**Dr Sheena Kumari**
D. Tech. (DUT)	D. Tech. (DUT)	D. Tech. (TUT)	PhD (Mangalore University)

DEDICATION

I am dedicating this project to Jehovah, the father of the whole Universe who made this project a reality. To the memories of my brother Enitan, Ibukunoluwa Olabisi—my friend and brother–an encourager and motivator, who passed on without witnessing the results of his pieces of advice and motivation. We shall meet again in Paradise.

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TABLE OF CONTENTS

This thesis is a compilation of different manuscripts, where each chapter is an individual entity and some repetition is unavoidable between chapters.

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ABBREVIATIONS

AD : anaerobic digestion
ANN : artificial neural network
ANN-GA: artificial neural network coupled with genetic algorithm
b : dimensionless kinetic parameter
B : Actual volume of methane produced (in litres) per gram of COD (substrate) added to the reactor at S.T.P.
B_o : ultimate methane yield coefficient under normal conditions of temperature and pressure per gram of substrate (COD) added for complete utilization of substrate or at an infinite hydraulic retention time
BOD : biological oxygen demand
CH_4 : methane
CO_2 : carbon dioxide
COD : chemical oxygen demand
CPMDE : Combined Pareto Multi-Objective Differential Evolution
Cq : quantification cycle
$\frac{dS}{dt}$: rate of substrate removal, (g/ L/ d)
$\frac{dX}{dt}$: rate of change in microbial mass, (g/ L/ d)
DE : differential evolution
EA : evolutionary algorithm
GA : genetic algorithm
HRT : Hydraulic retention time
gDNA : genomic deoxyribonucleic acid
K : biokinetic constant
K_d : endogenous decay coefficient, (/d)
MMGM : modified methane generation model
ng : nanogram
NH_3 : ammonia
OLR : organic loading rate
P : fraction of biodegradable COD,
Q : flow rate, (L /d)
S : concentration of substrate, (g COD/L)
S_e : effluent substrate concentration, (g/ L)
S_i : influent substrate concentration, (g/ L)
S_r : concentration of substrate in the reactor, (g/ L)
T : operational temperature, (°C)
TS : total solid
TSS : total suspended solid

TVS : total volatile solid
UASB : upflow anaerobic sludge bed
VFA : volatile fatty acid
VS : volatile solid
VSS : volatile suspended solid
V_r : reactor volume, (L)
X : microbial cell concentration, (g / L)
X_e : concentration of biomass in the effluent, (g/L)
X_i : concentration of biomass in the influent, (g/L)
X_r : concentration of biomass in the reactor, (g/L)
Y : growth yield coefficient, (g/g)
Y_v : volumetric methane production rate, (L methane/g COD $_{added}$/d)
θ_h : hydraulic retention time, (/Time),
μ_{max} : maximum growth rate of microorganisms when the substrate is being used at its maximum rate
μ : specific growth rate of microorganisms, (/d^1)

PREFACE

PUBLICATIONS

This work has resulted in the following publications.

(a) Book Chapter

1) **Enitan, A. M.,** Adeyemo, J., Olofintoye, O. O., Bux, F. and Swalaha, F. M. (2014). Multi-objective optimization of a methane producing UASB reactor using a combined Pareto multi-objective differential evolution algorithm. *EVOLVE - A Bridge between Probability, Set Oriented Numerics, and Evolutionary Computation V*. Advances in Intelligent Systems and Computing, Springer, 288: 321-334.

(b) Journal Articles

1) **Enitan, A. M.**, Kumari, S., Swalaha, F. M., Adeyemo, J., Ramdhani, N. and Bux, F. (2014). Kinetic modelling and characterization of microbial community present in a full-scale UASB reactor treating brewery effluent. *Microbial Ecology*, 67: 358–368.

2) **Enitan, A. M.,** Swalaha, F. M., Adeyemo, J. and Bux, F. (2014). Assessment of brewery effluent composition from a beer producing industry in KwaZulu-Natal, South Africa. *Fresenius Environmental Bulletin*, 23 (3): 693-701.

3) Adeyemo, J. and **Enitan, A.** (2011). Optimization of fermentation processes using evolutionary algorithms. *Scientific Research and Essays*, 6 (7): 1464-1472.

4) **Enitan, A. M.** and Adeyemo, J. (2011). Food processing optimization using evolutionary algorithms. *African Journal of Biotechnology*, 10 (72): 16120-16127.

(c) Conference Papers

1) **Enitan**, **A. M**., Swalaha, F. M., Adeyemo, J. and Bux, F. (2014). Evaluation of effluent composition from a beer producing industry in South Africa. Presented at the *International Journal of Arts & Sciences' (IJAS) American Canadian Conference at Ryerson University's International Learning Center*, Toronto, Canada, 19-22 May, 2014 (Oral presentation).

2) **Enitan, A. M.**, Kumari, S., Swalaha, F. M. and Bux, F. (2014). Real-time PCR for quantification of methanogenic Archaea in a UASB reactor treating brewery wastewater. Presented at *International Journal of Arts & Sciences' (IJAS) American Canadian Conference at Ryerson University's International Learning Center*, Toronto, Canada, 19-22 May, 2014. *Conference of the International Journal of Arts & Sciences*, CD-ROM. ISSN: 1943-6114 : 07(03):103–106.

3) Adeyemo, J. and **Enitan, A. M. (**2014). Multi-objective optimization of anaerobic digestion models for biogas production. *Presented at International Journal of Arts & Sciences' (IJAS) for academic disciplines Conference* at Harvard Medical School, 77 Louis Pasteur, Boston, Massachusetts, 26-30 May, 2014 (Oral presentation).

4) **Enitan**, A. M., Kumari, S., Swalaha, F. M., and Bux F. (2014). Use of *mcrA*-targeted real-time quantitative PCR for quantification of methanogenic communities in reactor treating brewery wastewater. Presented at *Water Institute of Southern Africa* (WISA) *Conference*, Mbombela, Mpumalanga, South Africa, May 25-29, 2014 (Oral presentation).

5) Swalaha F. M., **Enitan A. M.** and Bux, F. (2014). Efficiency of industrial scale anaerobic reactor treating brewery wastewater. Presented at *Water Institute of Southern Africa* (WISA) *Conference*, Mbombela, Mpumalanga, South Africa, May 25-29, 2014 (Oral presentation).

6) **Enitan, A. M.** and Adeyemo, J. (2014). Estimation of bio-kinetic coefficients for treatment of brewery wastewater. Presented at the *World Academy of Science, Engineering and Technology Conference*, New York, USA, June 5-6 . *International Science Index*, 8(6): 365-369.

CHAPTER ONE: INTRODUCTION

Industries produce millions of cubic meters of wastewater every year. The wastewater produced may be released into the surrounding rivers, or treated on site or at municipal treatment plants. With competing demand for water resources and water reuse, appropriate discharge of industrial effluents into the aquatic environment has become an important issue, which has led to considerable public debate (Phiri *et al.,* 2005; Baig *et al.,* 2010; Danazumi and Hassan, 2010; Bello-Osagie and Omoruyi, 2012). Some industries have been fined by their national water and municipal authorities for discharging poor quality effluents that do not meet the discharge standards into natural water bodies as well as municipal wastewater treatment plants (Ikhu-Omoregbe *et al*., 2005; Phiri *et al.,* 2005). Also, much attention has been placed on the impact of industrial wastewater on domestic wastewater treatment plants and water bodies worldwide due to accumulation of organic and inorganic compounds in the water bodies (Islam *et al.,* 2006; Kanu and Achi, 2011; Kovoor *et al.,* 2012).

Brewery industries, among others, produce millions of litres of various types of beers each year with global beer production in 2011 estimated to be about 192.71 million kilolitres of beer (Kirin Holdings, 2012) with an average consumption of 23 litres per person per year (Fillaudeau *et al*., 2006). As large volumes of water are being used by the industries in the production of beer, the amount of wastewater that is being discharged from the industries after production is very high in organic content and thus highly polluting to the environment (Jones *et al.,* 2011).

Anaerobic digestion (AD) technology has long been used for the treatment of industrial wastewater. It is a complex biological process that has been adopted for effective treatment of organic wastewater in the absence of oxygen by microorganisms. It is used not only as a pollution control tool, but also for energy recovery (Tiwari *et al.,* 2006; Stafford *et al.,* 2013). The success of AD technology in the removal of high chemical oxygen demand (COD) has led to its increasing application in treating many types of wastewater for bioconversion of organic matter to biogas and better effluent quality (Castillo *et al.*,1999; Liu *et al.,* 2003; Bhunia and Ghangrekar, 2008). The development of the upflow anaerobic sludge blanket (UASB) reactor, initially by Lettinga *et al.* (1980), has made AD the most competitive and

favoured treatment technology to process industrial wastewater in some parts of the world (Li *et al.,* 2014).

An UASB reactor is a biogas-producing digester that uses complex and sequential biochemical processes through the association of anaerobic microorganism (Lettinga and Hulshoff-Pol, 1991; Tiwari *et al.,* 2006). The methane content of biogas produced is known as an environmentally friendly, clean fuel which is part of nature's own cycle that can be used for lighting, cooking, and running internal combustion engines. Thus, the use of anaerobic waste fermentation to produce biogas is a promising and economical way of generating renewable energy at the industrial scale. In recent years, UASB reactors have been successfully applied to the treatment of different types of wastewater for better effluent quality and in turn to produce biogas as a source of renewable energy (Cronin and Lo, 1998; Parawira *et al.,* 2005; Manhokwe *et al*., 2009; Madukasi and Zhang, 2010; Muda *et al.,* 2011; Nacheva *et al.*, 2011). Anaerobic breakdown of organic compounds to biogas involves the action of several groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic bacteria) that grow in a syntrophic manner when the reactor is operated under optimum reaction conditions (Hulshoff-Pol *et al.,* 2004; Crocetti *et al.,* 2006; Mumme *et al.,* 2010; Amani *et al.,* 2011). Studies have shown that the microbial community in the UASB reactor responds to the changes in environmental and operational conditions (Klocke *et al.*, 2007; Khalid *et al.,* 2011; Ziemiński and Frąc, 2012; Enitan *et al,.* 2014b; Jang *et al.,* 2014). Thus, in order to optimize the performance of a UASB reactor, it is necessary to identify and quantify the microbial communities for better treatment efficiency and biogas production (Chen *et al.,* 2008). Effluent quality and the amount of biogas produced depend on the type of substrate, digester configuration and the environmental conditions (Traversi *et al.,* 2014).

Therefore, to understand and predict the phenomena occurring in AD processes, increase the plant performance and methane (CH_4) production, holistic mathematical models are required. To this effect, different AD models have been developed to describe and predict increased treatment efficiency and optimize the operating conditions of the digestion system (Batstone *et al.,* 2002; Parawira *et al.,* 2005; Parsamehr, 2012). Mathematical modelling is more effective in providing information on the interactive behaviour of various factors in fermentation processes compared to conventional one-at-a-time-optimization methods, which

are less effective at representing the interactive effects of all the factors involved in a complex bioprocess (Lakshmi *et al.,* 2009).

Mathematical modelling is useful for designing, predicting and controlling anaerobic processes. It can assist process engineers to design new configuration of reactors for higher efficiency and to improve the efficiency of an existing system. A process model can be either mechanistic or empirical (Thorin *et al.,* 2012; Estes *et al.,* 2013). Simple and sophisticated models for several systems of AD processes have been developed to fulfill the increasing need of understanding the parameters required to improve the efficiency of bioreactors (Batstone *et al.,* 2002; Parsamehr, 2012). Technical approaches for the development of these dynamic models to adequately describe the treatment processes and biogas production vary from one method to another. However, the integration of different parameters, linear and nonlinear equations with single and multi-objective functions under different constraints in large-scale engineering problems have contributed to the development of alternative solutions (Babu *et al.,* 2005; Iqbal and Guria, 2009, Abu Qdais *et al*., 2010). A new optimization and control strategy with immense benefits to manage AD of wastewater for energy generation and production of better effluent that may reduce pollution to the environment is essential for effective reactor operation.

Optimization can be defined as the art of finding one or more feasible solutions corresponding to extreme values of one or more objectives problems, while satisfying specific constraints (Babu *et al.*, 2005). Optimization problems are divided into two, namely single- and multi-objective optimization (Fister *et al.,* 2013). The single–objective optimization problem involves one objective function to which heuristic-based and gradient-based search techniques are applied in order to solve the single–objective optimization problem. It involves finding the minimum and maximum of a single–variable function. The single–objective optimization method is employed for finding an optimum of a first– and second–order derivative of a function. It may also involve finding the true optimum in the presence of constraints to get solutions to real world problems (Adeyemo and Otieno, 2009a).

On the other hand, multi–objective optimization problem (MOOP) is an optimization problem solving method that has more than one objective functions. It involves finding one or more optimum solutions to more than one objective optimization problems that are conflicting in nature (Deb, 2011). The aim of MOOP is to simultaneously optimize a set of conflicting objectives to obtain a group of alternative trade-off solutions called Pareto-optimal or non-inferior solutions which must be considered equivalent in the absence of specialized information concerning the relative importance of the objectives (Deb, 2011). With regards to all objectives, there is no best solution rather; the solutions are equally good solutions. Meanwhile, most real-world search and optimization problems are multi-objectives in nature with all the objective functions being very important (Fister *et al.,* 2013).

However, limited knowledge with highly complex and non-linear digestion processes was one of the underlying problems in AD due to lack of an online-measurements for most of the industrial biogas–producing plants. This problem has led to the development of new optimization and control strategies with respect to external influences and different process disturbances that are vital for efficient operation of AD treatment plants (Sendrescu, 2013). One approach to address this problem is to exploit the flexibility and power of computational intelligence of evolutionary algorithms (EAs).

Evolutionary algorithms as a class of direct search algorithms have proven to be an important tool to solve optimization problems and thus, have been employed more often during the last decade due to their ease way of handling multiple-objective problems (Woldesenbet *et al*., 2009). Constrained or unconstrained multi-objective problem may in principle be two different ways to pose the same underlying problem and can be solved by EAs (Karaboga, 2004). Evolutionary algorithms are proving robust in delivering global optimal solutions and helping to resolve limitations encountered in traditional methods (Enitan and Adeyemo, 2011).

Like many other natural world problems, problems of AD process are conflicting in nature. For instance, reduction of organic matter to meet the discharge standard and biogas production during anaerobic treatment of wastewater requires different conflicting objectives

such as maximization of desirable properties (such as biogas production for energy generation) and simultaneously minimizing its undesirable characteristics (such as a reduction of effluent substrate concentration or the organic pollutant loads in the final effluent to meet the discharge standards) (Babu *et al.,* 2005). Evolutionary algorithms are of interest in optimizing AD processes to generate Pareto-optimal solutions, although this may not be an easy task due to the complexity and variations in the organic content of the industrial wastewaters.

Recently, a few industries have started using sophisticated technologies to improve, monitor, optimize and control processing parameters in order to increase treatment efficiency (Rodríguez-Fernández *et al.,* 2007; Iqbal and Guria, 2009). However, expert knowledge is still needed to apply these techniques successfully. If the specific technique is not applicable to certain problem due to unknown system parameters, then multiple local minima or non-differentiability evolutionary algorithms have the potential to overcome these limitations (Karaboga, 2004) by using mathematical model-based techniques to make decisions about optimal production scenarios.

1.1 STUDY OBJECTIVES

1.1.1 Aim

The aim of this study was to monitor the performance and the microbial diversity especially the methanogens in a UASB reactor treating brewery wastewater, develop a dynamic model to describe the behaviour of a UASB reactor and optimize the model using an evolutionary algorithm called the Combined Pareto Multi-objective Differential Evolution (CPMDE) algorithm.

1.1.2 Objectives

In order to achieve our aim, the following specific objectives were set out:

- To monitor the parameters associated with the performance of a full-scale UASB reactor in order to establish kinetic constants to be used in further mathematical modelling.

- To determine the microbial community structure of the full–scale UASB reactor using different molecular techniques including; FISH, PCR and QPCR.

- To develop and simulate a dynamic multi-variable optimization model in order to predict the methane production rate during the anaerobic treatment of brewery wastewater using MATLAB object-oriented language.

- To optimize the developed (MMGM) and adopted modified Stover–Kincannon kinetic models using a combined Pareto multi–objective differential evolution (CPMDE) algorithm to maximize CH_4 production and enhance wastewater treatment efficiency.

1.2 THESIS OUTLINE

Chapter one begins with a general introduction with the study objectives and outline of the thesis. Each subsequent chapter is concluded with details of the research output(s) from the chapter. Chapter two presents the literature review relevant to the study. Chapter three presents the physico-chemical composition of brewery wastewater and the performance of the full–scale UASB reactor treating the brewery wastewater in KwaZulu-Natal, South Africa. Chapter four presents the identification and quantification of the microbial ecology of the full–scale UASB reactor. The Stover–Kincannon kinetic model was adopted to predict effluent substrate concentration, in order to reduce the pollutant load discharged into the environment and water bodies. In chapter five, development of kinetic model (MMGM) for CH_4 production during AD was presented. Chapter six is a continuation of the study in Chapter four and five where, a multi-objective constrained optimization problem was presented. A novel evolutionary algorithm called CPMDE algorithm was used as the optimization tool to integrate and determine the optimum CH_4 production rate, effluent substrate concentration and biomass washout from the UASB reactor treating brewery wastewater. Finally, Chapter seven presents the general conclusions of the study along with suggestions for future research and the significance of the study. The thesis ends with a list of references and appendices.

CHAPTER TWO: LITERATURE REVIEW

2.1 INTRODUCTION

Manufacturing industries produce wastes that contain high levels of organic materials which could adversely affect the environment should they be directly discharged. For industries to meet discharge requirements, economical and practical treatment methods are important factors that need to be considered. Therefore, there is an increasing need for industries to treat their wastewater before discharged into the environment using effective, eco-friendly, simple and inexpensive technologies, thereby minimizing the impact on the environment. Anaerobic treatments have gained more attention in developing countries due to the fact that traditional aerobic technologies, like the activated sludge process, require professional skills and high costs to operate and may not be able to handle high strength effluents (Bhatti *et al.*, 1993; Leitao *et al.,* 2006).

Anaerobic treatment involves the conversion of complex organic matter present in low to high–strength industrial wastewaters into simpler monomers and production of biogas in a closed system, through the activity of various anaerobic microorganisms (Bhatti *et al.,* 1996; Keyser, 2006; Ziemiński and Frąc, 2012; Enitan *et al.,* 2014a). Biogas recovery systems referred to as 'methane (CH_4) recovery systems', 'bioreactor/biodigester', 'methane digester', or 'anaerobic digester' can be used to treat industrial waste and capture CH_4 that can be used for on-site energy generation (Parawira *et al.,* 2005). However, the reduction of organic matter and quantity of biogas released depends on the conditions under which the reactor is operated (Gyalpo *et al.*, 2008), because any sudden changes in the performance of the system can have a damaging effect on the quality of effluents discharged and biogas recovery.

Several anaerobic digestion (AD) technologies have been designed and constructed for the treatment of high–strength wastewater (Demirel *et al.,* 2010; Abbasi and Abbasi, 2012). Anaerobic system such as an upflow anaerobic sludge blanket (UASB) reactors have received much attention due to their ability to treat industrial wastewaters at higher organic loading rate (OLR) and a lower hydraulic retention time (HRT) (Mata-Alvarez *et al.*, 2000; Nadais *et al.,* 2011).

2.2 ANAEROBIC TREATMENT OF WASTEWATER

Anaerobic digestion has received worldwide attention due to it being a simple, inexpensive technology to operate, that produces low biomass outputs and low energy input (Karagiannidis and Perkoulidis, 2009; Kaparaju *et al*., 2010). The treatment of high-strength industrial wastewater such as brewery wastewater using AD technologies has been employed in several instances throughout the world (Brito *et al.,* 2007; Demirel *et al.,* 2010; Simate *et al.,* 2011). It has been used widely as a source of renewable energy. The biogas comprising of carbon dioxide (CO_2), CH_4 and traces of other gases produced during the process of AD can be used directly as fuel in combined heat and power gas engines, thereby reducing the release of these biogases to the atmosphere (Ward *et al.,* 2008; Singh and Prerna, 2009). On the other hand, some disadvantages of AD processes includes long retention times (Chan *et al.,* 2009), bad odour and effluents that sometimes needing post-treatment to meet the discharge standards for nutrients levels, organic matter and pathogens content (Seghezzo *et al.,* 1998).

Over the past 25 years, different types of reactors have been developed and their installations have been commercialized. Along with the UASB reactor (Fang *et al.,* 1995a; Lettinga, 1995; Ryan *et al.,* 2010; Qiao *et al.,* 2011; Chong *et al.,* 2012), anaerobic sequencing batch reactor (ASBR) (Shao *et al.,* 2008; Won and Lau, 2011), hybrid upflow anaerobic sludge-filter bed (UASFB) (Rajagopal *et al.* 2009), continuous stirred tank reactors (CSTR) (Diaz *et al.,* 2006; Klocke *et al.,* 2007; Kaparaju *et al.,* 2010; Mirzoyan *et al*., 2010), expanded granular sludge bed (EGSB) (Seghezzo *et al.,* 1998), anaerobic baffled reactor (ABR), anaerobic fixed-bed reactors (AFBR) and membrane technology have been widely used for wastewater treatment (Figure 2.1a) (Lettinga *et al.,* 1980; Driessen and Vereijken, 2003; Parawira *et al.,* 2005; Zhou *et al.,* 2006). Among these UASB reactor configuration is the most widely used high–rate anaerobic reactor for the treatment of high-strength wastewater. Over one thousand UASB reactors have been installed worldwide due to its simple design and low operational cost (Tiwari *et al.,* 2006; Nigel and Sneeringer, 2011). An overview of the above-mentioned anaerobic treatment systems used for different industrial wastewater pre-treatment is presented in Figure 2.1(b) (International Energy Agency, 2001). Descriptions and further information on the different types of reactors can be found in the literature (Shao *et al.,* 2008; Sip*ma et al.,* 2010; Won and Lau, 2011; Rajagopal *et al*., 2013; Tauseef *et al.,* 2013).

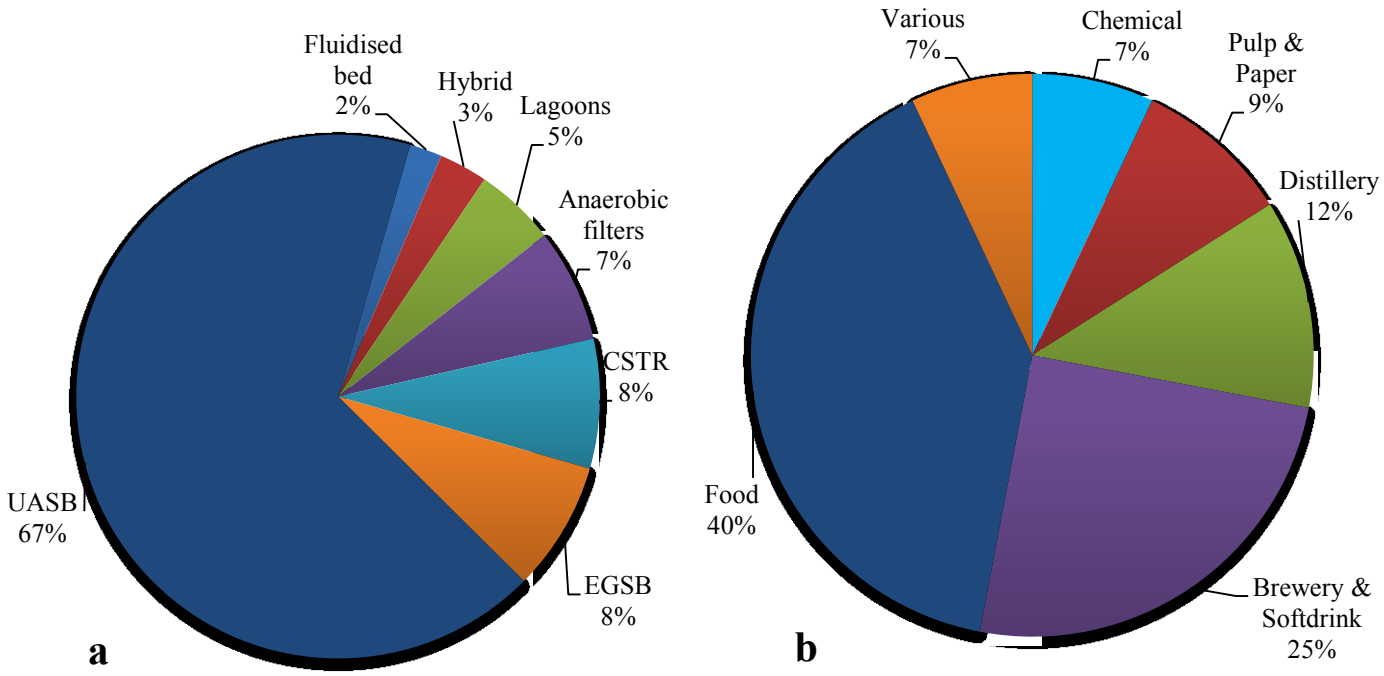

Figure 2.1: (a) Proportions and types of anaerobic digestion systems that have been installed and commercialized for the treatment of industrial wastewater (b) percentage of industries using anaerobic treatment technologies for industrial wastewater (International Energy Agency, 2001).

2.2.1 Upflow Anaerobic Sludge Blanket Reactors

The UASB reactor designed by Lettinga *et al.* (1980) has made AD the most competitive and favourable treatment technology for high–strength organic wastewaters (Ryan *et al.,* 2010; Abbasi and Abbasi, 2012). It has been widely employed to treat industrial and domestic wastes around the world due to features such as simple design, easy construction and maintenance, low operating cost, high removal efficiency, short retention time, stability, temperature and low energy demand (Alvarez *et al.,* 2006; Tiwari *et al.,* 2006). UASB reactors are highly dependent on its granular sludge as the core component during wastewater treatment for an effective conversion of organic matter to biogas (Batstone *et al.,* 2002; Liu *et al.,* 2003).

A schematic diagram of a typical UASB reactor is shown in Figure 2.2. In an UASB reactor, the influent enter through the bottom of the reactor, thereby helping in the aggregation of microbial biomass in the sludge bed and blanket to get in contact with the influent (Abbasi and Abbasi, 2012). Several investigations have been carried out at laboratory, pilot and full-scale level to optimize UASB reactors using different types of effluent including domestic (Atashi *et al*., 2010) and industrial wastewaters. Some of the industrial effluents treated include pharmaceutical (Herumurti *et al.,* 2008), pulp and paper (Ali *et al.,* 2009), sugar factories (Demirel and Scherer, 2008a; Hampannavar and Shivayogimath, 2010) brewery wastewater (Parawira *et al.,* 2005; Kovacik *et al.,* 2010; Madukasi and Zhang, 2010), slaughterhouse (Nacheva *et al*., 2011) and textile (Muda *et al.,* 2011).

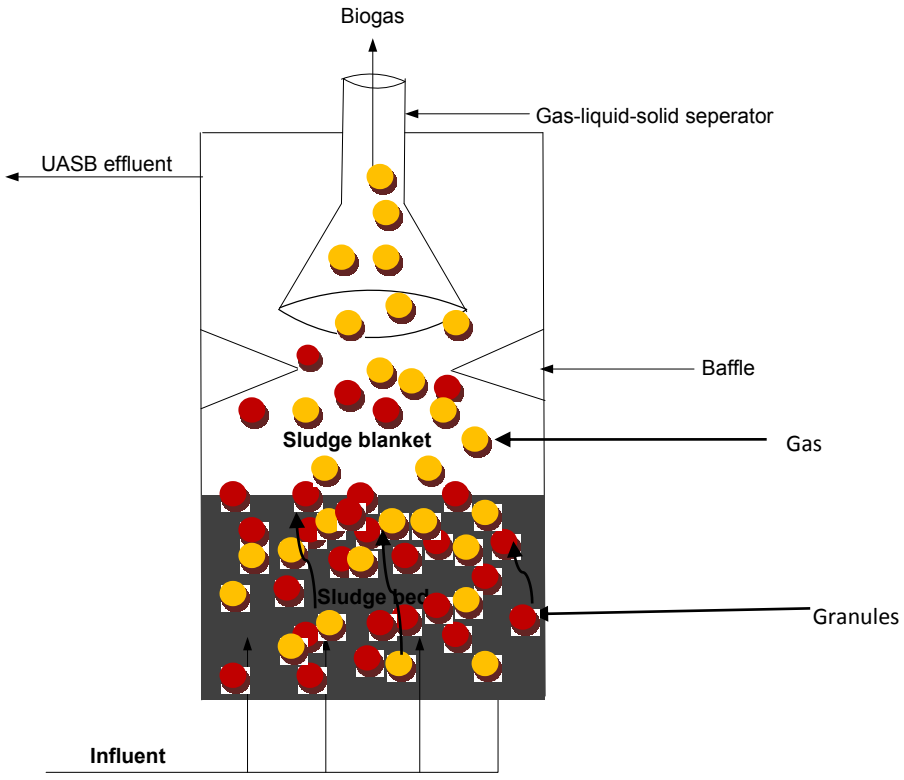

Figure 2.2: Schematic diagram of an upflow anaerobic sludge bed (UASB) reactor with red balls indicating granules and yellow balls indicating evolved biogas.

2.3 BIOGAS RECOVERY FROM ANAEROBIC DIGESTERS

Due to the increasing effect of climate change in the world, industrial waste management strategies and reduction of environmental effects caused by the industrial waste disposal has gained more attention. From the clean development mechanisms (CDM) point of view, 'mitigating CH_4 emissions' is very fascinating, since the global warming potential (GWP) of CH_4 is 21 times higher than that of CO_2. Under anaerobic conditions CH_4, CO_2, nitrogen (N_2), hydrogen (H_2), hydrogen sulphide (H_2S) and oxygen (O_2) called 'biogas' are produced (Wen *et al.,* 2009) with calorific values of 21-24 Mj/m^3, equivalent to 6 KWh/m^3 of CH_4 (Bond and Templeton, 2011). Moreover, the use of biomass for energy generation is classified as a 'carbon neutral' process because, the CO_2 released during this process is balanced by the CO_2 absorbed by plants during their growth (The Centre for Sustainable Environmental Sanitation the Centre for Sustainable Environmental Sanitation, 2009).

Furthermore, the use of CH_4 gas from AD as a renewable energy source has been widely adopted as one of the CDM in order to obtain a certified emission reduction (CER) credit under the Kyoto Protocol. This facilitates the promotion of biogas to reduce the greenhouse effect, through reduction of CH_4 emissions into the atmosphere. Biogas generation has been widely adopted in Asia, particularly in Bangladesh, China, India and Nepal (Dutschke *et al.,* 2006; NSWAI-ENVIS, 2007; The Centre for Sustainable Environmental Sanitation, 2009). The treatment of these wastes and biogas production with high CH_4 content as energy recovered is a good alternative to fossil fuel, since human activities and industries at large produce sufficient amounts of waste (Tauseef *et al*., 2013). The biogas that is used for fuel energy must contain more than 50% of CH_4 (Srisertpol *et al.,* 2010) which could be used for heating, cooking, lighting or to generate electricity for domestic and larger industrial plants (Bond and Templeton, 2011; Heffernan *et al.*, 2012).

However, South Africa and other African countries have placed less attention on the implementation of national biomass energy from wastewater when compared to world's implementation of AD technology. The application of AD technology in South Africa for the treatment of industrial wastewater has been reviewed by Ross and Louw, (1987) and Stafford *et al*., (2013). Their survey showed that the use of anaerobic reactors, especially UASB

reactors that could be used for recovery of energy is very low in South Africa compared to other countries.

In 2003, the South Africa Government set a ten-year target to produce 10 000 GWh (0.8 MTOE) from biomass, wind, solar and small-scale hydropower technology for renewable energy consumption by 2013. The energy from biomass could be used for power generation and non-electric technologies such as bio-fuels and solar water heating. It is approximately 1667 MW (4%) of the estimated electricity demand for 2013 (41539 MW)—which is equivalent to replacing two units of Eskom's combined coal fired power stations (2 x 660 MW) (Shabangu, 2004).

In another report for biogas generation for electricity in 2012, environmental engineering company Talbot & Talbot was employed to design and supply a biogas train in South Africa– for its first green energy project (Cloete, 2008). The R5-million biogas project, include the production of biogas from anaerobic wastewater digestion that can be converted to electricity or used as boilers fuel (Cloete, 2008). However, some industries and municipal wastewater treatment plants that have onsite anaerobic reactors still flare or vent the biogas that are produced during anaerobic treatment of wastewater (Stafford *et al.,* 2013). This demonstrates that energy use has been poorly integrated and the opportunities for mitigating greenhouse gas (GHG) emissions have not been realized.

Few industries in South Africa such as Cape Flats wastewater treatment plant in Cape Town, PetroSA's gas-to-liquids refinery in Mossel Bay and some isolated community, household and small-scale industries are using biogas generated during anaerobic treatment for energy generation (Stafford *et al.,* 2013). Stafford *et al.* (2013) further listed different types of industrial and domestic blackwater wastewaters being treated using anaerobic reactors in South Africa.

2.4 BIOCHEMISTRY AND MICROBIOLOGY OF THE ANAEROBIC DIGESTION PROCESS

The AD process is carried out by a group of facultative, obligate and strict anaerobic bacteria (Mshandete *et al,.* 2005; Appels *et al.,* 2008) that are divided into four groups (Figure 2.3) based on the biochemical processes and the metabolites they produce. Under ideal conditions, these microorganisms break down the complex organic compounds through a variety of intermediates into the components of biogas, such as CH_4 and CO_2 with small levels of H_2S, H_2 and N_2 (Appels *et al.,* 2008; Mirzoyan *et al.*, 2010; Amani *et al.* 2011). The overall reaction is shown in equation 2.1 (Bitton, 1994).

$$\text{Organic matter} \longrightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S \qquad (2.1)$$

About 70% of the total CH_4 production during AD is from acetic acid, while the remaining 30% comes from H_2 and CO_2 conversion (Ahring, 2003). It has been reported that about 80 - 90% CH_4 composition can be produced in reactors treating wastewater (Okonkwo *et al.*, 2013). The origin of the AD process and the biodegradable materials determines the composition of biogas produced.

The stability of the microbial ecosystem in the AD process has been shown to depend on the methanogenic activity, which is characterized by slow growth rates of microorganisms. These microorganisms have been found to be very sensitive to operational and environmental variations in the anaerobic wastewater treatment systems, such as salinity, sludge properties, temperature, pH, mineral composition, loading rate, HRT, carbon-to-nitrogen ratio and volatile fatty acids (VFAs). These factors in-turn influence the digestibility of the organic matter and production of biogas (Leitao *et al.,* 2006; Chong *et al.,* 2012).

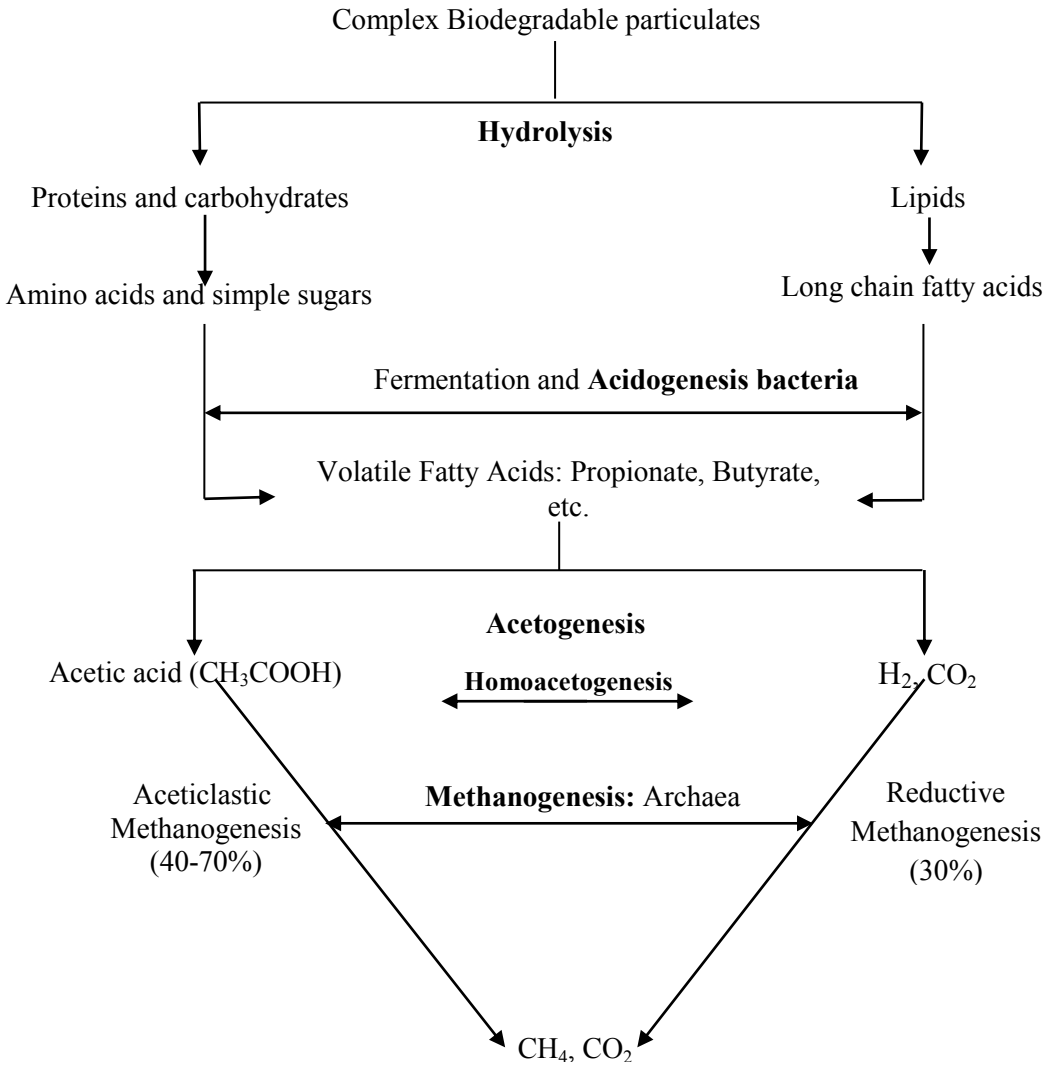

Figure 2.3: The key stages of anaerobic digestion of organic matter in the wastewater (Li *et al.,* 2011).

2.4.1 Hydrolytic Bacteria

The digestion process is initiated by the action of facultative and obligate fermentative anaerobic bacteria of mainly the genera *Bifidobacteria, Lactobacillus, Enterobacterium* and *Streptococcus* (Krzysztof and Frac, 2012). This stage has been found to be common to both aerobic and AD processes. The anaerobic bacteria were shown to catalyse the breakdown of large complex soluble and insoluble organic molecules present in the wastewater into smaller soluble monomers which could be transported into cells of non-hydrolytic fermentative bacteria and metabolized (Bitton, 1994). The rate of hydrolysis process has been shown to be

dependent on parameters such as: wastewater type, pH, size of particles, production of enzymes, diffusion and adsorption of enzymes on the particles of wastes subjected to the digestion process (Ziemiński and Frąc, 2012).

2.4.2 Fermentative Acidogenic Bacteria

The acidogenic bacteria are the largest trophic groups, and consist of about 90% of the total bioreactor population (Zeikus, 1980). Several microbial genera take part in acidogenesis, the first stage of AD (Krzysztof and Frac, 2012). Acidogenesis is the process during which more "simple" organic material is metabolized to form CO_2, H_2, acids and alcohols through the action of the genera viz, *Pseudomonas, Bacillus, Clostridium, Micrococcus* or *Flavobacterium* (Krysztof and Frac, 2012). This process has been divided into two stages: The hydrogenation and dehydrogenation. The acids forming bacteria convert sugars, fatty acids and amino acids to organic acids (including formic, acetic, propionic, butyric, lactic acids), ketones and alcohols, which causes an accumulation of electrons in response to an increase in H_2 concentration in the solution. The new products may not be used directly by methanogenic bacteria and must be converted by obligate anaerobes producing H_2 in the process called acetogenesis (Krzysztof and Frac, 2012). Other acid-forming bacteria facilitate the production of acetate, H_2 and CO_2 depending on environmental conditions such as pH, temperature for direct assimilation of the new metabolites as substrates and energy source by the methanogens (Keyser, 2006; Krzysztof and Frac, 2012).

2.4.3 Acetogenic Bacteria

Acidogenesis products are further oxidized to acetate, H_2, and CO_2 by the activity of acetogenic bacteria (acetate and H_2-producing bacteria) such as *Desulfovibrio, Syntrophobacter wolinii, Syntrophomonas wolfei, Syntrophus buswellii, Syntrophococcus, Natroniella* and *Acetigena* spp. (Bhatti *et al.*, 1996; Pitryuk and Pusheva, 2001; Karnholz *et al.,* 2002). The acetogens have been found to be obligate H_2-producing bacteria that can only survive at very low H_2 concentrations. They have been shown to help in the conversion of fatty acids and alcohols to acetate, H_2, and CO_2 at low H_2 partial pressure (Bitton, 1994). Therefore, for acetogenic bacteria to maintain a low partial pressure of H_2 less than 10^{-5} atmospheres, they live in symbiosis with the H_2-utilizing methanogens when the digester is

operated at optimum temperature and pH levels (Ziemiński and Frąc, 2012). Most digesters are normally operated at about pH 7 because; it favours all the groups that are involved in the conversion of organic matters to biogas including methanogens. Studies on the syntrophic reactions have been described in the literature with optimum pH levels and temperature between 6.3-8.5 at 25˚C and 45˚C respectively for syntrophic association of acetogenic and methanogenic bacteria (Schink, 2002; Amani *et al.,* 2011).

2.4.4 Methanogenic Archaea and their Taxonomy

Living organisms have been classified into three main taxonomies based on 18S and 16S rRNA analysis and comparison of conservative phylogenetic features. The phylogenetic domains include Archaea, Bacteria and Eukarya. Organisms belonging to domain Archaea are divided into two phyla namely Crenarchaeota and Euryarchaeota (Figure 2.4) (Anderson *et al*., 2009). The Crenarchaeota have been discovered to consist mainly of thermoacidophiles and thermophiles while the Euryarchaeota contains a wider variety of organisms including the methanogens, the extreme halophiles, thermoacidophiles and thermophiles. Recently the third phylum, Thaumarchaeota, was proposed to include the mesophilic organisms previously classified as Crenarchaeota (Brochier-Armanet *et al.,* 2008). The CH_4-producing organisms (methanogens) are classified to domain Archaea, and phylum Euryarchaeota based on the phenotypic and taxonomic classification (Ziemiński and Frąc, 2012).

Methanogenic bacteria are divided into four classes, five orders, nine families and 26 genera. They are different from each other in shape, membrane lipids, 16S rRNA sequence, structure, cell wall chemistry and other features (Demirel and Scherer, 2008b, Ziemiński and Frąc, 2012). Figure 2.4 shows the phylogenetic classification of methanogens. Methanogens are archeons, unlike bacteria, they do not have a typical peptidoglycan (mureinic) skeleton; rather several genera have pseudomurein, while others have walls consisting of lipids composed of isoprenoid hydrocarbons glycerol lipids with different metabolism (Ziemiński and Frąc, 2012). Methanogenic ribosomes exhibit a similar size to that of eubacteria ribosome, but their sequence of ribosomal RNA is completely different (Watanabe *et al*., 2004).

