A technological model for low energy domestic wastewater treatment

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November, 2015

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Abstract

This study evaluated the potential for efficient treatment of domestic wastewater, while satisfying energy efficiency requirements. Various treatment systems and the influences of their physical configurations and operational characteristics on wastewater treatment and energy efficiency were initially considered and evaluated. Review of literature identified high rate anaerobic systems as viable low energy systems for domestic wastewater treatment, with reported high removal of influent chemical oxygen demand (COD) and high net energy balance for the anaerobic baffled reactor (ABR). Low energy recovery is reported in literature as a limitation of anaerobic domestic wastewater treatment, and anaerobic domestic wastewater treatment systems have failed to meet effluent discharge standards, and post-treatment using aerobic processes have been recommended in order to ensure high effluent quality. Therefore, the ABR was selected as a feasible option that can be developed as the first stage of an anaerobic-aerobic low energy domestic wastewater treatment system. The literature review also identified the net energy consumption per cubic metre (m^3) of treated wastewater during the treatment process as an energy efficiency evaluation criterion.

Energy efficiency for domestic wastewater treatment facilities should be achieved if efficient treatment performance can be sustained at ambient temperature, instead of the fixed mesophilic temperature that is commonly adopted in anaerobic treatment processes. To identify an energy efficient design of the ABR in terms of hydraulic retention time and operational temperature, the performance efficiencies of ABR bench models were monitored at ambient temperature and 37^{o}C at hydraulic retention times (HRT) of 48, 36, 24, 12 and 6 hours, which corresponded to organic loading rates (OLR) of 1.25, 1.67, 2.5, 5.0 and 10.0 kg COD/m^3 day. 88.43, 90.00, 84.03, 77.01 and 59.35% of the influent COD (mean = 2479.50 mg/L) were removed at 48, 36, 24, 12 and 6 hour HRTs, respectively, in the 37^{o}C bench reactor, while 70.16, 70.36 and 74.99% of the influent COD were removed at 48, 36 and 24 hour HRTs, respectively, in the ambient temperature bench reactor. Steady state performance, in the form of stable pH values, was not observed in the ambient temperature reactor at 12 hours HRT before the end of the bench experiments. Retention of influent total solids was observed to correlate to hydraulic retention time, with increase retention of total solids corresponding to increase in hydraulic retention time. Furthermore, observed total solids retention in the ambient temperature reactor were less than the total solids retention in the 37^{o}C reactor.

Anaerobic reduction of domestic wastewater sludge and the corresponding methane production were also evaluated using bio-chemical methane potential (BMP) batch assays at ambient temperature and compared to a fixed mesophilic temperature of 37^{o}C. Low reduction of volatile solids was observed in the BMP assays, with 40% at ambient temperature compared to 56% at 37^{o}C for primary sludge, and 22% at ambient temperature compared to 38% at 37^{o}C for secondary sludge. Critical limitations of the anaerobic stage at ambient temperature were determined to be the biological reduction and conversion of the organic contaminants to soluble COD and volatile fatty acids (VFA). Also, achieving and maintaining steady state performance required a longer time period at ambient temperature than at 37^{o}C, potentially due to the slow growth of the anaerobic microorganisms at ambient temperature. These limitations indicate the need for long ($\geq$ 24 hours) retention periods for efficient operation at ambient temperature. The ABR bench models were evaluated for energy efficiency with the identified energy efficiency criteria, and the operational condition with the highest energy efficiency was determined to be 12 hours HRT at 37^{o}C. Finally, design criteria for the anaerobic stage of the anaerobic-aerobic system were proposed, along with a process model as a preliminary step for future process research.

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Abbreviations

ABR	Anaerobic baffled reactor
AD	Anaerobic digestion
AFB	Anaerobic fluidized bed reactor
AMBR	Anaerobic membrane reactor
BMP	Biochemical methane potential
BOD_5	5 day biochemical oxygen demand
CH_4	Methane gas
CO_2	Carbon dioxide gas
COD	Chemical oxygen demand
CSTR	Completely stirred tank reactor
DOC	Dissolved organic carbon
DPF	Dispersed plug flow
DWS	Domestic wastewater sludge
EGSB	Expanded granular sludge bed reactor
ELR	Environmental loading ratio
EQI	Effluent quality index
EU	European union
EYR	Energy yield ratio
FEI	Functional efficiency index
FOGs	Fats , oils and grease
hrs	Hours
HRT	Hydraulic retention time
ISO	International organisation for standardization
K	Kelvin
kg	Kilogram
kJ	Kilojoules
kW	Kilowatts
kWh	Kilowatts hour
L	Litre
LCA	Life cycle assessment
ln	Natural logarithm
MFC	Microbial fuel cell
min	Minutes
mm	Millimetre
nm	Nanometre
O_2	Oxygen gas
ODS	Organic dry solids of the sludge
OFMSW	Organic fraction of municipal solid wastes
OLR	Organic loading rate
PS	Primary sludge
R1	Reactor 1 operated at 37^{o}C
R2	Reactor 2 operated at ambient temperature
R^2	Square of the correlation between predicted and observed values
RAS	Return activated sludge
RTD	Residence time distribution
s	Seconds
SI	Sustainability index

SBR	Sequencing batch reactors
SRT	Solid retention time
SS	Secondary sludge
ThOD	Theoretical oxygen demand
TIS	Tanks in series
TS	Total solids
TSS	Total suspended solids
tWh	terawatts hours
UASB	Up-flow anaerobic sludge blanket reactor
UK	United kingdom
USA	United States of America
VFA	Volatile fatty acids
VS	Volatile solids
WAS	Waste activated sludge
WWTPs	Wastewater treatment plants

€	Euro
µL	microliter

Chapter One - General Introduction

1.0 Introduction

The use of water in households and municipalities generates substantial volumes of contaminated water as waste (Weiss *et al.* 2008). Improper discharge of this wastewater can lead to ecological problems in receiving natural environments due to high oxygen depletion potential and high concentrations of pollutants and odour (Chan *et al.* 2009). Also, from biodegradation of improperly discharged wastewater, the release of methane (CH_4) can constitute serious environmental hazards to natural ecosystems and also contribute to the increase in atmospheric concentrations of greenhouse gases (Daelman *et al.* 2012). Since early Roman times, systems for wastewater collection and management have been utilized in major cities like Rome (Wiesmann *et al.* 2007), and wastewater treatment by physical processes became common practice in most European municipalities by the 19th century. Wastewater treatment efficiency can be measured in terms of influence on the quality of natural environment receiving the discharge; the desired final water quality; economic and energy considerations (Khan *et al*. 2011).

Efficiency in terms of water quality is usually regulated by environmental protection agencies through treatment levels, which can be preliminary, primary, secondary and tertiary or advanced treatment processes (Helmer and Hespanhol 1997). Economic efficiency is achieved at the design stage through evaluation and comparison of process alternatives, operational requirements and costs (materials and manpower) versus benefits (corresponding water quality), and then selection of the most sustainable and financially feasible alternative (Tandukar *et al.* 2007). Energy

efficiency is achieved through reduction of the energy footprint of wastewater facilities (Mckeown *et al.* 2012), which is by reducing the levels of consumed electricity and fossil fuels and increasing energy recovery. Energy is the property of matter that expresses its capacity to perform work (Avallone *et al.* 2007), quantified in units of joules, and is usually manifested in a variety of forms such as chemical, electrical, mechanical, nuclear and thermal, and it is transformable from one form to another. Energy consumption has also been directly related to release of carbon dioxide (CO_2) and heat to the atmosphere, and is therefore considered as a climate change agent (Daelman *et al.* 2012).

Historically, the processes for water supply and wastewater management have proven to be important components of worldwide energy consumption (Rojas and Zhelev 2012). The percentage of total energy consumption for water and wastewater services is about 3% in the USA and UK (McCarty *et al.* 2011; Huggins *et al.* 2013), and 1% in Sweden (Jonasson 2007). In the UK, the water industry is the fourth largest energy consumer (Caldwell 2009), while in the USA, wastewater treatment services are estimated to consume about 60 – 110 tWh (terawatt hours) of electricity annually (Huggins *et al.* 2013). For domestic wastewater, it is rare to have a treatment process that satisfies all the efficiency criteria highlighted (Rojas and Zhelev 2012), and any design or operational decision usually needs to be carefully evaluated for each criterion (Hernandez-Sancho *et al.* 2011). Improving energy efficiency in domestic wastewater treatment can therefore result in ensuring environmental quality, protection of public health and reducing greenhouse gas release (Daelman *et al.* 2012; McCarty *et al.* 2011).