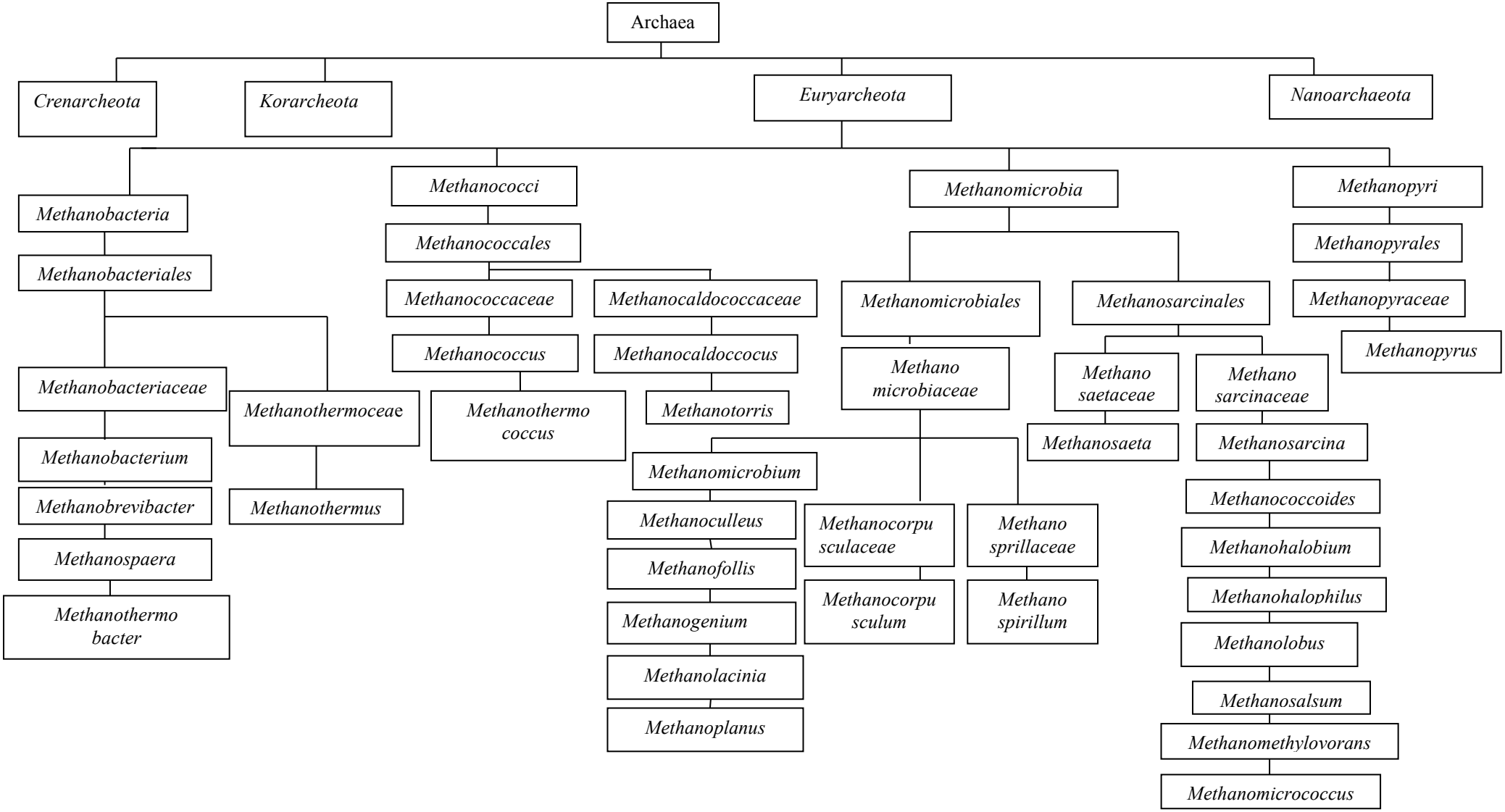

Figure 2.4: Classification of methanogens based on 18S and 16S rRNA analysis and comparison of conservative phylogenetic features (Demirel and Scherer, 2008b; Ziemiński and Frąc, 2012).

Methanogens are largely differentiated morphologically. They exhibit almost all shapes occurring in bacteria including cocci (*Methanococcus*), rods (*Methanobacterium*), short rods (*Methanobrevibacter*), spirillaceae (*Methanospirillum*), sarcina (*Methanosarcina*) and filiforms (*Methanothrix*). The size of these microorganisms ranges from 0.3 to 7.4 μm (Karakashev *et al*., 2006). They are strict anaerobes and contain neither catalase nor superoxide dismutase. Due to extraordinary sensitivity of methanogens to oxygen, their biochemistry, physiology and ecology have been reviewed (Ziemiński and Frąc, 2012). Some of their characteristics include, their sensitivity to changes in pH and temperature, inhibition of their growth by high level of H_2, sulphur, NH_3 and VFAs and other compounds, in the environment or in the bioreactor (Ziemiński and Frąc, 2012, Nakasaki *et al*., 2013).

Methanogens are slow-growing bacteria with a generation time between 3 days at 35˚C and 50 days at 10˚C (Bitton, 1994). Studies have shown that three different major pathways exist for CH_4 formation depending on the source of the reducing potential and the carbon compound used as substrate (Figure 2.5); which include hydrogenotrophilic, aceticlastic and the methylotrophic methanogens (Bapteste *et al*., 2005; Ziemiński and Frąc, 2012). Hydrogenotrophilic methanogens are H_2 using organism. They use H_2 as an electron donor to reduce CO_2 to CH_4 (Figure 2.5; Equation 2.2). This group helps in maintaining very low levels of partial pressure needed by the aceticlastic methanogens for the conversion of VFA and alcohols to acetate (Gerardi, 2003).

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O \qquad (2.2)$$

Abundance of *Methanobacterium*, *Methanobrevibacter* and *Methanococcus* of orders *Methanobacteriales, Methanomicrobiales and Methanococcales* in different types of anaerobic bioreactor treating wastewaters have been reported (Casserly and Erijman, 2003; Bhatti *et al*., 1993; Liu *et al.,* 2002a; Castro *et al*., 2004; Diaz *et al.,* 2006; Cardinali-Rezende *et al.,* 2009; Kovacik *et al.,* 2010a). The second group is the acetotrophic or aceticlastic methanogens which convert acetate to CH_4 and CO_2 (Zheng and Raskin, 2000). The overall reaction is;

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad (2.3)$$

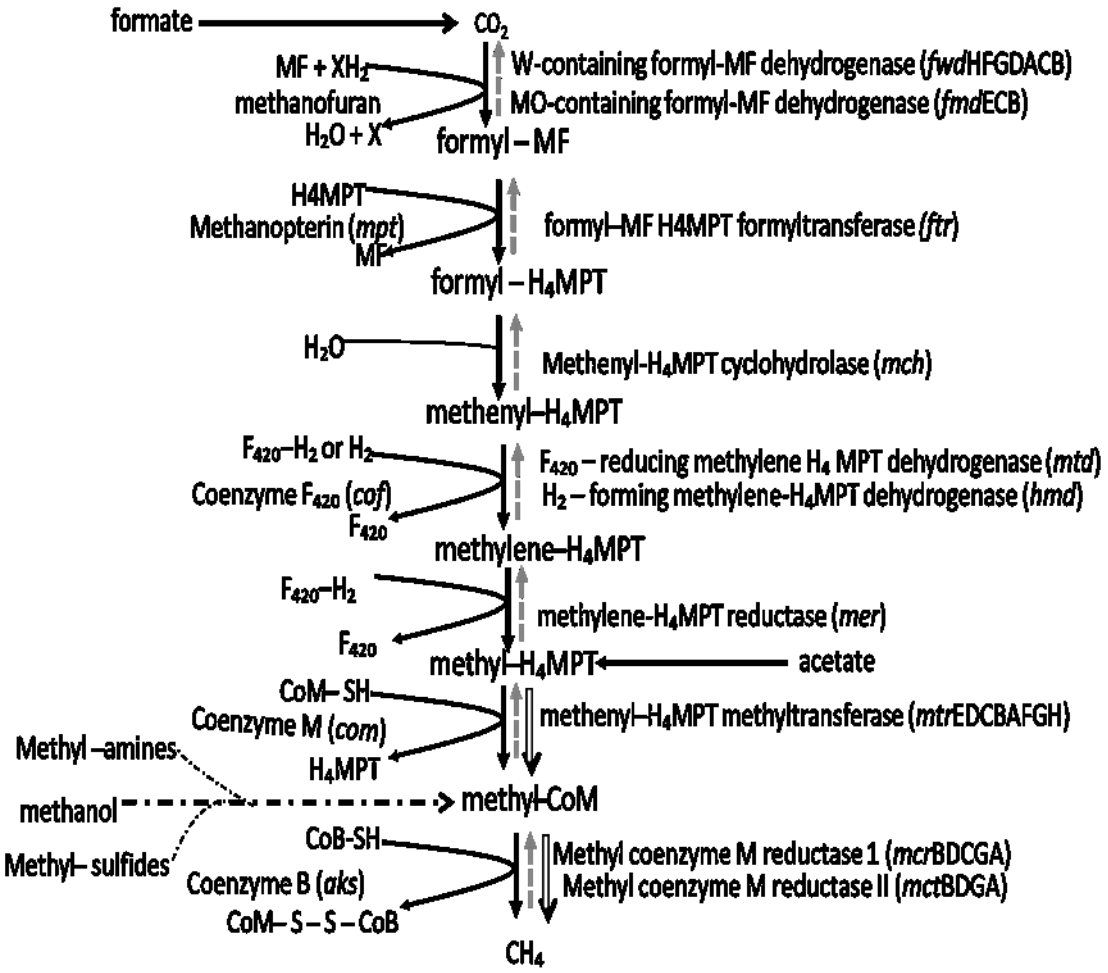

Figure 2.5: Pathways of methanogenesis: hydrogenotrophic (double-lined arrows), aceticlastic (solid arrows) and methylotrophic (broken gray arrows) (Bapteste *et al.,* 2005).

In the aceticlastic pathway, the CO_2 is oxidized to provide electrons and the methyl group converted from acetate is linked to methanopterin (or sarcinapterin, for *Methanosarcina*) before being reduced to CH_4 in two enzymatic reactions (Figure 2.5), the last two steps of the hydrogenotrophic pathway (Meuer *et al.,* 2002). The most commonly reported aceticlastic methanogens from bioreactors belong to the genera *Methanosaeta* (coccoid bacteria) and *Methanosarcina*–sheathed rods or long filaments bacteria (Keyser *et al.,* 2006). This group of methanogens helps in the production of about 70% of the total CH_4 generated during the AD of wastewater (Ahring, 2003). *Methanosaeta* sp. such as *M. thermophila* and *M. concilii* belonging to genus *Methanosaeta* utilize acetate, while *Methanosarcina* strains like *M. barkeri, M. mazeii* and *M. thermophila* utilize acetate, methanol, methylamines, H_2 and CO_2 as substrate (Keyser *et al.,* 2006). The abundance of *Methanosarcina* sp. at high acetate levels and *Methanosaeta* sp. at low acetate concentrations has also been reported (Keyser *et al.,*

2006). An abundance of *Methanosarcina* and *Methanosaeta* sp. in UASB granules treating different wastewaters at steady–state conditions have been reported in the literature (Fang *et al*., 1994, 1995b; Chan *et al.*, 2001; McHugh *et al.,* 2003b).

The third group is the methylotrophic methanogens. This group include orders *Methanosarcinales* and *Methanobacteriales* namely *Methanosarcina barkeri* and genus *Methanosphaera* (with several possible variants) respectively (van der Wijngaard *et al.,* 1991; Meuer *et al.,* 2002). They directly produce CH_4 from methyl groups (-CH_3), methylamines [$(CH_3)^3$-N] and methanol (CH_3OH) as substrate (Gerardi, 2003). Methanol is usually found as organic pollutant in several wastewaters and is a substrate for both methanogens and acetogens (Bapteste *et al*., 2005). Compounds such as methanol or methyl-amines can be used as both electron acceptor and donor respectively. In running the hydrogenotrophic pathway in the reverse direction from methyl-CoM to CO_2, one molecule of C-1 compound is oxidized to provide electrons for reducing three additional molecules to CH_4. However, some *Methanosarcinales* can reduce this C-1 compound in the presence of methanol and H_2/CO_2, using only the last step of hydrogenotrophic methanogenesis (methyl-CoM to CH_4), drawing electrons from H_2 (Bapteste *et al.*, 2005).

2.4.5 Techniques Used To Detect Microorganisms from Anaerobic Reactor Samples

The main aims of studying any microbial ecology include the identification, classification and determination of microbial activity in the granules of an anaerobic reactor (Ziemiński and Frąc, 2012). In the past, traditional identification methods have been used to determine the morphology and phenotypic characteristics (Smith, 1966; Zeikus, 1977; Grothenhuis *et al.,* 1991; Liu and Tay, 2002), which are time-consuming and limited. Many microorganisms especially the methanogens are difficult to culture using traditional methods, because they have slow growth rates, restricted environmental conditions and selective nutritional requirements (Grothenhuis *et al.,* 1991; Briones and Raskin, 2003).

The development of molecular techniques (Figure 2.6) to study the complex microbial populations in environmental samples has eliminated the use of more elaborate traditional

techniques of culturing microorganisms (Gonzalez *et al.,* 2003; Hofman-Bang *et al.,* 2003). Basically these techniques have been divided into two main types: quantitative and qualitative. Qualitative techniques that may be used include polymerase chain reaction based denaturing gradient gel electrophoresis (PCR-DGGE), temperature gradient gel electrophoresis (TGGE) and terminal-restriction fragment length polymorphism (T-RFLP) etc. Microbial profiling techniques involve amplifying the nucleic acids isolated from environmental samples, sequencing and comparing them to the known sequences in the GenBank database appropriate for identifying related microorganisms. These methods have been successfully employed to study complex microbial populations in the laboratory- and industrial-scale fermenters to study the shift in microbial community structure (McHugh *et al.,* 2003a; Wang *et al.,* 2010; Ziganshin *et al.,* 2011; Shen *et al.,* 2013).

Quantitative real-time PCR (QPCR) and fluorescence *in-situ* hybridization (FISH) are quantitative techniques used in the survey of microbial ecologies (Yu *et al*., 2006; Zhang and Fang, 2006; Demirel and Scherer, 2008b; Tabatabaei *et al.*, 2009; Bergmann, 2012; Traversi *et al.,* 2012). Fluorescence *in-situ* hybridization has also been used for the quantitative analysis and to understand the spatial distributions of microorganisms (Briones and Raskin, 2003). This technique is based on hybridization of whole cells with specific probes, and microscopic analysis of dyed hybridized cells using epifluorescence microscopy, flow cytometry or scanning electron microscopy (Demirel and Scherer, 2008b; Tabatabaei *et al.,* 2009).

Quantitative real-time PCR on the other hand, can be used to amplify and simultaneously quantify targeted DNA sequence by employing a PCR-based technique that enables one to quantify the number of gene copies or relative number of gene copies in a given sample. The reliability of QPCR results is strongly dependent on the quality of the extracted genomic DNA (Bergmann, 2012). The amplified gene copy number from bulk DNA reflects the relative abundance of the microorganisms in the community. The amplification principle of QPCR is similar to that of PCR. This technique monitors the concentration of the amplified target after each PCR cycle using a fluorescent dye or probe change in fluorescence intensity that reflects the concentration of amplified gene in real-time assay (Zhang and Fang, 2006; Bergmann, 2012). Either absolute or relative quantification can be used to determine the

concentration of DNA or RNA in an extracted sample. This technique has been widely used to quantify the microbial population and dynamics in anaerobic reactors in their natural environments (Yu *et al.,* 2005; Yu *et al*., 2006; Traversi *et al.,* 2012).

However, it is difficult to monitor specific groups or a domain using only one technique as each technique has its own merits and demerits. Therefore, a combination of qualitative and quantitative methods including PCR-DGGE, QPCR and microarrays could be used to overcome the limitations of one technique (Park *et al.,* 2009). A combination of different molecular techniques, such as electron microscopy, PCR-based DGGE, cloning and FISH to gain insight into the physical appearance, function and structure of microbial diversity of methanogenic granules from a full-scale UASB reactor treating brewery wastewater have been explored in the past (Diaz *et al.,* 2006). The PCR-based DGGE and FISH analyses were used to identify the microbial populations in a full-scale UASB reactor treating brewery wastewater (Chan *et al.*, 2001; Liu *et al.*, 2002a). Chan *et al*. (2001) reported *Delta* and *Gammaproteobacteria; Methanosaeta concilii* and *Methanobacterium formicicum as the* dominant bacterial and Archaea bands detected in the full-scale UASB reactor. Likewise, Keyser *et al.* (2006) used PCR-DGGE for the fingerprinting and identification of the microbial consortium present in different types of granules collected from the UASB reactor treating brewery wastewater. Diverse group of methanogens such as *Methanosarcina, Methanosaeta, Methanobacterium* and uncultured bacteria belonging to Archaea domain were identified and fingerprinted using PCR-DGGE technique (Keyser *et al.,* 2006).

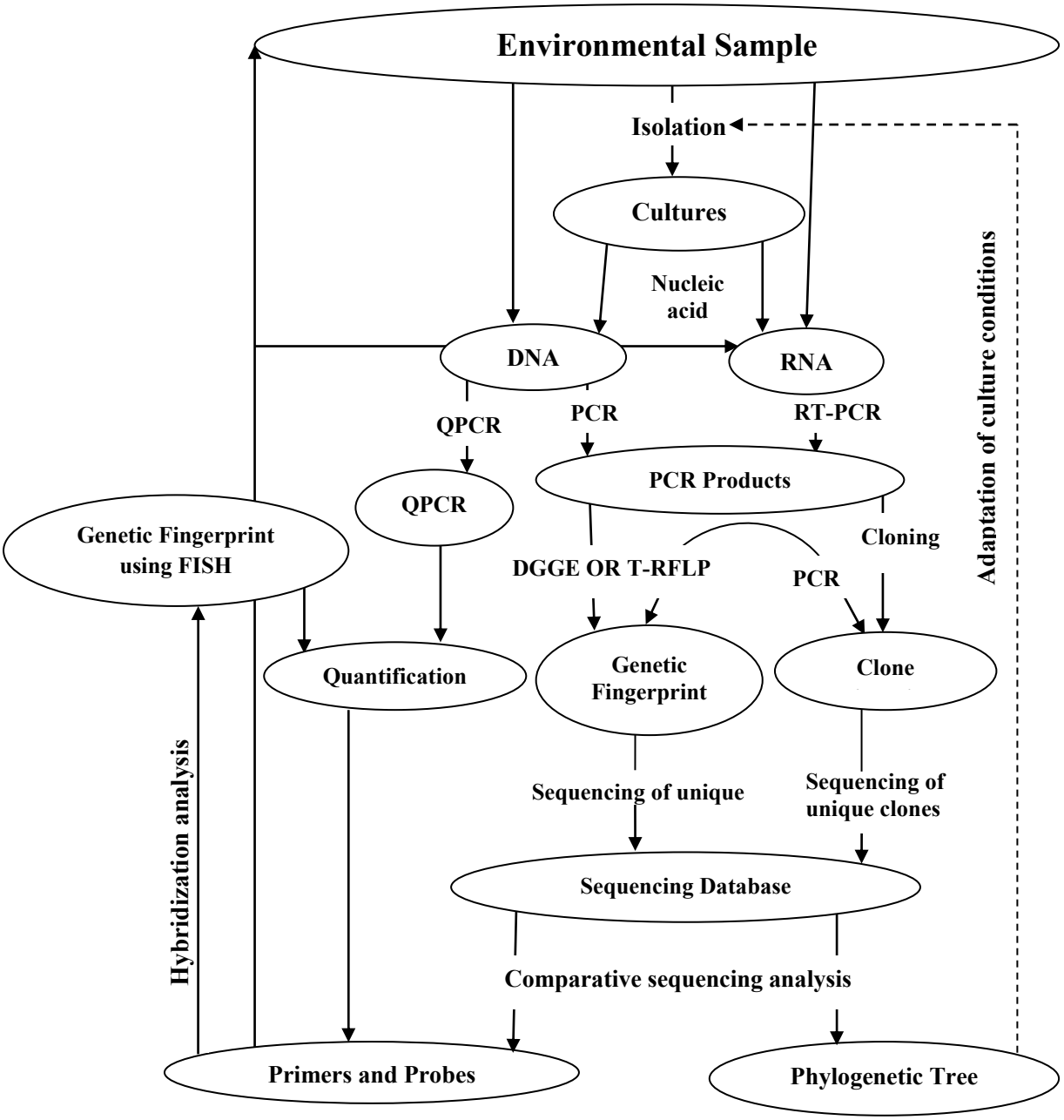

Figure 2.6: Flow diagram of different steps used in studying the structure and functions of microbial communities in an environmental sample.

2.5 FACTORS AFFECTING PERFORMANCE OF UASB REACTORS AND BIOGAS PRODUCTION

Even though the advantages of using anaerobic systems for pre-treatment of wastewater are recognized, anaerobic treatment plants are subjected to variations in one or more parameters, which affect or define the reactor's performance (Leitao, 2004; Keyser, 2006). Concerns still exist about reactor stability, effluent variability, the biological degradation of the adsorbed organic matter and activities. Due to these facts, several works on the operational variations and the stability of UASB reactor performance due to extreme transient conditions have been reported (Turovskiy and Mathai, 2006; Coelho *et al.,* 2007; Abbasi and Abbasi, 2012). The operational pH, temperature, nutrients availability, presence of VFA, influent COD concentration, influent type, sludge retention time (SRT), organic and hydraulic load variations are some of the parameters that should be monitored for the successful operation of any anaerobic reactor treating industrial wastewater (Fang *et al.*, 1995a; Leitao *et al.,* 2006; Coelho *et al.,* 2007; Abbasi and Abbasi, 2012).

2.5.1 Organic Loading Rate

Organic loading rate (OLR) is an important parameter that significantly affects the microflora and the performance of a UASB reactor. Fluctuations in organic load depends on the SRT, HRT, sludge properties, mixing intensity, duration of the variation, bacterial mass and activity (Rincón *et al.,* 2008; Abbasi and Abbasi, 2012). Different studies have shown that higher values of OLR can cause reduction in COD removal efficiency in a wastewater treatment system (Torkian *et al*., 2003; Sánchez *et al.,* 2005). Zhou *et al.* (2007) have reported that a higher loading rate could cause unrecoverable acidification, suppression of the methanogenic activity due to serious imbalance between the methanogens and the acidogens, as well as inhibition of methanogens by VFA production (Latif *et al.*, 2011).

2.5.2 Nutrients

The ability of anaerobic microorganisms to grow depends on the availability of the essential nutrients that are present in the wastewater (Speece *et al.,* 1983; Lettinga, 1995; Fermoso *et al.,* 2008; Mudhoo and Kumar, 2013). Lack of these nutrients could negatively affect their growth and the efficiency of the anaerobic degradation (Lettinga, 1995; Mudhoo and Kumar,

2013). The biochemistry of fermentation and CH_4 production involves many enzymes that contain different trace elements that need to be supplied as nutrients. Each anaerobic microorganisms involved in the degradation of complex organic matter to simple components are trace element specific, depending on the enzyme pathways (Zandvoort *et al.*, 2006).

Several studies on the impact of nutrients on the efficiency of AD and enhancement of granules in the bioreactors have been reported (Yu *et al.*, 2001; Zandvoort *et al.,* 2006; Fermoso *et al.,* 2008; Krishna, 2013; Zhang *et al*., 2013). Some bacteria, such as CH_4-forming bacteria in the reactors have relatively high internal concentrations of iron, cobalt and nickel (Zandvoort *et al.,* 2006; Zhang *et al.,* 2013), which may not be present in sufficient concentrations in the wastewater produced from the industries. Therefore, the addition of trace elements prior to treatment to improved reactors performance is highly recommended (Yenigun *et al*., 1996; Onodera *et al.*, 2013). The optimum C: N: P ratio to enhanced CH_4 yield was reported to be 100:2.5:0.5 (Rajeshwari *et al.,* 2000). This could be calculated based on the wastewater biodegradable COD concentration, nutrient concentration in bacterial cells and cell yield (Hulshoff-Pol, 1995).

2.5.3 Hydraulic Retention Time

The hydraulic retention time (HRT) has been defined as the average time that wastewater spends inside the reactor (Bitton, 1994). The flow rate and composition of wastewater entering the UASB reactor both affect the HRT (Cheng and Liu, 2002). High HRT increases the contact time of wastewater with the sludge, thus improving the effluent quality and biogas production rate. Therefore, a suitable HRT is very important for proper wastewater treatment in a UASB reactor for better treatment efficiency as well as quality and quantity of biogas concentration. From equation (2.5), the flow rate is inversely proportional to the HRT and directly related to the reactor volume (Liu *et al.,* 2003). This shows that volume is an important parameter that must be considered when designing a reactor.

$$Q = \frac{V}{HRT} \quad (2.5)$$

Where Q = Flow rate of influent stream (L/ d),

V = Volume of the reactor (L),

HRT = Hydraulic retention time (days).

Several studies have shown the effect of HRT on the microbial degradation in a single UASB reactor treating different types of industrial wastewater (Diamantis and Alexandros, 2007; Krakat *et al.*, 2010; Muda *et al.,* 2011).

2.5.4 Volatile Fatty Acids

Volatile fatty acids (VFAs) are important intermediate products in the formation of CH_4. The right concentration determines the efficiency of substrate removal from the reactor. For a typical reactor, overloading, or sudden variations in HRT and OLR could cause the accumulation of VFA and stressful conditions in the reactor during the break down of complex organic matter (Wang *et al.,* 2009). It can also affect the type of intermediates produced. This might cause a shift between acetogens and acidogens population (VFA producers), nitrogen reducing bacteria (NRB), sulphate reducing bacteria (SRB) and methanogens (consumers) leading to drastic changes in biogas production rates and compositions (Inanc *et al.*, 1999; Akarsubasi *et al.* 2006; Wang *et al.,* 2009). The toxic effects of all VFAs in the AD process especially propionate, on the activity of acetogens and methanogens have been investigated (Gallert and Winter, 2008; Uneo and Tatara, 2008; Wang *et al.,* 2009). Therefore, VFAs should be monitored and parameters adjusted in order to avoid their accumulation in the UASB reactor to prevent the inhibition of methanogenic organisms, thus reducing biogas production.

2.5.5 Operational Temperature

Operational temperature is an important parameter in anaerobic degradation processes. It determines the dominant bacterial flora and the growth rates of microorganisms present in a reactor (Khemkhao *et al.,* 2012). Different species of bacteria can survive at different

temperature ranges. Operational temperature greatly affects the biodegradation and the biogas production rate of any anaerobic reactor (Singh and Viraraghavan, 2000). The temperatures at which anaerobic reactors could operate include psychrophilic (0-25°C), mesophilic (25-40°C) and thermophilic conditions (40-60°C) (Sánchez *et al.,* 2006). Studies have shown that the performance of anaerobic reactors such as UASB reactors is temperature dependent (Kim *et al.,* 2006; Chen *et al.,* 2008; Thomas, 2010; Khemkhao *et al.,* 2012).

2.5.6 Operational pH

Microbial communities in the anaerobic digester have been shown to be highly pH dependent and require suitable conditions of pH to grow optimally (Table 2.1). pH far below or higher than the range required by the anaerobes could cause an accumulation of acetate, thereby inhibiting the methanogens and leading to conversion of COD to volatile acids instead of CH_4 production (Thomas, 2010). Therefore, most large scale AD reactors have been operated at pHs of between 6.5–7.5. The standard operating method to keep the pH in this range has been found to be the addition of lime and bicarbonate salts (Droste, 1997; Gerardi, 2003) or by reusing treated effluent in the reactor (Najafpour *et al.,* 2006; Espinoza-Escalante *et al.,* 2009). Therefore, controlling the pH of bioreactor is an essential factor for the growth of diverse group of microorganisms and high reactor performance.

Table 2.1: Optimum pH ranges for selected methanogens (Gerardi, 2003; Steinhaus *et al.,* 2007)

Genus	pH range
Methanothermus	6.5
Methanohalobium	6.5 – 6.8
Methanolacinia	6.6 – 7.2
Methanomicrobium	7.0 – 7.5
Methanosphaera	6.8
Methanogenium	7.0
Methanosprillium	7.0 – 7.5
Methanosaeta	7.6
Methanolobus	6.5 – 6.8
Methanothrix	7.1 – 7.8
Methanococcoides	6.5 – 7.5

2.6 MODELLING OF ANAEROBIC DIGESTION SYSTEMS

Mathematical models are analytical abstractions of the real world, representing the real system and can be used to simulate the behavior of any system under investigation. Models are typically a computer program, a set of mathematical formulas or an existing idea. Process modelling is the design and description of a real system that provides a better understanding of the processes, functions and its optimal working conditions (Pontes and Pinto, 2006). It can also be used to control a process, predict a system's behavior and outcomes; without a model, good predictions become difficult to make. Thus, among many other important monitoring and control strategies for proper understanding of the underlying phenomena in AD and biogas production is the development of suitable models, which adequately describe processes taking place in the AD bioreactor. It is an elegant and cost-effective tool to investigate certain engineering questions without wasting time and performing expensive laboratory tests (Thorin *et al.,* 2012). This has been found to be a good tool for process-control strategies and to enhance gas production. Mechanistic and empirical or data-based models are the two basic types of models available.

Mechanistic models are based on the underlying chemistry and physics governing the behaviour of a process. They have a structure that clearly represents the biological, chemical or the physical laws to propose one or more possible alternatives (Barampouti *et al.,* 2005). A mechanistic model assumes that a complex and real system can be understood by examining the working and manner in which individual parts are coupled (Batstone *et al*., 2002). Procedures for developing a mechanistic models include; the use of fundamental knowledge of the interactions between process variables to define the model structure, the determination of model parameters using experimental data, collection of data from the process to validate the model; then if the model is not satisfactory, one can re-examine process knowledge and restructure the model (Batstone *et al*., 2002; Mu *et al.,* 2008; Yetilmezsoy, 2012)..

Based on qualitative understanding of UASB process gained over the years, several attempts have been made to develop mechanistic models for quantitative descriptions of UASB reactors (Colussi *et al.,* 2012; Barampouti *et al.,* 2005; Zhao *et al*., 2010; Elnekave *et al.,* 2012; Yetilmezsoy, 2012). A comprehensive model of AD processes known as anaerobic

digestion model no. 1 (ADM1) was proposed (Batstone *et al*., 2002). This model divided the reactions that take place in the digester into two main types, biochemical and physico-chemical reactions. Detailed description of the model can be found elsewhere (Batstone *et al.,* 2002). This model has been widely used in anaerobic processes for CH_4 production (Mu *et al.,* 2008; Derbal *et al*., 2009; Zhou *et al.,* 2011).

Wu *et al.* (2005) applied the axial dispersion model developed by Gomes *et al.* (1998) to a laboratory scale UASB reactor using an orthogonal collocation algorithm. However, mechanistic models have been found to be insufficient to understand the UASB process due to several shortcomings in the models and to predict biogas production rates. These include inaccurate prediction of substrate availability to the methanogens, or the rate of VFA production or composition in the reactor (Elnekave *et al.,* 2012). Other deficiencies in formulation due to insufficient qualitative understanding of the process dynamics in reactor have been reviewed in the literature (Sinha *et al*., 2002). These may be overcome through the empirical observation and analysis of experimental data on UASB reactor performance (Elnekave *et al.,* 2012).

Empirical models are based on direct observation, measurement and extensive data records. They are frequently used as the basis for process control designs. Response surface methodology (RSM), fuzzy models and most recently, neural networks have emerged as one of the most efficient methods in empirical modeling, particularly for non-linear systems (Abu Qdais *et al.*, 2010; Thorin *et al.,* 2012). These models have been used to explain and predict the performances of UASB reactors treating different wastewater from domestic and industrial sources (Tay and Zhang, 1999; Holubar *et al.,* 2002; Cakmakci, 2007).

Empirical modeling depends on the availability of representative data for model-building and validation. Knowledge about the process is not needed for empirical modelling apart from cause-and-effect between variables; empirical modelling uses a trial and error approach (Thorin *et al.,* 2012). This type of model does not require much data. Once the structure of the model is defined, numerical techniques can be applied to parameterize the model. In this case, although the structure has been determined from process knowledge, the modelling

procedure becomes an empirical one. The numerical techniques that are used are also very different from those usually encountered in purely empirical modelling. They tend to be iterative, and are more complex (Khataee and Kasiri, 2011).

Kanat and Saral (2009) developed an artificial neural network (ANN) model to study biogas production from a thermophilic digester based on OLR, influent and effluent total VFA, alkalinity, pH and temperature of the reactor. A similar study was also conducted by Abu Qdais *et al.* (2010), where an ANN based model was developed to optimize CH_4 production using total and volatile solids, pH and temperature. Other studies on modeling of biological and wastewater treatment processes using an ANN was reviewed by Khataee and Kasiri (2011). The authors concluded that ANN models could predict the behaviour of the processes based on experimental data with high correlation coefficients. They further mentioned that additional information about the mechanisms and kinetics of the biological reactions was not necessary. However, none of the studies reviewed by Khataee and Kasiri (2011) had biogas production as an output parameter for their models. Ericson *et al.* (2010) modeled biogas production from a full-scale biogas digester using process data obtained from several years of running the digester using a statistically based ANN models.

Other model-based approaches to predict biogas production in an AD process have been reviewed in the literature (Lyberatos and Skiadas, 1999; Levstek and Lakota, 2012; Thorin *et al.,* 2012). Regression neural network (GRNN), feed-forward back (FFBP) and radial basis function-based neural networks (RBF) were designed and trained to predict the effluent COD, TSS, and biogas production from a full-scale UASB reactor treating juice wastewater (Elnekave *et al.,* 2012). The ANN results reported for the prediction of both COD concentration and biogas production were more accurate and closely related to the actual biogas produced, while relatively larger discrepancies existed for the TSS concentration (Elnekave *et al.,* 2012).

2.7 OPTIMIZATION TECHNIQUES USING EVOLUTIONARY ALGORITHMS

In recent times, due to problems in evaluating the first derivatives, to locate the optimal for many rough and discontinuous optimization surfaces, several free derivative–optimization algorithms have been developed. This optimization problem is represented as an intelligent search problem, where one or more agents are used to represent the constrained surface and finding the optimal point on the search landscape (Das *et al.,* 2008; Adeyemo and Otieno, 2009a). This includes restricting some variables of the system to be within certain ranges.

Evolutionary algorithms (EAs) as computer-based, biologically-inspired optimization algorithms are stochastic searching methods commonly used for solving non-differentiable, non-continuous and multi-modal optimization problems based on Darwin's natural selection principle (Enitan and Adeyemo, 2011; Thorin *et al.,* 2012; Sendrescu, 2013). They imitate the process of natural evolution and are becoming more important optimization tools for several real world applications for finding global optimum solutions regardless of initial parameter value (Kachitvichyanukul, 2012.). General steps for evolutionary algorithm development are shown in Figure 2.7. Evolutionary algorithms operate on a population of potential solutions, applying the principle of survival of the fittest method to produce successful and better solutions using evolutionary resembling operations (selection, reproduction and mutation), which are applied to individuals in a population (Ronen *et al.,* 2002; Shaheen *et al*., 2009). The use of EAs in conjunction with a simulated model for an optimization is an important factor for efficient and stable biogas production, especially CH_4 (Adeyemo and Enitan, 2011; Sendrescu, 2013).

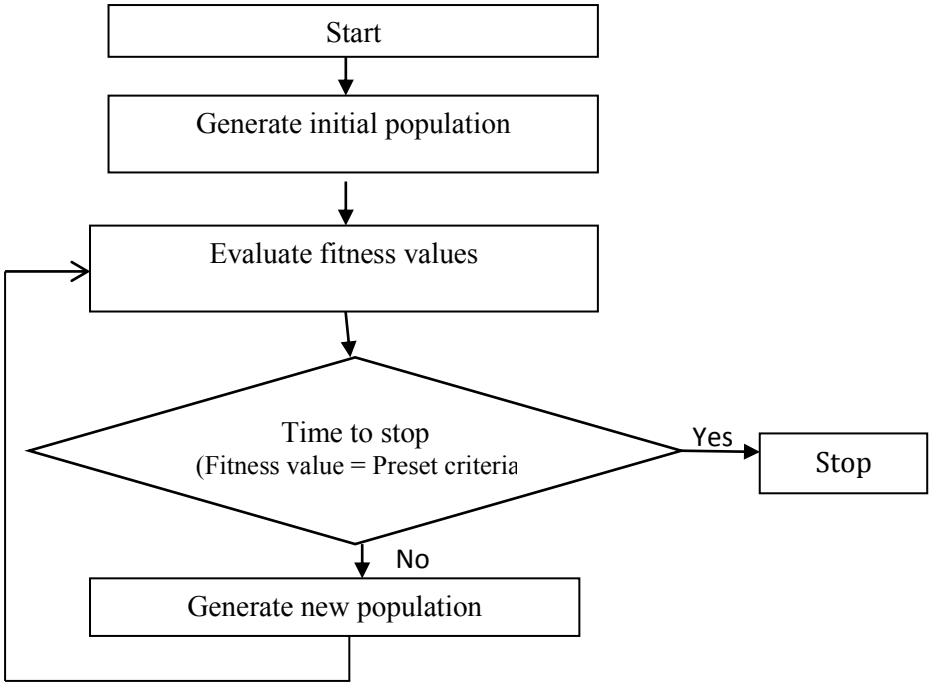

Figure 2.7: Flowchart for evolutionary algorithm development.

Genetic algorithms are computerized search and optimization heuristics based on the mechanics of natural genetics and selection (Deshmukh and Moorthy, 2010). They mimic the natural evolution to make a search process. The solutions are commonly represented as strings of binary digits. These algorithms require long processing times for a near-optimum solution to evolve. These algorithm types have been successfully used in science and engineering applications to reach near-optimum solutions to a variety of problems since its introduction by Holland (1975). Details on the principal steps of a typical GA have been reviewed (Mukhopadhyay *et al.,* 2009; Enitan and Adeyemo, 2011). New GA techniques that use real numbers for coding and genetic operators to generate new solutions until a stopping criterion is satisfied have emerged (Mohebbi *et al.,* 2008). It involves repeated procedures with an initial population of potential solutions, a fitness evaluation via the application of genetic operators and the development of a new population (Goñi *et al.,* 2008). There are different improved versions of the original genetic algorithm that have been reported in the literature such as elitist non-dominated sorting genetic algorithm (NSGA-II) (Deb *et al.,* 2000), compressed-objective genetic algorithm (COGA-II) (Boonlong, 2013) and multi-objective uniform-diversity genetic algorithm (MUGA) (Nariman-Zadeh *et al.,* 2010).

In an attempt to reduce the processing time and improve the quality of solutions, a differential evolution (DE) strategy was introduced by Storn and Price (1995) for faster optimization. Differential evolution is a population based algorithm like genetic algorithms using similar operators; crossover, mutation and selection of optimization problems. The basic steps of a DE algorithm are summarized in Figure 2.8. Differential evolution generates a new solution by combining several solutions with the candidate solution. The population of solutions in DE evolves through repeated cycles of three main DE operators: mutation, crossover, and selection. Details on the DE operators and operation of the DE algorithm are discussed by Deng *et al.* (2013) and Huang and Chen (2013). Unlike conventional GA that uses a binary coding for representing problem parameters, the DE algorithm represents each variable in the chromosome by a real number. Differential evolution selection process and its mutation scheme make DE self–adaptive. Differential evolution has efficient straight forward features that make it very attractive for numerical optimization. The basic approach of differential evolution algorithm works as follows:

1. Initialize the number of a potential population (NP) at random, the maximum numbers of evolution, the crossover rate (CR) and the scale factor, (F).
2. Initialize the population (pop), by some repair rules such that 'variables' values are within their boundaries.
3. Following the DE/rand/1/bin strategy, and production of new generation of individual solutions:
    a) Implementation of differential strategy on the individual mutation for each target vector. The mutation component is a different vector of the parent.
    b) With the crossover probability, each variable in the main parent is perturbed by adding to it a ratio F of the difference between the two values of this variable in the other two supporting parents. At least one variable must be changed. This process represents the crossover operator in DE.
    c) Selection operation of best solutions, if the resultant vector is better than the trial solution, it replaces it; otherwise the trial solution is retained in the population.
    d) Go to 2 above.
4. If the termination conditions are met go to 5, else go to 2 above
5. End.

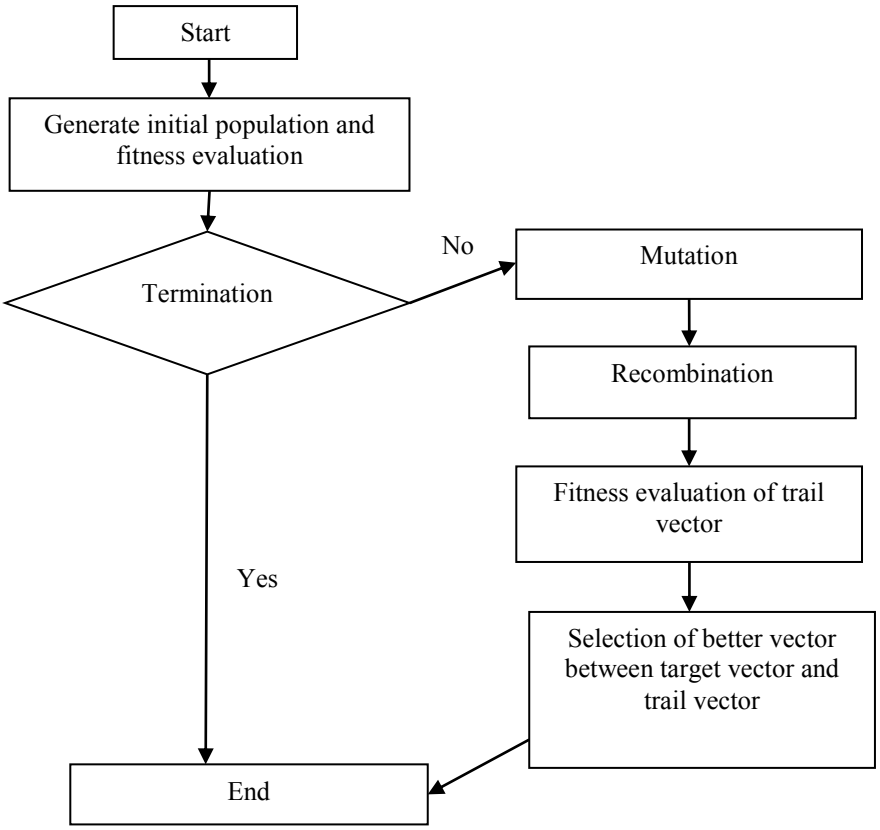

Figure 2.8: Flowchart for the main steps in DE algorithm development.

The principal difference between DE and GA is that GA relies on crossover, a mechanism of probabilistic and useful exchange of information among solutions to locate better solutions, while DE uses mutation as the primary search mechanism (Enitan and Adeyemo, 2011). However, the operators are not all exactly the same as those with the same names in GA (Kachitvichyanukul, 2012). Differential evolution uses non-uniform crossover and tournament selection operators to create new solution strings. In GA, two parents are selected for crossover and the child is a recombination of the parents. In DE, three parents are selected for crossover and the child is a perturbation of one of them. The new child in DE replaces a randomly selected vector from the population only if it is better than it. In simple GA, children replace the parents with some probability regardless of their fitness. All solutions in DE have the same chance of being selected as parents without depending on their fitness value. Differential evolution employs a greedy and less stochastic approach to solve problems than the classical evolutionary algorithms (Babu and Chaurasia, 2003; Karaboga, 2004;

Mariani *et al.*, 2008; Liu and Wang, 2010). The crucial idea behind DE is a scheme to generate the trial parameter vectors that are completely self-organizing which adds the weighted differences between two population vectors to a third vector, therefore no separate probability needs to be used (Adeyemo and Enitan, 2011).