1.1 Research justifications

Wastewater management operators have to minimize costs and reduce energy footprints due to continuous rise in treatment quality requirements as a result of new regulations; high importance accorded to environmental considerations; increasing energy prices and operational costs (Brissaud 2007; Shoener *et al.* 2014). The consequences of ignoring these identified challenges will be an increase in inefficient treatment and disposal of wastewater into the environment, and the subsequent depletion and deterioration of natural environmental systems (Daelman *et al.* 2012; Gomec 2010). Climate change is also another complication (Singh *et al.* 2012), with decreased resilience of ecosystems, increased variability in renewable freshwater resources, and increase in greenhouse gas emissions. Additionally, domestic wastewater volumes and compositions are variable, depending mainly on time of day, climate, season, life style and nature of the collection (sewer) system (Mara 2003; Davis 2011), and there is usually a corresponding variable influence on the environment, especially when the treatment facilities are not adequate.

Current conventional wastewater management and treatment facilities in developed societies consists of systems of pipes to transport wastewater from source, and energy intensive treatment processes in order to satisfy regulatory standards (Rojas and Zhelev 2012; Brissaud 2007). This makes wastewater treatment highly sophisticated and capital intensive and not readily affordable in many countries (Kassab *et al.* 2010). A large section of the global population presently live with poor access to efficient wastewater treatment facilities (World Health Organisation 2014), and the reliance on energy-intensive and expensive processes for treatment and disposal of wastewater is not tenable. As a result, the ability for some communities

and municipalities, particularly in developing countries, to ensure reliable protection of public health and the environment through efficient wastewater management is under threat (McKeown *et al.* 2012). Therefore, there is a need to develop new (or improve existing) treatment systems so that wastewater management can become less energy-intensive, easy to operate, flexible and can be rapidly deployed (Gomec 2010).

1.2 Research question

In line with the need for a low energy, sustainable and efficient technology for wastewater treatment, the principal research question to be addressed is:

> Which domestic wastewater treatment system is capable of achieving both energy and treatment efficiency, and can be a sustainable and stable process with relatively low capital, operational and maintenance requirements?

Specific areas of interests in this research are the consideration of the options for physical configuration and operational characteristics of treatment systems and how they influence the treatment processes. Also of interest is the influence of temperature on treatment processes and efficiency. Finally, with consideration of the natural variations in wastewater flows and characteristics, the treatment process that will ensure low energy requirements and efficient domestic wastewater treatment needs to be examined.

1.3 Research aim and objectives

The aim of this research is to develop and characterise an efficient low-energy domestic wastewater treatment system. The specific research objectives to be addressed are:

1. To identify and review the key requirements for energy efficient treatment of domestic wastewater.
2. To evaluate the relationship between temperature and anaerobic reduction of domestic wastewater sludge, and corresponding production of methane.
3. To identify relationships between the anaerobic removal of organic pollutants in domestic wastewater and the operational hydrodynamics and treatment temperature.
4. To investigate the energy efficiency of treatment of domestic wastewater at ambient temperature.
5. To propose a design and operational model for a low energy domestic wastewater treatment system.

The expected outcome of the stated aim and objectives should be a comparison of the common processes of domestic wastewater treatment based on their operational energy requirements. Also, there should be an identification and characterisation of the performance of a low energy domestic wastewater treatment system, and the presentation of process and operational criteria for efficient low energy domestic wastewater treatment.

1.4 Organisation of the thesis

Chapter Two presents a review of relevant literature on energy efficiency in domestic wastewater treatment and treatment processes. **Chapter Three** presents details of the experimental plan and methodology adopted. **Chapter Four** presents an evaluation of the influences of temperature on the anaerobic digestion of domestic wastewater sludge and the corresponding production of methane gas. **Chapter Five** presents an analysis of the relationships between temperature, hydrodynamic characteristics and the removal and retention of organic contaminants under anaerobic conditions in ABR bench models. **Chapter Six** presents the evaluation of the energy efficiency of the ABR bench models and proposed design criteria and process model. Finally, **Chapter Seven** presents a summary of the research in the form of key research outcomes, conclusions and recommendations for future research.

Chapter Two – Literature review

2.0 Introduction

The composition of domestic wastewater usually includes various solid contaminants, organic compounds and microorganisms in a mixture with characteristics that are mainly influenced by the source of the wastewater, the amount of water use, organic waste produced and the life style of the community (Davis 2011). The organic compounds in wastewater normally consist of complex polymers such as proteins, carbohydrates and cellulose (Morgenroth *et al.* 2002; Olvera and Lopez-Lopez 2012) which are dissolved, suspended, settleable, or as fats, oils and grease (FOGs). Also, domestic wastewater usually has concentrations of chemicals, such as detergents, soaps and pesticides from laundries, kitchens and gardens (Appels *et al.* 2008; Foresti 2002). Domestic wastewater can be characterized in terms of the oxygen demand, per unit volume of wastewater, for biochemical oxidation of organic matter (Davis 2011), which can be biochemical oxygen demand (BOD), chemical oxygen demand (COD) or theoretical oxygen demand (ThOD).

According to Hernandez-Leal *et al.* (2011), reported COD concentrations in municipal wastewater range from 171 – 4770 mg/L, with the low concentrations reported in urban areas in Europe and the high concentrations in rural areas of developing countries, for example Jordan and South Africa. The most important factor for consideration in the selection of a wastewater treatment system is performance efficiency, in terms of removal of the contaminants (Mara 2003). However, the associated costs (construction and operational) along with overall

system sustainability and the potential for environmental impact will also always play a major role (Kassab *et al.* 2010; Hu *et al.* 2012). With respect to wastewater treatment, sustainability can be defined as the potential for maintaining long-term performance efficiency of processes in terms of environmental, economic and social standards (Hu *et al.* 2012). Sustainable wastewater treatment technologies that will overcome the challenges identified in Section 1.1, as well as recover or produce energy during wastewater treatment are the focus of developments and research in wastewater management (Khan *et al.* 2011; Krozer *et al.* 2010). The objective of this chapter is to identify and review the key requirements for energy efficient treatment of domestic wastewater.

2.1 Treatment of domestic wastewater

For domestic wastewater treatment, the main objectives are removal of organic pollutants in order to satisfy discharge standards and resistance to shock loads due to variation in wastewater characteristics (Appels *et al.* 2008; Mara and Horan 2003). Standards for discharge of wastewater are usually established through regulations and environmental protection laws, for example in India the discharge tolerance limits to surface water systems is 20 mg/L for BOD_5 and 30 mg/L for total solids (Singh *et al.* 2013). Other desirable objectives include high solids retention with low overall sludge production; hydraulic flexibility with high operational efficiencies at low and high hydraulic retention times (HRTs) during high and low flows, respectively (Vuono *et al.* 2013). Also desirable are systems that can achieve improved natural mixing without using any form of mechanical process, and resource recovery from the wastewater (McCarty *et al.* 2011; Zhu *et al.* 2015), usually in the form of methane/biogas, nutrients and water reuse.

Most of the contaminants in domestic wastewater are reduced through physical, biological or chemical processes (Appels *et al.* 2008; Morgenroth *et al.* 2002; Tilley *et al.* 2008; Olvera and Lopez-Lopez 2012), however, biological systems have received widespread adoption as the main units in wastewater treatment. This is mainly because biological treatment systems have reliable performance and are cheap compared to the land and power requirements of physical processes or the cost implications of chemical reagents in chemical processes (Davis 2011). The most commonly applied biological process is aerobic wastewater treatment, which is based on the utilization of the natural processes of various microorganisms in the presence of oxygen (Tchobanoglous *et al.* 2003). Among the aerobic systems, the activated sludge process has shown great process stability and performance efficiency, usually with high effluent quality that meets most effluent standards (Tchobanoglous *et al.* 2003; Davis 2011). The major disadvantage of the activated sludge process is intensive energy requirements in order to achieve the performance targets, and there is also the generation of large volumes of sludge wastes from the process which will require further treatment before disposal (Khan *et al.* 2011).