Differential evolution algorithm is a stochastic optimization method minimizing an objective function that can model the problem's objectives while incorporating constraints. It can be used for optimizing functions with real variables and many local optima (Pierreval *et al.,* 2003). There are different improved versions of original differential evolution that have been reported in the literature, such as hybrid differential evolution (HDE) (Tsai and Wang, 2005), Pareto differential evolution approach (PDEA) (Madavan, 2002), multi-objective differential evolution algorithm (MDEA) (Adeyemo and Otieno, 2009b), multi-objective differential evolution algorithm (MODEA) (Ali *et al.,* 2012) and a combined Pareto multi–objective differential evolution (CPMDE) (Olofintoye *et al.,* 2014).

However, only few studies have been reported on the applications of evolutionary algorithms in the optimization of anaerobic reactor for CH_4 production. Recently, artificial neural network coupled with genetic algorithm (ANN-GA) has emerged as one of the most efficient methods in empirical modeling and optimization, particularly for non-linear systems (Abu Qdais *et al.*, 2010). Once an ANN-based and mass balance-based process model with fairly good generalization capability is constructed, its input space can be optimized appropriately to secure the optimal values of process variables.

Modelling and optimization of biogas production on mixed substrates of banana stem, cow dung, saw dust, paper and rice bran waste, using ANN coupled with GA was reported (Gueguim Kana *et al.,* 2012). In another study, simulation and optimization of the effect of operational pH, temperature, total volatile solids (TVS) and total solids (TS) on biogas yield using ANN and GA was studied by Abu Qdais *et al.* (2010). The integration of the ANN model with GA as the optimization tool resulted in identification of the optimal operational digester parameters that lead to increase in CH_4 yield by 6.9%. The study demonstrated that optimization of models using evolutionary algorithms such as GA will help in better

prediction of process output such as biogas production, especially CH_4 yield (Abu Qdais *et al.,* 2010).

A mathematical model of a laboratory-scale plant for slaughterhouse effluents biodigestion for biogas production was formulated with the objective of obtaining a liquid effluent of low COD and to generate CH_4 as a byproduct, stored and then used as an energy source (Martinez *et al.,* 2012). Parameters of this model were fitted into a two-step algorithm. The authors first adjusted the parameters that are directly related to the measured variables using GA, while a gradient descendent algorithm was used for fine adjustment of all the whole parameters to optimize for maximum CH_4 yield. The results reported showed that the model used was able to predict four system variables and CH_4 generation (Martinez *et al.,* 2012).

Wei and Kusiak (2012) used a data-driven approach for optimization of a biogas production process in a wastewater treatment plant. A multi-layer perceptron neural network was used for the construction of an optimization model. High computational complexity of the model led to the use of a particle swarm optimization (PSO) algorithm to maximize biogas production, by finding the optimal settings of controllable variables. The model solution has resulted in an increase in biogas production under an optimized operational condition using the PSO algorithm. However, two major challenges in this field of parameter estimation of non-linear dynamic biological systems were numerical integration of differential equations and finding global parameter values (Tsai and Wang, 2005).

The particle swarm optimization technique was used to identify the parameters for an offline estimation of yield and kinetic coefficients in a non-linear dynamical model for anaerobic wastewater treatment bioprocesses (Sendrescu, 2013). The identification scheme was formulated based on the optimization problem. The error between the simulated response of a parameterized model and the actual physical measured response of the system was optimized. The multimodal function and classical iterative methods used in their study as reported, failed due to its inability to find global optimum solutions. However, the parameters estimation for the system was achieved by the PSO algorithm for the minimization of error function. The

Table 2.2: Anaerobic model and optimization tools for different types of wastewater

System modelled and wastewater types	Model type	Evolutionary algorithm type	Input	Output	References
UASB reactor treating poultry wastewater	Empirical	-	HRT, OLR	Daily biogas production rate, effluent COD concentration.	Yetilmezsoy and Sakar (2008)
UASB reactor treating potato wastewater	-	-	pH, influent and effluent COD, temperature, VFA, alkalinity	Biogas production rate.	Barampouti *et al*. (2005)
Anaerobic hybrid reactor for treating alkali rice straw	Stover-Kincannon	-	OLR, HRT	Biogas production and effluent COD concentration.	Narra *et al.* (2014)
Distillery wastewater	Stover-Kincannon	-	OLR, HRT	Effluent concentration.	Acharya *et al.* (2011)
Olive mill solid waste	Chen-Hashimoto	-	Substrate Concentrate, HRT	Volumetric methane production rate.	Borja *et al.* (2003)
Poultry wastewater treatment in UASB reactor	Chen-Hashimoto and Stover-Kincannon	LOQO/AMPL algorithm	Influent concentration, temperature, OLR, HRT, reactor volume and flow rate	Methane production rate, effluent substrate concentration and net operating cost	Yetilmezsoy (2012)
Dry–thermophilic anaerobic digestion of organic fraction from municipal solid waste	Model based on Romero García (1991)	-	Influent and effluent concentration, HRT	Methane production.	Fdez-Güelfo *et al.* (2012)
Biogas production digester	-	ANN-GA	Temperature, TS, TVS, pH	Methane production and biogas yield.	Abu Qdais *et al.* (2010)
Real cotton textile wastewater treatment in UASB reactors	-	ANN	HRT, influent COD concentration, pH, VFA concentration, operating temperature, dilution ratio, alkalinity, TSS and OLR	COD removal efficiency.	Yetilmezsoy and Sapci-Zengin (2009)

strategy of PSO algorithm could still converge to accurate results, even in the presence of measurement noise. The authors reported that PSO algorithms can be used in the optimization of parameters during model identification (Sendrescu, 2013). Further studies on anaerobic model and optimization tools for different types of wastewater are summarized in Table 2.2.

Models applied to describe AD have been shown to be an effective tool and could be used in the near future for tracking and predicting the development of biogas production especially CH_4 yield. This could help in accelerating the speed of digester start-up, as well as biogas (CH_4) production from biomass and wastes for energy generation. Likewise, data-base system could be developed for the simulated results in the field of renewable energy for gathering and sharing information on the suitable technology, provide an appropriate operational conditions for AD of wastes and thus, improve the amount of biogas formation.

2.8 RESEARCH OUTPUT

Journal Articles

1) Adeyemo, J. and **Enitan, A.** 2011. Optimization of fermentation processes using evolutionary algorithms. *Scientific Research and Essays,* 6(7):1464-1472.

CHAPTER THREE: PERFORMANCE EVALUATION OF AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR TREATING BREWERY WASTEWATER

3.1 INTRODUCTION

The brewing process involves series of batch operations on raw materials to the final product. The production process includes the blending and fermentation of maize, malt and sorghum grits using yeast, which requires large volumes of water as the primary raw material. Traditionally, the amount of water needed to brew beer has been found to be several times the volume actually brewed (Simate *et al.,* 2011). For instance, an average water consumption of 6.0 hectolitres is required to produce one hectolitre of clear beer (South African Breweries, 2001). Large volumes of water are being used by the industry for production of beer for two distinct purposes; as the main ingredient of the beer itself and as part of the brewing process for steam raising, cooling, washing of floors, cleaning of the brew house, packaging and cleaning after the completion of each batch operation. The amount of wastewater that is being discharged from the industry after the production of beer also contributes to this large volume of water (Simate *et al.,* 2011). This wastewater is very high in organic content and is highly polluting to the environment if discharged without prior treatment (Raposo *et al.,* 2010; Inyang *et al.*, 2012; Mata *et al.,* 2012). Furthermore, most industries discharge their effluents into municipal treatment plants or to the environment without adequate characterization, quantification and pre-treatment due to economic and technological constraints (Ikhu-Omoregbe *et al.,* 2005). This may have an adverse effects on the municipal treatment plants by overloading these systems thus, reducing the efficiency of the treatment plants.

Among the brewery industries, South African Breweries (SAB Ltd) has been reported to be the second largest beer producer in the world and uses about 10.5 million cubic metres of water per annum at one of its breweries and approximately 70% of this is discharged as wastewater (Jones *et al.,* 2011; Kirin Holdings, 2012). With the competing demand on water resources and water reuse, discharge of industrial effluents into the aquatic environment has become an important issue (Islam *et al.,* 2006; Danazumi and Hassan, 2010; Kanu and Achi, 2011; Simate *et al.,* 2011; Kovoor *et al.,* 2012). Much attention has been placed on the impact of industrial wastewater on water bodies worldwide due to the accumulation of organic and

inorganic suspended matter, nitrite, nitrate and soluble phosphorus (Phiri *et al.,* 2005; Islam *et al.,* 2006; Baig *et al.,* 2010; Ipeaiyeda and Onianwa, 2012).

A considerable number of environmental pollution problems have emerged recently, which has led to monitoring and controlling of the quality and quantity of liquid effluents being discharged into natural water bodies or municipal treatment plants especially by the industry (Kanu and Achi, 2011; Kovoor *et al.,* 2012). The effects of contamination on water bodies include change in pH, electrical conductivity, temperature and eutrophication of rivers and dams due to high concentrations of inorganic and organic matter from the industrial activities. Some industries have been fined by the national water authorities and municipal authorities for discharging poor quality effluents that do not meet the discharge standards into the natural water bodies, as well as the municipality water treatment plants (Ikhu-Omoregbe *et al*., 2005; Parawira *et al.,* 2005; Worldwide Brewing Alliance, 2011). In order to meet regulatory standards, many industries including brewery industries pre-treat their effluent using different AD technologies before its release into municipal treatment plants (Parawira *et al.,* 2005; Melamane, 2007).

High concentrations of pollutants load in the brewery wastewater are greatly reduced by the use of high-rate anaerobic digestion (AD) technology. It has helped the industry to comply with stricter pollution control regulations, satisfy the search for greater efficiency and improves effluent quality (Parawira *et al.,* 2005; Li *et al.*, 2011). AD process produces less sludge than aerobic treatments, hence reducing effluent disposal costs. The UASB system has successfully been used to treat different types of wastewater (Nery *et al*., 2001; Manhokwe *et al*., 2009).

In the last few decades, much attention has been paid to the use of AD processes for the treatment of brewery wastewater due to the nature and strengths of the brewery wastewater (Parawira *et al.,* 2005; Nizami and Murphy, 2010). Benefits of using UASB reactors include the production of sufficient amounts of biogas as a natural source of energy that can be used as electricity to power the entire brewery wastewater treatment process (Bocher *et al.,* 2008).

The aim of this study was to monitor, characterize and quantify the brewery wastewater pollution load from one of the brewery industry in KwaZulu-Natal, South Africa. Thereafter, the efficiency of a full-scale UASB reactor for the treatment of brewery wastewater and the composition of biogas produced during AD was determined. This UASB reactor is being used for on-site treatment of brewery wastewater to reduce the organic load before discharge. This study will help in generating data for both the industry and the local authority, as well as assess the level of compliance by the industry to the local legislative guidelines for effluent disposal. The data obtained from the full-scale UASB reactor will also be used in the course of this study to determine model coefficients to predict CH_4 production and effluent quality.

3.2 MATERIALS AND METHODS

3.2.1 Description of Full-Scale UASB Reactor

The full-scale UASB reactor was constructed from concrete with a series of settlers and baffle plates arranged at the bottom for even distribution with a pre-conditioning tank (Figure 3.1) and 20% effluent recycle. The operating capacity of this UASB reactor is 1480 m^3 excluding the pre-conditioning tank (Ross and Louw, 1987); however the total capacity increases up to 1700 m^3 including the pre-conditioning tank (Isherwood, 1991). The pre-conditioning tank is used to retain effluent for hours for solid settlement. The pre-conditioning tank was used to homogenize the incoming effluent and balance the variations in pH, organic loads and flow resulting from the brewing process operation to desired levels of anaerobic treatment. The volume of the reactor was based on the average volumetric loading rate of about 10 kg COD /m^3 per day. Nitrogen from nutrient supplements are added into the conditioning tank in the form of urea and $FeCl_3$ to provide the biomass with necessary nutrients for nitrogen and iron as well as to help in flocculation of the biomass in the reactor. The adjustment of acidic influent to neutral pH is currently being done by the addition of soda ash (Enitan *et al.,* 2014a).

The operational temperature and pH of the reactor was maintained between 33 ± 2°C and 6.5-7.2 respectively. Retention time varies with influent flow rate between 8-12 h for bacteria

to make use of the influent substrate. The biogas produced in the reactor is separated from the effluent and the biomass in three-phase separators at the top of the reactor. The gas passes through a defoam tank to remove any solids present, and was then flared. The biomass separated from the gas and effluent is retained in the reactor and settled back into the sludge bed. Off gas produced at the surface of the weirs in the UASB reactor is currently collected and treated through a biofilter prior to being vented to the atmosphere (Enitan *et al.,* 2014a). The treated UASB effluent is disposed to the municipal wastewater treatment plant for further treatment.

3.2.2 Wastewater and Biogas Sampling Procedure

Raw brewery wastewater, influent (pre-conditioned wastewater) and effluent (post-reactor treatment) wastewater samples were each collected in one-litre sterile glass bottles (Figure 3.2) and transported to the laboratory at 4°C for analysis. Physico-chemical analyses were carried out within 48 hours of sample collection over a period of one year with necessary preservation techniques adapted from Standard Methods (APHA–AWWA–WPCF, 1998). Biogas was collected into a gas holder (Tedlar bag, Sigma-Aldrich) for analysis. At first, sample was taken bi-weekly but changed to monthly basis from the third month

3.2.3 Wastewater Characterization

Wastewater samples were analyzed for total dissolved solids (TDS), total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), volatile solids (VS), temperature, pH, oxidation-reduction potential (ORP), alkalinity, total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), biological oxygen demand (BOD_5), conductivity (mS/cm), crude protein, sulphates, orthophosphate, total oxidised nitrogen (TON), nitrite (NO_2), nitrates (NO_3) and NH_3 according to Standard Methods for Examination of Water and Wastewater (APHA–AWWA–WPCF, 1998). The TS and TSS were determined gravimetrically by drying well homogenized samples respectively at 103°C for 24 h. The VS and VSS fractions were determined gravimetrically by incineration in a muffle furnace at 550 °C for 1 h (APHA–AWWA–WPCF, 1998). Alkalinity was measured by potentiometric titration using 0.02N H_2SO_4 to an end-point pH value of 4.5. The aim of measuring alkalinity

was to evaluate the buffering capacity of the UASB reactor treating brewery wastewater and the effect on the granular sludge (APHA–AWWA–WPCF, 1998). Tests were carried out in duplicate, means and standard deviations are presented where appropriate.

3.2.3.1 Conventional and instrumental methods used for analysis

The TDS, conductivity (mS/cm) and oxidation-reduction potential (O/R potential) were measured using calibrated electrode (YSI 556MPS, *Yellow Springs,* USA). The pH and temperature were measured using a pH meter (Beckman pH 211 Microprocessor, USA). The BOD_5 measurement was done using the respirometric method for five days (OxiTop TS 606/2-i system). The COD concentration in the wastewater was determined by close refluxing according to the standard method, 5220D (APHA–AWWA–WPCF, 1998). The protein concentration was analyzed using a UV/VIS Spectrophotometer (Merck, Spectroquant Pharo 300, Germany) according to the protocol of Lowry *et al.* (1951). Sulphates, orthophosphate, NH_3, TON, NO_2 and NO_3 were measured using Thermo Gallery photometric analyser (Thermo Scientific, UK) (APHA–AWWA–WPCF, 1998). The composition of biogas produced was analyzed using a gas chromatograph (Shimadzu GC-2014, Japan) equipped with a thermal conductivity detector (TCD). The column used was Porapak Q 1.8 m × 2.10 mm with the column oven, injector and detector temperatures set at 40°C, 100°C and 100°C, respectively. Helium gas was used as the carrier at 20 ml/min.

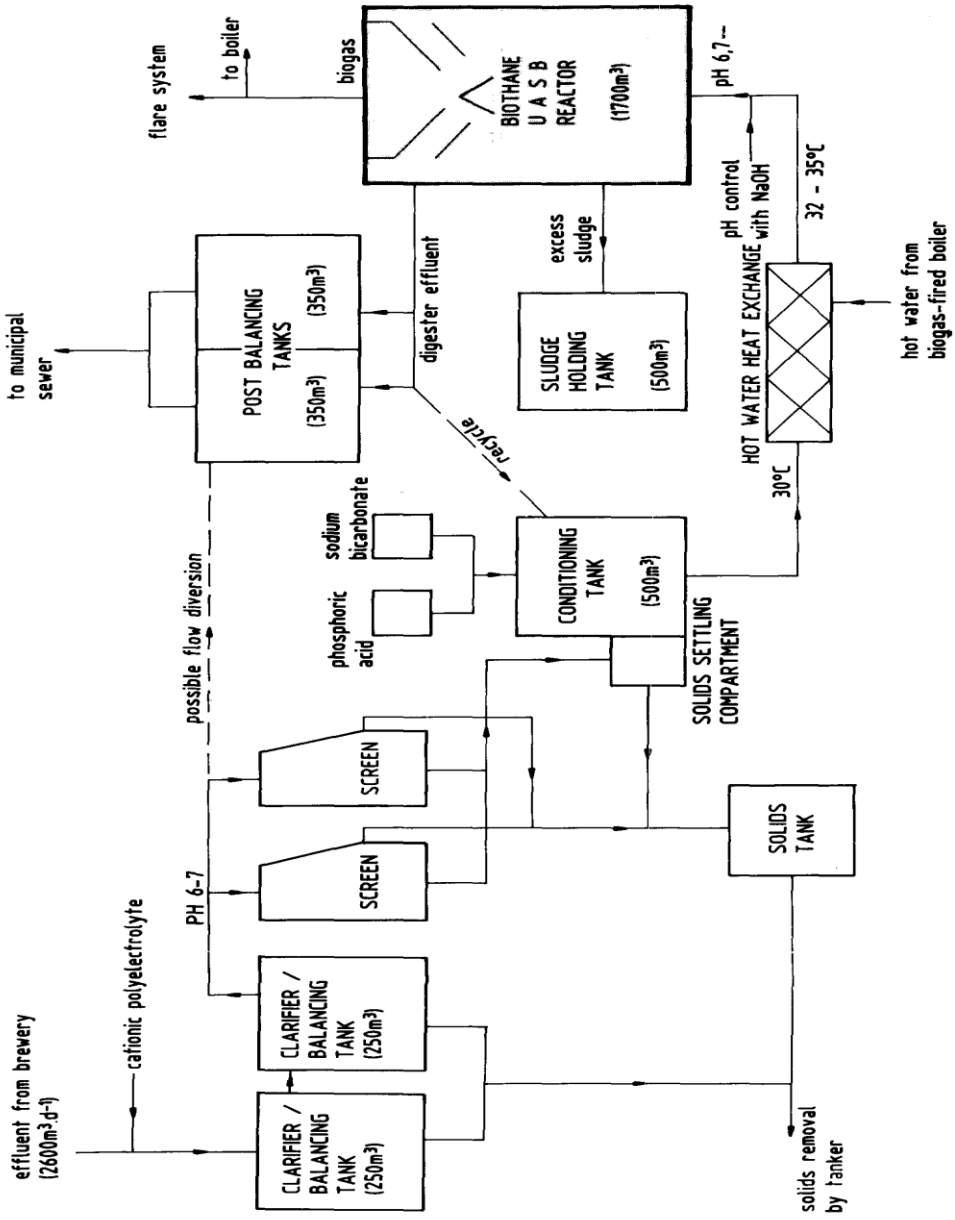

Figure 3.1: Layout of full–scale UASB reactor treating brewery wastewater (Hoffmann, 1985; Ross, 1989).

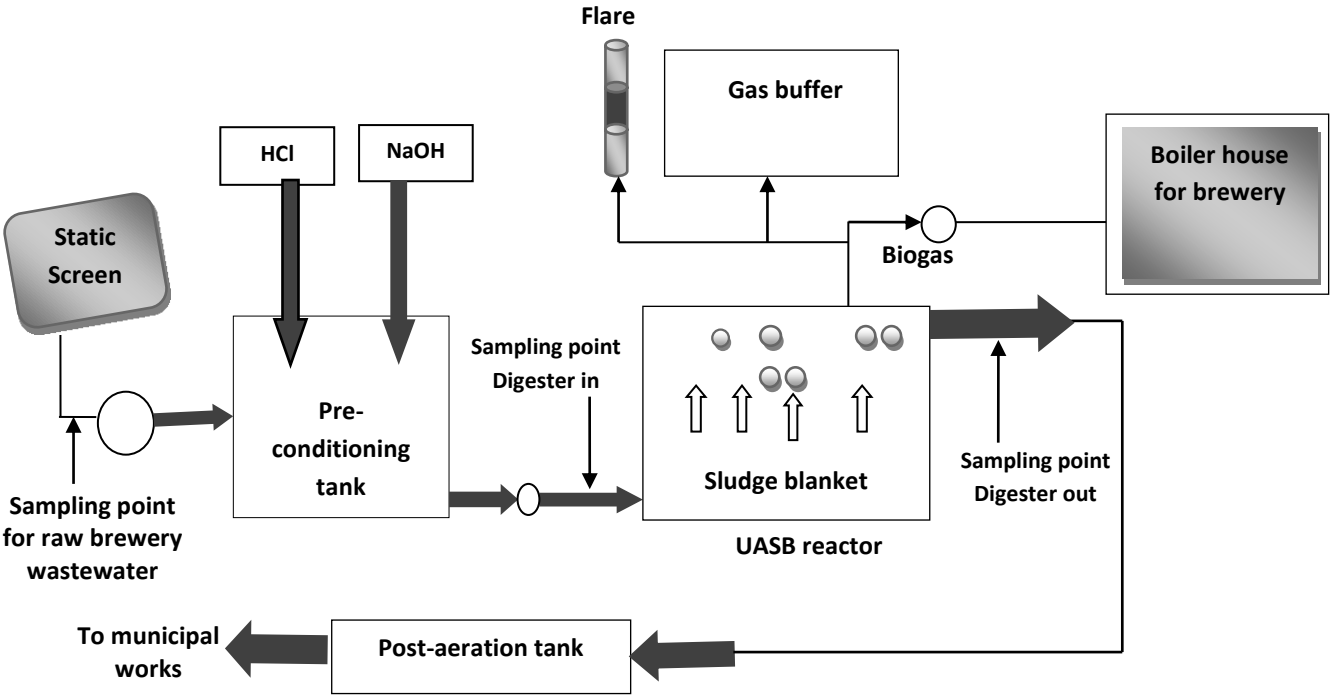

Figure 3.2: Schematic diagram of the sampling points from which samples were collected for this study to monitor the full-scale UASB reactor treating brewery wastewater.

3.2.4 Analytical Quality Assurance and Statistical Analysis

Both reagent and sample blanks were used for all the methods that required the use of the spectrophotometer and Aquakem Gallery discrete auto analyser. Standard solutions were prepared for the analysis of COD and protein content. Instruments were first calibrated before using standard solutions. Sample bottles were thoroughly cleaned, 1:1 HCl, triple rinsed with distilled water and a final triple rinse was done with the sample as suggested by Fatoki and Mathabatha (2001). The data obtained was used to calculate mean, ranges, and standard deviations. Graphs and statistical analysis were performed using GraphPad Prism v 5.0, software package for Pearson's correlation coefficient and analysis of variance (ANOVA) of the parameters measured.

3.2.5 Estimation of Pollutant Removal Efficiency

The organic load, nutrient and suspended solid removal efficiency of the UASB reactor were calculated using Equation 3.1 (Clara *et al*., 2005).

$$\text{Removal efficiency (\%)} = \frac{C_{influent} \times C_{effluent}}{C_{influent}} \times 100 \quad (3.1)$$

Where, $C_{influent}$ = initial parameter concentration and $C_{effluent}$ = final parameter concentration.

3.3 RESULTS AND DISCUSSION

3.3.1 Brewery Wastewater Composition

The results of the physico-chemical analyses and the summary of the statistical analysis of the raw brewery wastewater composition investigated for over a period of one year are shown in Table 3.1. The results showed that the effluent produced from the brewery industry did not meet the discharge limit for wastewater disposal to water bodies according to the European Union (EU) discharge limits (Driessen and Vereijken, 2003). Although, the local effluent discharge standards do vary from one location, region and country to another, as shown in Table 3.1 (Department of Public Works Republic of South Africa, 2012). Furthermore, the discharge limits are less stringent when the effluent is to be discharged to a municipal wastewater treatment plant (Adeniyi, 2002).

The results of the analysis indicated that the qualities of the raw brewery wastewater from the industry prior to treatment in terms of total and soluble COD as well as the BOD_5 are higher than the discharge standards (Table 3.1). The trends and variability of the values as well as large standard deviations from the means shows that the pollution level of the wastewater was high. The average and standard deviation of the total and soluble COD values of wastewater prior to treatment were 5340.97 ± 2265.11 mg/L and 3902.28 ± 1644.25 mg/L respectively. The trends of total and soluble COD during the course of the brewery wastewater composition monitoring showed fluctuation in the strength and composition of the brewery wastewater with the range being between 1096.41 to 8926.08 mg/L for TCOD and 1178.64 to 5847.74 mg/L for SCOD (Enitan *et al.,* 2014b). The variations in the COD concentration for each week could be as a result of variation in the activities and housekeeping practices of the

brewery plant. The observed values are within the range reported for some brewery wastewater plants as shown in Table 3.2 (Ikhu-Omoregbe *et al*., 2005; Parawira *et al.,* 2005; Rao *et al.,* 2007; Inyang *et al*., 2012).

The variation in BOD_5 and SCOD contents of the brewery wastewater. The BOD_5 values range between 1609.34 – 3980.61 mg/L with a mean value of 3215.27 mg/L and standard deviation of ± 870.90 (Table 3.1). Low COD: BOD_5 ratios of 1.932 ± 0.543 obtained in this study were in accordance with past reports, which suggested that the wastewater content is biodegradable (Kilani 1993; Dupont, Theodore and Ganesan 2000). Effluent from the brewery plant is regarded as a biodegradable industrial wastewater and the COD concentration of brewery effluent that is more than 800 mg/l has been reported to be more suitable for treatment using anaerobic digestion (Dupont, Theodore and Ganesan 2000; Ikhu-Omoregbe, Kuipa and Hove 2005). Further work on the characterization of brewery wastewater during the monitoring period could be found in the literature (Enitan *et al.,* 2014b).

Table 3.1: Summary of raw brewery wastewater composition from the industry prior to anaerobic treatment and indicative discharge limits in South Africa (SA) and the EU (Driessen and Vereijken, 2003)

Parameters	Range	Average value*	SA Discharge limits	EU Discharge Limits
Temperature (˚C)	24-30.5	27.90 ± 2.23	< 44	-
pH	4.6-7.3	6.0 ± 1.44	Between 5.0 and 9.5	-
Total COD	1096.41- 8926.08	5340.97 ± 2265.11	75	125
Soluble COD	1178.64 - 5847.74	3902.28 ± 1644.25	-	-
BOD_5	1609.34 – 3980.61	3215.27 ± 870.90	Determined by the treatment capacity of the receiving sewerage treatment plant	25
Crude protein	61.67-754.42	273.47 ± 233.63	-	-
Orthophosphates	7.51 -74.10	23.71 ± 21.88	10	1-2
TON	0 - 5.36	1.81 ± 1.66	-	-
NH_3-N	0.48 - 13.05	8.62 ± 10.40	3	-
Nitrate	1.14 -11.55	4.30 ± 3.41	15	-
Nitrite	0-0.24	0.37 ± 0.18	15	-
ORP (mv)	-27.10 to -84.91	-47.80	-	-
Conductivity (mS/cm)	1.04-1.62	1.52	70-150	-
TS	1289.26 – 12248.13	5698.11±2749.06	-	-
VS	1832.82 – 4634.31	3257.33± 1074.34	-	-
TSS	530.67 – 3728.02	1826.74± 972.46	25	35
VSS	804.11 -1278.43	1090.86 ± 182.74	-	-
Alkalinity($mgCaCO_3$/ L)	500- 10000	2450.33± 3034.19	-	-

* All values are in mg/L except otherwise stated.

*An average of 14 samples ± std deviation.

Table 3.2: Brewery wastewater characterization and the efficiency of the UASB reactor as compared to the literature

Parameter	Units	This study	Parawira *et al.* (2005)	Ahn *et al.* (2010)	Rao *et al.* (2007)	Diaz *et al.* (2006)	Rüffer *et al.* (1991)	Inyang *et al.* (2012)
pH	-	4.6-7.3	3.30-6.30	6.3-6.9	3-12	7.2	-	11.97
Temperature	ºC	24-30.5	25-35	-	18-40	-	-	-
NH_4-N	mg/L	0.48 - 13.05	-	2.2-7.0	-	15	-	-
TN	mg/L	0 - 5.36	0.0196-0.0336	17-36	-	15	30-100	0.39
TP	mg/L	-	16-124	8.4-17	-	-	10-30	0.462
COD	mg/L	1096.41- 8926.08	8240 ≥ 20000	910-1900	2000-6000	4000	1120-1500	471
TSS	mg/L	530.67 – 3728.02	2020-5940	140-320	2901-3000	1300	10-60ml/l	81
VSS	mg/L	804.11 -1278.43	-	90-180	-	-	-	-
TS	mg/L	1289.26 – 12248.13	5100-8750	1300-2000	5100-8750	-	-	-
$COD_{removal}$	%	78.97	57	80	-	80	-	-
Total COD quantity in reactor	g	13210.48	10,000	-	-	-	-	-
Total COD removal	g	10436.28	5700	-	-	-	-	-

* All values are in mg/L except otherwise stated

3.3.2 Efficiency of UASB Reactor Treating Brewery Wastewater

3.3.2.1 Effect of pH and temperature on UASB reactor performance

Raw wastewater from the brewery industry was acidic and adjustment to neutral pH was done by the addition of soda ash inside the conditioning tank prior to treatment. This was done because the anaerobic reactor is very sensitive to changes in pH and if wastewater is not buffered, it could lead to accumulation of VFA concentrations in the reactor and thus, affect the activity of microorganisms (Rosenwinkel *et al*., 2005). The concentration of substrate in the pre-conditioned brewery wastewater and the pollution effect on the treatment plants are presented in Figure 3.3. This figure shows the observed pH values of the pre-conditioned wastewater (digester inlet) and effluent from the reactor (digester outlet) with the corresponding substrate COD concentrations. The reactor's pH was stable throughout the monitoring period between 6.6 and 7.3 at an average operating temperature of 29˚C under various organic loading rates.

Several studies have reported reactor failure or under performance of their anaerobic treatment system due to low pH values and changes in reactor temperature (Visser *et al.*, 1993; Poh and Chong, 2009; Tabatabaei *et al.,* 2011). In a study conducted by Tai *et al.* (2006), a similar trend in the pH of effluent from the UASB reactor with pH values between 6.9 and 7.5 was reported. This condition is considered optimal for the growth of methanogens (Gerardi, 2003). Steinhaus *et al.* (2007) studied the optimum growth conditions of *Methanosaeta concilii* using a portable anaerobic microtank. They reported an optimum pH level of 7.6 for the growth of methanogens and any deviation from this optimum pH could lead to the inhibition of methanogens in the anaerobic reactor as well as CH_4 production.

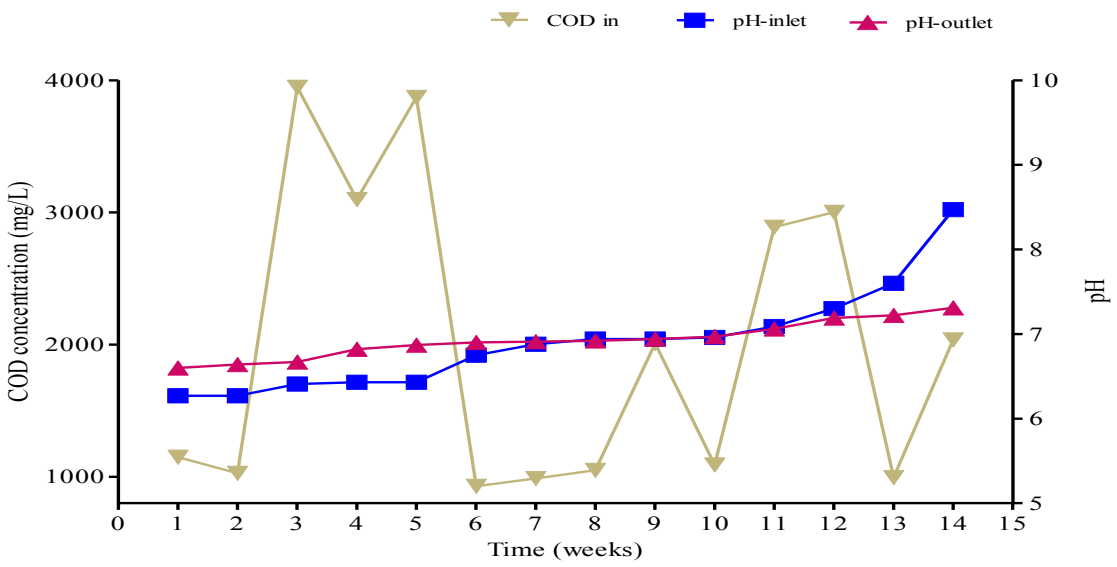

Figure 3.3: The effect of inlet COD variations on the pH of the full-scale UASB reactor treating brewery wastewater.

However, in this study, it was observed that operational temperature affected the pH of the reactor, which in turn determined the amount of biogas produced and CH_4 content respectively. Figure 3.4(a) presents the operational temperatures of the reactor against the final pH values of the AD of brewery wastewater using the full-scale UASB reactor. A simple linear regression was performed on the data to determine if there was a significant relationship between pH and temperature. A poor positive relationship between the final pH

of the treatment unit and the operational temperature was shown by a low Pearson's correlation coefficient of R = 0.177 (Figure 3.4b). The statistical result indicated that there was a weak positive relationship between these two parameters.

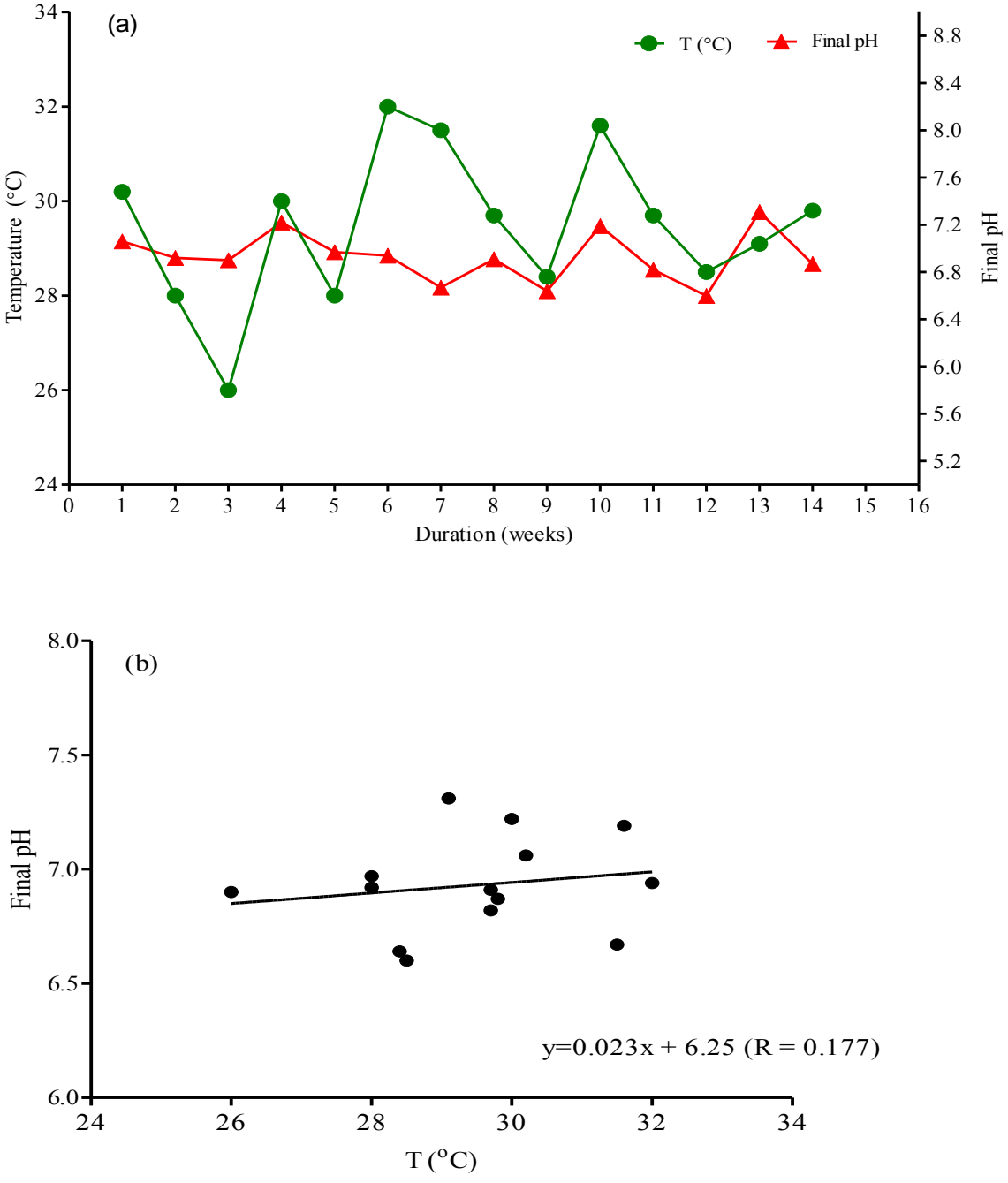

Figure 3.4: (a) Change and (b) the relationship between reactor temperature and final pH value of UASB reactor treating brewery wastewater

3.3.2.2 COD removal efficiency and solids concentration

In this study, the characterized raw brewery wastewater required additional nutrient nitrogen for the anaerobic microorganisms due to a low COD: N ratio. Urea was added as additional nitrogen. The UASB reactor was fed with pre-conditioned wastewater with an average COD concentration of approximately 2005.73 ± 1139.85 mg/L at 28˚C. During the monitoring period (Figure 3.5), the effluent from the UASB reactor had a considerably low level of COD concentration remaining after treatment (457.25 ± 272.41 mg/L). COD removal efficiency was 78.97% on average (Table 3.3).

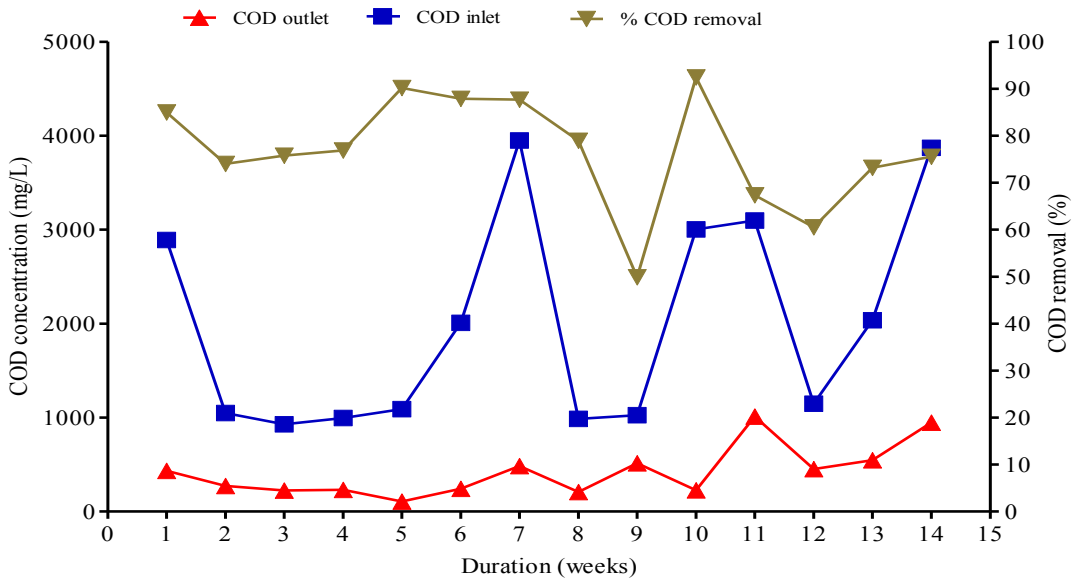

Figure 3.5: Performance of the full-scale UASB reactor treating brewery wastewater in terms of COD removal efficiency.

Ochieng *et al.* (2003) and Parawira *et al.* (2005) reported a high COD removal efficiency for brewery wastewater enriched with nitrates and phosphates, compared to the wastewater without enrichment. COD removal efficiencies ranging from 80 to 90% have been achieved using different industrial effluents in UASB reactors (Britz *et al.,* 2002; Manhokwe *et al*., 2009; Atashi *et al.,* 2010). In a similar study carried out by Kilani (1993), the effect of dairy and brewery effluents on the treatment efficiency of a domestic sewage system was investigated. An average COD removal of 60% was reported using a laboratory scale reactor. Atashi *et al.* (2010) reported about 90% COD removal efficiencies from a pilot-scale UASB

reactor treating sugar mill wastewater. Table 3.2 showed earlier presented few examples of brewery wastewater characterization studies from the literature and the efficiency of the anaerobic treatment units in organic matter removal.

Table 3.3: Composition of influent (brewery wastewater after pre-conditioning) and UASB effluent

Parameters (Average values)	Digester inlet	Digester outlet	% Decrease	% Increase
Temperature (˚C)	29.21	29.46	-	-
pH	6.87	6.93	-	-
COD	2005.73	457.25	78.97	-
TSS	2449.46	3268.97	-	33.46
TS	4520.10	3295.67	27.09	-
TDS	1792.80	2043.20	-	13.97
Protein content	134.40	71.39	46.88	-
Orthophosphates	21.25	25.34	-	19.21
TON	0.52	0.48	7.65	-
NH_3	21.64	53.85	-	148.85
NO_2	2.30	1.99	13.53	-
NO_3	0.07	0.34	-	-
Sulphate	178.25	826.28	-	-
ORP (mv)	-144.78	-73.15	-	42.89
Conductivity(mS/cm)	2.18	2.59	-	18.49

* All parameters are in mg/L except otherwise stated.

Figure 3.6 shows the values of TSS removal in the UASB reactor with an inlet and outlet TSS concentration of the brewery wastewater. An increase in the effluent total suspended solids was observed with an average increase of 33.46%. This shows that the discharged effluent was higher in TSS concentration than the influent. Furthermore, there was a marked decrease in COD removal efficiency and total solids at week 9, with low COD removal efficiency of 49.90% recorded (Figure 3.5). This might have been as a result of an increase in total suspended solids (TSS) of the influent composition from 3063.41 mg/L to 11176.38 mg/L of effluent from the reactor (Figure 3.6a). A second order quadratic polynomial regression between %TSS and %COD removal showed a strong non-linear relationship with an R^2 of 0.910 (Figure 3.6b). This could be attributed to the high concentration of the protein in the influent before treatment. The protein can easily be converted to biomass which in turn increases the reactor TSS, thus leading to sludge wash out from the reactor as shown in the effluent TSS value. Structural problems in the 3 phase separator and effluent weirs could be another contributing factor to biomass wash out.

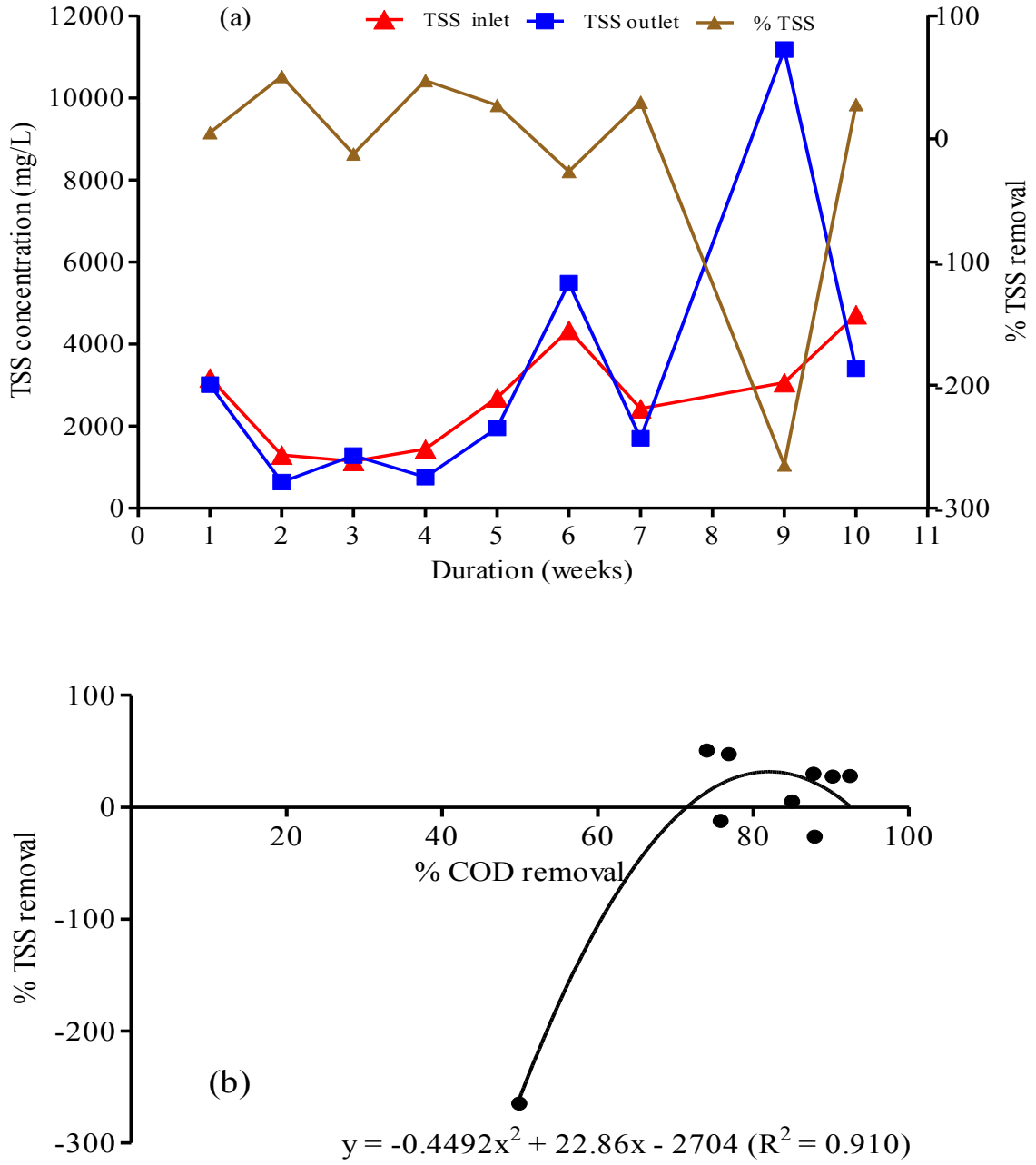

Figure 3.6: (a) Performance of the UASB reactor treating brewery wastewater in terms of total suspended solids removal and (b) the second order quadratic polynomial regression between %TSS and %COD removal efficiency of the UASB reactor.

Uyanik *et al.* (2002) and Zhou *et al*. (2006) mentioned the importance of extracellular polymeric substances (EPS) in granule formation (Miksch and Beata, 2012). Taking into account the results of the percentage degradation of COD, TSS, biogas and high biomass formation in the UASB reactor studied, the results suggested that some of the biodegradable COD is converted to biomass with biomass profile of 800-1000 ml rather than biogas formation. This confirmed the problem often encountered in the treatment of brewery wastewater (Zvauya *et. al*., 1994). Hence, it is very important to improve the performance of this UASB reactor in terms of COD removal and TSS concentration in the final effluent.

3.3.2.3 Nitrogen and phosphate concentrations in the wastewater

There was variation in inlet and outlet concentrations of nitrite for the treatment of brewery wastewater using UASB reactor. The nitrite-nitrogen load was reduced to 1.99 mg/L from an influent concentration of 2.30 mg/L nitrite-nitrogen (Table 3.3). Very little residual nitrate (0.34 mg/L) was detected in the effluent at an influent concentration of 0.07 mg/L, which shows that there was an increase in nitrate-nitrogen in the reactor during organic matter degradation, which in turn favours both the denitrification and methanogenesis processes (Atashi *et al.*, 2010). However, the performance of the reactor may be disturbed by the increase in nitrate-nitrogen load, thereby having an inhibitory effect towards the anaerobic biodegradation of biomass which in turn reduces the CH_4 production (Sternenfels, 2012). Thus, low nitrate concentration in this study might have contributed to high CH_4 content produced in the reactor.

However, the digester effluents still have considerable amounts of NH_3 content. Many studies have shown that free NH_3 and not ammonium is responsible for inhibiting the methanogenic activity during AD (Sawayama *et al.,* 2004; Calli *et al.,* 2005; Garcia and Angenent, 2009). Ipeaiyeda and Onianwa (2012) explained that the presence of NH_3 concentrations in the effluent has its origin from the proteins and chitins contained in the brewery waste, because most nitrogen in the waste are in the form of NH_3 following the degradation of proteins and amino acid (Inanc *et al.*, 2000). Ouboter *et al.* (1998), further mentioned that almost all of the proteins in brewery effluent is mineralised through the activity of proteolytic and deaminative bacteria, initially hydrolysing protein to peptides and amino acids and finally by deamination

to ammonium (NH_4). This explains the major source of NH_3 in the effluent after treatment in the UASB reactor.

The NH_3 content of the influent and effluent of the UASB reactor during the monitoring period is shown in Figure 3.7. The concentration increased by 148.85% from an influent concentration of 21.64 ± 10.70 to 53.85 ± 21.08 mg/L on average (Table 3.3). This showed that there was production of NH_3–N during the treatment of brewery wastewater in the UASB reactor. The release of NH_3–N in the bioreactor during treatment of waste was also observed by Inanc *et al.* (2000) and Govahi *et al.* (2012). Furthermore, the NH_3 concentration detected in this study can be said to be within an acceptable level for the growth of the methanogenic bacteria and biogas production (Tabatabaei *et al.,* 2011). However, excess concentrations of free NH_3 can inhibit these microorganisms. Tabatabaei *et al*. (2011) reported a wide range of total NH_3 nitrogen concentrations that can inhibit the growth of methanogens.

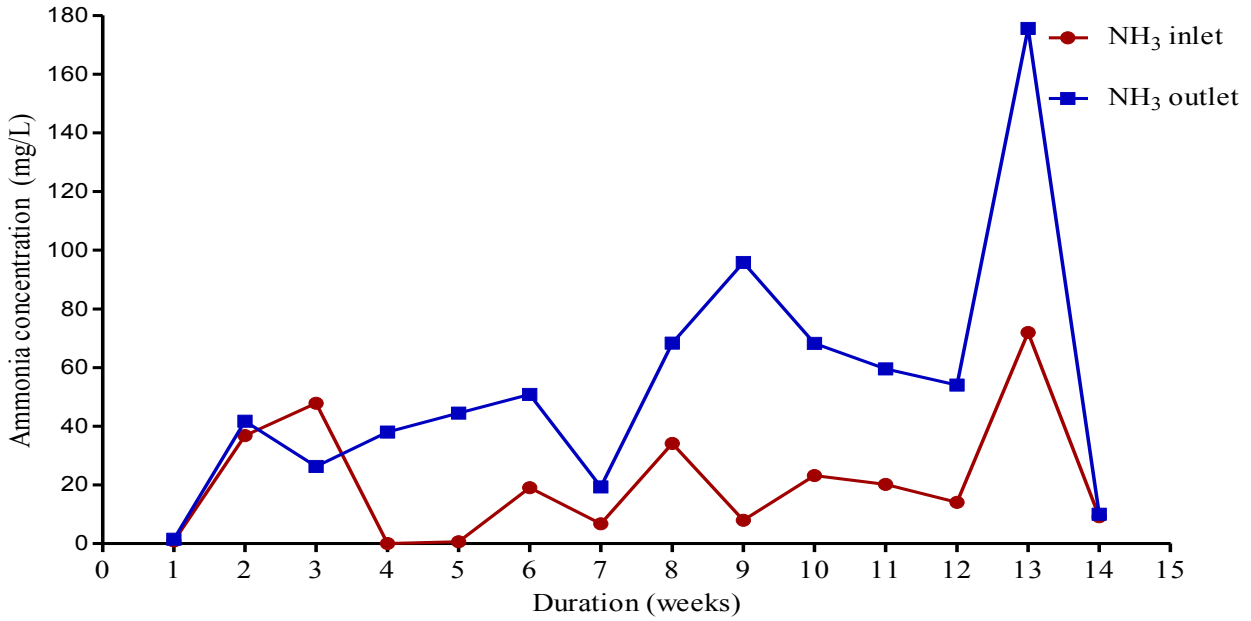

Figure 3.7: Variation in average inlet and outlet concentrations of ammonia nitrogen during anaerobic treatment of brewery wastewater using the UASB reactor.

The average influent and effluent orthophosphate concentration during anaerobic treatment of brewery wastewater by the UASB reactor is shown in Figure 3.8. The reactor was fed with an average influent orthophosphate concentration of 21.25 ± 9.30 mg/L that was increased to 25.34 ± 11.21 mg/L. This shows that orthophosphate was produced during the degradation process. Parawira *et al.* (2005) reported low removal efficiency of nitrogen and phosphorus during the AD of brewery wastewater in a UASB reactor. This shows that phosphate was released by the microorganisms in the reactor during the anaerobic wastewater treatment process.

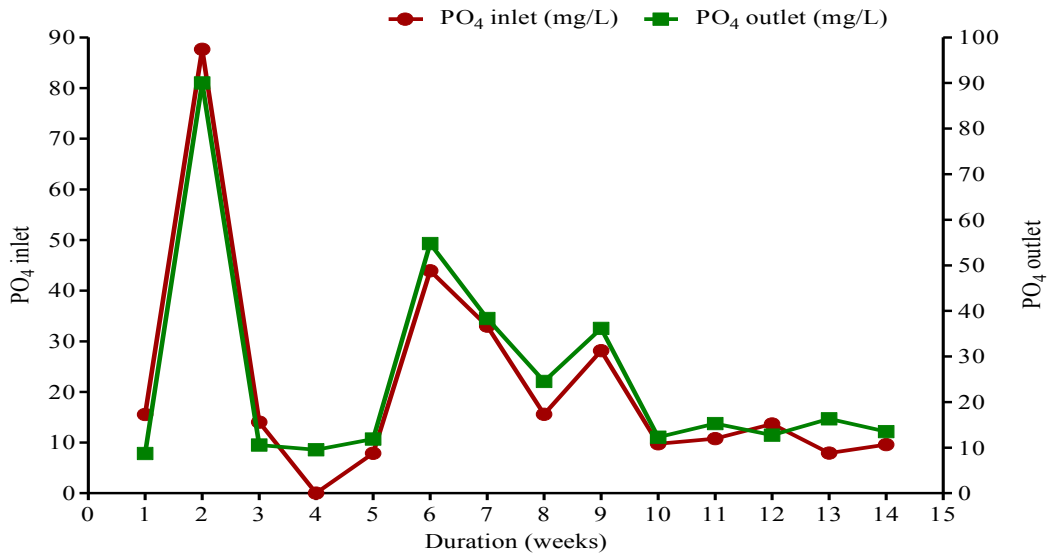

Figure 3.8: Average orthophosphates concentration in the reactor during treatment of brewery wastewater.

3.3.2.4 Correlation between methane production and operational variables

Methanogenesis was active in the reactor during the treatment of brewery wastewater, which is shown by the efficiency of the UASB reactor in terms of biogas production with CH_4 content of 60-69% of the total gas throughout the study (Figure 3.9). The relationship between the percentage COD removal and biogas yield (CH_4 and CO_2) during anaerobic degradation is shown in Figure 3.9. The results of analysis carried out using ANOVA showed that biogas yield depended on the substrate present in the wastewater in terms of COD

removal efficiency (Appendix 1). There was a strong positive correlation between the percentage COD removal and biogas yield (CH_4 and CO_2 production) with an R value of 0.975; which showed that significant portion of the organic matter presence in the brewery wastewater was converted to biogas (Figure 3.9). The performance of the reactor as a function of quantity of COD per reactor volume showed that high concentration of organic matter in the reactor was used for biogas production as shown in Figure 3.9. Apart from the composition of biogas produced, the quantity of COD removed in term of COD removed per reactor volume shows the efficiency of the investigated full-scale UASB reactor during the monitoring period.

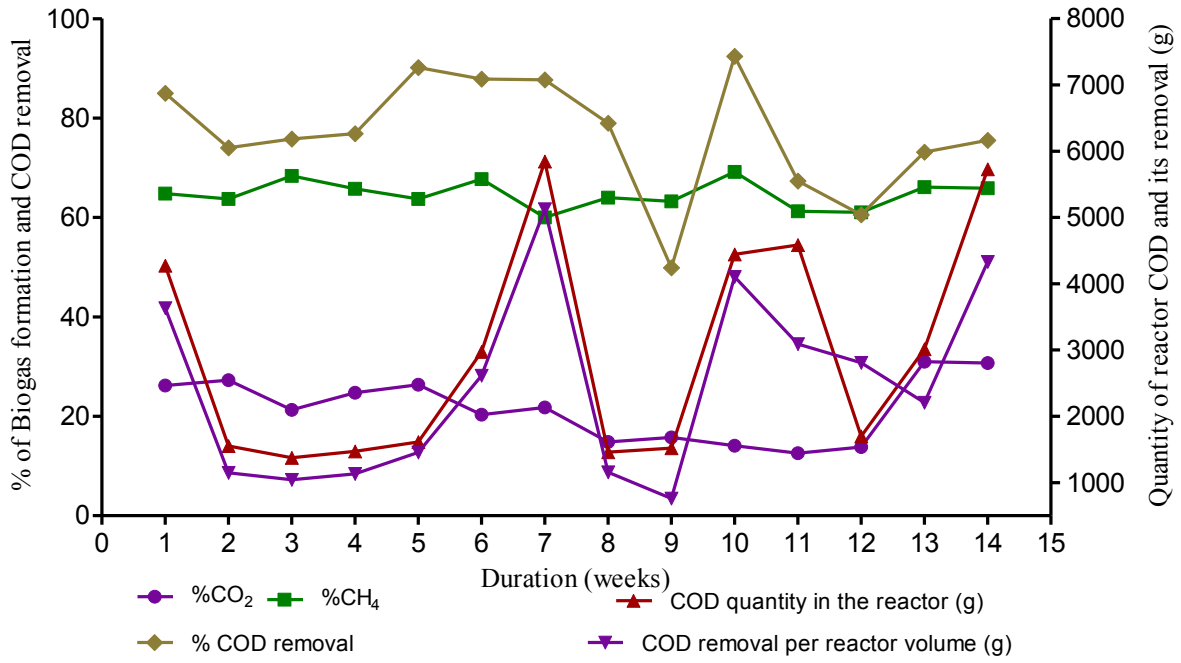

Figure 3.9: Efficiency of organic matter removal (COD quantity) as function of reactor volume to produce biogas during anaerobic treatment of brewery wastewater.

As shown in Figure 3.10, CH_4 gas production rate increased from 6.32 L/day to 19.47 L/day as the OLR increased from 2.10 L/day to 9.30 g/L/day in UASB reactor; however an increase in loading rates above 11.00 g COD/L/day resulted in a decrease in CH_4 production to 15.43 L/day. Habeeb et al. (2011) and Govahi et al. (2012) earlier reported a negative correlation between CH_4 production rate and OLR when the latter was increased. Furthermore, increase in OLR could cause VFA accumulation, which in turn could result in decrease in pH of the

reactor, thus inhibiting CH_4 production (Habeeb *et al.,* 2011). The result from the comparison of the final pH of effluent from the UASB reactor treating brewery wastewater and the CH_4 content of the biogas produced from this reactor shows that there was a moderate positive correlation (R = 0.664) between these two parameters [Figure 3.11 (a and b)]. Thus, the pH of the reactor had a significant effect on the CH_4 production ($P < 0.001$). This is because CH_4 producing Archaea or methanogens are known to be affected by pH (Poh and Chong, 2009; Habeeb *et al.,* 2011) and they could only survive a very narrow pH range as discussed in section 2.5.6 (Gerardi, 2003; Tabatabaei *et al.,* 2011).

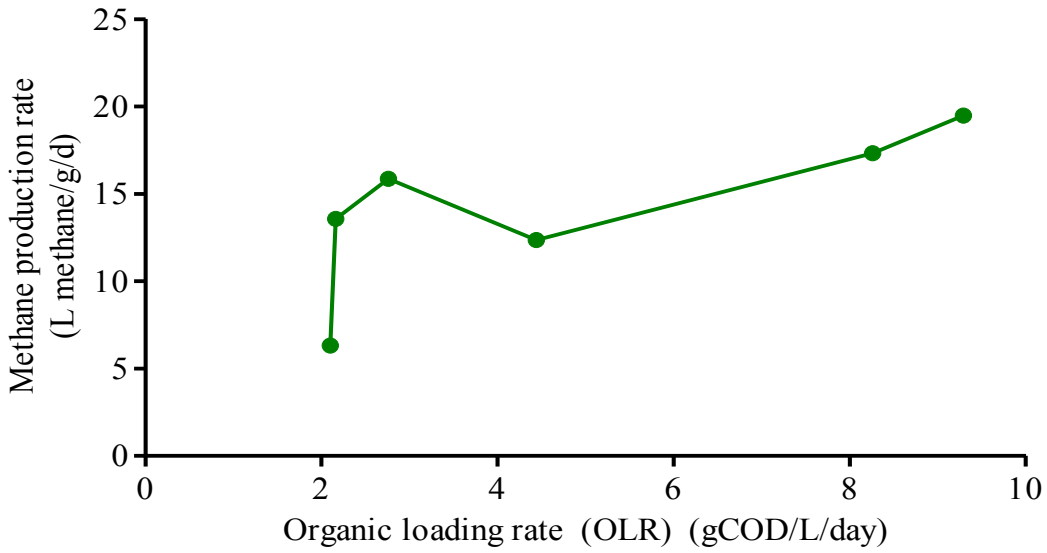

Figure 3.10: Effect of organic loading rate on methane production rate in the UASB reactor treating brewery wastewater.

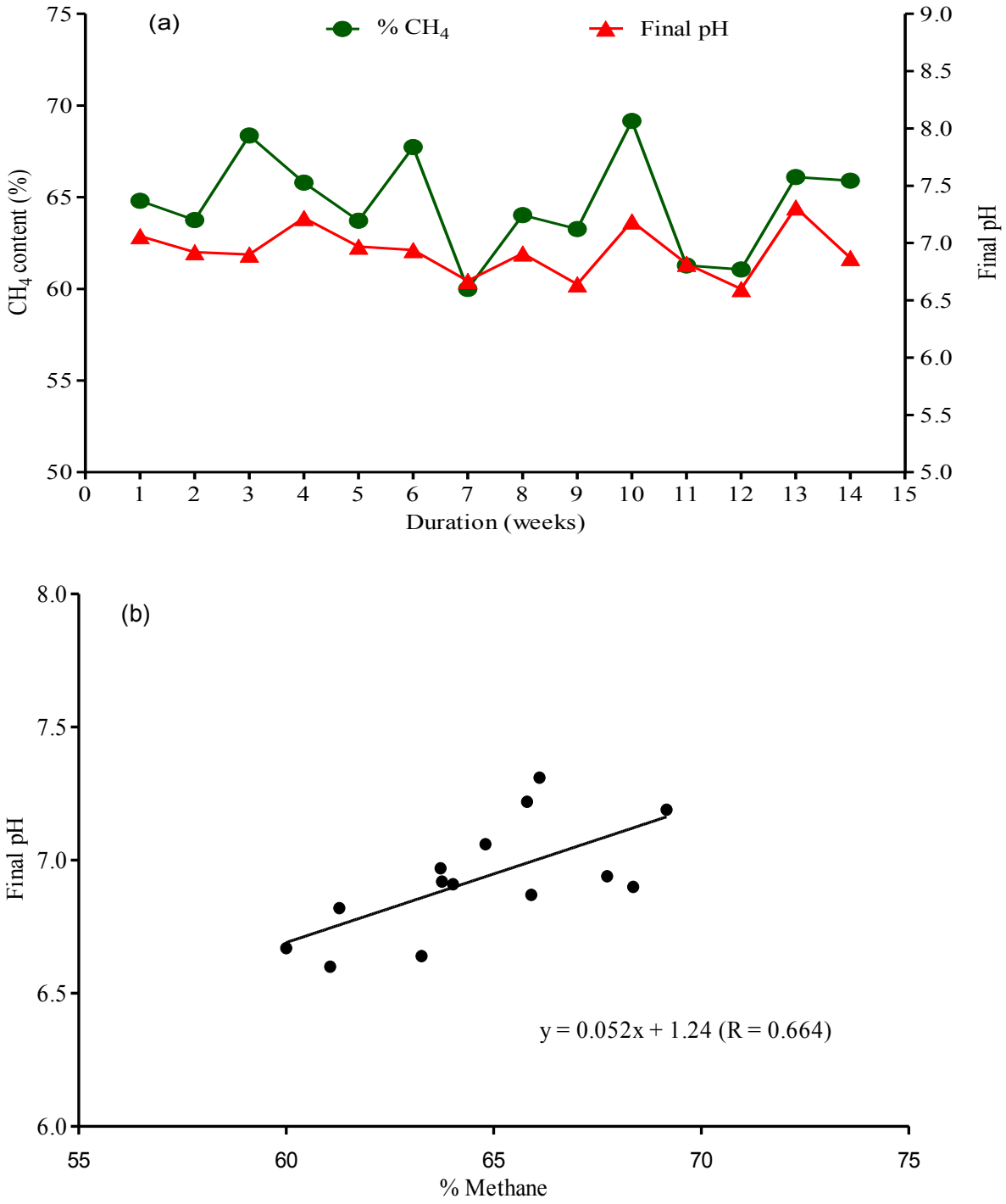

Figure 3.11: Graph showing (a) the effect of reactor's pH on the methane content and, (b) the relationship and linear regression analysis showing a significant moderate positive correlation between these two parameters during the treatment of brewery wastewater using the UASB reactor.

3.4 CONCLUSIONS

- Raw brewery wastewater characterization results showed that the process wastewater from the brewery industry was high in COD, BOD_5, TSS, NH_3 and protein content and did not meet the required effluent regulatory standards during the period of sampling. From the results obtained in this study, the BOD_5: COD ratio indicated that the raw wastewater was high in organic matter which is biodegradable. Therefore, this effluent could easily be degradable by the microorganisms in AD technology.

- The full-scale UASB reactor was able to reduce the organic content in the brewery wastewater to a reasonable level (average 457.25 ± 272.41 mg COD/L in effluent), which could be discharged into the municipal wastewater plant for further treatment. However, the results of the percentage removal efficiency of NH_3 and phosphorus showed high concentrations of these nutrients in the final effluent; therefore secondary treatment was highly recommended.

- The composition of biogas, especially the CH_4 yield, showed that considerable amounts of substrate was being converted to biomass due to an increase in the concentration of total suspended solids and total dissolved solids in the final effluent. A very strong non-linear relationship between the percentage solids and COD removal was observed. The CH_4 yield as a percentage of total biogas was between 60-69%. This showed that the performance of the UASB reactor in terms of biogas (CH_4) production could be improved for energy generation, since CH_4 production depends on the rate of organic matter degradation.

- As observed in this study, the UASB reactor needs optimization to improve the treatment efficiency and post treatment of the final effluent is required for nutrient removal, in order to meet the discharge standards. Also, the microbial population structure within the anaerobic digester need to be investigated in order to determine the contribution to effluent treatment and biogas production. Further optimization using mathematical models to improve the efficiency of the reactor was required.

3.5 RESEARCH OUTPUTS

(a) Journal Articles

1) **Enitan, A. M**., Swalaha, F. M., Adeyemo, J. and Bux, F. 2014b. Characterization of brewery effluent composition from a beer producing industry in KwaZulu-Natal, South Africa. *Fresenius Environmental Bulletin,* 23(3): 693-701.

(b) Conference Papers

1) **Enitan, A. M.,** Swalaha, F. M., Adeyemo, J. and Bux, F (2014). Evaluation of effluent composition from a beer producing industry in South Africa. Presented at the *International Journal of Arts & Sciences' (IJAS) American Canadian Conference* at Ryerson University's International Learning Center, Toronto, Canada, 19-22 May, 2014 (Oral presentation).

2) Swalaha, F.M., **Enitan A.M.** and Bux, F. (2014). Efficiency of industrial scale anaerobic reactor treating brewery wastewater. Presented at *Water Institute of Southern Africa* (WISA) *Conference*, Mbombela, Mpumalanga, South Africa, 25-29 May, 2014 (Oral presentation).

CHAPTER FOUR: KINETIC MODELLING AND CHARACTERIZATION OF THE MICROBIAL COMMUNITY PRESENT IN AN UASB REACTOR TREATING BREWERY EFFLUENT

4.1 INTRODUCTION

The anaerobic breakdown of complex organic compounds involves the action of several groups of microorganisms which results in a variety of intermediates including biogas such as H_2, CH_4 and CO_2 (Appels *et al.,* 2008; Mirzoyan *et al.,* 2008; Amani *et al.,* 2011). Microbial species involved in the conversion of organic material in anaerobic digesters are grouped based on their biochemical activities. This group includes hydrolytic, acidogenic, acetogenic and methanogenic organisms (Hulshoff-Pol *et al.,* 2004). These organisms grow in a syntrophic manner when the digester is operated under optimum reaction conditions (Chulhwan *et al.,* 2005; Crocetti *et al*., 2006; Mumme *et al*., 2010). Studies have shown that the microbial community in the UASB reactor responds to any sudden change in the environmental conditions, thus leading to a shift in the type of microbial species found in the reactor, their population size and activities (McHugh *et al.,* 2003a; Diaz *et al.,* 2006; Keyser *et al.,* 2006; Zhang *et al.,* 2012). Therefore, an in-depth understanding of the microbial consortia and the associated activities are essential for an effective reactor operation.

It is difficult to assess the diversity, colonization and topological distribution of these microorganisms using conventional methods due to the structural complexity of the granular sludge (Liu *et al*., 2002b). Recently, molecular techniques such as denaturing gradient gel electrophoresis (DGGE), fluorescence *in-situ* hybridization (FISH), quantitative polymerase chain reaction (QPCR) and pyrosequencing have been successfully adopted to study these complex microbial populations (McHugh *et al.,* 2003a; Diaz *et al.,* 2006; Keyser *et al.,* 2006; Ziganshin *et al.,* 2011; Zhang *et al.,* 2012).

Furthermore, development and use of suitable mathematical models, which adequately describe the overall process performance in the bioreactor have shown to be an important tool for process control strategies resulting in better effluent quality and biogas production (Pontes and Pinto, 2006). Mass balances, kinetic and stoichiometric models are some of the methods that are being employed in describing the operating principles of different anaerobic digesters

(Acharya *et al*., 2008; Yetilmezsoy, 2012). Simple and more sophisticated models such as the Monod, Chen and Hashimoto, Contois, Michaelis-Menten, Haldane, Grau second-order and anaerobic digestion model 1 (ADM1) have also been developed to improve the reactor performance (Batstone *et al.,* 2002; Parsamehr, 2012).

Kinetic modelling is an acceptable method to describe and predict the performance of any biological treatment unit (Yetilmezsoy and Sakar, 2008; Debik and Coskun, 2009). It can be applied to the optimization and control of anaerobic wastewater treatment processes, to determine the relationship between fundamental parameters needed for anaerobic reactions (Acharya *et al.,* 2011; Yetilmezsoy, 2012). Among several kinetic models developed for organic substance removal in the UASB reactor, the Stover- Kincannon model has been well documented (Acharya *et al.,* 2011; Yetilmezsoy, 2012). The modified form of this model is one of the most widely adopted methods for the determination of kinetic constants and has been successfully applied for anaerobic treatment of poultry slaughterhouse waste (Debik, and Coskun, 2009), municipality wastewater (Turkdogan-Aydinol and Yetilmezsoy, 2010), distillery wastewater (Acharya *et al.,* 2011) and poultry manure wastewater (Yetilmezsoy, 2012).

Thus, monitoring of environmental conditions and identification of the functional microbial population, as well as analysing the kinetic process of UASB reactors is crucial for reactor design, maintenance and its efficient operation to increase CH_4 production as a source of renewable energy and for better effluent quality. This chapter focused on determining and quantifying the microbial composition in the granules collected from the full-scale UASB reactor treating brewery wastewater in KwaZulu-Natal, South Africa using FISH, PCR and QPCR techniques to detect and quantify the Bacteria and Archaea concentrations in the reactor samples. The bio-kinetics of the degradable organic substrates present in the brewery wastewater using Stover-Kincannon kinetic model to predict the effluent quality was further considered. It is hoped that the characterization of eubacteria and methanogenic Archaea in the granules used for this study will bridge the gap of knowledge on the microbial ecology of the full-scale UASB reactor investigated.

4.2 MATERIALS AND METHODS

4.2.1 Sample Collection from the Full-Scale UASB Reactor

Well-suspended granular samples were obtained for microbial analysis from the UASB reactor compartments as shown in the flow diagram (Figure 4.1). Prior to sample collection, the sampling valves were opened for 5 minutes in order to flush out the sampling tubes and valves. Thereafter, granular sludge samples were collected in sterile glass bottles and flushed with nitrogen gas and sealed immediately to maintain anaerobic conditions during transportation to the laboratory. Both granular sludge samples and wastewater samples collected were transported to the laboratory at 4°C for analysis. Physico-chemical analyses were done within 48 hours of collection with necessary preservation techniques adapted from Standard Methods for Examination of Water and Wastewater (APHA–AWWA–WPCF, 1998). For microbial analyses, the aliquots were centrifuged at 9,600 x g at4˚C for five minutes. Supernatants were discarded and the pellets were washed with phosphate buffered saline (1 x PBS) and stored at -20˚C before analysis.

Volatile fatty acids (VFA) (acetic, propionic, isobutyric, butyric, valeric and isovaleric acids) were quantified using HPLC (Model LC-20AT, Shimadzu, Japan) equipped with a UV detector (SPD-20A) and analysed using a Metrosep organic acid column (250×7.8 mm) at a flow rate of 0.6 ml/min and an injection volume of 20 µl at 210 nm. The mobile phase consisted of a 0.5 mM H_2SO_4 solution. Biogas was collected in a gas holder (Tedlar bag, Sigma-Aldrich) for analysis using gas chromatography (Shimadzu GC-2014, Japan) as described in section 3.2.3.1.

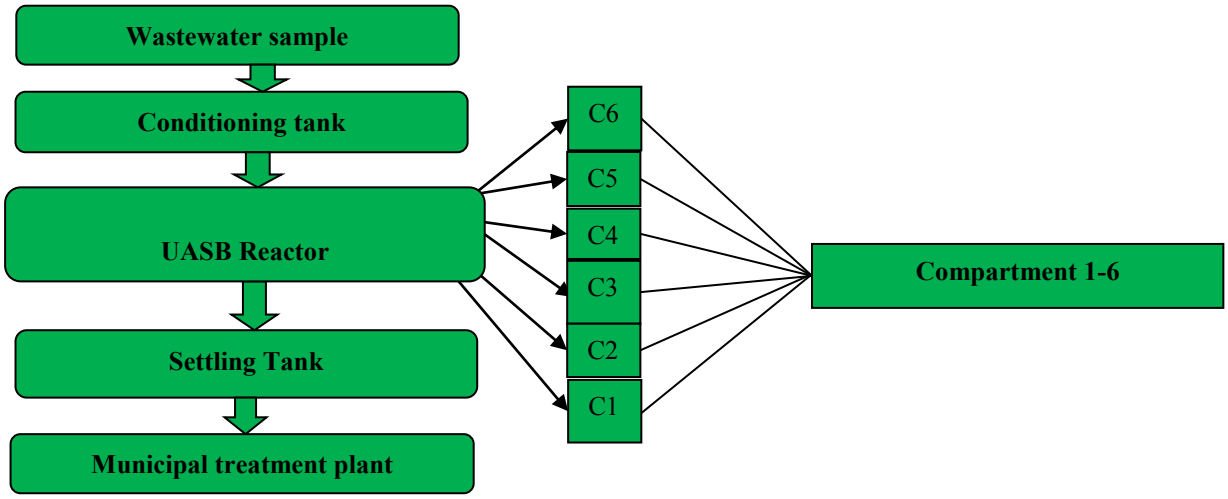

Figure 4.1: Flow diagram showing the six sampling points from the UASB reactor compartments where granular samples were obtained for microbial analysis.

4.2.2 Fluorescence *In-Situ* Hybridization (FISH)

Fluorescence *in-situ* hybridization was carried out according to the protocol described by Amann *et al.* (1995) with minor modifications using the oligonucleotides probes given in Table 4.1 (Enitan *et al.,* 2014b). Sludge granules were fixed in 4% paraformaldehyde (Gram negative) and in PBS-ethanol (Gram positive) (Amann *et al*., 1995). Fixed samples were then sonicated at 2 W for 5 minutes using an Ultrasonic Liquid Processor (Misonix XL-2000 Series). Thereafter, granules were treated with 10 µl of lysozyme (10 mg/ml) at 37°C for 30 minutes; then with Proteinase K (10 mg/ml) at 50°C for 45 minutes. Samples were diluted further by the addition of 500 µl of sterile water for even dispersion and quantification with the group specific, Archaea and bacteria domain probes (Table 4.1). A volume of 5–10 µl of the treated samples were fixed on poly-L-lysine coated slides and allowed to air-dry at room temperature and dehydrated by a series of ethanol washes (50, 80 and 100%). The oligonucleotide probes were labeled with rhodomine (FAM) and tetramethylrhodomine-5-isothiocyanate (TAMRA) dye at the 5'-end respectively (Table 4.1). The hybridisation and wash buffers were prepared according to the formamide stringency as listed in Table 4.1. Samples were hybridised by the addition of 9 µl of hybridisation solution (10% SDS, 1 M Tris/HCl (pH 8), 5 M NaCl and formamide concentrations; Table 4.1); together with 1 µl of oligonucleotide probe, incubated in the hybridisation oven at 46°C, overnight. After hybridisation, the slides were washed with pre-warmed wash buffer (1 M Tris/HCl, 10% SDS, 0.5 M EDTA and 5 M NaCl; Table 4.1) for 1 h at 48°C; subsequently rinsed with distilled water and then air dried. The slides were counter-stained with 4′-6-diamino-2-phenylindole (DAPI) for 10 minutes at room temperature. Slides were rinsed in pre-warmed distilled water and air-dried in the dark. The samples were then mounted with an anti-fading solution (Vectashield, Vector Laboratories, Inc. Burlingame).

4.2.2.1 Microscopy and image analysis

The hybridized slides were viewed using a Zeiss Axio-Lab HB050/AC microscope (Carl Zeiss, Jena, Germany) equipped with an HBO 50W Hg vapour lamp, with appropriate filter sets, specific for TAMRA (43 HE, Zeiss) and FAM (Filter set 09, Zeiss) using a 100x Plan Apochromat objective. Images were captured using a Zeiss AxioCam MRC camera (Carl Zeiss, Gottingen, Germany) and analysed using the Zeiss Axio Vision Release 4.8 imaging system.

Table 4.1: 16S rRNA oligonucleotide probes with the corresponding formamide stringency and NaCl concentrations used in this study

Target group	Oligonucleotides			
	Probe name	**Probe sequence (5′-3′)**	**Formamide concentration (%)/ NaCl (µl)**	**References**
Archaea[a]	ARC915	GTGCTCCCCCGCCAATTCCT	30 / 1020	Stahl and Amann (1991)
Methanosarcina[a]	MS821	CGCCATGCCTGACACCTAGCGAGC	40 / 460	Raskin *et al.* (1994)
Methanosaeta[a]	MX825	TCGCACCGTGGCCGACACCTAGC	50 / 180	Raskin *et al.* (1994)
Eubacteria[b]	EUB338	GCTGCCTCCCGTAGGAGT	30 / 1020	Amann *et al.* (1990)
	EUB338 II	GCAGCCACCCGTAGGTGT	30 / 1020	Daims *et al.* (1999)
	EUB338 III	GCTGCCACCCGTAGGTGT	30 / 1020	Daims *et al.* (1999)

a = Rhodomine, b = Tetramethylrhodomine-5-isothiocyanate.

4.2.3 Total Genomic DNA Extraction from Granular Sludge Samples

The full-scale UASB reactor has six different compartments (C1, C2, C3, C4, C5 and C6) and samples were taken from each for microbial analysis. The direct isolation of total genomic DNA from granular sludge samples was carried out using a phenol extraction method (Sekiguchi *et al.,* 1998; Klocke *et al.,* 2007) with modifications. Two millilitres of the sample were centrifuged at 9,600 x g for 5 minutes to release the microorganisms entrapped within the granules and other undigested particles; after which the supernatant was discarded. The pellets were washed twice with 1 x PBS and centrifuged again at 9,600 x g for 5 minutes to collect the pellets. Total genomic DNA was recovered by lysis of the cells by adding 700 µl lysis buffer (0.5mol^{-1} EDTA, 0.1 mol^{-1} NaCl, 0.5 mol^{-1} Tris/HCl at pH 8.0) with 0.2% *β*-mercaptoethanol and 30-40 mg PVPP, then homogenised with 0.6 g sterile glass beads at 600 x g for 5 minutes using bead beater machine. The granules were further treated with 20 µl of Proteinase K (10 mg/L), vortexed to mix and incubated for 30 minutes at 37°C. The suspension was incubated for 2 h at 65°C. Thereafter, the cells were freeze-thaw in series (in dry ice: ethanol slurry for three minutes) and thawed at 65°C in a water bath for three minutes. After cell lysis, debris, RNA and proteins were separated from the aqueous phase containing the DNA by performing two-step phenol–chloroform–isoamyl alcohol extraction (25:24:1) followed by 24:1 chloroform–isoamyl alcohol and centrifuged for 10 minutes at

13,800 x g to remove the phenol; this step was repeated until a clean interface was seen. Precipitation of genomic DNA was done by the addition of 1 x volume of isopropanol and stored at -20°C overnight for complete precipitation. The DNA was collected by centrifugation at 13,800 x g for 20 minutes, washed twice with 90% ice-cold ethanol followed by 70% ice-cold ethanol, air dried and dissolved in 100 µl TE buffer (0.5 mol^{-1} EDTA, 1 mol^{-1} Tris/HCl at pH 8.0). The concentration of the DNA was checked by Nanodrop (ND-1000) Spectrophotometer. The purified DNA were stored at -20 °C and used for further construction of the 16S rDNA clone library. DNA extraction was carried out in duplicate for the UASB granules collected.

4.2.4 Amplifications using Polymerase Chain Reaction (PCR)

Polymerase chain reaction amplification conditions were optimized for methyl coenzyme-M reductase gene (*mcrA*), domain Archaea, (ARC) and bacterial (BAC) genes using the corresponding primer sets as listed in Table 4.2 (Giovannoni, 1991; Chan *et al.,* 2001; Luton *et al*., 2002). The PCR mixture contained 25 µl reaction volume of 0.3 µl of Taq DNA polymerase (5 U/ml), 2.5 µl of PCR reaction buffer, 1 µl of each of the primers (10 mM), 0.5 µl of dNTPs (10 mM), 2 µl of the extracted DNA (10 µl) and PCR-grade water. The modified PCR amplification conditions of Luton *et al.* (2002) was used as follows: initial denaturation was performed at 94°C for 5 minutes; followed by 40 cycles of denaturation at 92°C for 1 minute; primer annealing at 52°C for 1 minute for *mcrA* and 53°C for 1 minute (Archaea and bacteria), elongation at 72°C for 1 minute and a final extension was performed at 72°C for 5 minutes. The PCR amplification was carried out in an automatic thermal cycler Veriti (Applied Biosystems).

4.2.4.1 Agarose gel electrophoretic detection of PCR products

The PCR amplified products were resolved on 0.8-1.0% (w/v) agarose-Tris-borate EDTA gel (ABgene, UK) (10 mM Tris-HCl, 10 mM boric acid, 2.5 mM EDTA, pH 8.0), visualized and photographed under the BioDoc-It transilluminator system. An appropriately sized marker (1 kb DNA smart ladder) was included on each gel as a standard. Purification of the PCR products was carried out for subsequent cloning with a commercial kit following the manufacturer's instructions.

4.2.4.2 Cloning

4.2.4.2.1 Preparation of competent cells, ligation, transformation and clone analysis using colony PCR

Escherichia coli DH5-*α* was selected as the competent cells for ligation. A single colony of *E. coli* DH5-*α* was subcultured from the stock plate onto prepared Luria Bertani (LB) agar antibiotic agar plates supplemented with 50 mg/ml of Ampicillin and incubated overnight at 37˚C. A day before the transformation, 2 ml of LB antibiotic broth was seeded with single overnight bacteria colony and incubated overnight at 37°C in a shaker. Selected PCR amplicons were ligated into the pTZ57R/T vector using T4 DNA ligase of the insTAclone PCR cloning kit (Invitrogen) according to the manufacturer's instructions (Thermo Scientific, InsTAclone PCR Cloning Kit). Ligation and insertion were carried out in a 30 µl reaction volume constituting of 6 µl of ligation buffer, 3 µl PCR product containing the DNA, and 3 µl digested plasmid DNA, 1 µl enzyme T4 DNA ligase and 17 µl nuclease-free water in an Eppendorf tube. The mixture was briefly vortex and centrifuge for 3-5 s at 9,600 x g. This was incubated overnight at 4°C. In a separate micro-centrifuge tube, 2.5 µl of the ligated mixture was directly transformed into the prepared competent cells and incubated on ice for five minutes. These were then, plated onto pre-warmed LB antibiotic agar plates containing X-gal (20 mg/ml) and IPTG (100 mM) stock solutions and incubated overnight at 37°C using standard procedures (Sambrook and Russell, 2001). White clones were randomly selected on LB antibiotic agar plates containing X-gal and IPTG stock solutions and positive clones were confirmed by colony PCR using appropriate primer-sets and resolved on agarose gel for further confirmation of plasmids containing the targeted inserts.

Table 4.2: Primer sets used in this study for both conventional and quantitative real-time PCR

Target group	Target microorganism	Primer name	Sequences(5'→3')	Amplicon length (bp)	References
16S rDNA	Archaea	ARC622f	TGAAATCYYRTAATCCC	246-250	Chan *et al.* (2001)
		ARC915r	GTGCTCCCCGCCAATTCCT		
McrA	Functional gene for methanogenic Archaea	MLf	GGTGGTGTMGGATTCACACARTAYGCWA	464-491	Luton *et al.* (2002)
		MLr	CAGCTTCATTGCRTAGTTWGGRTAGTT		
16S rDNA	Bacterial	27f	AGAGTTTGATCMTGGCTCAG	~1500	Giovannoni (1991)
		1492r	TACGGYTACCTTGTTACGACTT		

4.2.4.3 Sequencing and phylogenetic analysis

Positive clones from compartments 1, 3 and 6 of the six compartments were selected for sequencing to assess organisms at the top, middle and the bottom of the reactor (Inqaba Biotechnical Industries Laboratory, South Africa). The obtained bacteria, archeon and *mcrA* gene sequences were manually edited and similarity searches for the DNA sequences were carried out using the Basic Local Alignment Search Tool (BLAST) program to search in the (http://www.ncbi.nlm.nih.gov/BLAST) National Centre for Biotechnology Information (NCBI) sequence database. The nucleotides sequences obtained from the GenBank were converted to amino acid sequences and then aligned in CLUSTAL X. The aligned amino acid gene sequences were edited using BioEdit and exported to MEGA version 5.10. Evolutionary analyses were conducted in MEGA version 5.10 software (Tamura *et al.,* 2011). The phylogenetic trees were constructed from the alignments and bootstrap analyses were performed using 1000 replicates by the neighbour-joining method (Saitou and Nei, 1987).