Sludge is a by-product of wastewater treatment, generated through sedimentation of particulate compounds (Mara 2003), and its disposal is a critical aspect of domestic wastewater treatment. Conventional wastewater treatment plants usually produce two main types of waste sludge (Davis 2011), which are the primary sludge (PS) and the secondary sludge (SS). Primary sludge is collected in primary settling tanks in the treatment process, while secondary sludge is the waste from aeration tanks or trickling filters, and is also referred to as waste activated sludge (WAS) for sludge

from activated sludge plants. For over 100 years, anaerobic digestion has been an acceptable means for the disposal of sludge from wastewater treatment (Stillwell *et al.* 2010), and it is a proven process that can degrade sewage sludge and generate low quantities of disposable sludge compared to other sludge treatment alternatives, for example aerobic composting and disposal to landfill (van Lier *et al.* 2001; Seghezzo *et al.* 1998). In a study by Aitken *et al.* (2005), fermented (anaerobically digested) primary sludge was observed to have volatile solids destruction efficiency of 45% and unfermented sludge had volatile solids destruction below 38%.

With increasingly tighter discharge standards and regulations on wastewater disposal, the conventional processes, such as the activated sludge systems, have continued to be adapted by having additional unit processes that require more energy and technical understanding (Chan *et al.* 2009). For example, biological nitrification through effluent recycling in order to retain critical biomass is now part of the treatment process due to new effluent nitrogen standards (Hu *et al.* 2012). As alternatives to the aerobic systems, anaerobic treatment systems have been developed (Chan *et al.* 2009; Gomec 2010), based on the reduction of complex organic wastes into methane, carbon dioxide and water in the absence of oxygen. The main advantages of anaerobic systems over aerobic systems are the production of biogas containing methane and generation of considerably low quantities of sludge compared to aerobic systems (Shoener *et al.* 2014). These advantages have made the anaerobic process the method of wastewater treatment that is commonly favoured with respect to low energy (Verstraete *et al.* 2009).

Common anaerobic systems, for example anaerobic ponds and covered lagoons are systems operated in batch processes with requirements for large treatment volumes and long treatment retention times (Khan *et al.* 2011). Since wastewater generation is a continuous process, research and development efforts have been directed at continuous treatment systems which can achieve rapid and effective treatment of wastewater when compared to batch systems (Chan *et al.* 2009). Also, the need to retain useful microorganisms is critical for system efficiency, which is not easily accomplished in batch processes (Barber and Stuckey 1999). For these reasons, high rate anaerobic reactors, operated with short retention times (1 – 48 hours) and high organic loading rates, were developed (Abbasi *et al.* 2012). These systems can be either suspended growth systems, for example anaerobic membrane reactor (AMBR); attached growth systems, for example anaerobic filters, or sludge-based systems, for example the up-flow anaerobic sludge blanket (UASB) reactor (Khan *et al.* 2011). Recent increase in reliance on anaerobic treatment of wastes is due to advancement in understanding the processes involved (Gomec 2010).

There have been advancements in understanding the influence of contact between the organic material in wastes and the active microorganisms in anaerobic processes, and the increase in retention of active microorganisms due to modified designs for example with the UASB reactors (van Haandel *et al.* 2006). The improvement in design of reactors like the UASB led to a disentanglement of the hydraulic retention time from the solids retention time, thereby allowing for increased retention of active biomass with short hydraulic retention times (van Lier *et al.* 2001). The most commonly used high rate anaerobic reactor units for treatment of wastewater are the

UASB reactors, the Expanded Granular Sludge Bed (EGSB) reactors and the fluidized bed reactors (van Haandel *et al.* 2006; Luostarinen and Rintala 2005).

2.2 Energy in wastewater treatment

Using conventional treatment systems, energy from electricity (or fossil fuels) is used to treat wastewater, and the energy contained in the influent flow (chemical, kinetic and thermal) is never recovered during the treatment process (Mo and Zhang 2013). Lazarova *et al.* (2012) provided a review of the relationship between water and energy, and energy sustainability of wastewater systems, and it is evident that most conventional domestic wastewater management systems have no facility for resource recovery. Wastewater treatment processes can be operated as energy-positive systems where energy is recovered from the treatment process and returned to the local or national community. An energy positive system is where the energy produced from available resources onsite, by the processes in the system, is greater than the energy required to operate the system (Mo and Zhang 2013).

The recovery of heat by using heat pumps to harness the excess heat from wastewater and make it available for heating and cooling purposes can be a reliable and efficient way to recover energy (Mo and Zhang 2013). Biogas from anaerobic digesters fed to combined heat and power (CHP) units to generate electricity/heat has been reported as capable of meeting the onsite energy requirements of individual WWTPs in Austria (Wett *et al.* 2007), making the facilities energy-neutral. In the period 2005 to 2006 the UK water industry generated 6.4% of its water treatment facilities energy requirements from digestion processes (Caldwell 2009).

In conventional domestic wastewater treatment systems, the largest proportion of energy consumption is usually by biological processes, generally in the range of 30 - 60% of total plant energy consumption (Hernandez-Sancho *et al.* 2011). Energy demand in biological wastewater treatment is usually for pumping (oxygen for aerobic processes, sludge removal and to maintain pressure) and mixing (Lazarova *et al.* 2012). In a study by the Water and Environment Federation (Water Environment Federation 2009) on the electricity consumption of wastewater treatment processes, aeration, pumping, anaerobic digestion and dissolved air flotation processes were found to be the major energy consumers.

For example, in a 37,850 m^3/day (10 MGD) activated sludge plant, aeration and pumping required an average of 5320 and 1402 kWh/day, respectively (Water Environment Federation 2009). Anaerobic digestion and dissolved air flotation processes required daily averages of 1400 and 1805 kWh/day, respectively. With an average of 3500 kWh/day energy recovery in the form of biogas, the total average net electricity consumption for the treatment process without advanced treatment was 8,532 kWh/day (Water Environment Federation 2009). A 69% increase was observed when advanced treatment processes and biological nitrification were added making the total average daily energy consumption = 14,412 kWh/day. The anaerobic digestion component of the activated sludge plant is an energy positive component since it consumes 1402 kWh/day and produces 3500 kWh/day in the form of biogas.

Another aspect of energy consumption in wastewater treatment is the influence of the size of the facility, with large facilities having relatively low energy consumption per cubic metre of wastewater when compared to smaller facilities (Klein *et al.* 2005).

The Electric Power Research institute (EPRI) and the Institute for Diversification and Energy Saving of Spain (IDEA) have observed high energy consumption per cubic metre of wastewater in treatment facilities with small influent flow compared to large plants (Hernandez-Sancho *et al.* 2011). This means small treatment plants will have high unit energy consumption footprints compared to large treatment plants, as shown in Table 2.1, based on data in a study by Goldstein and Smith (2002).

Table 2.1: Electricity Consumption in kWh/m^3 for Wastewater Treatment based on plant capacity (Goldstein and Smith 2002)

Capacity (m^3/day)	**Trickling filter** (kWh/m^3)	**Activated sludge** (kWh/m^3)	**Advanced treatment** (kWh/m^3)	**Advanced treatment Nitrification** (kWh/m^3)
3,785	0.479	0.591	0.686	0.780
18,925	0.258	0.362	0.416	0.509
37,850	0.225	0.318	0.372	0.473
75,700	0.198	0.294	0.344	0.443
189,250	0.182	0.278	0.321	0.423
378,500	0.177	0.272	0.314	0.412

In terms of sustainability and economic costs, the continual rise in energy costs in the 27 EU states from an average of 0.0756 €/kWh in 2005 to 0.1023 €/kWh in 2009, and then 0.1100 €/kWh in 2011 (Hernandez-Sancho *et al.* 2011) is a sign of the increasing pressure on wastewater treatment facilities (Hernandez-Sancho *et al.* 2011). This is making energy-neutrality a necessity for the sustainability of wastewater treatment facilities.