4.2.4.3.1 Nucleotide sequence accession number for samples obtained from the full-scale UASB reactor

The obtained nucleotide sequences for methyl coenzyme-M reductase gene (*mcrA*), domain Archaea, (ARC) and bacterial (BAC) obtained from the full-scale UASB reactor treating brewery wastewater were submitted to the National Centre for Biotechnology Information

website (NCBI) under the accession numbers KF715644–KF715648 for *mcrA* gene, KM191135–KM191137 for Archaea and KM065733–KM065740 for bacterial clones.

4.2.5 Quantitative Real-time PCR

Quantification of gene copy numbers in the extracted DNA samples were performed using real–time PCR machine (C-1000 Touch, CFX 96, Bio-Rad Laboratories Pty Ltd, USA) with two primer sets targeting the Archaea and bacteria domain, Table 4.2 (Steinberg and Regan, 2009). For each reaction mixture, amplification was carried out in a final volume of 20 µl containing 10 µl of the Sso fast Eva green Master Mix (Bio-Rad Laboratories Pty Ltd, USA) 1 µl of each primer (final concentration, 10 µM), 4 µl of template DNA and PCR-grade water was added to a final volume of 20 µl. Two-step amplification of the target DNA were carried out using the modified protocol described by Steinberg and Regan (2009) as follows: initial denaturation for 3.5 minutes at 94°C followed by 40 cycles of 30 s at 95°C and annealing for 30 s at 55°C and final extension with image capturing at 72°C for 30 s. For melting curve analysis, the temperature was increased at 0.5˚C every 10 s from 40 to 95˚C. Each QPCR assay was conducted in duplicate. For all experiments, appropriate negative controls containing no genomic DNA were subjected to the same procedure to exclude any possible contamination or carry-over.

Standard curve was obtained by plotting quantification cycle (Cq) as a function of log of copy number of target DNA. Standard curves were constructed from purified PCR amplicons for Archaea and bacteria primers. Standard curve for bacteria was constructed from a series of 10-fold dilution of target DNA using the primer sets of 27f and 1492r targeting the 16S rDNA gene of bacterial at a concentration of 2.77×10^3 to 2.77×10^{10} copies/ng DNA. A second standard curve for Archaea was constructed from a series of 10-fold dilution of target DNA using the primer sets of ARC622f and ARC915r targeting the 16S rDNA of the domain Archaea at a concentration of 1.64×10^4 to 1.64×10^{11} copies/ ng DNA.

For each QPCR assay, the value of the logarithmic starting quality for the different 16S rDNA gene were plotted against the threshold cycle (Cq) numbers and the linear ranges of

the standard curves were selected based on the R^2 of the slope greater than 0.990. For quantification of 16S rDNA gene concentration that were present in the DNA obtained from the different compartment, the Cq values for each sample were compared with the corresponding standard curves. Equation 4.1 was used to calculate the target 16S rDNA gene copy numbers in each sample (Yu *et al*., 2006; Tan *et. al.*, 2013). An average molecular weight of 660 Da with the 6.02×10^{23} Avogadro's numbers are assumed for a base pair in the double-stranded DNA (He *et al.,* 2003).

$$16\text{S rDNA (copy/ml)} = \frac{16\text{S rDNA concentration (g/ml)} \times 6.02\times10^{23}\text{(copy/mole)}}{16\text{S rDNA amplicon size (bp)} \times 660\text{ (g 16S rDNA mol/bp)}} \quad (4.1)$$

4.2.6 Kinetic Analysis Using Stover–Kincannon Model

According to the Stover–Kincannon model (Kincannon and Stover, 1982), the organic substrate utilization rate in a UASB reactor process can be expressed as a function of organic loading rate. The substrate consumption rate can be expressed as (Acharya *et al*., 2008; Turkdogan-AydInol and Yetilmezsoy, 2010; Yetilmezsoy, 2012);

$$\frac{dS}{dt} = \frac{Q}{V_r}(S_i - S_e) \quad (4.2)$$

The original Stover-Kincannon model is described in equation (4.2) as;

$$\frac{dS}{dt} = \frac{Q(S_i - S_e)}{V_r} = \frac{U_{max}\left(\frac{QS_i}{V_r}\right)}{K_B + \left(\frac{QS_i}{V_r}\right)} \quad (4.3)$$

Where dS/dt is the substrate removal rate (g COD/L/day) in the UASB reactor, S is the reactor substrate concentration (g/L), U_{max} is the maximum utilization rate constant (g/L/day), V_r is the working volume of reactor (L), K_B is the saturation constant (g/L/day), Q is the flow rate (L/day), S_i and S_e are the influent and effluent substrate concentrations (g/L) respectively.

Combining equation (4.2) and (4.3) gives the modified Stover- Kincannon model for a UASB reactor at steady state.

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{V_r}{Q(S_i - S_e)} = \frac{K_B V_r}{U_{max}(QS_i)} + \frac{1}{U_{max}} \quad (4.4)$$

$$Y = \lambda X + \lambda_0, \quad Y = \frac{V_r}{Q(S_i - S_e)}, \quad \lambda = \frac{K_B}{U_{max}}, X = \frac{V_r}{Q(S_i)}, \quad \lambda_0 = \frac{1}{U_{max}}$$

Considering the mass balance of substrate present in wastewater that flows into the reactor and out of the reactor plus the total amount of substrate degraded, at a specific flow rate, control volume and time, then the mass balance can be written as;

$$QS_i = QS_i + V_r\left(\frac{dS}{dt}\right) \quad (4.5)$$

Substituting dS/dt from the equation (4.4) into the equation (4.5) and by rearranging the expression, it will give equation (4.6) and (4.7).

$$S_e = S_i - \frac{U_{max} S_i}{K_B + \left(QS_i / V_r\right)} \quad (4.6)$$

$$V_r = \frac{QS_i}{\frac{U_{max} S_i}{S_i - S_e}} \quad (4.7)$$

At a given influent concentration, organic loading rate (QS_i/V_r) and known volume of anaerobic reactor, equations (4.6) can be used to estimate the concentration of substrate present in the reactor effluent when K_B and U_{max} values are obtained. Equation (4.7) can be used to determine the required volume of anaerobic reactor needed to reduce effluent substrate concentration in order to meet the discharge standard. Equation (4.4) can be used to determine the K_B and U_{max} of the reactor. The inverse of loading rate [$V_r/Q(S_i-S_e)$] can be

plotted against the total loading rate of the reactor V_r/QS_i. The slope and intercept of the straight line are K_B/U_{max} and $1/U_{max}$ respectively.

4.2.7 Statistical Analysis

Statistical analyses were performed on the measured parameters as well as to test the differences between the measured and predicted results at an alpha level of 0.05. Graph Pad Prism v.5, software package was used for statistical analyses and graphs.

4.3 RESULTS AND DISCUSSION

4.3.1 Profiling of Microbial Community Structure of a Full-Scale UASB Reactor Granules Based on 16S rDNA Analysis

4.3.1.1 Characteristics of granular sludge used for the molecular analysis

The physico-chemical characteristic of granular sludge collected for microbial analysis in this study were determined using standard methods as described in section 3.2.3 (Table 4.3).

Table 4.3: Characterization of granular sludge used for molecular analysis

Parameter	Concentration (mg/L)
TCOD	1700
SCOD	1220.58
TSS	70.54
VSS	62.27
TS	83.42
VS	70.38
PO_4	70.59
NO_2	0.12
NH_3	1.5
pH	6.78
Temperature (°C)	28

4.3.1.2 Methanogenic Archaea and bacteria detected from the granular sludge using FISH technique

The preliminary analysis of granular sludge was carried out using FISH technique with probes targeting Eubacteria and Archaea domains (Table 4.1). *In-situ* hybridization analysis of the samples stained with ARC 915 and EUB 388 mix probes revealed the dominance of both rod and coccoid-shaped methanogens in the reactor (Figure 4.2a-c). Thick cell wall with long and short curved rods, cocci and irregular cocci packet shapes indicated the presence of diverse groups of acetoclastic methanogenic Archaea belonging to the order *Methanobacteriales, Methanococcales* and *Methanomicrobiales*. Detection of rod and cocci packet shapes by ARC915 probe shows that *Methanosaeta* and *Methanosarcinales*-like species are also present in the UASB reactor. The presence of cocci with thick cell wall and packet-like shape, typical to the genus *Methanosarcina* was further confirmed by the MS821 probe. Furthermore, the positive hybridization of MX825 probe confirmed the presence and dominance of acetoclastic *Methanosaeta* group in the samples (Figure 4.2d-e), which is distinguished by their typical rod-shape (Raskin *et al.,* 1994; Sekiguchi *et al.,* 2001; Gomec *et al.,* 2008; Vavilin *et al.,* 2008). These groups of methanogens have previously been reported to be present in anaerobic reactors which showed more than 70% CH_4 production (Krzysztof and Frac, 2012). The detection is in agreement with the previous findings where the genus *Methanosarcina* were detected in granular sludge samples (Sekiguchi *et al.,* 1999; Jupraputtasri *et al.,* 2005; Kovacik *et al.,* 2010).

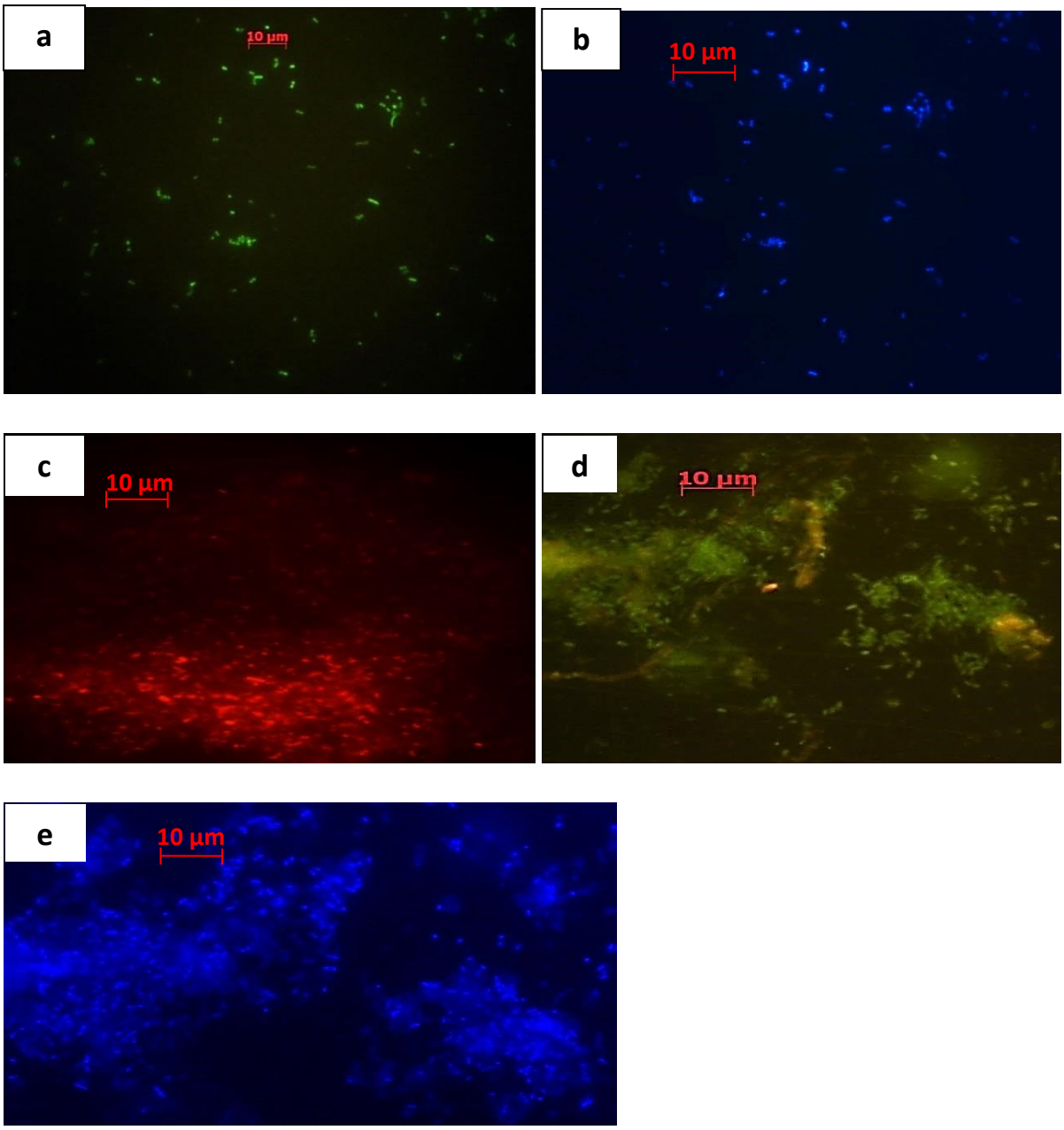

Figure 4.2: (a) Images of granules hybridized by highly rhodomine labeled archaeal-domain oligonucleotide probes (ARC915) showing diverse species of methanogens (green) at 1000 x magnification; (b) corresponding image of ARC915 granules showing diverse species of methanogens stained with DAPI (blue), (c) granular sludge of FISH labeled with tetramethylrhodomine-5-isothiocyanate using the universal probes for eubacteria (EUB338), (d) the MX825 probe labeled sample to confirmed the acetoclastic *Methanosaeta* group and (e) the corresponding DAPI stained cells for EUB mix.

4.3.2 Community of the Granular Sludge Using PCR

The results of FISH were further confirmed using PCR. The phylogenetic structure of the bacterial, archaeal and methyl coenzyme-M reductase (*mrcA*) gene communities was investigated by 16S rDNA gene-cloning analysis. The full-scale UASB reactor has six different compartments (bottom to the top; C1, C2, C3, C4, C5, and C6; Figure 4.1), of which PCR amplicons of both eubacteria and methanogenic Archaea obtained from compartments 1, 3 and 6 were selected for cloning and analysis. The results obtained are discussed in details below.

4.3.2.1 Bacterial diversity within the reactor compartments

The bacterial populations in the granule samples were analyzed using the domain specific primer set 27f/1492r that target eubacteria 16S rDNA genes (Giovannoni, 1991). Figure 4.3 shows the bands on agarose gel corresponding to each of the six compartments. The phylogenetic analysis of the PCR products revealed an abundance of three major bacterial phyla belonging to the *Proteobacteria, Firmicutes* and *Chloroflexi* within the reactor compartments. The other major phylum detected was an uncultured candidate division WS6 (Table 4.4). Class *Gamma* and *Deltaproteobacterium*, *Clostridia, Syntrophorhabdus aromaticivorans* and *Dehalococcoidetes* were also present in abundance in the reactor samples (Figure 4.4; Table 4.4).

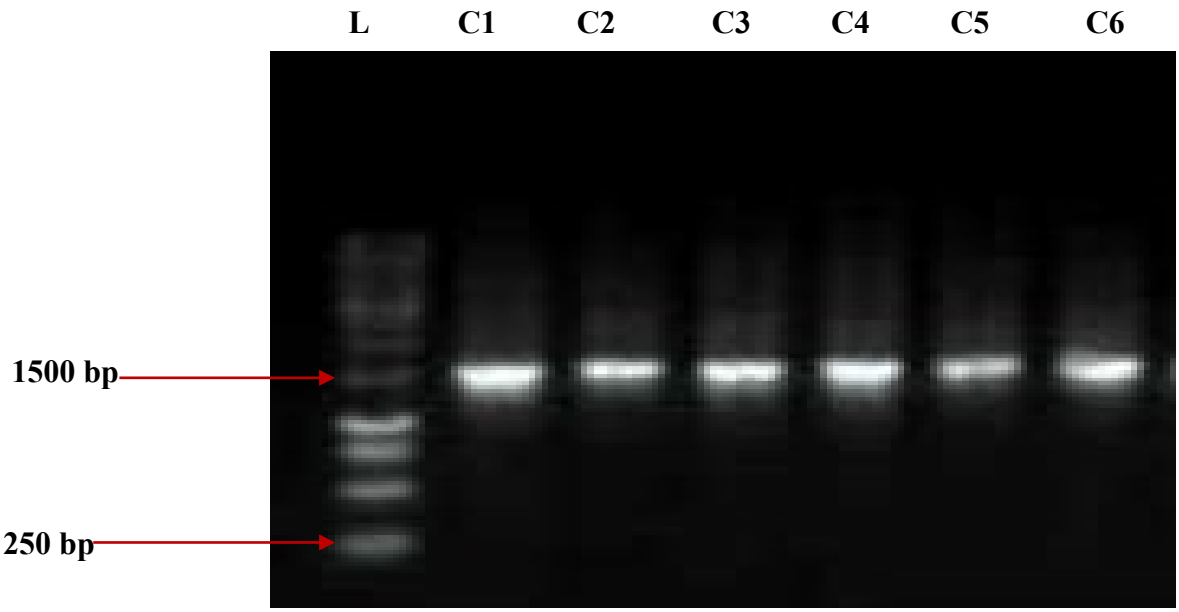

Figure 4.3: Agarose gel depicting PCR products for the bacterial fragments (1500 bp). The bands corresponding to lanes C1–C6 represent the bacterial fragments from the six compartments of the UASB reactor when PCR amplification was performed using 27f/1492r specific primer set. Lane L corresponds to the 1 kb DNA marker used in this study.

Table 4.4: Bacterial community profiles of the clones retrieved from granular sludge samples taken from the UASB reactor, as compared to the known sequences in the GenBank database

Source/Habitat/Microorganism	Hits	Sequence length	Identity (%)	Accession number/Reference
Anaerobic digestion of beet silage	1	1473	95-98	Krakat *et al.* (2011)
Forest musk deer intestine	6	1451	95	JF690890, JF690880, JF690878, JF690871, JF690869, JF690882
Bacterial communities in sediment of shallow lake Dong ping	1	-	95	Song *et al.* (2012)
Fiber degrading bacteria from pig faeces		-	95	FJ753786, FJ753832
Swine faeces, human faeces, cellulose/xylan degraded	1	-	98	JX120100, JX006776
Psychrophilic methanogenic community of wastewater treatment EGSB bioreactor	4	970	96	EU722393, McKeown *et al.* (2009)
Granular sludge of full-scale reactor treating corn straw	13	1470	95	Qiao *et al.* (2013)
Microbial community composition as affected by substrate types of anaerobic digesters	3	1027	92	JX023221
Hydrocarbon and chlorinated-solvent contaminated aquifer undergoing intrinsic bioremediation	1	1470-1472	95	Dojka *et al.* (1998)
Anaerobic swine lagoons	1	1428	95	AY953166
Methane production from hydrocarbon in oil sand tailings		1366	86	Siddique *et al.* (2012)
Microbial fuel cells	1	1474	85	Dunaj *et al.* (2012)
Biological wastewater treatment plant integrated with constructed wetland for the treatment of tannery effluent			95	KC110172
Anaerobic digestion of food waste		1506	90	KF699851
Toluene-degrading methanogenic consortium	1		85	Ficker *et al.* (1999)
Biogas slurry		1514	95	GU112185

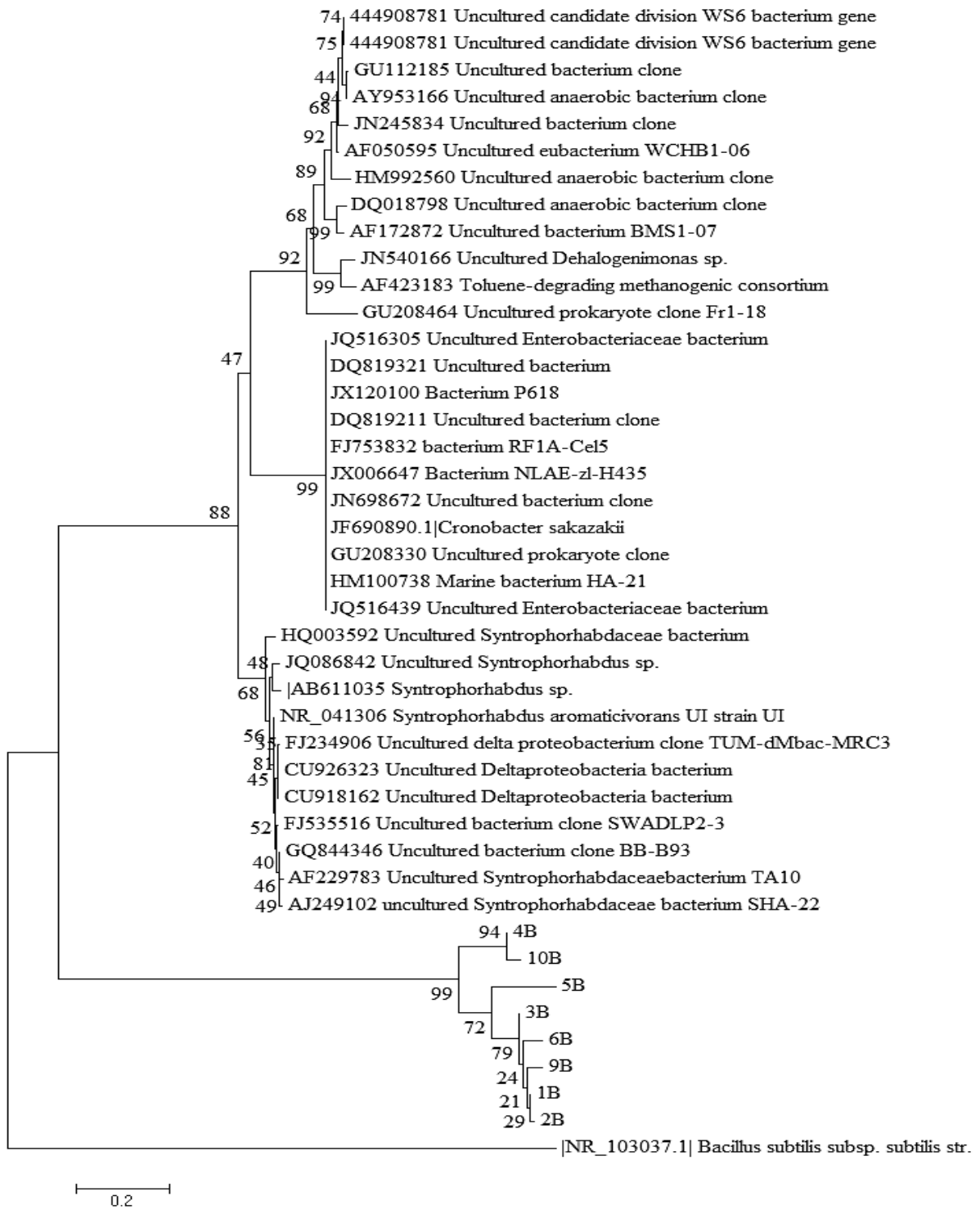

Figure 4.4: Phylogenetic tree of bacterial clones obtained from granular sludge of UASB reactor treating brewery wastewater using universal 27f/1492r bacterial primer set. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The nucleotide sequences were submitted to the National Centre for Biotechnology Information website under the accession numbers KM065733 – KM065740 corresponding to the selected clones (1B-10B) from compartments C1, C3 and C6.

Detection of four major phyla (*Bacteroidetes, Chloroflexi, Firmicutes* and *Proteobacteria*) with relative differences in the bacterial population in AD systems has been reported previously (Nelson *et al.*, 2011; Lee *et al.,* 2012; Lee *et al.*, 2014; Jang *et al.,* 2014). A similar pattern of diverse phylogenetic fingerprint for the bacteria at phylum and genus level were reported for anaerobic degradation of brewery wastewater, corn straw, as well as birch and conifer pulp (Werner *et al.,* 2011; Nissilä *et al.,* 2012; Novak *et al.,* 2013; Qiao *et al.,* 2013).

The clones obtained from compartment 1 showed more than 90% sequence similarity to uncultured bacterial. Clone B1 from compartment 1 showed 98% similarity with cellulose, amylase and protease enzyme-producing bacterium P618 in the GenBank as shown in Table 4.4 (JX120100). These enzymes are excreted by hydrolytic and fermentative bacteria during the hydrolysis stage of anaerobic conversion of complex organic matter in the wastewater into soluble monomers (Arsova, 2010; Ralph and Dong, 2010; Krzysztof and Frac, 2012). Clone B8 obtained from compartment 1 was found to be closely related to uncultured bacterium and uncultured *Enterobacteriaceae* bacterial clones in the phylum *Proteobacteria*. These organisms are involved in the direct production of methanogenic substrates, such as CO_2, H_2, formate and acetate. The clones in this compartment also showed 98% sequence similarity with known sequences of *Escherichia ferusonii* (NR074902) *and Escherichia coli* (JX041515) in the GenBank database.

In compartment 3, the major groups of bacteria were closely related to class *Gammaproteobacteria* and uncultured *Enterobacteriaceae* bacterium (JQ516439). Few clones were similar to *Cronobacter sakazakii* (JF690890)*,* formerly known as *Enterobacter sakazakii*, a Gram-negative, non-spore–forming, motile and peritrichous rod of the *Enterobacteriaceae* family. Furthermore, few other clones showed similarity to uncultured prokaryote (GU208330) bacteria, uncultured eubacterium WCHB1-06 (AF050595) of the phylum *Firmicutes* and class *Clostridia* and uncultured *Dehalogenimonas* sp. (JN540166) in phylum *Chloroflexi,* toluene-degrading methanogenic consortium bacterium (AF423183) (96% sequence similarity).

Enumeration of *Cronobacter sakazakii* from sewage sludges has been reported in the literature (Iversen *et al.*, 2008; Kucerova *et al.,* 2010). The importance of this *Cronobacter sakazakii* strain in the treatment of winery effluent using UASB reactor was demonstrated by Keyser *et al.* (2003). Its ability to degrade recalcitrant compounds of anaerobically digested spent wash from an effluent discharge site (Rajasundari and Murugesan, 2011) and its relevance in the production of H_2 as a metabolite that can be used by CH_4 producing Archaea during dark fermentation were mentioned in an earlier study (Kang *et al.*, 2012). Phylum *Firmicutes*, genus *Clostridium* are known to be directly involved in the conversion of complex organic matter in the industrial waste to the metabolites that can be used directly by the methanogenic Archaea. They are efficient in the degradation of complex organic matter and acetic or lactic acid fermentation to CO_2 and H_2 (Nelson *et al*., 2011; Wirth *et al.,* 2012). Other closely related genera of this phylum were observed in compartment 3. Similar observations were noticed in other studies as reported in the literature (Keyser *et al.,* 2006; Rincón *et al.,* 2008; Krzysztof and Frac, 2012; Wirth *et al.,* 2012; Sundberg *et al.,* 2013).

In compartment 6, the largest proportion of bacteria belonged to phylum *Proteobacteria* of class *Delta* and *Gammaproteobacteria* that contain mostly Gram-negative bacteria in their lineages. Sequence similarity (99%) with known sequences in the GenBank database further showed that the clones from this compartment belong to class *Deltaproteobacteria* of family *Syntrophorhabdaceae.* They are closely related to the clustered sequence of anaerobic environmental clones belonging to phylum *Deltaproteobacteria* (formally known as *Deltaproteobacteria* group TA), family *Syntrophorhabdaceae* and *Syntrophorhabdus aromaticivoran*s (Figure 4.4).

The abilities of *Syntrophorhabdaceae* bacteria to digest recalcitrant compounds of spent wash during anaerobic degradation have been reported (Qiu *et al.,* 2008; Nakasaki *et al.,* 2013; Shen *et al.,* 2013; Nobu *et al.,* 2014), especially, in brewery wastewater (Werner *et al.,* 2011). The family *Syntrophorhabdaceae* contains well-known species of syntrophic substrate-degrading anaerobes such as those of the genera *Syntrophus*, *Smithella and Syntrophobacter* (Qiu *et al.,* 2008; Nobu *et al.,* 2014). They are known as amino acids degraders and sulphate–reducing bacteria (SRB) (Shen *et al.,* 2013). Species of the genus *Syntrophobacter* has the

ability to utilize sulphate as an external electron acceptor, but their growth by sulphate reduction is known to be very slow (Shen *et al.,* 2013; Nobu *et al.,* 2014). Detection of sulphate–reducing bacteria in this study explained the low to no sulphate in the brewery effluent (treated wastewater) from the UASB reactor as observed in this study (Figure 4.4). Raskin *et al*. (1996) also noticed about 15% of SRB in a methanogenic reactor, even in the absence of sulphate in the reactors influent. Competition and coexistence of sulphate-reducing bacteria, acetogens and methanogens in an anaerobic bioreactor was investigated by Dar *et al.* (2008). The SRB may be competing with methanogenic organisms for the available electrons and utilization of acetate particularly at high organic loading rates (Casserly and Erijman, 2003; Ince *et al.,* 2010).

Furthermore, members of the *Syntrophorhabdaceae* family isolated from anaerobic treatment of industrial wastewater have been reported to play an important role in degradation of aromatic compounds present in the industrial wastewater, especially *Syntrophorhabdus aromaticivorans* (Qiu *et al.,* 2008; Nakasaki *et al.*, 2013; Shen *et al.,* 2013; Nobu *et al.,* 2014). *Syntrophorhabdus aromaticivorans* is an obligate anaerobic, syntrophic substrate-degrading mesophilic organism capable of oxidizing *p*-cresol, phenol, benzoate, isophthalate and 4-hydroxybenzoate in association with an H_2-scavenging methanogen partner (hydrogenotrophic methanogen) (Shen *et al.,* 2013). The 16S rDNA gene sequence analysis of clones in compartment 6 were closely related to *Syntrophorhabdus aromaticivorans* strain UI of group TA in the class *Deltaproteobacteria* isolated in granular sludge taken from an UASB reactor treating manufacturing wastewater (Qiu *et al.,* 2008). Relatively large numbers of this type of bacteria isolated mainly from methanogenic environments especially UASB sludge samples have been reported in the literature (Sekiguchi *et al.*, 1998; Wu *et al.,* 2001; Lykidis *et al.,* 2011).

4.3.2.2 Archaea composition in the granular sludge

The Archaea community, as group of CH_4–producing organisms is assumed to be dominant in the granules obtained from a biogas-producing UASB reactor. Figure 4.5 showed the Archaea bands on agarose gel corresponding to each of the six compartments using a universal ARC622f/ARC915r primer set.

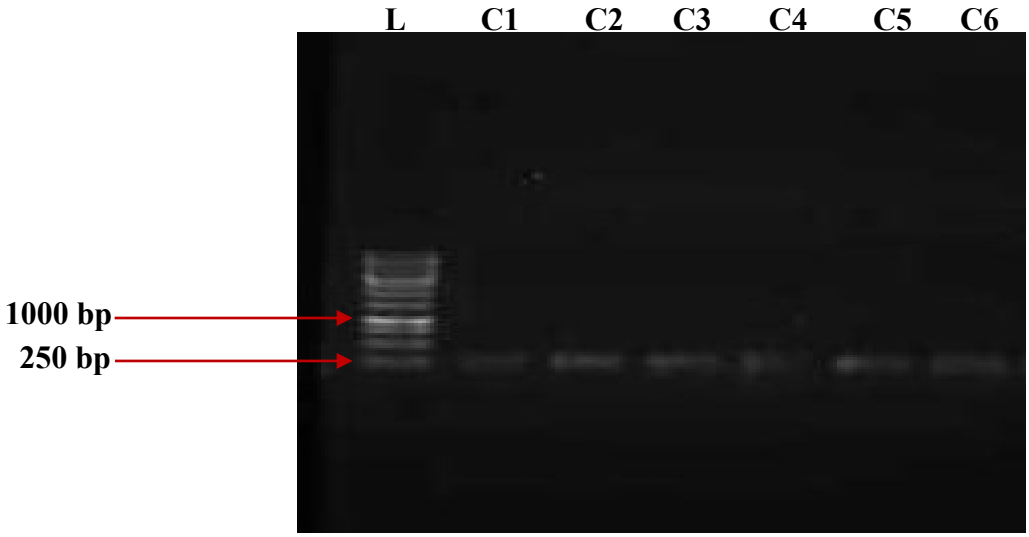

Figure 4.5: Agarose gel showing 16S rDNA gene PCR fragments obtained from the amplification of genomic DNA extracted from the granular sludge samples using ARC primer set. Bands corresponding to lanes C1–C6 represent the Archaea fragments from the six compartments of the UASB reactor between 243–250 bp using 1 kb DNA marker (lane L) in the analysis.

Analysis of the clones obtained from the different compartments showed 98-100% sequence similarity to known sequences of methanogenic Archaea in the GenBank (Table 4.5). The detected 16S rDNA sequences were affiliated to the *Methanobacteriales, Methanomicrobiales* and unclassified archaeon clones. However, members of the *Methanococcales* were not detected in the granules using this primer set as shown in the phylogenetic analysis from the gene sequences obtained from the GenBank database (Table 4.5). This may be due to their growth requirement of high–salt concentration (0.3-0.9% NaCl (w/v)), which are not normally found in anaerobic reactors (Bialek *et al.,* 2011).

Table 4.5: Sequence similarity of Archaea from the full-scale UASB reactor with the GenBank database sequences

Clone	Most closely related organisms	Accession number	Source/Habitant	Reference
C1(KM191135)	Uncultured archeons	DQ262487,	Methanogens from biogas plant	Unpublished
		HF966604,	Methanogens in anaerobic digesters	Sousa *et al.* (2013)
		JN038003, KC442808, KC352709, KC182519, AB775722, AB710147, AB818554	Environmental sample	Unpublished
		HE648051, HE648045, HE648044	Anaerobic bioreactors treating oleate-based wastewater	Salvador *et al.* (2013)
	Uncultured *Methanobacterium* archeons	JQ247412, JQ247417, JF754565, JF754562	Ody sludge and its enrichment amended with alkanes incubated for over 500 days	Unpublished
	Methanobacterium sp.	JF732736 AB288281	Microbial fuel cell	Unpublished
	Methanobacterium formicicum	JN566059, JN243318, JN205061, JN205059, JN205052, NR115168	Mesophilic corn-fed on-farm biogas plants and lab scale biogas fermenters	Unpublished
		JX042445	Methanogen Isolated from the anaerobic batch reactor of pig slurry	Unpublished
	Methanoculleus sp	AB288272	Deep subsurface groundwater from sedimentary rock	Unpublished
	Uncultured euryarchaeote clone ANT2-EFL	GU969413	Brazilian Antarctic Station wastewater	Unpublished
C3 (KM191136)	*Methanobacterium formicicum*	HQ591420	Anaerobic microorganisms involved in methanol transformation in an underground gas storage facility	Unpublished
		Z29436 (DSM 3636)		
	Methanobacterium palustre	NR_041713	Methanogenic rod isolated from a Philippines rice field	Joulian *et al.* (2000)
	Uncultured archaeon clone	HQ438759 AB598266	Soil microcosms contaminated with phenanthrene	Unpublished
	Euryarchaeote clone	GU969419	Brazilian Antarctic Station wastewater	Unpublished
C6 (KM191137)	Uncultured archeons	JN617328	Methanogenic archaeal community in Lake Taihu	Unpublished

In compartment 1, clones obtained from the granular sludge were closely related to phylum *Euryarchaeota,* genus *Methanobacterium* with 98% similarity to *Methanobacterium formicicum* (Table 4.5). Genus *Methanoculleus* sp. (AB288272) of order *Methanomicrobiales* was found to be similar to clones obtained from this compartment. This group comprised of ~ 85% of the total Archaea clones, while 12% belonged to uncultured archaeon clones (98-99% similarity). The clones obtained from compartment 3 granular sludge were affiliated to known DNA sequences of *Methanobacteriaceae* archaeon, *M. formicicum* (DSM 3636; Z29436), *Methanobacterium palustre* (NR_041713), *Methanobacterium* sp. clone ARC and uncultured archaeon (99% sequence similarity) in the GenBank database.

Similar pattern was observed in the clones obtained from the last compartment (C6). The clones showed 99% similarity with archaeon sp. and *Methanobacterium* species. The majority of the clones detected in this compartment were affiliated to the order *Methanobacteriales* (CU466652; 99% similarity), family *Methanobacteriaceae* obtained from environmental 16S rDNA sequence from Evry wastewater treatment plant anoxic basin (Chouari *et al.,* 2010), uncultured prokaryote (EU717078; 100% sequence similarity), and uncultured archeons.

Sequence analysis of the Archaea community showed that closely related species belonging to the *M. formicicum* and *M. palustre* were abundant in the reactor, especially in compartments 1 and 3. Thus, the presence of this hydrogenotrophic Archaea in sufficient amounts in the reactor and in the compartments indicated that the rate of H_2 conversion produced by the acetogenic bacteria to CH_4 is high in these compartments. This further confirmed the syntrophic association between acetogenic and the methanogenic bacteria in the studied UASB reactor (Amani *et al.,* 2011; Ziemiński and Frąc, 2012).

Dominant hydrogenotrophic methanogens of order *Methanobacteriales* has previously been reported in a mesophilic reactor (Bialek *et al.,* 2011; Traversi *et al.,* 2011; Zhu *et al.,* 2011; Salvador *et al.,* 2013). The findings of Leclerc *et al.* (2004) showed the abundance of *Methanobacteriales* among the diverse group of the archaeal community in a UASB reactor treating brewery wastewater. During brewery wastewater degradation, production of acetate

and H_2 from ethanol normally occur through the interaction of H_2 utilizing Archaea and H_2 producing syntrophic bacteria (Ince *et al.,* 2010). As discussed earlier, the UASB reactor studied was very high in sulphate-reducing bacteria and they are the major competitor with *Methanobacteriales* species for H_2 in the absence of sulphate (Casserly and Erijman, 2003). Likewise, *Methanobacteriales* are the most abundant hydrogenotrophic and acetoclastic methanogens that were detected in the UASB reactor investigated in this study.

4.3.2.3 Detection of methyl coenzyme-M reductase gene A (*mcrA*) in the granular sludge

A novel method of metagenomics coupled with FISH is increasingly used to link the genetic identity of microorganisms to their ecological function in this field (Nercessian *et al.,* 2005). Characterization of methanogens based on the methanogenic approach using a small subunit of ribosomal RNA has been used in many studies and their limitations in providing a direct link to physiology, metabolic capacities, as well as difficulties to determine the functions of the unknown organism was mentioned (Pycke *et al.,* 2011; Supaphol *et al.,* 2011; Niu *et al.,* 2013, Buriánková *et al.,* 2013). Identification of methanogens based on 16S rRNA as a marker is generally limited as methanogens from several different major lines of descent can only be found within the kingdom Euryarchaeota. The use of a functional marker (*mrcA*) genes-based approach encoding the key enzymes of characteristic metabolic pathways that is exclusive to the methanogenic Archaea to identify the methanogenic population in the treatment process is well documented (Nercessian *et al.,* 2005; Nettmann *et al.,* 2008; Rastogi *et al.,* 2008; Krober *et al.,* 2009; Steinberg and Regan, 2009; Traversi *et al.,* 2011; Zhu *et al.*, 2011; Kampmann *et al.,* 2012; Traversi *et al.*, 2014).

The successfully amplified PCR products using methanogenic specific primers (*mcrA*) after sequencing and phylogenetic analysis showed 96 to 100% similarity to methanogenic Archaea belonging to the order *Methanobacteriales* and *Methanomicrobiales* (Figure 4.6). Similar results were previously reported from the UASB reactor granules treating brewery wastewater (Diaz *et al.,* 2006) and also from other anaerobic reactors producing biogas (Castro *et al.*, 2004; Cardinali-Rezende *et al.,* 2009; Kovacik *et al.,* 2010).

The *mcrA* sequences clustered around the *Methanobacteriales* such as *Methanobacterium beijingense* strain, *Methanobacterium aarhusense* and *Methanothermobacter crinale* showed 96% sequence similarity (Shlimon *et al.,* 2004; Cheng *et al.,* 2011; Kampmann *et al.,* 2012). This further confirms the dominance of hydrogenotrophic *Methanomicrobiales* within the UASB reactor granules. However, in this study, the amplification of the *mcrA* primer sets using PCR did not detect the *Methanosarcina and Methanosaeta* sp. in the granular samples as reported by Luton *et al.* (2002). Most clones belonged to the order *Methanomicrobiales* and few clones were *Methanosarcina* sp., while none was reported for *Methanosaeta* sp. (Luton *et al.,* 2002). Similar observations were also made by Castro *et al.* (2004) and Smith *et al.* (2007).

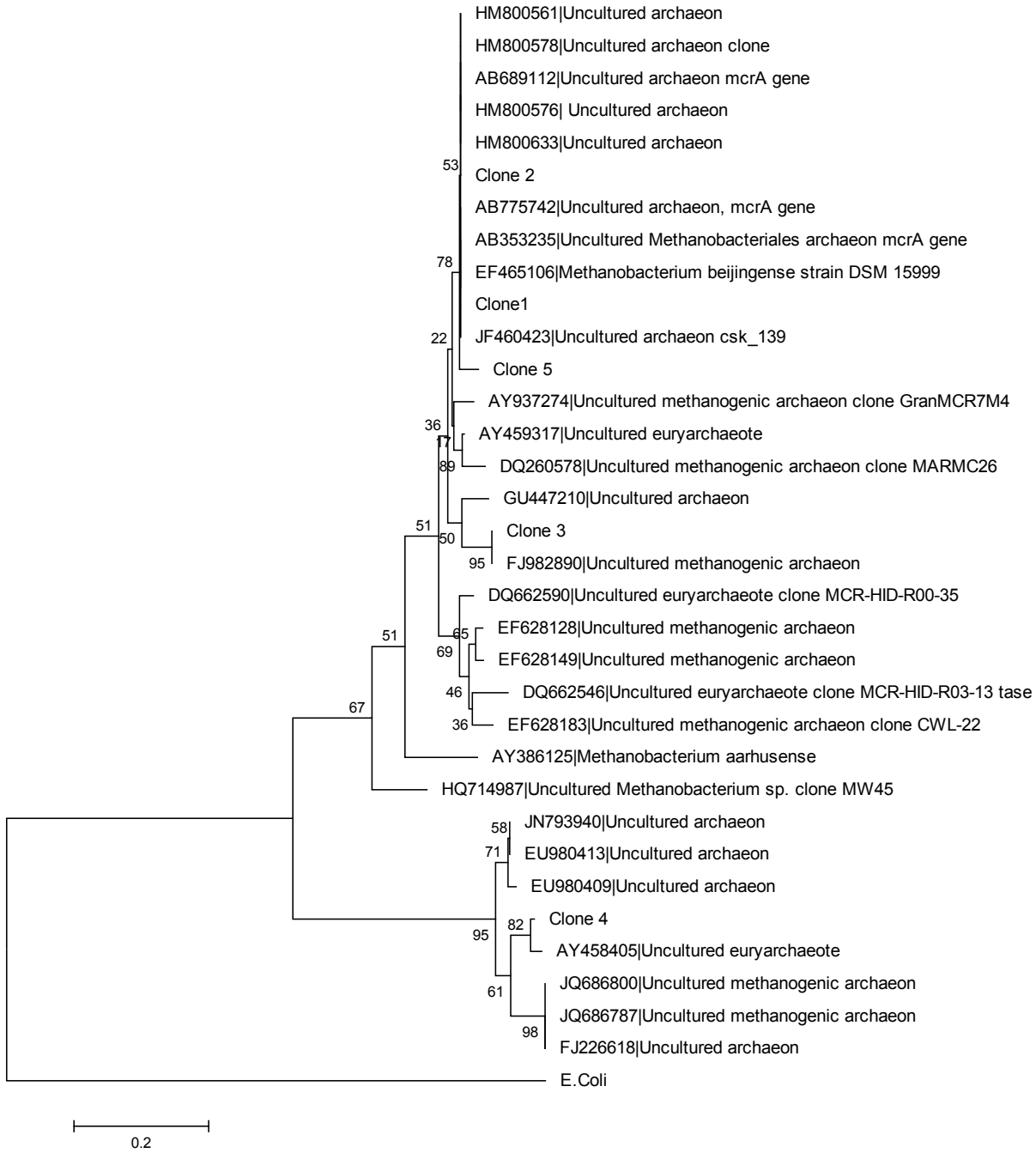

Figure 4.6: Phylogenetic tree for methanogenic Archaea obtained from granular sludge of UASB reactor treating brewery wastewater using methyl coenzyme-M reductase (*mcrA*) gene primer set. The evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The GenBank accession numbers are KF715644–KF715648 corresponding to the selected clones.

4.3.3 Optimization of QPCR for Quantification of Microbial Communities Present in the Granular Sludge Samples

The abundance of Archaea and bacterial 16S rDNA copies was quantified within the different compartmental of the UASB reactor using the quantitative real-time PCR (QPCR) based assay. This was done to quantify and compare the microbial populations in the UASB reactor and to establish the phases of anaerobic fermentation correlation to the microbial data within the compartments. In contrast to the conventional end-point detection PCR, QPCR technology has better sensitivity and reproducibility than conventional PCR and can easily be used in studies requiring a large number of samples (Talbot *et al.,* 2008).

Firstly, the universal primer set for the domain Archaea was tested with the suggested PCR mixture and thermocycling conditions as modified from the protocol described by Steinberg and Regan (2009) and this protocol was applied to all primer sets. Due to different amplicon lengths of the 16S rRNA gene fragment an additional annealing step was included in the cycling protocols for the ARC and BAC assays to obtain an optimum standard curve.

Known concentrations of standard DNA were used to validate all real-time PCR assays with determination coefficient (R^2) values of 0.991 and 1.000 respectively (Table 4.6). Table 4.6 shows the statistical analysis derived for the constructed standard curves with the corresponding primer sets. There were no significant differences in the slopes of the standard curves at 95% confidence interval for each set of primers used regardless of their amplicon size. This shows the feasibility and accuracy of QPCR assays for the quantification of microbial 16S rDNA gene copy numbers in the granular sludge samples. This approach has previously been employed for quantifying microbial DNA from the samples collected from biogas producing UASB reactors (Brinkman *et al*., 2003; Hermansson and Lindgren, 2001; Nadkarni *et al.,* 2002; Suzuki *et al.,* 2000),

The average values of intercept and slope for each primer set were used to quantify the amount of targeted 16S rDNA copy numbers in the granules (Table 4.6). Average amplification efficiencies for bacteria and Archaea were 97.6% and 98.8% respectively,

which further showed the consistency of the QPCR assay. At the end of each QPCR run, primer dimer was checked to confirm that there was no non-specific binding during each reaction using melting curve analysis. The abundance of microbial communities as determined by QPCR was reported as DNA copy numbers of 16S rDNA genes per nanogram of genomic DNA isolated from reactor samples.

Table 4.6: Description of QPCR standard curves parameters for 16S rDNA copy number for ARC as the universal Archaea and BAC as the universal bacterial primer sets that are responsible for biological conversion of complex organic matter in the brewery wastewater into simple monomer and CH_4 production

Parameter	**Primer set**	
	ARC-set	BAC-set
Linear range (copies/ng DNA)	$1.64 \times (10^4 \sim 10^{11})$	$2.77 \times (10^3 \sim 10^{10})$
Slope (standard deviation)	-3.565 (0.019)	-3.485 (0.011)
R^2 of slope	1.000	0.991
Intercept	43.93	41.05

4.3.3.1 Comparison of concentration of Archaea and bacterial communities in the different reactor compartments

The average 16S rDNA gene copies of Archaea in the samples were calculated against the total bacterial 16S rDNA gene copies. The compartment showed a noticeable disparity in terms of the composition of bacteria and methanogenic Archaea population using real-time PCR (Figure 4.7). It was observed that the concentration of Archaea decreased with an increase in bacterial concentration along the reactor compartments (1 to 6) as shown in Figure 4.7. There was a correlation between species diversity using PCR and gene copy number using QPCR. Identification and quantification of the 16Sr DNA using PCR and QPCR confirmed the variation in the concentration of bacteria and Archaea down the reactor compartments. There was a reduction in bacteria and an increase in Archaea concentrations at

the bottom of the reactor in compartment 1 when compared with compartment 6 using QPCR. Similarly, the PCR results showed high methanogenic diversity at the bottom of the reactor (C1) with few known bacteria clones, when compared with identified bacterial and Archaea species in compartment 6.

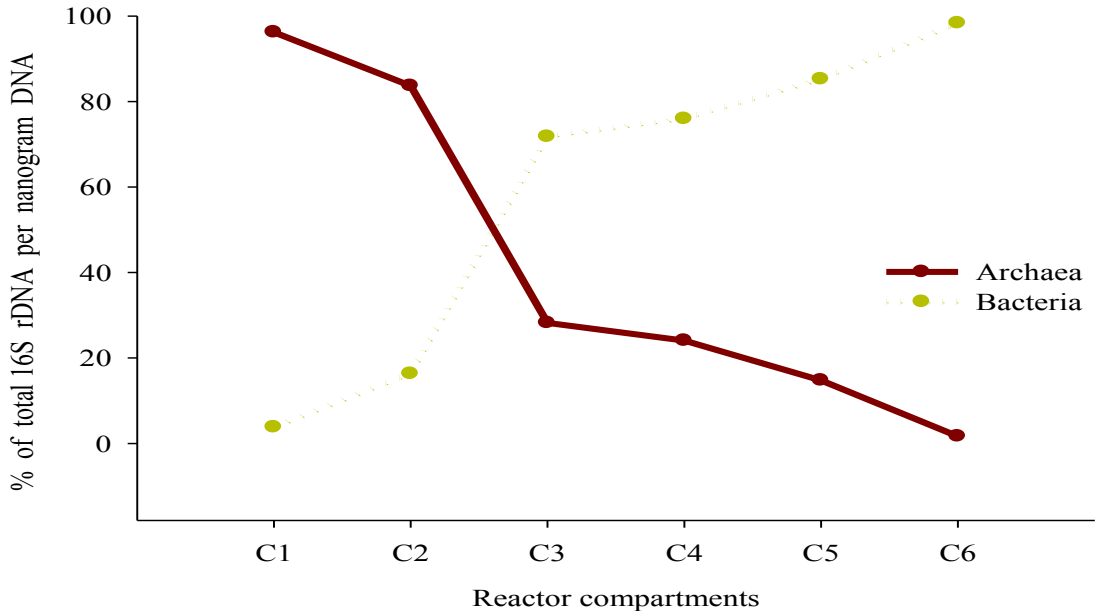

Figure 4.7: Variation in the percentage of bacteria and Archaea communities in the granules collected at the different reactor compartments (C1–C6) using universal primer sets for the quantitative real-time PCR assay, in this study.

In compartment 1, the percentage of Archaea in the sample was much higher (96.28%) than the percentage of bacteria (3.78%) (Figure 4.7). However, the percentage of bacterial increased to 98.34% in compartment 6 with decrease in Archaea percentage (1.66%). There was a change in the quantity of bacteria along the reactor compartment throughout the monitoring period.

The results showed variation in the microbial population in each compartment. It can further be deduced that different compartments in the reactor may have been involved in different

phases of anaerobic degradation of organic matter in brewery wastewater with different concentration of metabolic products been produced as confirmed by the DNA sequencing results and the QPCR assays. Ye *et al.* (2009) noticed the abundance of the Archaeal 16S rDNA gene in the total prokaryotic community quantified from sediment of Lake Taihu using QPCR. Similar variation in the quantity of archaeal genes along the length of the reactor was recorded by Kubota *et al.* (2014) with Archaea colonizing the lower and middle parts of the reactor as observed in the present study.

Figure 4.8 shows the results of the QPCR assays in terms of gene copy number using the gDNA samples extracted from the granules obtained from each compartments of the full-scale UASB reactor using each primer set. The concentration of bacteria as revealed by QPCR assays showed that bacterial gene copies was dominant and abundant in compartment C6, but decreases down the reactor compartments (Figure 4.8). Compartment 1 has a relatively low concentration of bacterial gene copy number, followed by an increase in cell number in compartment 2 and thereafter, gradual increase in concentration from compartment 3 to 6.

The total concentration of bacteria using QPCR in this study ranged between 2.58×10^3 to 3.43×10^6 copies/ng DNA. The highest concentration of bacterial per nanogram of sample was observed in samples taken from compartment 6 (3.43×10^6 copies/ ng DNA) and decreased to 2.58×10^3 in compartment 1. However, fluctuation in the quantity of bacteria in the different compartments was also noticed. This might be due to competition among the bacteria for available nutrients or as a result of inhibition of some bacteria through the activities of other group of bacteria in the reactor or the influence of digestion temperature (Lee *et al.,* 2012; Welte and Deppenmeier, 2013; Yuan *et al*., 2014). Apart from the intermediate metabolites that are produced during the conversion of complex organic matter, some intracellular material is released when bacteria die which serves as nutrients for other organisms (Aquino and Stuckey, 2004; Ghosh, 2013).

On the other hand, quantification of 16S rDNA genes using ARC915r/ARC622f revealed that the proportion of total Archaea varied along the reactor compartments with Archaea

colonizing the lower part (C1 and C2) and the middle (C3) of the reactor (Figure 4.8). The concentration of Archaea decreased from C1 to C6 with higher DNA copies in compartment 2 and lowest concentrations were found in compartment 6. Specifically, on average, the total concentration of Archaea during the study ranged between 5.80×10^4 to 1.45×10^6 copies/ng DNA. The concentration of Archaea per nanogram of sample was much higher in compartment 2 (1.45×10^6 copies/ ng DNA) and decreased to 5.80×10^4 with an increase in the reactor's compartment (C6). Quantification in terms of percentage of microbial community showed that the reactor had higher percentage of Archaea in compartment 1 due to small amount of bacterial concentration in compartment 1; however, compartment 2 had higher concentration of Archaea and bacteria when compared with compartment 1. The Archaea domain consists of sensitive organisms; their increase in the lower compartments 1 to 3 also correlates with the presence of low concentrations of toxic substances in those compartments (Gerardi, 2003; Ali Shah *et al.,* 2014). A reduction in Archaea concentration or cell number indicated the production of intermediates metabolites that did not favour or inhibit the growth of methanogens (Botheju and Bakke, 2011). Langenhoff and Stuckey (2000) also observed a higher methanogenic activity of the bottom part of an anaerobic reactor treating low strength wastewater.

Thus, a combination of both PCR-based and FISH (RNA-based methods) techniques produced a better understanding of the microbial consortia present in the UASB reactor treating brewery wastewater. These techniques helped us to identify and quantify the microbial population and possible phases at which anaerobic fermentation takes place in the reactor. This study extends our knowledge on the different hydrolytic, acidogenic, acetogenic bacteria and methanogenic Archaea present in the granules of the full-scale reactor investigated.

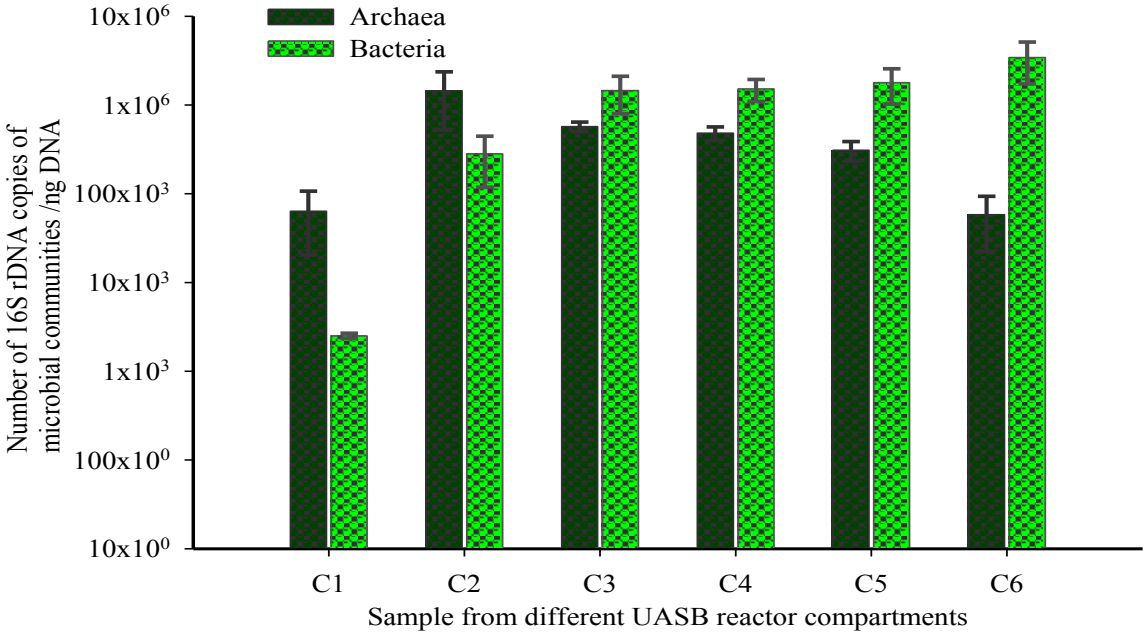

Figure 4.8: Abundance of Archaea and bacterial DNA copy numbers of 16S rDNA genes per nanogram of genomic DNA extracted from the granular samples obtained from each compartments of the full-scale UASB reactor using QPCR assays for the primer sets used in this study.

4.3.4 Performance of UASB Reactor and Biogas Production

The characteristics of the influent brewery wastewater are shown in Table 4.7. The average influent COD concentration was 2005.73 ± 1139.85 mg/L at 28˚C (Table 4.7) with a COD removal efficiency of 78.97 %. The average effluent substrate concentration (S_e) from the UASB reactor was lower (457.25 ± 272.41 mg/L) than the influent substrate concentration (S_i). This might be due to low levels of total solids introduced into the reactor which helped the reactor performance (Section 3.3.2.2).

Table 4.7: Biochemical properties of pre-conditioned brewery wastewater entering the UASB reactor before treatment

Parameters	Average concentration values*
Temperature (˚C)	29.21
pH	6.87
COD	2005.73
TSS	2449.46
TS	4520.00
TDS	1792.80
TON	0.52
NH_4	21.64
NO_2	2.30
NO_3	0.07
ORP (mv)	-144.78
Sulphate	178.25
Protein content	134.40
Orthophosphates	21.25
Conductivity (mS/cm)	2.18
Alkalinity (mg $CaCO_3$/ L)	2880.52

* All parameters are in mg/L unless otherwise stated.

Table 4.8: Average composition of biogas produced in this study

Biogas composition	Values (%)
CH_4	65.9
CO_2	30.7
N_2	3.4
H_2S	Not Detected
H_2	Not Detected

The efficiency of COD removal and the methanogenic activity were further shown in the composition of biogas generated from the UASB reactor with CH_4 content of 60-69% (Table 4.8). The ANOVA results showed that CH_4 yield depended on the substrate present in the wastewater in terms of COD removal efficiency as shown in section 3.3.2.4. In addition, the microbial characterization and biogas production results further confirmed the presence of methanogens in the UASB sludge.

The influent characterization confirmed the presence of significant amount of VFAs in the brewery wastewater, which could serve as substrate for the methanogens to produce biogas (Karakashev *et al.,* 2006). Volatile fatty acids such as acetate (538.30 mg/L), propionic acid (237.50 mg/L), butyric acid (50.06 mg/L) and valeric acid of 16.54 mg/L were detected in the influent wastewater with no detection of these acids in the effluent. This shows that the methanogens metabolized the VFA present in the brewery wastewater to produce CH_4. Among all the methanogens detected using FISH, *Methanosaeta* sp. and *Methanosarcina* sp. were reported to possess the ability to metabolize acetate (Ferry, 1993; Buriánková *et al.,* 2013). Presence of *Methanosarcina* in the granular sample can be explained by the acetate concentration and high biogas production from the reactor (Traversi *et al.,* 2011). The significance and abundance of *Methanosarcina* s*p.* at high acetate level was in agreement with previous studies (Karakashev *et al.*, 2006; Ariesyady *et al.*, 2007; Rincón *et al.,* 2008; Vavilin *et al.,* 2008). It is known that members of this genus grow by obligate methyl reduction with H_2 or CO_2 reduction with H_2 or methylotrophic catabolism of methanol dimethylsulfide and methylated amines as well as aceticlastic fermentation of acetate (Maeder *et al.,* 2006; Trzcinski *et al*., 2010). Delbès *et al.* (2001) reported that species closely related to the family *Methanobacteriales* and *Methanobacterium formicicum* were found dominant in an anaerobic bioreactor during acetate accumulation. However, the current study has shown a reduction in methanogenic activities when there was a high nitrogen and ammonium-nitrogen content in the effluent. This could be as a result of unfavourable conditions in the reactor leading to a reduction or inhibition of methanogenic growth in the reactor. There was almost no nitrites and very low concentrations of nitrates (less than 25mg/L) in the reactor effluent. This shows that nitrate reduction took place in the reactor because many Archaea and bacteria can utilize nitrate as a source of cellular nitrogen (Trzcinski *et al.,* 2010).

Studies have shown the dynamics and structures of methanogenic populations at various volatile fatty acid concentrations (Wang *et al.*, 2009) during digestion processes (Yu *et al*., 2006). All of these studies discussed the capacity of the microbial communities to respond to changes in anaerobic environments, such as altered feeding (Kovacik *et al.*, 2010) and temperature (Sasaki *et al*., 2011), among others. In addition, the FISH analysis of these samples have also shown a poor fluorescent signal during hybridization which could be attributed to a high protein content of the granules due to the low methanogenic activities (Wikström *et al.,* 2012).

4.3.5 Kinetic Modelling and Model Validation

Kinetic studies are critical for the design and operation of any full–scale reactor to determine the substrate removal rates. Various kinetic models viz., Monod, Contois, modified Stover-Kincannon and Grau second order have been tested (Kincannon and Stover, 1982; Vasant and Barsoum, 2009). Among these, the modified Stover–Kincannon kinetic model was selected for this present study which has been widely employed for high strength wastewater samples (Kapdan and Erten, 2007; Turkdogan-Aydinol and Yetilmezsoy, 2010; Yetilmezsoy, 2012). From equation 4.6, the saturation constant (K_B) and the maximum utilization rate constant U_{max} in the model was estimated to be 13.64 and 18.51 (g/L/day) respectively. The application of equation (4.6) by regression analysis showed that the utilization rate was directly proportional to the reactor efficiency (R^2= 0.978; Figure 4.9). The comparison studies exploring the modified Stover-Kincannon model for anaerobic treatment of different types of wastewater under different experimental conditions are shown in Table 4.9. From Table 4.9, the maximum utilization constant (U_{max}) values (11.83 and 1.996 g/L/day) reported by Yetilmezsoy (2012) was lower than the value obtained in this study, however, lower than the estimated value obtained for synthetic-based wastewater (Ahn and Forster, 2000). The high U_{max} in the synthetic wastewater could be attributed to the presence of readily biodegradable substrates that are easily accessible to microorganisms (Ahn and Forster, 2000).

Table 4.9: Comparison of different types of anaerobic wastewater treatment processes using the modified Stover-Kincannon model

Digester types	Type of substrates	Operating temperatures (°C)	Modified Stover-Kincannon model kinetic and estimated coefficients			
			K_B (g/L/day)	U_{max}(g/L/day)	R^2	References
UASB	Brewery wastewater	28-32	13.64	18.51	0.978	Present study
UASB	Poultry manure wastewater	30-34.5	13.02	11.83	0.991	Yetilmezsoy (2012)
Anaerobic biphasic fixed film reactor	Distillery wastewater	37	1.69(kg/m^3/d)	2 (kg/m^3/d)	0.992	Acharya *et al.* (2011)
UASB	Municipal wastewater	17.1-21	1.536	1.996	0.972	Turkdogan-Aydinol and Yetilmezsoy (2010)
UASB	Synthetic wastewater (2,4-dichlorophenol)	-	0.0098 (mg/L/day)	0.01 (mg/L/day)	0.992	Sponza and Uluköy (2008)
Anaerobic filter	Synthetic wastewater (saline)	37	5.3	7.05	0.910	Kapdan and Erten (2007)
Mesophilic anaerobic filter	Synthetic wastewater (starch)	35	50.6	49.8	0.998	Ahn and Forster (2000)
Mesophilic anaerobic filter	Paper pulp liquor	35	6.14	6.71	0.998	Ahn and Forster (2000)

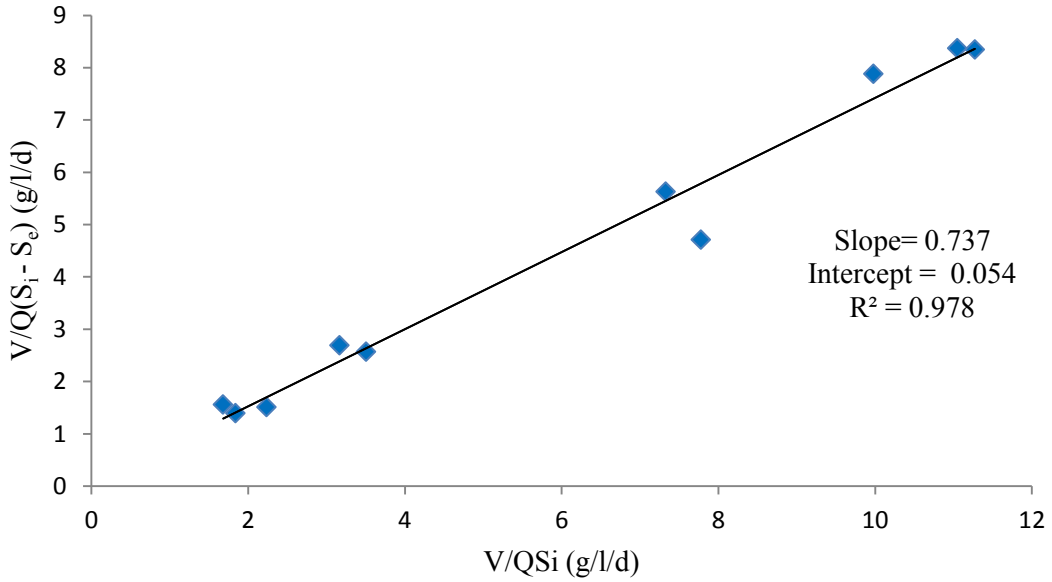

Figure 4.9: Effect of organic loading rate on COD removal rate using the modified Stover-Kincannon model to determine the kinetic constants.

Industrial scale wastewaters such as brewery effluent might contain different recalcitrant and more complex compounds that are less degradable (Yetilmezsoy, 2012). Furthermore, the operating conditions of anaerobic reactors could also influence the activity of microorganisms which can affect the kinetic rates. The biochemical and the kinetic data obtained in this study confirms the efficiency of the microbial community present within the UASB reactor in degrading the organic matter present in brewery wastewater to produce optimum biogas that can serve as source of energy. Further, to test the validity of the model, the observed effluent COD values and predicted values obtained from the model were compared (Figure 4.10). The results indicated high significance of the model with an excellent fit between the predicted effluent COD concentrations and the observed concentrations ($P < 0.001$) (Figure 4.10). High R^2 value of 0.957 between the observed and predicted values suggested that the predicted results are in accordance with the observed results (Figure 4.10). This further showed the suitability of the modified Stover-Kincannon model to predict effluent concentrations from this anaerobic treatment system treating brewery wastewater.

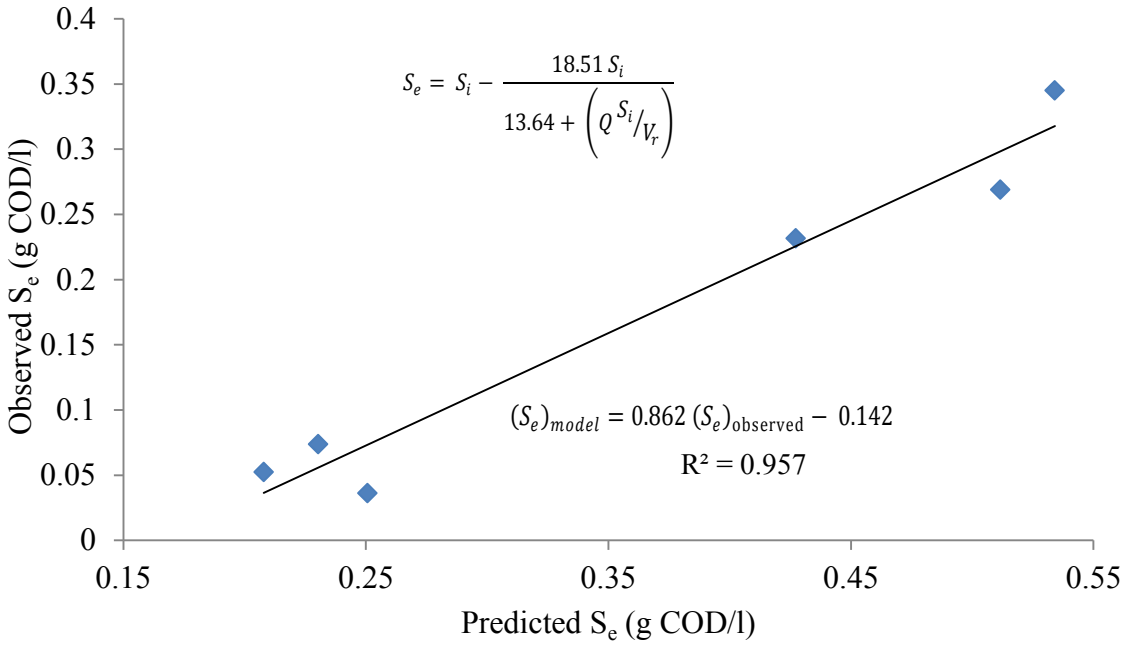

Figure 4.10: Relationship between the observed and predicted effluent COD concentrations by modified Stover-Kincannon model.

4.4 CONCLUSIONS

- A combination of FISH and PCR techniques helped to identify diverse group of microbial populations in each compartment. In addition, microbial fingerprinting showed syntrophic interactions between different bacterial groups and the methanogenic Archaea present in the reactor.

- *In-situ* hybridization analysis revealed the dominance of methanogenic Archaea of the orders *Methanobacteriales, Methanococcales, Methanomicrobiales* and *Methanosarcinales*-like species in the granular sludge samples.

- Bacterial groups that are required for the decomposition of organic matter in the brewery wastewater into simple monomers and for production of acetate as the major substrate for CH_4-producing Archaea were detected in this study. The major bacterial communities in the reactor include the representative from the phyla *Proteobacteria*, *Firmicutes* and *Chloroflexi.*

- Diverse groups of biogas-producing methanogens within the UASB reactor granules treating brewery wastewater were observed. *Methanobacteriales, Methanomicrobiales* and unclassified archaeon clones were detected in the granular sludge taken from the UASB reactor using PCR. Species detected include *Methanobacterium beijingense*, *Methanobacterium aarhusense, Methanobacterium formicicum, Methanoculleus* sp., *Methanobacterium palustre* and *Methanothermobacter crinale* using PCR.

- The modified Stover–Kincannon model was found to be applicable to predict effluent COD concentrations from the anaerobic reactor treating brewery wastewater.

- It is hoped that the characterization of eubacteria and methanogenic Archaea in the granules used for this study will bridge the gap of knowledge on the microbial ecology of the UASB reactor investigated. This will further help engineers to apply appropriate operational and environmental conditions that will select appropriate microbial community for efficient reactor performance.

4.5 RESEARCH OUTPUTS

a) Journal articles

1. **Enitan, A. M.,** Kumari, S., Swalaha, F.M., Adeyemo, J., Ramdhani, N. and Bux, F. (2014). Kinetic modelling and characterization of microbial community present in a full-scale UASB reactor treating brewery effluent. *Microbial Ecology*, 67:358–368.

b) Conference Papers

1. **Enitan, A. M.,** Kumari, S., Swalaha, F.M. and Bux, F. Real-time PCR for quantification of methanogenic Archaea in a UASB reactor treating brewery wastewater (2014)r. Conference of the International Journal of Arts & Sciences, CD-ROM. ISSN: 1943-6114: 07(03):103–106.

2. **Enitan, A. M.,** Kumari, S., Swalaha, F.M. and Bux, F. Use of *mcrA*-targeted real-time quantitative PCR for quantification of methanogenic communities in reactor treating brewery wastewater. Presented at *Water Institute of Southern Africa* (WISA) *Conference*, Mbombela, Mpumalanga, South Africa, 25-29 May, 2014 (Oral presentation).

CHAPTER FIVE: DEVELOPMENT OF A MATHEMATICAL MODEL TO DESCRIBE THE BEHAVIOUR AND PERFORMANCE OF A UASB REACTOR TREATING BREWERY WASTEWATER FOR BIOGAS PRODUCTION

5.1 INTRODUCTION

Recovery of bioenergy from spent biomass, industrial wastewaters and other types of waste is commonly achieved through the conventional anaerobic digestion (AD) process (Demirel *et al.,* 2010). AD technology, such as the upflow anaerobic sludge blanket (UASB) reactor technology is used for the treatment of different types of wastewaters for biogas production. The efficient functioning of biogas production systems provides different benefits to users and the community, resulting in energy and cost savings, environmental protection and conservation of resources (Tiwari *et al.,* 2006; Rajput *et al.,* 2012). However, bioconversion of organic substances to biogas depends on many operational factors (Oktem and Tufekei, 2006). Sometimes reactors may fail or encounter serious problems, depending on factors such as influent composition, pH, temperature, OLR, HRT and carbon to nitrogen ratio of the source material. These factors also affect microorganisms that are responsible for the degradation of organic matter in the bioreactors (Senturk *et al.*, 2013).

A UASB reactor depends on granular sludge as the core unit in order to convert the organic component of wastewater to biogas (Batstone *et al.,* 2002; Liu *et al.,* 2003). The sludge granules consist of dense microbial communities that typically include various bacterial communities in the sludge bed (Enitan *et al.,* 2014a) and the gas-liquid-solid phase at the top of the UASB reactor helps in sludge retention. Optimal operational conditions such as HRT, upflow velocity, influent COD, pH and temperature are needed for efficient biological treatment of wastewater to produce biogas in the UASB reactor (Wiegant, 2001). Thus, it is important to improve the operational parameters in order to enhance the efficiency of the UASB digestion process particularly for the production of methane (CH_4)-rich biogas. This could be done by several methods such as predicting and optimizing the operational conditions; satisfying the nutritional requirements of microbes by using different biological and chemical additives and manipulating the feed proportions (Yadvikaa *et al.,* 2004). Some other ways include the recirculation of digested slurry, returning microbes back into the reactor and modifying existing biogas plant design (Yadvikaa *et al.,* 2004). Hence, an in-

depth understanding of process dynamics including the (i) feedstock characteristics, (ii) operational and environmental parameters, (iii) reactor design and (iv) the microbial ecology are important for the optimization of AD systems.

A simple mathematical model that describes some of the conditions that define the anaerobic treatment process is a generally accepted approach in defining the specific parameters of system performance. Models based on process kinetics can be used to understand the underlying biological and transport mechanisms within the reactor (Acharya *et al.,* 2011) thus, giving more useful information on the state of the reactor and any impending failure.

Recently, mathematical modelling of bioreactors has greatly helped in controlling and improving the treatment efficiency of such systems, as well as in facilitating the experimental procedure to enhance the degradation of organic material in the waste feedstock used for biogas production (especially CH_4) (Blumensaat and Keller, 2002; Jeong *et al.,* 2005; Lübken *et al.,* 2007; Mu *et al.,* 2008; Zhou *et al.,* 2011) and to improve the effluent quality (Acharya *et al*., 2008). Models have been used to account for reactor performance along with the associated principles and conditions that affect CH_4 production (Reungsang *et al.*, 2012). In addition, models could be used to predict the compounds that are produced or consumed as well as the rate of production (Nadais *et al.,* 2011; Thorin *et al.,* 2012). The results of modelling can be used to estimate treatment efficiencies and system characteristics of full–scale reactors operating under similar conditions.

To study the kinetics of biogas formation from complex organic matter, two approaches can be adopted. The first approach is to find the rate-limiting substrate for the kinetic evaluation and the second approach is the use of COD or volatile solids concentration as an indicator of substrate concentration (Chen and Hashimoto, 1978). Methane production is said to be directly related to COD removal, and biogas yield is not the same as CH_4 yield because the composition of biogas comprises of CH_4, CO_2, water vapour, and a few other gases, such as hydrogen sulphide and hydrogen gas (Krishna, 2013).

Several studies have been carried out on the development of suitable models that best explain the conditions that will enhance the conversion of organic substances present in the wastewaters to biogas (CH_4) production during AD (Batstone *et al.,* 2000; Batstone *et al.,* 2002; Colussi *et al.,* 2012; Parsamehr, 2012). However, one of the main drawbacks of the available mathematical models for anaerobic reactors is their complexity. Several models based on different concepts and parameters have been reported to be difficult to apply to a UASB reactor, because they involve many variables (Zhao *et al.,* 2010; Colussi *et al.,* 2012; Thorin *et al.,* 2012). The application of these models is limited by the parameters needed to describe them.

For this reason, the development of an applicable model for a UASB reactor with the aim of reducing the complexity will be helpful for better understanding of the behaviour of the reactor and to enhancing bioenergy generation. More studies are needed to derive simple and convenient models that can predict and optimize biogas yield, especially CH_4. This paper presents a model that describes the kinetics of an intermittent-flow UASB reactor treating brewery wastewater based on mass balance principles. We considered that untreated COD as the primary substrate with no additional oxidizing agents added into the reactor would be converted to biogas (CH_4 and CO_2) (Zainol, 2012). We considered the reduction of COD to hydrogen gas and hydrogen sulphide insignificant in this study (Chen and Hashimoto, 1978). At standard temperature and pressure (STP), the digestion of 1 g COD added is equal to the formation of 0.35 L of CH_4. Thus, knowing the influent COD concentration and quantity, we could deduce the volume of CH_4 produced from a reactor. The remaining COD in the reactor could then be calculated and the energy equivalent released through AD of the wastewater could be determined, because most of the energy contained in biogas is represented by CH_4. Thus,the developed model describes the behaviour of the reactor with respect to substrate degradation and the effect of endogenous decay rate on CH_4 production based on modified Chen-Hashimoto equations by Ghaly *et al*. (2000).

5.2 MATERIALS AND METHODS

5.2.1 Ghaly *et al*. (2000) Model

Various mathematical models have been proposed to describe substrate and biomass concentrations as well as biogas production in a batch, or continuous process reactor (Colussi *et al.,* 2012; Fdez-Güelfo *et al.,* 2012; Zainol, 2012). Among these models for AD, Ghaly *et al.* model (2000) was found to be suitable forthis study. The governing equations for the process are obtained from the mass balance of substrate and concentration of biomass in the reactor compartment. The model follows Monod kinetics. The principle of the process is based on modified Chen-Hashimoto equations, in which the concentration of biomass in the system depends on the growth and decay rate of microorganisms under steady–state conditions for an intermittent flow of organic matter into the biological treatment unit.

5.2.1.1 The microbial mass balance

The microbial mass balance of an UASB reactor (Figure 5.1) was described as follows by Ghaly *et al.* (2000):

Microbial change rate = Microbial input rate + Microbial growth rate - Microbial death rate- Microbial output rate (5.1)

The microbial growth rates in a batch experiment have traditionally been measured, in which a single species of microorganisms passes through a logarithmic growth phase during the conversion of the organic substrate. The microbial growth rate, dX/dt, is described by;

$$\frac{dX}{dt} = \mu X, \tag{5.2}$$

which can be written as;

$$\frac{dX}{dt} = QX_i + \mu X_r V - K_d X_r - QX_e. \tag{5.3}$$

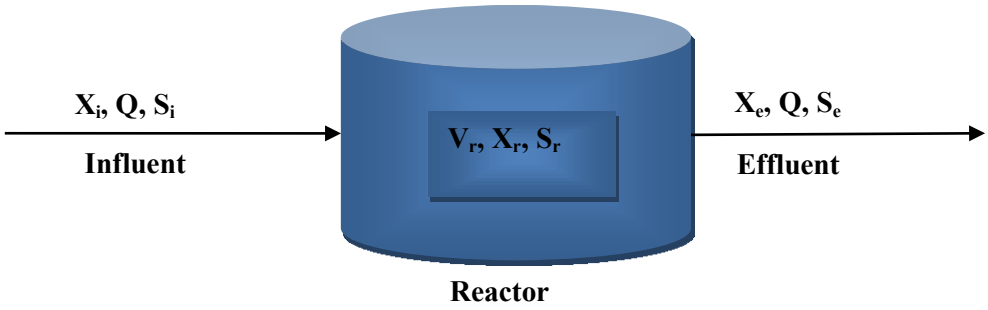

Figure 5.1: Schematic diagram of a single compartment of an upflow anaerobic sludge blanket reactor (See abbreviations for definition of symbols).

During steady-state conditions, the biomass concentration in the influent is negligible ($X_i \approx 0$), compared to the biomass concentration in the reactor. In addition, X_r is equal to X_e due to perfect mixing in a completely mixed reactor. The rate of substrate removal from the reactor is therefore neglected. In steady-state conditions, dX/dt = 0 and equation (5.3) can be rearranged to obtain equation (5.4). Thus,

$$QX_i \approx 0$$

$$0 = X_e(\mu V - K_d V - Q)$$

$$Q = V(\mu - K_d) \tag{5.4}$$

Equation (5.4) can be rewritten as;

$$\frac{Q}{V} = \mu - K_d. \tag{5.5}$$

The hydraulic retention time, θ_h, is defined as V/Q. The inverse of θ_h can be substituted into equation (5.4) as;

$$\mu - K_d = \frac{1}{\theta_h}. \tag{5.6}$$

As shown in equation (5.6), the net specific growth rate is $\mu - K_d$.

5.2.1.2 Substrate mass balance and effluent substrate concentration

The rate of substrate balance in the UASB reactor can be expressed using equation (5.7) and mathematically as equation (5.8) (Ghaly *et al.*, 2000):

[Substrate change rate] = [Substrate input rate] – [Substrate utilization rate] – [Substrate output rate] (5.7)

Mathematically, equation (5.7) can be written as;

$$V\frac{dS}{dt} = QS_i - (\mu - K_d)\,V\frac{X_r}{Y} - QS_e \quad . \tag{5.8}$$

At steady state, equation (5.8) was divided by V, and Q/V was substituted for θ_h. At equilibrium the substrate balance of a working system was obtained as;

$$\frac{S_i - S_e}{\theta_h} = (\mu - K_d)\frac{X_r}{Y}. \tag{5.9}$$

Thus, under perfect mixing of the reactor content ($X_r = X_e$), the microbial mass concentration in the effluent can be written as equation (5.10). This gives the concentration of microorganism in the effluent as;

$$X_e = \frac{Y\,(S_i - S_e)}{\theta_h(\mu - K_d)}, \tag{5.10}$$

Where, $(S_i - S_e)/\theta_h$ is the rate of substrate utilization. Contois (1959) defined the relationship between limiting substrate concentration and specific growth rate for effluent substrate concentration as;

$$\mu = \frac{\mu_{max} S_r}{bX_r + S_r} \quad . \tag{5.11}$$

Under perfect mixing ($S_e = S_r$ and $X_e = X_r$), the association between the rate–limiting substrate concentration and specific growth rate can be expressed as;

$$\mu = \frac{\mu_{max} S_e}{bX_e + S_e} \quad . \tag{5.12}$$

Equations derived from the combination and rearrangement of equations (5.6), (5.10) and (5.12) are;

$$S_e = \frac{S_i K}{\frac{\mu_{max}\theta_h}{K_d\theta_h + 1} + (K-1)} \quad (5.13)$$

$$\frac{S_e}{S_i} = \frac{K}{\frac{\mu_{max}\theta_h}{K_d\theta_h + 1} + (K-1)} \ , \quad (5.14)$$

Where, equation (5.14) shows that the influent substrate concentration is inversely proportional to the substrate concentration in the final effluent.

5.2.1.3 Biogas production

In the reactor, the biodegradable COD is proportional to (B_o–B). B_o is directly proportional to the biodegradable COD loading rate (Zainol, 2012). Therefore, from equation (5.14), the CH_4 yield (B) can be described by;

$$\frac{B_o - B}{B_o} = \frac{K}{\frac{\mu_{max}\theta_h}{\mu_m\theta_h + 1} + (K-1)} . \quad (5.15)$$

Methane production per gram of substrate (COD) added, B can be described by;

$$B = B_o \left[1 - \frac{K}{\frac{\mu_{max}\theta_h}{K_d\theta_h + 1} + (K-1)}\right]. \quad (5.16)$$

Since B is equal to the volume of CH_4 produced per unit of COD added, the volumetric CH_4 production rate, Y_v is equal to B, multiplied by the organic loading rate, S_i/θ_h. The equations describing the theoretical CH_4 output rate per unit of reactor volume therefore, are written as equations (5.17) and (5.18):

$$Y_v = \frac{BS_i}{\theta_h} \quad (5.17)$$

$$Y_v = \frac{B_o S_i}{\theta_h}\left[1 - \frac{K}{\frac{\mu_{max}\theta_h}{K_d\theta_h + 1} + (K-1)}\right]. \quad (5.18)$$

5.2.2 Modified Methane Generation Model (MMGM)

Ghaly *et al.* (2000) model does not consider temperature or the amount of non-biodegradable COD of the feedstock, which are important factors in wastewater treatment. We now describe a new Modified Methane Generation Model (MMGM), which integrates the effect of temperature and non-biodegradable COD with the model described above (Ghaly *et al.,* 2000; Equation 5.18) for a UASB reactor under anaerobic conditions. The assumptions made include the following:

- The UASB reactor was treated as a single compartment.
- It was considered as a completely mixed system with continuous influent flow into the reactor and no return of microbial solids back into the reactor (it is non-recycling).
- The substrate was a single biodegradable substance.
- Substrate consumers were uniformly distributed in the reactor (bed and blanket) under perfect mixing.
- Reactor operation is at steady state.
- The kinetic model follows first–order kinetics using the Monod model with respect to substrate and biomass concentration.

The developed model's outcomes include the quantification of the growth rate of biomass, substrate consumption and the effect of endogenous decay on biogas formation. The ultimate CH_4 yield coefficient B_o is assumed to be constant based on the literature survey. Studies have shown that B_o depends on the OLR, sludge or HRT used during the treatment of brewery wastewater (Oktem and Tufekei, 2006; Fdez-Güelfo *et al.,* 2012). Oktem *et al.* (2006) investigated a pilot–scale UASB reactor for the treatment of brewery wastewater in the mesophilic range. An increase in CH_4 yield of 0.25–0.30 $m^3CH_4/kgCOD_{removed}$ was observed when OLR was increased with a rise in COD removal efficiency from 60% to 95%.