2.2.1 Methodologies for evaluation of energy in wastewater treatment

A simple audit of the energy consumption of wastewater treatment facilities is a method that is commonly adopted to evaluate the sustainability of the systems, based

on the energy demand of the technological units or equipment within the wastewater treatment processes (Balkema *et al.* 2002). This analysis is usually limited to the analysis of the main forms of energy consumption in wastewater treatment facilities, usually the electricity and heating demands (Mo and Zhang 2013). Energy audits provide simple and straightforward energy balance evaluations using energy consumption data assessments, and this can be used to compare treatment systems in terms of their process energy consumptions in the form of energy benchmarks (Remy *et al.* 2011). Energy benchmarking is a way of establishing an energy baseline and defining energy related goals (Remy *et al.* 2011), usually in terms of energy consumption per m^3 of wastewater treated (kWh/m^3).

The use of the kWh/m^3 benchmark can provide a reliable evaluation of the functional units in the treatment process (Heidrich *et al.* 2011); however, this does not give any consideration for the influence of the variable nature of wastewater composition. Heidrich *et al.* (2011) suggested that in order to ensure the energy efficiency of a treatment process, a qualitative audit of all the various forms of energy involved in the treatment process is necessary. Such an analysis requires the identification of the components of total energy consumed during the treatment process such as electrical, manual (human labour), chemical (fossil fuels and reagents) and mechanical forms, each calculated in terms of kWh/m^3 of wastewater treated. Also, the energy contained in the influent and effluent flow of the treatment process is evaluated in order to determine the total energy in the system (Lazarova *et al.* 2012).

Influent energy can be in the form of chemical, thermal, kinetic or potential energy (McCarty *et al.* 2011), where the chemical component is the energy content stored in

various organic materials in the wastewater. The thermal component is the heat energy contained in the wastewater, and the kinetic and potential components are the energy related to the flow of water throughout the treatment plant. For the qualitative energy audit, an alternative benchmark can be adopted based on the energy consumed (kWh/g $COD_{removed}$) per organic load removed (Merlin and Lissolo 2010). Also, by relating the energy to organic loading removal, different phases of the treatment process and life cycle of the facilities can be evaluated and compared (Merlin and Lissolo 2010).

Energy audits are the most commonly adopted methods in the analysis of energy in wastewater treatment systems, but life cycle assessment (LCA) methods have been proposed as comprehensive and systematic environmental assessment methodologies (Remy *et al.* 2011). The life cycle assessment (LCA) is defined by ISO 14040, and it is a tool which can provide a cradle to grave assessment of various aspects of the treatment process (Weiss *et al.* 2008). LCA can be data intensive, but the method provides the possibility to quantify and assess all categories of energy demands using a set of consistent reference units and the inclusion of a carbon footprint and an environmental impacts evaluation (Remy *et al.* 2011).

The identification of the system boundaries of the LCA is a critical step in the method (Lundie *et al.* 2004) unlike in the simple energy analysis where the boundaries are normally defined only by the extent of the internal processes within the treatment facility. For the LCA, the boundaries need to include all relevant processes, for example the provision of electricity, the recycling of nutrients to agriculture, the production of biofuels and also greenhouse gas emissions from

energy and material consumptions and transportation (Remy *et al.* 2011). The LCA methodology can be modified to become the life cycle cost analysis, by considering operations and maintenance costs in the evaluation methods (Mo and Zhang 2012).

The life cycle cost analysis requires the evaluation of the cost of any equipment or facility over an expected lifetime, including any potential energy and maintenance costs that will be consumed over that time. Ko *et al.* (2000) reported that the variation of socio-economic settings both temporally and geographically is considered a major disadvantage to the use of cost/economic models for energy analysis. However, according to Crawford and Sandino (2010) and Hernandez-Sancho *et al.* (2011), substantial differences have not been observed in reported average kWh/m^3 for energy consumption data from several countries and different treatment technologies. This is probably due to the lack of a standardized method for evaluation of energy consumption and recovery by wastewater treatment systems (Mo and Zhang 2013).

2.2.2 Low energy domestic wastewater treatment

Anaerobic and phototrophic systems are considered to provide the best conditions with respect to the recovery of energy from wastewater treatment (Shoener *et al.* 2014). Phototrophic systems are based on processes that use energy from sunlight and the nutrients in the wastewater to cultivate algae, bacteria or plant biomass (Kothari *et al.* 2012). Systems with combinations of anaerobic digestion with algae or wetland processes are reported to have energy consumption levels between 0.3 – 0.6 kWh per m^3 of wastewater treated, and are capable of achieving energy recovery

in the range of 5.0 – 9.2 kWh per m^3 of wastewater treated (Shoener *et al.* 2014), making them energy-positive systems.