Similar observation was reported by Chen and Hashimoto (1978) and Yetilmezsoy (2012), on the value of B_o. The authors mentioned that the value of B_o depends on the type of waste that is being treated and environmental conditions. Most especially, bioreactor temperature was mentioned to affect the ultimate CH_4 yield coefficient (Chen & Hashimoto, 1978;

Yetilmezsoy, 2012) hence, we added operational temperature to our equation (5.18). Chen and Hashimoto (1978) defined an empirical relationship between the maximum specific microbial growth rate (μ_m) and temperature (T) for temperatures between 20˚C and 60˚C on the analysis of a data set obtained from the literature as equation (5.19) (Yetilmezsoy, 2012).

$$\mu_{max} = 0.013\ (T) - 0.129 \tag{5.19}$$

Studies have shown that maximum specific microbial growth rate in the Chen and Hashimoto equation (5.19) depends on operational temperature and it increases linearly as the temperature increases (Yetilmezsoy, 2008; Turkdogan-Aydinol *et al.,* 2010). Therefore, equation (5.19) can be substituted into equation (5.18) to obtain equation (5.20).

$$Y_v = \frac{B_o S_i}{\theta_h} = \left[1 - \frac{K}{\frac{[\theta_h(0.013(T) - 0.129)]}{K_d \theta_h + 1} + (K-1)}\right] \quad . \tag{5.20}$$

According to equation (5.20), the theoretical CH_4 output for any given values of S_i and θ_h is determined by the specific characteristics of the biodegradation of substrate and the kinetic constants (μ_{max} and K). In addition, the value of K, according to the Monod equation, may be associated with the ability of microorganisms to degrade the substrate present in the waste to produce CH_4. A high K value is an indication that the microorganisms present in the reactor have greater difficulty in converting the organic matter to CH_4 (Fdez-Güelfo *et al.,* 2012). The physicochemical parameters such as temperature have been shown to be the primary factors affecting μ_{max}. The effect of temperature on μ_{max} could be described by the empirical relationship mentioned in equation (5.19); for K, the concentration of the organic matter in the substrate and for B_o the kind of substrate. The biodegradable substrate in the reactor in terms of its COD concentration is considered to be directly proportional to the actual CH_4 generated under normal conditions of temperature and pressure and the fraction of non-biodegradable COD was included in the model. The fraction of the non-biodegradable COD

($_{nb}$COD) was written as equation (5.21) with respect to the initial substrate concentration and P, as the fraction of the biodegradable COD removed:

$$_{nb}COD = (1 - P) \qquad 0 \leq P \leq 1 \qquad (5.21)$$

Hence, equation (5.20) can be written as shown below, which indicates that the biodegradable substrate concentration in the reactor is directly proportional to the actual CH_4 volume. Then, the governing equation (5.22) for modified methane generation model (MMGM) can be obtained as:

$$Y_v = \frac{(1-P)B_o S_i}{\theta_h}\left[1 - \frac{K}{\frac{[\theta_h(0.013(T)-0.129)]}{K_d\theta_h+1}+(K-1)}\right] . \qquad (5.22)$$

The kinetic constant K shows the level of microbial growth in the digestion process. This is an extension of Ghaly *et. al*. (2000) model. This model can be used for anaerobic processes at steady–state operation under perfect mixing and also takes into consideration the material balance for a mixed reaction; the substrate being the rate-limiting factor. The design and operation of an AD system is based on fundamental knowledge of kinetics and stoichiometry of biological reactions. Thus, prediction of industrial–scale anaerobic reactor performance based on UASB technology in treating brewery wastewater depends on the estimated values of parameters such as K, μ_{max}, K_d , Y and B_o. However, the kinetic values estimated from laboratory–scale data are inadequate to describe the actual plant performance (Sykes, 1995; Iqbal and Guria, 2009). Thus, it is important to determine these parameters from the actual full–scale treatment plant data, such as the influent and effluent COD concentration, VSS concentration in the reactor, flow rate and reactor volume. The determination of model coefficients (K, B_o, μ_{max}, and K_d) is important for the validation of the model, to predict and to optimize not only the volumetric CH_4 production rate of any UASB reactor treating brewery wastewater, but other different wastewater sources.

5.2.3 Determination of MMGM Parameters (K, $\mu_{max,}$ K_d , Y and B_o)

The determination of a first-order reaction is represented by Chen and Hashimoto (1978). They developed a kinetic model based on substrate utilization of the Contois model as;

$$\frac{\mu_{max}}{\mu} = K\frac{S_i - S_e}{S_e} + 1 \ . \tag{5.23}$$

This model has been widely adopted and used in many studies in the investigation of anaerobic treatment of high strength wastewater (Cecchi *et al.,* 1992; Yetilmezsoy, 2012; Zainol, 2012). Equation (5.23) becomes equation (5.24) when divided by $\mu_{max.}$

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K}{\mu_{max}}\frac{S_i - S_e}{S_e} \tag{5.24}$$

In a completely mixed system,

$\frac{1}{\mu_{max}}\ \theta_h$ and

$$\theta_h = \frac{1}{\mu_{max}} + \frac{K}{\mu_{max}\frac{S_i - S_e}{S_e}} \tag{5.25}$$

Let,

$$\frac{S_i - S_e}{S_e} = S \tag{5.26}$$

Then, the first–order kinetic constant coefficients K and μ_{max} can be determined by plotting θ_h against S using equation (5.25). The ultimate CH_4 yield (B_o) can be determined using a least–squares method through nonlinear regression of $1/\theta_h$ versus CH_4 yield. The endogenous decay constant, K_d can be determined as a function of HRT and VSS values using equation proposed by Bhunia and Ghangrekar (2008), equation (5.27) or (5.28). These equations can be used to obtain the values of K_d by plotting a linear regression of $1/\theta_h$ against $(S_i - S_e)/(X_e\theta_h)$. The intercept is equal to K_d and Y is the slope of the straight line that passes through the plotted points.

$$\frac{1}{\theta_h} = \frac{Y.Q(S_i - S_e)}{V_b.X_e.\theta_h} - K_d \quad (5.27)$$

Or

$$\frac{Q(S_i - S_e)}{V_b.X_e} = \frac{1}{Y}.\frac{1}{\theta_h} + \frac{1}{Y}.K_d \quad (5.28)$$

5.2.4 Software Used and Statistical Analysis

Data obtained from the full-scale reactor were used to derive the parameters in the developed model. Nonlinear and linear regressions were fitted to data using the GraphPad Prism v5.0 program as the statistical software. Nonlinear regression was conducted based on a least-squares method to analyzed the predicted CH_4 yield and volumetric CH_4 production rate. Correlation using the coefficient of determination between the observed and the predicted production values was carried out, the probability of fit was calculated and accepted when $p < 0.05$. The MMGM governing equation (5.22) was coded and simulated using the MATLAB 7.14 software (R2010a, The MathWorks, Inc. Natick, Massachusetts, USA).

5.2.5 Description of the UASB Reactor System Used and Wastewater Sampling

An industrial full–scale UASB reactor treating brewery wastewater was used as described in section 3.2.1. The biogas produced in the reactor was separated from the effluent and the biomass in three-phase separators at the top of the reactor was collected in a gas holder (Tedlar bag, Sigma-Aldrich) for analysis (Section 3.2.3.1). A series of pre-screened brewery wastewater (reactor influent) and the full–scale UASB reactor effluent ready to discharge into the municipal sewer system were collected in one–litre sterile glass bottles and transported to the laboratory at 4°C. Physico-chemical analyses were conducted within 48 hours of collection with the necessary preservation techniques adapted from Standard Methods (APHA–AWWA–WPCF, 1998). Physico-chemical tests were carried out as mentioned in section 3.2.3. Tests were carried out in duplicate.

5.2.6 Calculation of Methane Potential and Yield (United Nations Economic Commission for Europe, 2004)

$$\mathrm{CH_4\ production\ (L/d)} = \frac{\text{Biogas production(L/d)} \times \mathrm{CH_4\ content(\%)}}{1000} \quad (5.29)$$

$$CH_4 \text{ yield (L/d)} = \text{Load of COD to digester (g/day) x COD}_{\text{removed}}\ (\%) \times 0.362\ L\ \mathrm{CH_4COD_{removed}} \quad (5.30)$$

$$\text{Volumetric } \mathrm{CH_4}\ \text{potential} = \frac{\text{Volume of } \mathrm{CH_4}\ \text{produced}}{\text{Volume of substrate added}} \quad (5.31)$$

5.3 RESULTS AND DISCUSSION

5.3.1 Estimated MMGM Parameters

Removal efficiencies for both BOD_5 and COD were found to be ~80% and 79% respectively and the mean biogas (CH_4 content) produced was 65.9%. This indicated that the organic matter in the industrial wastewater was converted to usable biogas with good effluent composition. Figure 5.2 shows the time–course for the performance of full–scale UASB reactor in treating brewery wastewater during the monitoring process (Section 3.2.2), in–terms of COD and BOD_5 removal efficiencies over the time period.

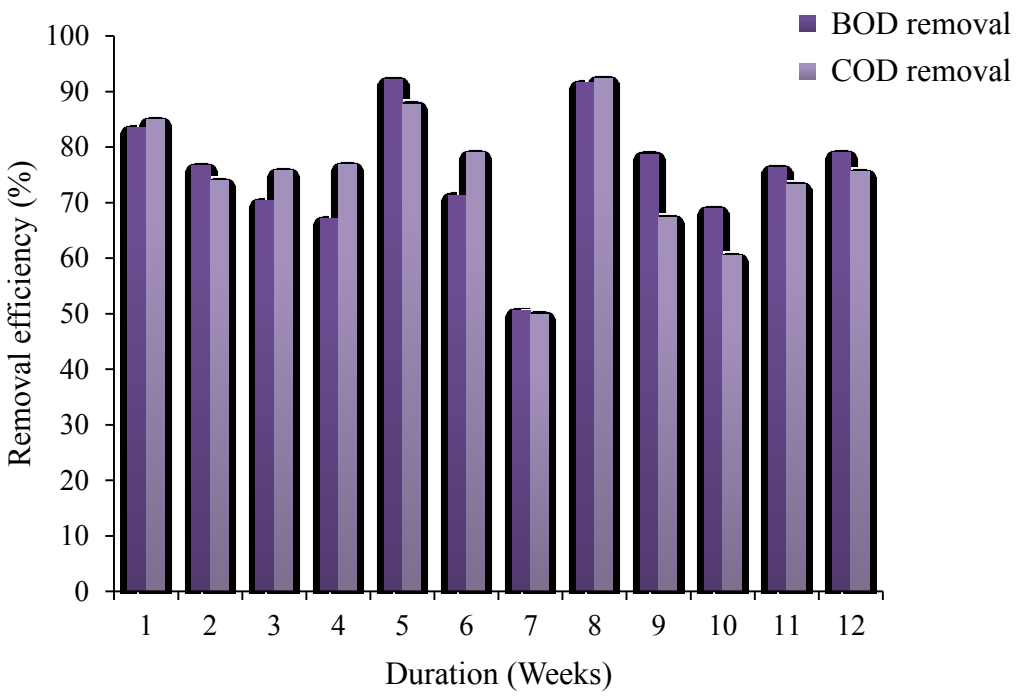

Figure 5.2: The time–course of COD and BOD_5 removal efficiencies for the full–scale UASB reactor treating brewery wastewater in this study.

Table 5.1 shows the experimental data used to determine MMGM parameters as shown in Table 5.2. The first-order kinetic coefficients K and μ_{max} were determined by plotting θ_h against S using equation (5.25) (Figure 5.3). The graph produced a straight line with μ_{max} given by 1/intercept and K as slope/intercept. The values of μ_{max} and K derived in this study were 0.117 d^{-1} and 0.046 g/g, respectively (Table 5.3). The kinetic parameters could then be used to determine the behaviour of a system or bioreactor, which would help to characterize the microbial-substrate interaction for better treatment efficiency. The ultimate CH_4 yield (B_o) was determined using a least–squares method through the nonlinear regression of $1/\theta_h$ and CH_4 yield.

Table 5.1: Average data obtained from the full-scale UASB reactor treating brewery wastewater

θ_h (h)	Q (L/h)	COD loading rate (g/L)	S_i (g/L)	S_e(g/L)	X_e (g/L)	CH_4 production (L/h)	CH_4 yield (L/g COD_{added})
8	167	171.43	1.03	0.51	0	224.30	0.18
9	180	167.24	0.93	0.23	2.19	44.35	0.27
9	300	929.53	3.10	1.01	4.40	219.11	0.24
11	180	520.05	2.89	0.43	1.00	154.67	0.30
12	250	248.66	1.00	0.23	6.11	66.88	0.27
12.1	156	170.16	1.10	0.11	4.00	53.70	0.32
13	300	900.62	3.00	0.23	1.73	291.34	0.32

Table 5.2: Data used for the determination of MMGM parameters

θ_h	$1/\theta_h$	X_e	$X_e\theta_h$	S_i	S_e	S_i-S_e	$X_e\theta_h/(S_i-S_e)$	$(S_i-S_e)/(X_e\theta_h)$	$S=(S_i-S_e/S_e)$
8	0.13	0	0	1.03	0.51	0.52	0	0	1.00
9	0.11	2.19	19.71	0.93	0.23	0.70	28.00	0.04	3.13
9	0.11	4.40	39.60	3.10	1.01	2.09	18.98	0.05	2.06
11	0.09	1.00	11.03	2.89	0.43	2.46	4.49	0.22	5.66
12	0.08	6.11	73.34	1.00	0.23	0.76	95.96	0.01	3.32
12.1	0.08	4.00	48.40	1.10	0.11	0.98	49.21	0.02	9.18
13	0.08	1.73	22.52	3.00	0.23	2.78	8.12	0.12	12.20

Table 5.3: Estimated MMGM parameters as obtained using the data collected from the full–scale UASB reactor treating brewery wastewater

Parameter	Estimated value	Units	R^2	P-value
μ_{max}	0.117	d^{-1}	0.709	0.017
K	0.046	g/g	0.709	0.017
K_d	0.083	d^{-1}	0.767	0.009
B_o	0.516	L CH_4/g COD_{added}	0.988	0.006
Y	0.357	g/g	0.7670	0.0088

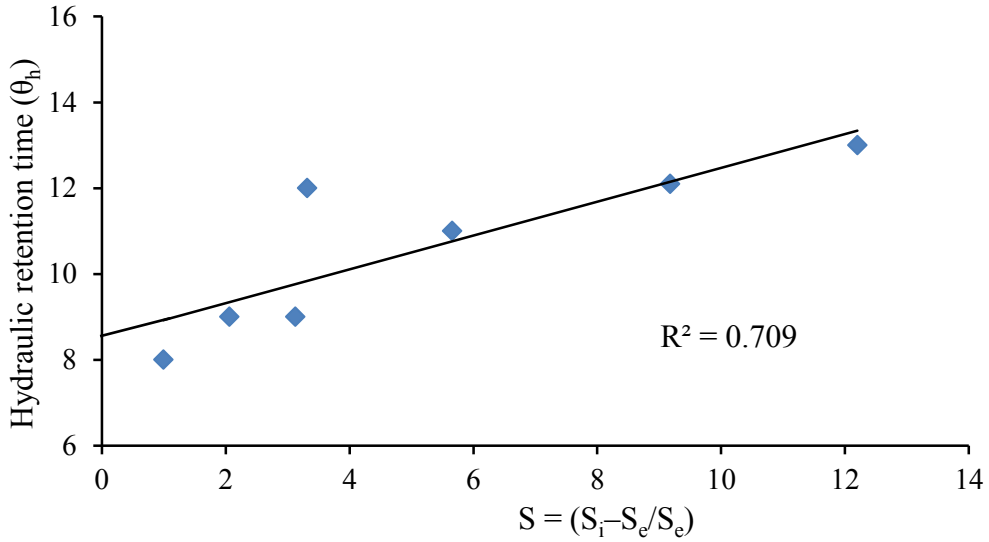

Figure 5.3: Estimation of the kinetic parameter K and the maximum growth rate of microorganism's μ_{max}, from data collected from the full–scale UASB reactor treating brewery wastewater. The plot of θ_h against S [where, S = $(S_i–S_e/S_e)$] gives a straight line with 1/intercept as μ_{max} and slope/intercept as K.

Figure 5.4 shows the graph of CH_4 yield against $1/\theta_h$ with the intercept, B_o corresponding to 0.516 L CH_4/g COD_{added}. The estimated endogenous decay coefficient, K_d value is represented by the intercept of the straight line graph shown in Figure 5.5 as 0.083 d^{-1}, while the slope Y, corresponds to 0.357 g/g. The estimated model coefficients for brewery wastewater used in this study are shown in Table 5.3. The values are within the range of values reported in the literature for mesophilic AD for waste types that include wastewater, banana stem and peel waste, palm oil mill wastewater, dairy manure and the organic fraction of municipal solid waste from a full-scale plant (Table 5.4). The value of μ_{max} obtained from the full-scale UASB reactor treating brewery wastewater was higher than the value (0.111 d^{-1}) reported by Zainol (2012) and lower than 0.135 d^{-1} reported by Fdez-Güelfo *et al.* (2012). However, the value of B_o is very similar to those reported in the literature (Table 5.4). Hence, the values of coefficients K, B_o, μ_{max} and K_d were used to validate the model and to predict treatment efficiency, determine the HRT for treatment of wastewater, and predict volumetric CH_4 productivity of an UASB reactor treating brewery wastewater.

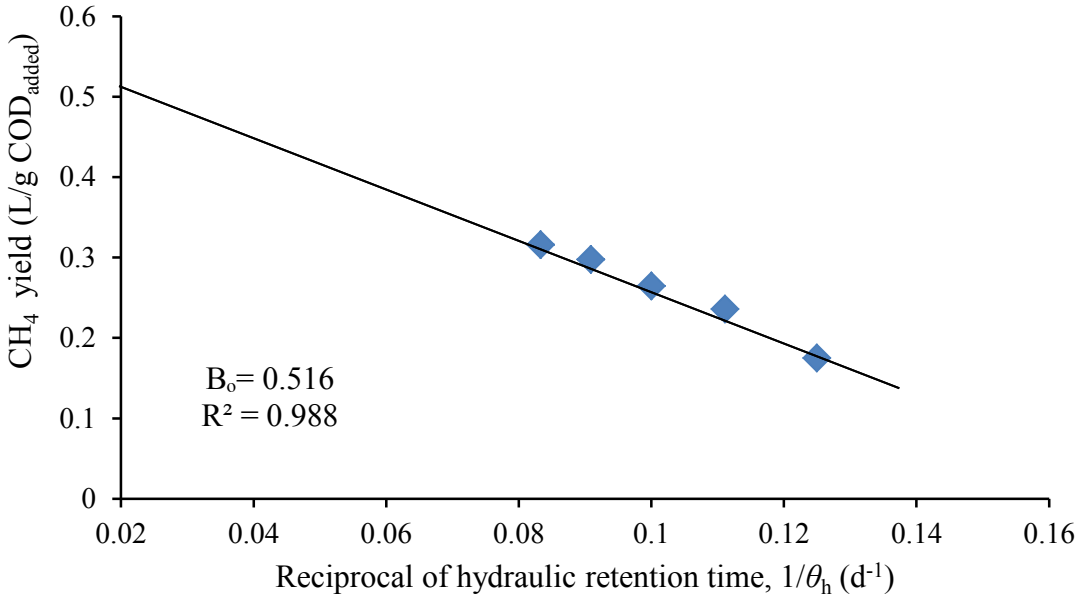

Figure 5.4: Ultimate methane yield (B_o) obtained from data collected from the full–scale UASB reactor treating brewery wastewater by plotting methane yield against the reciprocal of hydraulic retention time.

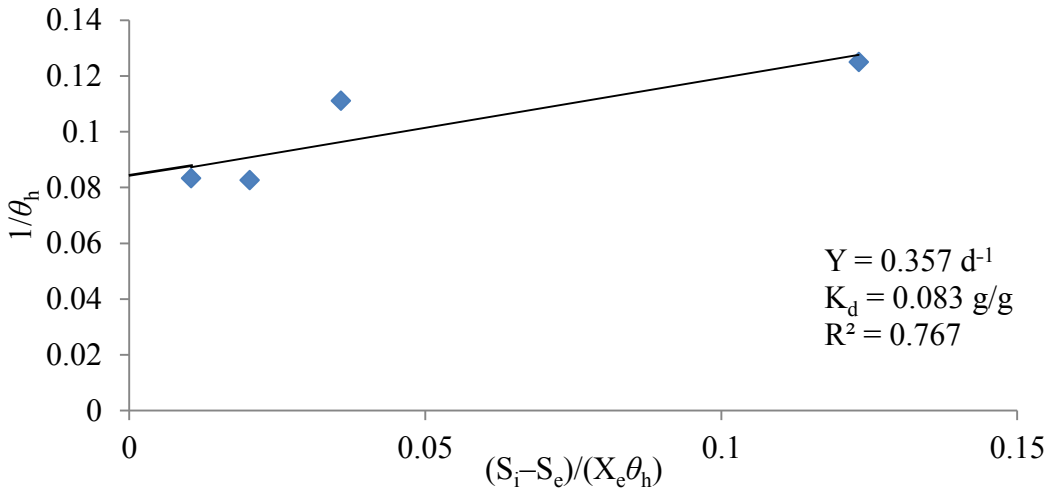

Figure 5.5: The endogenous decay coefficient, K_d and the growth yield coefficient, Y were calculated from the intercept and slope of the straight line of the plotted graph using the data obtained from the full–scale UASB reactor treating brewery wastewater.

Table 5.4: Kinetic parameters obtained in this study compared to other studies

Substrate	B_o (L CH_4 /g COD_{added})	K (g/g COD_{added})	μ_{max} (d^{-1})	K_d (d^{-1})	Reference
Brewery wastewater	0.516	0.046	0.117	0.083	This study
Banana stem waste	0.326	0.33	0.111	-	Zainol (2012)
Synthetic organic fraction of municipal solid waste	1.167[a]	-	0.238	-	Fdez-Güelfo *et al.* (2012)
Organic fraction of municipal solid waste from a full-scale composting plant	1.15[a]	-	0.135	-	Fdez-Güelfo *et al.* (2012)
Distillery spent wash	-	-	2[b]	-	Acharya *et al.* (2011)
Vegetable product—pea	0.36	-	-	-	Maya-Altamira *et al.* (2008)
Vegetable product—leek & fried onion	0.36	-	-	-	Maya-Altamira *et al.* (2008)
Banana peel	0.277[c]	-	0.089	-	Gunaseelan (2007)
Palm oil mill wastewater	0.381	-	0.304	-	Faisal and Unno (2001)
Dairy manure at 25°C	0.230[c]	0.883[c]	0.279	0.038	Ghaly *et al.* (2000)
Dairy manure at 35°C	0.230[c]	0.883[c]	0.317	0.036	Ghaly *et al.* (2000)
Brewery wastewater	-	-	0.022	0.037	Anderson *et al.* (1996)

a = l methane /g DOC ; b = Kg $m^{-3}d$[c] = l methane/g VS added
*All abbreviations are in abbreviation section.

5.3.2 Validation of the Modified Methane Generation Model

The values of K, μ_{max}, K_d, B_o and θ_h presented in Table 5.3 were used in the model to simulate methane yield. The simulations were carried out for a fixed substrate concentration at different hydraulic retention times based on equation 5.16. The simulation indicated methane yield as a function of hydraulic retention time. The application of the model was shown by regression analysis of the predicted methane yield with determination coefficient of 0.991 at 95% confidence range with P value of 0.0001. Only 0.009% of the total variations could not be explained by the regression analysis. A high coefficient of determination R^2 of

0.991 shows a strong Goodness of fit of the model. Figure 5.6 showed the expected behaviour when compared with experimental values obtained from the full-scale reactor investigated. There was a strong correlation coefficient of 0.747 between the predicted and the observed values for methane yield, which showed the applicability of the model to predict methane yield.

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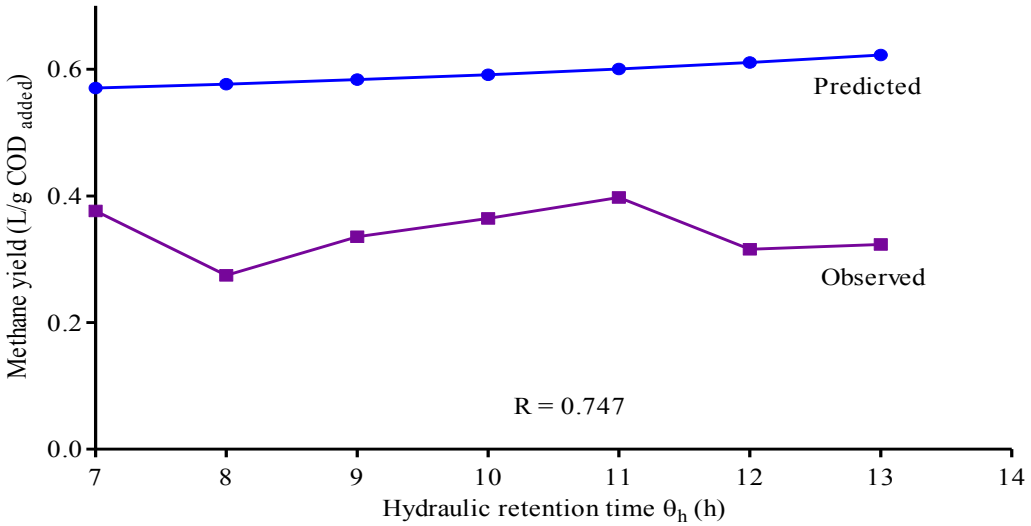

Figure 5.6: Observed and predicted methane yields at different hydraulic retention times.

In order to further validate this model, the observed volumetric methane production rate and predicted values obtained from MMGM were compared at different temperatures and OLR. For a randomly selected operating scenario, a volumetric organic loading rate between 2.0 and 11.8 g COD/L/day and the initial substrate concentration, S_i = 6 to 12 g COD/L, (B_o = 0.516, T = 26°C and 32°C) using MMGM (Equation 5.22) showed that increasing the volumetric OLR to 8.26 g COD/L/day would stimulate the methane yield better (corresponding to the maximum volumetric methane production rate of Y_v = 1.46 L CH_4/g COD_{added}/day).

In order to evaluate the fitness of MMGM, the predicted values of the volumetric methane production rates were plotted against the observed values for different organic loading rates (Figure 5.7a) and when the OLR increased from 2.0 to 8.26 g COD/L/day, the predicted Y_v increased from 0.29 to 1.46 L CH_4/g COD_{added}/day. However, Y_v decreased as the OLR rose to 11.80 g COD/L/day. The coefficient of determination value (R^2 = 0.994) for the methane production rate showed the goodness of fit of the developed model (MMGM). The coefficient of determination (R^2 = 0.994) showed that 99.4% of the variance in the model can be explained by the model and the model was shown to be extremely significant with $p < 0.0001$.

A similar trend was noticed in the observed methane production rates although; there was fluctuation in the observed values due to operational and environmental parameters. However, the highest value for the observed Y_v (0.51-0.83 L CH_4/g COD_{added}/day) was recorded at OLR between 4.4 to 9.29 g COD/L/day, and the observed Y_v decreased when the OLR reached 11.80 g COD/L/day. The data indicates that the volumetric methane production rate fluctuate with an increase in OLR, hence values higher than 0.8 g COD/L/day were not included in the relationship shown in Figure 5.7b. Up to this point, the correlation between the predicted and the observed Y_v was very strong (R^2 = 0.990), showing a linear relationship between these parameters at different OLR (Figure 5.7b). A noticeable decrease in Y_v as observed at higher organic loading rates suggested that OLR could influence the kinetic parameters due to the presence or accumulation of inhibitors or toxic compounds in the reactor and also reduce volatile solids removal, thus affecting the volumetric methane production rate (Babaee and Shayegan, 2011). However, at higher OLRs the values between observed and predicted methane production rate vary considerably and the MMGM overestimated the methane production rates.

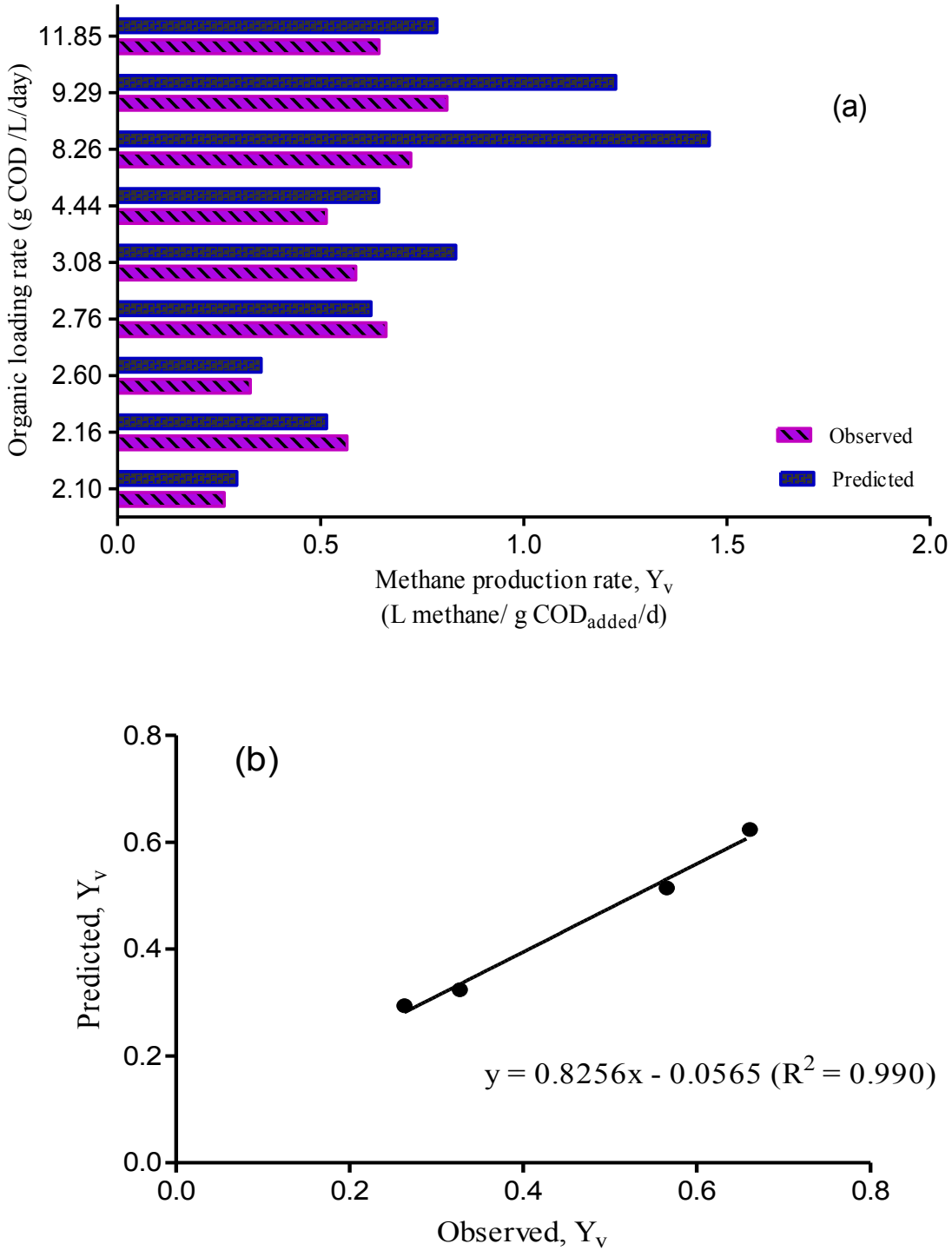

Figure 5.7: (a) The trend between observed and predicted volumetric methane production rates at different organic loading rates using the newly developed model and (b) the scatter plot of predicted vs observed volumetric methane production rates at lower organic loading rates.

A similar trend was reported in the AD of banana stem waste (Zainol, 2012) and a UASB reactor treating poultry manure wastewater (Yetilmezsoy, 2012). However, overloading the bioreactor with a high substrate concentration has been reported to be one of the factors contributing to the reduction in the methane production rate. A reduced methane production rate signifies the presence of a possible inhibiting factor in the process, such as a decrease in pH as a result of an increase in the concentrations of VFAs (Tiwari *et al.,* 2006; Yetilmezsoy, 2012).

The influence of HRT and OLR on the microbial communities and the performance of an anaerobic reactor to treat olive waste at steady state have also been investigated (Rincón *et al.,* 2008). The authors observed the maximum methane production rate of 1.7 L CH_4 STP/L day when the OLR was increased from 1.50 to 9.29 g COD/L/day at 17 days HRT. However, when the OLR was increased to 11.0 g COD/L/day at HRT of 15 days, there was a reduction in the pH value (from 7.5 to 5.3) as well as increase in the effluent total VFA by about 400% (Rincón *et al.,* 2008). This further confirmed that the OLR affects the value of methane production rate.

The effect of operational temperature on the activity, survival and growth of the microbial consortium in an AD system was reported by Khalid *et al*. (2011). The effect of operational temperature (26–32°C) on the volumetric methane production rate (Y_v) was simulated using the developed model (equation 5.22). The predicted volumetric methane production rate at 29°C was higher than that at other temperatures (Figure 5.8). The regression analysis showed the goodness of fit of the developed model with strong determination coefficient of 0.862 and the adjusted determination coefficient of 0.882. This confirms the applicability of the modified methane generation model to predict volumetric methane production from a UASB reactor treating brewery wastewater.

Several studies have shown the crucial effects of even a slight change in the operating temperature on biogas production, especially its CH_4 content. Any sudden change might lead

to a drastic decrease in biogas production due to change in microbial populations and reduced CH_4 content and volume (Chae *et al.,* 2007; Ward *et al.,* 2008). Chae *et al.* (2007) reported the maximum CH_4 yield at 35°C when compared to that at 30°C and 25°C. Therefore, for better treatment efficiency and high volumetric methane production rate, operating temperature should be optimized for the reactor design and operation (Ward *et al.,* 2008; Yetilmezsoy, 2012).

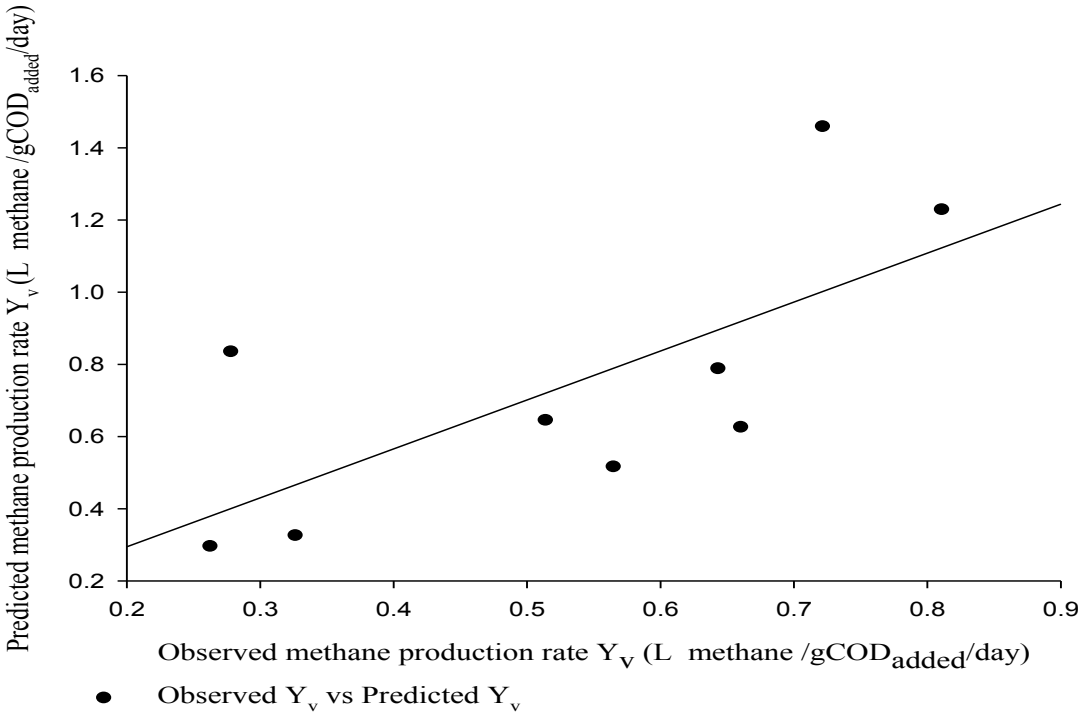

Figure 5.8: The predicted and observed volumetric methane production rates at different temperatures using the developed model (MMGM).

5.4 CONCLUSIONS

- We developed a modified methane generation model (MMGM) for an UASB reactor that treats brewery wastewater and validated it with respect to substrate degradation and the effect of endogenous decay rate on the methane production.
- Quantification of model parameters indicated that the composition of the wastewater strongly affects the kinetics of the digestion process.

- The developed model (MMGM) predicted the rate of methane production for AD of brewery wastewater at different temperatures and OLRs.
- There was a strong correlation between the predicted and the observed measured values for methane production rate. The predicted results further showed that a change in operational conditions (OLR, influent substrate concentration, the HRT and operational temperature) could significantly affect methane production rate.
- The model is easy to use due to its simplicity with only a few variables that facilitate the calibration of the model. It is believed that this model could be used to predict methane production rate of anaerobic digestion process treating brewery wastewater.

5.5 RESEARCH OUTPUT

1) **Abimbola M. Enitan** and Josiah Adeyemo. Estimation of Bio-kinetic Coefficients for Treatment of Brewery Wastewater. Oral presentation at the *World Academy of Science, Engineering and Technology Conference*, New York, USA, June 5-6, 2014. International Science Index, 8(6): 365-369.

CHAPTER SIX: MULTI-OBJECTIVE OPTIMIZATION OF A METHANE–PRODUCING UASB REACTOR USING A COMBINED PARETO MULTI-OBJECTIVE DIFFERENTIAL EVOLUTION ALGORITHM

6.1 INTRODUCTION

Environmental pollution, especially water and air pollution, has become a challenging task for both engineers and scientists in the world. Currently, research has shifted to biofuels as alternative renewable sources due to depletion of fossil fuels. Biofuels produced by AD of organic materials in industrial wastes through the synergistic metabolic activities of microbial consortia include biogas (Gueguim Kana *et al.*, 2012). In several European countries, AD is employed to treat more than 10% of organic matter present in the industrial wastes thereby, saving chemicals (Gueguim Kana *et al.*, 2012). However, the industrial viability of this process requires a suitable combination of chemical and physical process parameters and a low-cost substrate; hence there is a need for process optimization for efficient system performance to produce sufficient biofuel.

There are many optimization problems in science and engineering that require maximization of system desirable properties and simultaneously minimizing its undesirable characteristics. A significant portion of research and applications in the field of AD optimization has focused on single–objective optimization problems, whereas most of the natural world problems involve multiple-objectives which are conflicting in nature (Babu *et al.*, 2005; Iqbal and Guria, 2009; Kusiak *et al*., 2009; Abu Qdais *et al*., 2010). Multi–objective optimization problem (MOOP) involves finding one or more optimum solutions to more than one objective optimization problem (Deb, 2001). The aim of MOOPs is to simultaneously optimize a set of conflicting objectives to obtain a group of alternative trade-off solutions called Pareto-optimal or non-inferior solutions which must be considered equivalent in the absence of specialized information concerning the relative importance of the objectives (Adeyemo and Otieno, 2010; Deb, 2011).

Currently, optimization problems are represented as an intelligent search problem, where one or more agents are employed to determine the optimal on a search landscape, representing the constrained surface for the optimization problem (Das *et al.,* 2008). A large portion of control

problems exhibits multiple stage and multiple objective (MSMO) characteristics. Likewise, AD processes also involve several decision making branches resulting in many objective functions and constraints. Despite this prevalence, there are few methods with the capability to solve general large-scale conflicting multi-objective optimization problems.

Evolutionary algorithms (EAs) are computational-based biological–inspired optimization algorithms. They are stochastic searching methods, commonly used for solving non-differentiable, non-continuous and multimodal optimization problems based on Darwin's natural selection principle (Enitan and Adeyemo, 2011; Sendrescu, 2013). Evolutionary algorithms are widely used for single and multi-objective optimization in AD processes in relation to methane production (Babu *et al*., 2005; Iqbal and Guri, 2009; Wei and Kusiak, 2012).

Evolutionary algorithms use several variables of a problem to provide an optimum solution. Evolutionary algorithms can generate Pareto optimal solutions for different AD models with equally good solutions with respect to all objectives; none of the solutions should dominate another (Deb, 2001; Enitan and Adeyemo, 2011). Studies have shown that EAs are good alternative methods for monitoring state variables in biotechnological processes (Babu *et al*., 2005; Soons *et al.,* 2008; Iqbal and Guria, 2009).

Some of the most frequently used evolutionary multi-objective optimization algorithms for AD include non–dominated sorting genetic algorithm (NSGA), multi-objective genetic algorithm (MOGA), multi-objective differential evolution algorithm (MDEA), multiobjective differential evolution (MODE) and multi-objective particle swarm optimization (MOPSO) (Srinivas and Deb, 1994; Babu *et al*., 2005; Adeyemo and Otieno, 2010; Wei and Kusiak, 2012).

Successful applications of DE to batch fermentation process, optimization of non-linear chemical processes, optimization of process synthesis and design problems, optimization of biomass pyrolysis and optimal design of shell and tube heat exchangers have been reported in the literature (Babu and Chaurasia, 2003; Babu *et al.,* 2005; Angira and Babu, 2006). Among

other improved versions of differential evolution that have been reported in the literature include hybrid differential evolution (HDE) (Tsai and Wang, 2005), Pareto differential evolution approach (PDEA) (Madavan, 2002), MDEA (Adeyemo and Otieno, 2009b), multi-objective differential evolution algorithm (MODEA) (Ali *et al*., 2012) and more recently, a Combined Pareto Multi–Objective Differential evolution (CPMDE) algorithm (Olofintoye *et al.*, 2014).

Olofintoye and coworkers (2014) developed a combined Pareto multi-objective differential evolution algorithm for solving multi-objective optimization problems. The CPMDE algorithm has strength in multi-modal function optimization as demonstrated by Adeyemo *et al.* (2014). The algorithm combines methods of Pareto ranking and Pareto dominance selections to implement a novel selection scheme at each generation. The algorithm employs harmonic average crowding distance measure as against NSGA that implements a crowding distance. The superiority of harmonic average crowding distance has been demonstrated by Huang *et al.* (2005).

The CPMDE algorithm has been successfully applied to various engineering problems (Olofintoye *et al.*, 2014; Adeyemo *et al.,* 2014), where the ability of CPMDE in solving unconstrained, constrained and real-world optimization problems was also demonstrated. Their simulation results show that the CPMDE approach can generate a better Pareto-front for the selected problems.

The main aim of this chapter is to optimize a methane producing UASB reactor using a CPMDE algorithm and provide parameter settings for operating the reactor for more efficient methane generation and better effluent quality. It will be interesting to investigate if the algorithm will perform better using real-life optimization problems such as anaerobic treatment of wastewater for better and more robust solutions for the decision makers. In recent times, a slightly similar problem was solved using different industrial wastewater and algorithm, but we have now used an improved algorithm (CPMDE) to solve the problem to have better solutions.

The modified methane generation and adopted Stover-Kincannon kinetic models (Enitan *et al.,* 2014a) were used for the optimization. This is the first application of a CPMDE algorithm in the area of anaerobic treatment. It is also the first reported multi-objective optimization study on a brewery wastewater treatment plant for methane production, effluent COD reduction and biomass concentration.

6.2 METHODS

6.2.1 Optimization of UASB Reactor

The optimization problem was formulated for multi-objective optimization problem of an existing plant that has been scaled-down for easy optimization and simulation in pilot-scale reactor. The optimization problem was formulated for maximization of methane production rate (Y_v; Equation (6.1)), minimization of effluent biomass concentration (X_e; Equation (6.2)) and effluent COD concentration (S_e; Equation (6.1)). The constrained optimization problem is written as;

$$\text{Maximize } f_1 (P, \theta_h, S_i, T) = Y_v \tag{6.1}$$

$$\text{Minimize } f_2 (S_i, Q) = S_e \tag{6.2}$$

$$\text{Minimize } f_3 (S_i, \theta_h, Q, S_e) = X_e \tag{6.3}$$

Model equations

$$Y_v = \frac{(1-P)0.516S_i}{\theta_h}\left[1 - \frac{0.046}{\frac{[\theta_h(0.013(T)-0.129)]}{(0.083\theta_h)+1} - 0.956}\right] \tag{6.4}$$

$$X_e = \frac{0.357\,(S_i - S_e)}{0.034\theta_h} \tag{6.5}$$

$$S_e = S_i - \frac{18.51S_i}{13.64 + \left({}^{QS_i}/_{V_r}\right)} \tag{6.6}$$

The decision variables were bounded as;

$$S_{i,L} \leq S_i \leq S_i^U \quad (6.7)$$

$$Q_L \leq Q \leq Q^U \quad (6.8)$$

$$\theta_{h,L} \leq \theta_h \leq \theta_h^U \quad (6.9)$$

$$P_L \leq P \leq P^U \quad (6.10)$$

$$T_L \leq T \leq T^U \quad (6.11)$$

Subject to constraints $X \leq X_e$ (6.12)

$$S \leq S_e \quad (6.13)$$

$$V = V_r \quad (6.14)$$

Where, Y_v is the volumetric methane production rate, θ_h is the mean hydraulic retention time, S_i and S_e are the influent and effluent COD concentration respectively, while P is the COD removal efficiency. X_e is the concentration of biomass in the discharge effluent (biomass wash-out) and OLR is the organic loading rate. Q represents the influent flow rate of wastewater; T is the operational temperature, while V is the desired reactor volume.

Equation (6.4) is the governing equation to optimize volumetric methane production rate in a given reactor volume (V_r) in the multi-objective optimization problem formulated. The important decision variables and inequality constraints are shown in Table 6.1. In this problem, plant treatment efficiency depends on the biomass concentration in the reactor. Therefore, prevention of sludge or biomass washout from the reactor is needed for effective treatment and to meet the environmental discharge requirements, as well as increasing the methane production rate for biofuel. In equation (6.5), the desired value for biomass wash out from the reactor was considered as 0.025 g/L. The desired reactor volume of the existing plant is 1400 m^3, but for easy optimization and simulation in the pilot-scale reactor, it was scaled–down to 35 m^3. The results of the optimization can then be scaled-up to the actual volume of the large-scale UASB reactor.

The discharge of low effluent COD concentration to meet the standard limits is another important factor for environmental monitoring, as well as the production of substantial amount of biogas that is rich in methane. Therefore, it was logical to get an optimum operating condition that minimized the effluent discharge COD for any OLR, Q and S_i. Thus, the minimization of effluent substrate concentration using the modified Stover-Kincannon kinetic model (equation (6.6)) was included in the optimization. In equation (6.6), the desired effluent COD concentration was considered as 0.05 g/L.

Table 6.1: Details of model-based multi-objective optimization problem studied using CPMDE algorithm

Objective function	**Problem**
First	Maximixe Y_v
Second	Minimize S_e
Third	Minimize X_e
Inequality Constraints	
V_r (L)	= 35
S_e (g/L)	≤ 0.05
X_e (g/L)	≤ 0.025
Bounds	
S_i (g/L)	$1 \leq S_i \leq 10$
Q (L/day)	$1 \leq Q \leq 20$
θ_h (h)	$1 \leq \theta_h \leq 12$
P	$0.8 \leq P \leq 1$
T (˚C)	$10 \leq T \leq 35$

The boundary conditions for the decision variables based on the scaled-down industrial process are shown in Table 6.1. The lower and upper limits on θ_h were decided based on the HRT of the industrial treatment plant. The microbial consortia in the treatment plant have been found to be sensitive to temperature changes (Akarsubasi et al., 2006; Krakat et al., 2010; Khemkhao et al., 2012), which in turn can affects the rate of methane production. Therefore, operating temperature should be considered as one of the important factors. In this regard, the minimum and maximum values of temperature were selected based on the operating range of the industrial plant.

The lower and upper limits for the influent substrate concentration were set based on the capacity of the treatment plant. The minimum and maximum values for the efficiency of substrate utilization of the reactor at the end of the treatment period in terms of COD removal were considered. This was to ensure maximum conversion of organic matter to methane and good effluent quality in order to meet the discharge standard. The lower and upper limits for influent flow rate were chosen based on the industrial activities and wastewater that the industry is producing; however the volume in this study was scaled-down to 35 m^3 for a pilot-scale reactor.

6.2.2 Combined Pareto Multi-Objective Differential Evolution (CPMDE) Algorithm

6.2.2.1 The CPMDE algorithm

In this study, a combined Pareto multi-objective differential evolution (CPMDE) algorithm was used to optimize the formulated mathematical models. The algorithm combines methods of Pareto ranking and Pareto dominance selections to implement a novel selection scheme at each generation (Olofintoye *et al*., 2014). At each iteration of the CPMDE, the combined population of trial and target solutions is checked for non-dominated solutions. Solutions that will proceed to the next generation are selected using a combined Pareto ranking and Pareto dominance selection scheme (Mezura-Montes *et al.*, 2008). After generating a trial population, tournaments are played between trial solutions and their counterparts in the target population at the same index. Diversity among solutions in the obtained non-dominated set is promoted using a harmonic average crowding distance measure (Huang *et al.,* 2005; Olofintoye *et al.*, 2014) to select the solution that will proceed to the next generation, if solutions are feasible and non-dominated with respect to each other.

In the CPMDE, boundary constraints are handled using the bounce-back strategy (Price *et al*., 2005). This strategy replaces a vector that has exceeded one or more of its bounds by a valid vector that satisfies all boundary constraints. In contrasts to random re-initialization, the bounce-back strategy takes the progress towards the optimum into account by selecting a parameter value that lies between the base vector parameter value and the bound being violated (Babu *et al.*, 2005). Equality and inequality constraints are handled using the constrained-domination technique suggested by Deb (2001). The DE/rand/1/bin variant of

DE is used as the base for CPMDE. The CPMDE algorithm is summarized as follows (Olofintoye *et al*., 2014):

1. Input the required DE parameters such as number of individuals in the population (Np), mutation scale factor (F), crossover probability (Cr), maximum number of iterations/generations (gMax), number of objective functions (k), number of decision variables/parameters (D), upper and lower bounds of each variable, etc.
2. Initialize all solution vectors randomly within the limits of the variable bounds.
3. Set the generation counter, g =0
4. Generate a trial population of size Np using DE's mutation and crossover operations (Price *et al*., 2005)
5. Perform a domination check on the combined trial and target population and mark all non-dominated solutions as "non-dominated" while marking others as "dominated".
6. Play domination tournament at each population index.
    i. If the trial solution is marked "non-dominated" and the target is marked "dominated" then the trial vector replaces the target vector.
    ii. If the trial solution is marked "dominated" and the target is marked "non-dominated" then the trial vector is discarded.
    iii. If both solutions are marked "dominated", then replace the target vector if it is dominated by the trial vector or if they are non-dominated with respect to each other.
    iv. If both vectors are marked "non-dominated", then note down the index and proceed to the next index. When all solutions marked "non-dominated" from steps i – iii above are installed in the next generation, then sort out all solutions noted in step iv one at a time using the harmonic average crowding distance measure (Huang *et al.* 2005). The solution with a greater harmonic average distance is selected to proceed to the next generation.
7. Increase the generation counter, g, by 1. i.e. g = g+1.
8. If g < gMax, then go to step 4 above else go to step 9
9. Remove the dominated solutions in the last generation
10. Output the non-dominated solutions.

*Note domination checks are performed using the naive and slow method suggested by (Deb, 2001).

Source: (Olofintoye *et al.*, 2014).

Olofintoye *et al.* (2014) evaluated the performance of CPMDE using common difficult test problems obtained from multi-objective evolutionary computation literature. The ability of the algorithm in solving unconstrained, constrained and real optimization problems was demonstrated and competitive results obtained from its application suggested that it is a good alternative for solving multi-objective optimization problems. Furthermore, based on an argument by Deb (2001) that most of these test problems are not tuneable and it is difficult to establish the feature of an algorithm that has been tested, the CPMDE has further been tested using on tuneable multi-objective test problems (Adeyemo *et al.,* 2014). CPMDE has been applied to solve real world multi-objective problems and results obtained corroborate the efficacy of CPMDE in solving multi-objective optimization problems.

6.2.2.2 Implementation of CPMDE algorithm for optimization of an UASB reactor

The ability of CPMDE in solving unconstrained, constrained and real-world AD optimization problems is demonstrated herein. The principle of CPMDE algorithm includes coding of the models, decision variables, the constraints as well as evaluation of the fitness function and improvement of the fitness function using differential evolution operators such as tournament selection, crossover and the harmonic average crowding distance measure. The crossover constant, (Cr) and the mutation scaling factor, (F) were set at 0.1 and 0.9 respectively. Population size, Np was set to 50 and the algorithm was run for a maximum number of generations, gMax from 300-5000 on different optimization problems. Harmonic average crowding distances were computed using two nearest neighbours. Further details on the implementation of CPMDE may be found elsewhere (Adeyemo *et al.*, 2014; Olofintoye *et al.*, 2014).

6.3 RESULTS AND DISCUSSION

The kinetic model for methane production rate, effluent substrate COD and biomass concentration were simultaneously optimized in this study to obtain global optimal solutions from the conversion of organic matter in the brewery wastewater. The kinetic coefficients for the model equations used are summarized in Table 6.1. These models were optimized by using the CPMDE algorithm on a computer with dual core processor and 8GB RAM

processor. The model equations were first coded and tested with MATLAB software to ensure that the codes were free of error (Chapter Four and Five). Subsequently, CPMDE algorithm was used to solve the models as multi-objective optimization problem.

A multi–objective optimization problem involving three-objective functions was solved simultaneously using the CPMDE algorithm. These include (i) maximization of volumetric methane production rate, (ii) minimization of effluent discharge COD and (iii) minimization of biomass wash-out from the treatment plant. For this problem, the constraints and decision variables used are shown in Table 6.1. The best value of CPMDE optimization parameters for the three–objective functions are shown in Table 6.2.

Table 6.2: The CPMDE parameters used for multi-objective optimization problem

Parameters			**Value**
Number of Vectors:			50
Number of Parameters:			5
Number of DE generations:			5000
DE control parameters:	Cr	F	
Value	0.1- 0.9	0.1- 0.9	
Step	0.1	0.1	
Optimization			
Number of objectives:			3
Number of constraints:			4
Number of nearest neigbours:			2
Number of non-dominated solutions in final current population			50
Computational time, min			3.17

Cr- crossover constant, F- the mutation scaling factor

Figure 6.1 shows the Pareto optimal solutions for these three-objective functions. Equally good solutions with regard to all objectives were obtained for this problem; none of the solutions dominated another. It was found that as the volumetric methane production rate increased (improved), both the effluent discharge COD and biomass wash-out from the treatment plant also increased (worsens) over the entire Pareto optimal surface (Deb, 2001;

Liu and Wang, 2008; Iqbal and Guria, 2009; Enitan and Adeyemo, 2011). From these results, none of the solutions dominated any other. All the solutions on the Pareto front were found to be equally good and were expected to provide flexibility for the solutions on the Pareto front. Each point on the Pareto optimal front corresponds to a set of decision variables as shown in Table 6.1. Some of the advantages of using these three-objective optimization problem include to have a wide choice of solutions and operating points in the Pareto set, because each point on the Pareto set is obtained from a set of decision variables.

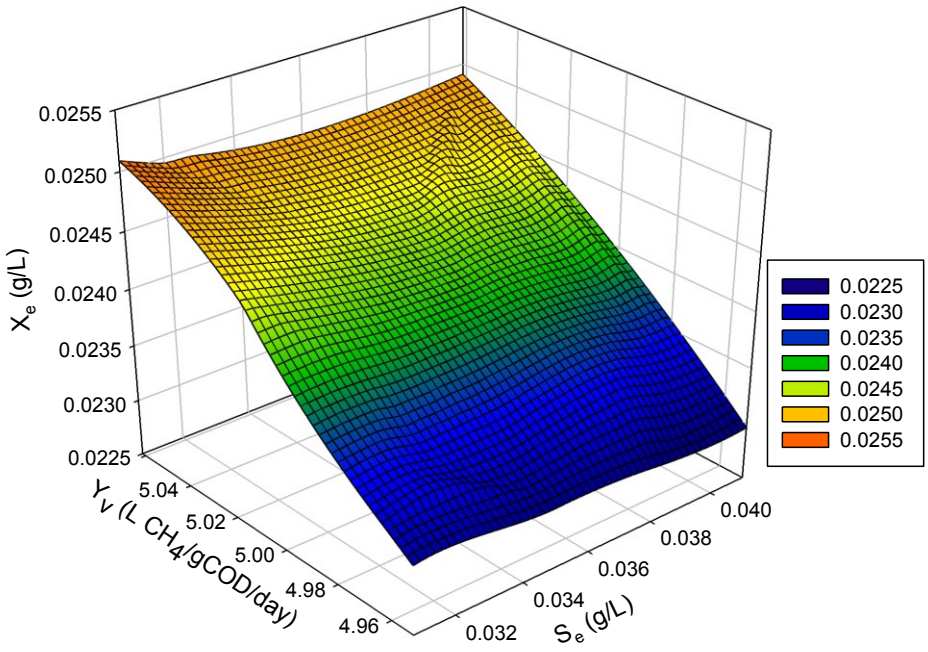

Figure 6.1: Pareto optimal set of solutions obtained for the simultaneous optimization of volumetric methane production rate (Y_v), effluent biomass concentration (X_e) and effluent substrate concentration (S_e) as a multi–objective optimization problem.

The decision variables were further plotted against volumetric methane production rate and effluent biomass concentration to determine the conflicting variables (Figure 6.2a-d). However, we noticed a nearly constant decision variables (T, S_i and Q) over the range of Pareto set, thus the results were not plotted. In addition, the degree of scatter of θ_h and P were found to be slightly higher with unsmooth Pareto front for the simultaneous optimization of these objective functions. A similar result was reported by Babu *et al.* (2005) when MODE

and NSGA algorithms were employed for solving multi–objective optimization problems of industrial adiabatic styrene reactor. Iqbal and Guria (2009) explained that scattered optimal values of the decision variables compensate for each other due to additional objective function and decision variable to the optimization problem.

However, CPMDE is able to give a more uniform distribution of solutions, than those reported by Yee *et al.* (2003) and Babu *et al.*(2005) using NSGA and MODE respectively. Furthermore, several other studies have been reported to have encountered scattered decision variables (Sareen and Gupta, 1995; Tarafder *et al.*, 2005; Khosla *et al.*, 2007). Better spread shows that CPMDE algorithm found more operating policies that were not discovered by any other algorithms from which the decision maker could choose from. That is, we have more options for operating the reactor to produce more methane during anaerobic degradation of industrial wastewater.

In addition, the methane production rate is observed to increase due to an increase in hydraulic retention time. This suggested that the higher the time the wastewater spent in the reactor, the higher the gas production in the reactor. The optimal values of methane production rate take the upper bound at different θ_h and high substrate removal efficiency. At higher effluent flow rate (Q = 14 L/day, V_r = 35), optimal θ_h took almost the lower limit between 8-9 h, and increase in Y_v was observed as θ_h decreased. In Figure 6.2(c), it was noted that the X_e decreased with increase in HRT as the COD removal efficiency (P) remained high (Figure 6.2d). It may be deduced from the optimal results that high P value between 85-87% and 8-9 h HRT at 30-31˚C were responsible for the low and almost constant effluent substrate and biomass concentration with a high methane production rate. This suggested that at high influent substrate concentrations and flow rate, high COD removal efficiency and Y_v depended on the time the wastewater spent in an anaerobic reactor. The results further showed that the decision variables at mesophilic temperature are responsible for the scattered Pareto solutions in the optimized problems for the three–objective functions as shown in Figure 6.2(a-c). Hence, the simulation models could be used to check the operational parameters for getting the best effluent quality, biomass washout and the highest methane production rate in the UASB reactor for the treatment of brewery wastewater.

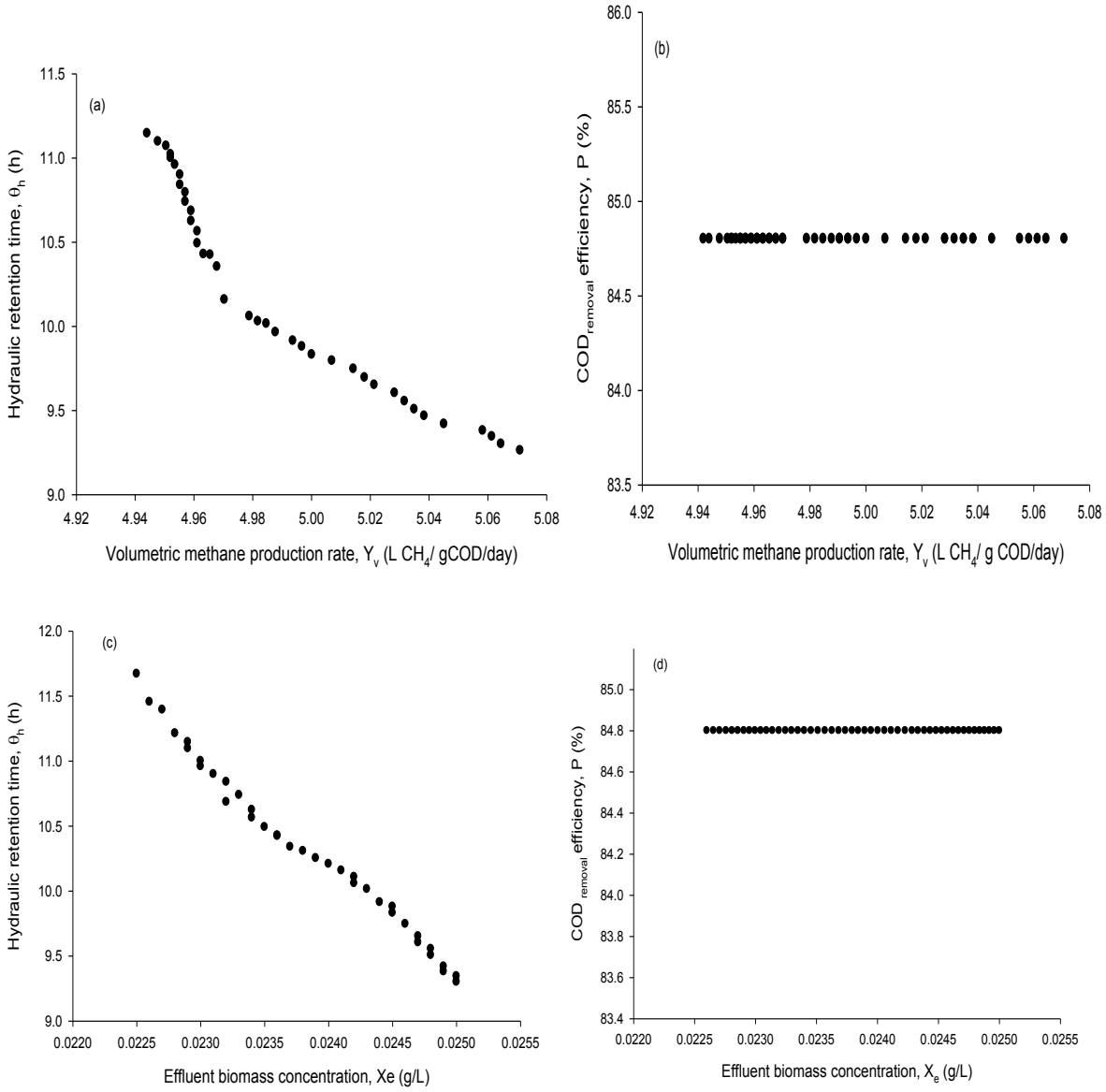

Figure 6.2: The Optimal decision variables (a) θ_h and (b) P plotted against volumetric methane production rate (Y_v), as well as (c) θ_h and (d) P plotted against effluent biomass concentration (X_e) for the optimized problem.

Consequently, the multi–objective optimization conditions within the framework of objective functions based on the holistic kinetic models using the CPMDE algorithm demonstrated a useful instrument for simultaneous optimization of various operational parameters needed for successful running of an UASB reactor. The strength of the integrated multi-objective optimization approach in this study can be applied to for large-scale applications (from pilot–to full–scale reactor). It should be noted that the holistic approach presented in this study is restricted by some boundary conditions and assumptions. However, it can readily be used as a preliminary analysis before transferring the initial concepts to the full–scale reactor, since the model coefficients are obtained from the data collected from the full–scale reactor. Based on the present optimization study, a set of optimal operating conditions was obtained which can enhance the plant performance without affecting the plant configuration. With regards to these facts, future works can consider scaling-up the results obtained in this study to the full–scale system.

6.4 CONCLUSIONS

- In this study, optimization of industrial wastewater treatment plant was carried out using combined Pareto multi-objective differential evolution algorithm.
- Modified methane generation and the Stover–Kincannon kinetic models were used for the optimization of anaerobic reactor treating brewery wastewater for better effluent and methane production.
- A multi–objective optimization problem was solved in this study using CPMDE algorithm as the optimization tool in order to determine the overall optimal operating conditions of anaerobic reactor treating brewery wastewater.
- The associated objective functions were: (i) the maximization of volumetric methane production rate, (ii) minimization of discharge effluent substrate concentration and (iii) the minimization of biomass washout from the reactor. Pareto–optimal sets of equally good non-dominated solutions were obtained for the multi–objective optimization problem considered. The decision variables followed the same trend that further proved the reliability of the results obtained in this study. It also showed that the objectives can further be improved. However, it is difficult to compare the results obtained in this study

with other studies in the literature due to different substrate and decision variables involved, as well as the algorithm used.

- This study is the first application of using combined Pareto multi–objective differential evolution algorithm for AD optimization of brewery wastewater for better methane production and effluent quality.
- The simulation results showed that the CPMDE algorithm can generate a better Pareto-front for the selected problem. Its ability to solve unconstrained, constrained and real-world optimization problem was also demonstrated.
- This will benefit the existing reactors and the design of new reactors treating brewery wastewater, in order to use an optimum environmental condition that will favour the growth of desired microorganisms to desirable end-products.
- The optimization method presented in this chapter has been found to be quite general and flexible to improve the reliability of design and performance of an existing anaerobic treatment plant or a new plant. It can be applied to an UASB reactor to enhance its robustness and performance for better discharge effluent quality and biogas production with high methane content.

6.5 RESEARCH OUTPUT

(a) Book Chapter

Enitan, **A.M.,** Adeyemo, J., Bux F. and Swalaha F. M. 2014. Multi-objective optimization of a methane-producing UASB reactor using a combined Pareto multi-objective differential evolution algorithm. *EVOLVE - A Bridge between Probability, Set Oriented Numerics, and Evolutionary Computation V.* Advances in Intelligent Systems and Computing, Springer, 288: 321-334.

CHAPTER SEVEN: GENERAL CONCLUSIONS AND RECOMMENDATIONS

In summary, the composition of raw brewery wastewater obtained from beer producing industry in KwaZulu-Natal, South Africa was characterized and the efficiency of the full–scale UASB reactor treating the wastewater was monitored over a period of one year. The microbial diversity of the granular sludge samples obtained from the UASB reactor were analyzed using latest molecular techniques with the domain and group-specific rRNA-targeted oligonucleotide probes and primers. The identification of microbial community structure was carried out using FISH and PCR techniques, while QPCR was employed for the quantification of 16S rDNA gene copy numbers in the given samples. A modified methane generation model (MMGM) in terms of kinetics of an intermittent-flow UASB reactor to convert brewery wastewater to biogas with high methane content on the basis of mass balance principles was developed with respect to substrate degradation and the effect of endogenous decay rate on the CH_4 production. In addition, a modified Stover–Kincannon kinetic model was adopted to predict the final effluent quality and a model-based multi-objective optimization was carried out using a CPMDE algorithm with set of constraints and decision variables for the overall optimization of the UASB reactor.

The raw wastewater from the brewery industry was found to be very high in organic matter, nutrients and solids content which does not meet the required effluent regulatory standards, however, it was suitable for microbial degradation with pH adjustment. The performance of the on-site full-scale UASB reactor that treats the above-mentioned raw wastewater was monitored and the results showed the efficiency of the reactor to reduce the concentration of organic matter to a permissible level for discharge. However, there is a need to improve the performance of the reactor in terms of biogas production (methane content), as well as reducing the ammonia and orthophosphate concentration of the final effluent (after treatment). The effect of an increase in VFA concentration as a result of decrease in pH was observed to have a negative effect on methane concentration and reactor's efficiency. The pH of the reactor effluent was within the optimal range for anaerobic bacteria (6.6 and 7.3) at mesophilic temperatures with a 12 h HRT. Volatile fatty acids were detected in the influent wastewater with no detection of these acids in the effluent. These acids served as substrates

for methanogens to produce biogas during anaerobic degradation of complex organic matter in the brewery wastewater.

The preliminary analysis of the granular sludge samples using fluorescence *in-situ* hybridization technique employing domain specific and group specific probes (ARC 915 and EUB 388 mix) revealed the dominance of both rod and coccoid-shaped methanogens and eubacteria in the reactor. Diverse group of methanogenic Archaea belonging to the order *Methanobacteriales, Methanococcales* and *Methanomicrobiales,* as well as *Methanosaeta* and *Methanosarcinal*-like species were detected using ARC 915 and MX825 probes.

The study of the different compartments of the full-scale reactor using PCR analysis demonstrated a substantial variation and changes in microbial populations. Symbiotic relationships between the bacteria that are involved in conversion of complex organic matter to simple monomers were observed using 16S rDNA sequence analysis. The major bacterial phyla belonging to *Proteobacteria, Firmicutes* and *Chloroflexi* needed to convert complex organic matter in the brewery wastewater to the simple metabolites required by methane-producing Archaea were detected in the analysed compartments. The sequence obtained showed (99%) similarity to *Enterobacteriaceae* bacterium clone, *Cronobacter sakazakii*, uncultured *Dehalogenimonas* sp., uncultured *Syntrophorhabdaceae* bacterium and *Syntrophorhabdus aromaticivorans*.

The microbial fingerprint of the functional gene (*mcrA*) and the universal Archaea primer sets revealed the diversity of the methanogenic populations in the granular sludge samples using PCR analysis. All samples from the different compartments showed positive results for the primer sets used and produced PCR products of high number of cells with the *mcrA* genes. The clones after sequencing and analysis displayed similarity (>97%) to the order *Methanomicrobiales, Methanobacteriales* and *Methanosarcinales* belonging to hydrogenotrophic and aceticlastic methanogens. Species detected include *Methanobacterium beijingense*, *Methanobacterium aarhusense, Methanobacterium formicicum, Methanoculleus* sp., *Methanobacterium palustre* and *Methanothermobacter crinale*.

To understand the distribution and activity of eubacteria, methanogenic Archaea within the different compartments of the reactor (Figure 4.8), a quantitative real-time PCR (QPCR) approach was employed. The results of QPCR assays revealed that the bacteria copy number was dominant and abundant at the upper part (C6) of the reactor (Figure 4.8), and decreased down the reactor compartments (C1). On the other hand, quantification of Archaea 16S rDNA copy number revealed that the proportion of total Archaea varied along the reactor compartments with Archaea colonizing the lower and middle part of the reactor. Lower concentrations of methanogenic Archaea were observed in compartment 6 using the domain specific primer set. In this study, variation in the microbial populations at various levels with the depth of the UASB reactor using PCR and QPCR suggested that each compartment is responsible for different phases during anaerobic fermentation of organic matter presence in the brewery wastewater. We could thus conclude that hydrolytic to methanogenic organisms are present in the reactor and the four stages of AD process do occur at the different compartment of the full-scale UASB reactor investigated.

In order to improve the effluent quality, the adopted Stover–Kincannon kinetic model was coded using MATLAB object-oriented language and the predicted values showed the applicability of the model. The simulated data were in good agreement with the observed results, which indicated high correlation of the model to predict effluent COD concentration. Likewise, the developed MMGM model was used to predict methane production from AD of industrial wastewater and the results showed the applicability of the developed model to predict usable methane component of biogas produced during AD of brewery wastewater. Finally, a combined Pareto multi–objective differential evolution algorithm was successfully applied to optimize multi-objective anaerobic treatment problem. Its performance was very encouraging when compared with other multi–objective evolutionary algorithms using common benchmark tests for the optimization of anaerobic treatment problem. The algorithm was tested on the multi-objective anaerobic treatment problem using the modified methane generation and the adopted Stover–Kincannon models. The results of CPMDE algorithm as compared with some multi-objective evolutionary algorithms reported in the literature in terms of convergence and diversity showed that CPMDE algorithm was able to solve multi–objective high dimensional anaerobic treatment problem with few control parameters.

The results further showed that the developed CPMDE algorithm was successful in searching the feasible solution space for good operational conditions of complex biological processes that involves multi-objective and multiple constraints operation in a closed system. The non-dominated solutions generated converge to a Pareto optimal front. The algorithm provided a useful instrument for simultaneous optimization of various operational parameters needed for successful running of an UASB reactor; improve methane production rate and effluent quality. Based on the present optimization study, the CPMDE algorithm produced set of optimal operational conditions to enhance the plant performance without affecting the plant configuration. Multi-objective optimization using this evolutionary algorithm was shown to be a good choice for simultaneous optimization of methane production, biomass washout and effluent substrate concentration during AD of industrial wastewater—using operational parameters as possible constraints and decision variables.

This work would help industries using AD technology to design, optimize and control their on-site anaerobic treatment plants for higher efficiency and renewable energy production. These results will further help reactor operators or environmental engineers to be more aware of operating parameters for anaerobic reactor, particularly the studied full-scale UASB reactor. It will help to set-up optimum operational parameters that will enhance the abilities of the microbial communities in the treatment unit. Furthermore, the results of the benchmark test using CPMDE algorithm in this study will be a good tool for process control strategies in AD operation. It is an elegant, convenient and cost effective tool to investigate certain engineering questions without using physical experimental time and performing expensive laboratory tests. Thus, the prediction and optimization of methane production and effluent quality under different operational conditions could improve the microbial community in order to increase the efficiency of anaerobic bioreactors.

7.1 SIGNIFICANCE AND NOVELTY OF THE RESEARCH FINDINGS

- The research findings in this study are significant in that, bacteria and methanogenic Archaea populations, as well as methane producing gene concentrations were identified and quantified in the full-scale UASB reactor and then correlated with the reactor's operational parameters. This study provides an insight for the first time into the diversity

of the microbial ecology present in the full-scale UASB reactor granules using different molecular techniques. DNA-based studies (PCR and QPCR) as used in conjunction with FISH in a complementary manner provided accurate information about active members of microbial populations or cells present in the reactor.

- An AD process model (MMGM) was developed to improve methane production in an AD system. To the best of our knowledge, MMGM is the first reported developed model that serves as both predictive and optimizing tools for brewery wastewater treatment plant in the literature, as well as multi-objective optimization study using a CPMDE algorithm for simultaneous optimization of methane production, biomass washout and effluent substrate concentration from anaerobic reactor treating brewery wastewater.
- Furthermore, this may be the first study that reported the identification of microbial communities in the granular sludge taken from the investigated UASB reactor using different molecular techniques, as well as a model-based multi-objective optimization study using CPMDE algorithm for brewery wastewater treatment plant in the literature.
- This study increases our knowledge of the microbial communities, especially the methanogens' ability to transform intermediate metabolites during the degradation of organic matter into biogas at the optimum reactor performance. It is hoped that the results of this study will help in environmental protection and energy generation during AD of wastewater in South Africa and, thus contribute to a sustainable long-term clean development mechanism to generate high methane content in a biogas producing UASB reactor. The captured methane can then be used as fuel, hence mitigating greenhouse gas emissions in order to obtain a certified emission reduction credit under the Kyoto Protocol.

7.2 RECOMMENDATIONS

- Due to increase in demand for fresh water by both domestic and industrial users, more work on post treatment of effluent from anaerobic treatment plant using advanced technologies should be considered to obtain almost zero pollutant discharge hence, reduce environmental and freshwater contamination. As observed in this study, treated effluent was still very high in total suspended solids, nitrogen, ammonia and orthophosphate concentration when compared with the discharge standards, thus, further treatment is required in this regard (post treatment).

- Competition between sulphate-reducing bacteria and methanogenic Archaea using different molecular techniques should be explored, in order to increase methane production from bioreactors. Further work on using DGGE and high throughput sequence could also be done to further elucidate the ecology in each compartment of the UASB reactor.
- In order to meet the demand for energy and reduce the consumption of fossil fuel more work should be carried out on the economical and sustainability of methane for energy generation. From the clean development mechanisms point of view, the use of biologically produced methane for energy generation is classified as a 'carbon neutral' process and the CO_2 released during this process is balanced by the CO_2 absorbed by plants during their growth. Therefore, further work should be carried out in this area.
- In addition, government should encourage industries with on-site anaerobic treatment plants that produce biogas to utilize this for energy generation and conversion to electricity (green electricity) instead of flaring these gases into the atmosphere. This will help in mitigation of greenhouse gases into the environment by recycling under-utilized biogas resources.
- Calibration and validation of the developed model (MMGM) using laboratory or pilot-scale processes treating industrial wastewater should be carried out under different operational conditions. Thereafter, the techno-economic analysis of biogas production in a full-scale system for energy generation should be carried out, before upgrading to the full scale system.

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APPENDICES

APPENDIX ONE: Analysis of variance-test (Chapter 3)

Table A1: One way ANOVA for percentage COD removal and biogas yield during anaerobic degradation

Table Analyzed	Data 1		
One-way analysis of variance			
P value	< 0.0001		
Are means signif. different? (P < 0.05)	Yes		
Number of groups	3		
F	634.0		
R squared	0.9702		
Bartlett's test for equal variances			
Bartlett's statistic (corrected)	8.462		
P value	0.0145		
Do the variances differ signif. (P < 0.05)	Yes		
ANOVA Table	SS	df	MS
Treatment (between columns)	34290	2	17150
Residual (within columns)	1055	39	27.04
Total	35350	41	
Post test for linear trend			
Slope	34.65		
R squared	0.9510		
P value	< 0.0001		
Is linear trend significant (P < 0.05)?	Yes		

APPENDIX TWO: Publications

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ENVIRONMENTAL MICROBIOLOGY

Kinetic Modelling and Characterization of Microbial Community Present in a Full-Scale UASB Reactor Treating Brewery Effluent

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Abstract The performance of a full-scale upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater was investigated by microbial analysis and kinetic modelling. The microbial community present in the granular sludge was detected using fluorescent in situ hybridization (FISH) and further confirmed using polymerase chain reaction. A group of 16S rRNA based fluorescent probes and primers targeting Archaea and Eubacteria were selected for microbial analysis. FISH results indicated the presence and dominance of a significant amount of Eubacteria and diverse group of methanogenic Archaea belonging to the order *Methanococcales*, *Methanobacteriales*, and *Methanomicrobiales* within in the UASB reactor. The influent brewery wastewater had a relatively high amount of volatile fatty acids chemical oxygen demand (COD), 2005 mg/l and the final COD concentration of the reactor was 457 mg/l. The biogas analysis showed 60–69 % of methane, confirming the presence and activities of methanogens within the reactor. Biokinetics of the degradable organic substrate present in the brewery wastewater was further explored using Stover and Kincannon kinetic model, with the aim of predicting the final effluent quality. The maximum utilization rate constant U_{max} and the saturation constant (K_B) in the model were estimated as 18.51 and 13.64 g/l/day, respectively. The model showed an excellent fit between the predicted and the observed effluent COD concentrations. Applicability of this model to predict the effluent quality of the UASB reactor treating brewery wastewater was evident from the regression analysis (R^2−0.957) which could be used for optimizing the reactor performance.

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Introduction

Brewery industries produce millions of litres of beer each year which results in the release of large amounts of wastewater with high organic content. The reduction of this high-strength wastewater is mandatory to protect the environment as well as to reduce the cost of penalties that might be incurred due to unlawful effluent discharge. Recently, the use of anaerobic treatment technology such as upflow anaerobic sludge blanket (UASB) reactors has become a popular biological treatment method for both industrial and domestic waste treatment [1].

The anaerobic breakdown of the complex organic compounds involve the action of several groups of microorganisms which results in a variety of intermediates including biogas such as hydrogen, methane, and carbon dioxide [2–4]. The microbial species involved in the conversion of organic material in anaerobic digesters are grouped based on their biochemical activities. The group includes hydrolytic, acidogenic, acetogenic, and methanogenic organisms [5]. These organisms grow in a syntrophic manner when the

ASSESSMENT OF BREWERY EFFLUENT COMPOSITION FROM A BEER PRODUCING INDUSTRY IN KWAZULU - NATAL, SOUTH AFRICA

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ABSTRACT

The objective of the study was to assess the physico-chemical composition and process variations of the effluent from a brewery industry located in KwaZulu - Natal, South Africa during the months of September 2011 to May 2012. The parameters monitored for the quantitative analysis of brewery wastewater include the total and soluble chemical oxygen demand (TCOD and SCOD), biological oxygen demand (BOD_5), total solids (TS), volatile solids (VS), total suspended solids (TSS), volatile suspended solids (TSS), pH, ammonia (NH_3), total oxidized nitrogen, nitrate, nitrite, phosphorus, electrical conductivity (EC), crude protein and alkalinity content. On the average, the TCOD and SCOD concentrations of the brewery effluent were 5340.97 and 3902.24 mg/L, respectively, with average pH values of 4.0 to 6.7. The BOD and the solids content of the effluent from the brewery industry were high indicating that the effluent is of biodegradable type. This suggests that the effluent is very rich in organics, and its discharge into the water bodies or the municipal treatment plant can cause environmental pollution or damage the treatment plant. In addition, there were variations in the effluent composition throughout the period of monitoring which might be due to the activities that take place during the production process and the effects of peak periods of beer production. Thus, there is a need for an on-site effluent treatment plant in order to reduce the high pollution of the effluent prior to its discharge to the municipal wastewater treatment plants.

KEYWORDS: Biodegradable, brewery wastewater, environmental pollution, organic content, physico-chemical composition

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1. INTRODUCTION

Brewery industries produce million L of various types of beers each year (global beer production in 2011 of 192.71 10^2 million L (50.9 billion gallons; Kirin [1]) corresponding to an average consumption of 23 L per person per year [2]. The brewing process involves a series of batch operations on raw materials to the final product. The production process includes the blending and fermentation of maize, malt and sorghum grits using yeast, which requires large volumes of water as the primary raw material. Traditionally, the amount of water needed to brew beer is several times the volume actually brewed. For instance, the average water consumption of 6 hectolitres is required to produce 1 hectolitre of clear beer [3]. Large volumes of water are being used by the industry in production of beer for two distinct purposes; the main ingredient of the beer itself and as part of the brewing process for steam raising, cooling, and washing of floors, cleaning of the brew house, packaging and cleaning after the completion of each batch operation, and also, the amount of wastewater being discharged from the industry after the production of beer. These wastewaters are very high in organic content and highly polluting to the environment [4-6]. Furthermore, it is a known fact that brewing of beer in most countries is a big business, and very few breweries make attempts to treat their wastewater. Most industries dispose their effluents without adequate characterization, quantification and pre-treatment due to economic and technological constraints [7], which may have adverse effects on the municipal treatment plant by reducing the efficiency of the treatment plant and overloading the system.

Thus, in recent times, a considerable number of environmental pollution problems have emerged which had led to monitoring and controlling of quality and quantity of liquid effluents discharged into the natural water-bodies or municipal treatment plants, especially by the industry [8-12]. The effects of water-body contamination include the eutrophication of rivers and dams due to the high inorganic and organic matter, namely, nitrogen and phosphorus compounds from industrial activities and organic matter, such

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Review

Optimization of fermentation processes using evolutionary algorithms - A review

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Optimization of multiple objectives and constraints of fermentation process problems have been extensively studied in recent years. High-performance, robust, cost-effective and reliable computing models that provide innovative solutions to fermentation problems have been developed. This paper reviews the problems of the optimal design of batch fermentation technology and the development of computer-based solving problems using evolutionary algorithms (EAs) to generate optimal or near-optimal solutions to the problems in the fermentation industries. In this review, the latest developments of evolutionary algorithm techniques are focused and optimization of fermentation processes improvement by the techniques is presented.

Key words: Evolutionary algorithms, fermentation, optimization, optimal solution, multiple objectives, constrained optimization.

INTRODUCTION

Biotechnology industries such as pharmaceutical, agricultural, food and chemical industries are rapidly developing during the past few decades where batch operations such as cooking, drying, fermentation, evaporation and sterilization are usually carried out in batches to produce a product with uniform, consistent and reliable characteristics (Curt et al., 2007). Although optimization problems arise in a variety of situations, process optimization has been the key issue to biotechnological scale production to maintain operating conditions, increase product yields and to ensure product quality (Schmidt, 2005).

Fermentation is the basis of many industrial activities. Its processes are important field of interest for system engineering due to its complex, biological, non-linear phenomena and dynamic processes (Andres-Toro et al., 2010). Some among major problems of fermentation processes include fluctuations in the quality of the raw material, influence of temperature, long process time and biomass concentration (Liu et al., 2010; Oonsivilai and Oonsivilai, 2010). Therefore, optimization method or model is an important step to decide suitable parameters for fermentation processes which help in determining the concentration of the medium components and most suitable reaction conditions in maximizing the fermentation products and minimizing important process variables or inputs (Singh et al., 2008). Optimum design methods that combine the optimization algorithms with the computer simulations have been reported (Mohebbi et al., 2008; Abakarov et al., 2009; Guo et al., 2010).

In recent years, an important branch of biotechnology is devoted to the development of proper fermentation processes and efficient steps in the utilization of fermentation technology (Desai et al., 2008). To this effect, evolutionary algorithms (EAs) have been developed and extensively used by many researchers in the fermentation optimization (Dehuri and Mall, 2006). EAs are computer based problem–solving systems of evolutionary computation field based on principle of evolution theory. They are biological–inspired optimizing algorithms, imitating the process of natural evolution and are becoming important optimization tools for several real world applications (Rakesh and Babu, 2005). They provide a robust optimizing technique to find multiple Pareto-optimal solutions in one single simulation run

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Multi-objective Optimization of Methane Producing UASB Reactor Using a Combined Pareto Multi–objective Differential Evolution Algorithm (CPMDE)

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Abstract. Multi–objective optimization of an operating industrial wastewater treatment plant was carried out using combined Pareto multi–objective differential evolution (CPMDE) algorithm. The algorithm combines methods of Pareto ranking and Pareto dominance selections to implement a novel selection scheme at each generation. Modified methane generation and the Stover–Kincannon kinetic mathematical models were formulated for optimization. The conflicting objective functions that are optimized in this study include, maximization of volumetric methane production rate in the biogas produced at a lower hydraulic retention time and optimum temperature; minimization of effluent substrate concentration in order to meet the environmental discharge requirements based on the standard discharge limit, and finally, the minimization of biomass washout from the reactor. Wastewater flow rate, hydraulic retention time, efficiency of substrate utilization within the reactor, influent substrate concentration and operational temperature are the important decision variables related to this process. A set of non-dominated solutions with the high methane production rate at lower biomass and almost constant solution for the effluent concentration was obtained for the multi-objective optimization problem. In this study, the simulation results showed that the CPMDE approach can generate a better Pareto-front of the selected problem and its ability to solve unconstrained, constrained and real-world optimization problem was also demonstrated.

Keywords: Differential evolution, methane production, multi–objective optimization, Pareto, wastewater treatment plant.