Shoener *et al.* (2014) reviewed the energy recovery by anaerobic wastewater treatment systems, and observed values ranging from 0.48 kJ/g COD for microbial fuel cell (MFC) to the highest which was 7.3 kJ/g COD for the anaerobic baffled reactor (ABR). Only four anaerobic systems among those reviewed by Shoener *et al.* (2014) proved capable of achieving energy-positive operation, these are the UASB, ABR, AFB and MFC. UASB reactors are considered the most appropriate systems for low energy treatment of domestic wastewater because of their low investment and operational costs and simplicity in terms of operational and technical requirements (Gomec 2010; Foresti 2002).

The UASB reactor is highly efficient due to the passage of the wastewater through the sludge bed where treatment takes place (van Haandel *et. al.* 2006). There are presently over 200 UASB reactors operating in several countries around the world, such as Brazil, Mexico, Colombia, Italy, Eygpt and India (Gomec 2010; Foresti 2002), with some of the reactors operating since the 1980s and reporting organic loading removal efficiencies higher than 75% of the influent oxygen demands. Table 2.2 provides a summary of reported operational UASB reactors in terms of HRT, COD removal efficiencies and operating temperatures (Gomec 2010).

Table 2.2: UASB reactors for domestic wastewater treatment (Gomec 2010)

Volume (m3)	Temp. (°C)	Influent COD (mg/L)	HRT (hours)	COD removed (%)	Country
12000.0	18–32	1183	8	51–63	India
3360.0	24	380	5	45–60	Colombia
2200.0	20	600	20.3	75–80 (BOD)	Mexico
1200.0	20–30	563	6	74	India
810.0	~ 31	549	9.4	75	Brazil
477.0	-	600	13	68	Brazil
336.0	7–27	205–326	12–42	31–56	Italy
120.0	>13	391	2–7	16–34	Netherlands
120.0	18–28	188–459	5–15	60	Brazil
100.0	20–25	500	12	70–80	Mexico
64.0	25	267	8	75–82	Colombia
60.0	18–25	1531	23–27	51	Jordan
20.0	11–19	150–550	6.2–18	31–49	Netherlands
20.0	20	300	6	70	Mexico
6.0	10–18	100–900	9–16	46–60	Netherlands
0.14000	15	721	6	44	Netherlands
0.14000	-	1159–1701	10	43–69	Palestine
0.14000	-	770–1525	10	5–57	Palestine
0.12000	27 ± 1	816	6	57	Brazil
0.12000	27 ± 1	195	6	53	Brazil
0.03000	12–27	≤30 – 700	5.08	70	Turkey
0.02100	13–25	312	4.7	69	Japan
0.00800	20	350-500	10–40	60–75	Canada
0.00645	13 ± 2	165–270	7.5	24–54	Turkey
0.00400	13	456	8	67	Netherlands
0.00375	25 ± 1	700–1000	15	81 ± 11	Egypt
0.00375	25 ± 1	700–1000	15	76 ± 10	Egypt
0.00375	25 ± 1	700–1000	4	87 ±3	Egypt
0.00375	25 ± 1	700–1000	4	89 ±4	Egypt
0.00350	15	310	12	48	Slovak Republic
0.00350	9	310	12	37	Slovak Republic

9 of the systems in Table 2.2 have average COD removal efficiencies greater than 70%, while the remaining 22 systems have average COD removal efficiencies ranging from 5 – 70%. Elmitwalli *et al.* (2007) achieved 79% removal of influent COD with a UASB reactor treating domestic wastewater by using recirculation of effluents and operating the system as a batch flow reactor. However, the observed COD removal efficiency was between 31 – 41% when the UASB system was

operated as a continuous flow reactor with a HRT of 8 – 20 hours at 14 – 28^{o}C. Generally, COD removal efficiencies for UASB reactors reported in literature were below 70%, and effluent suspended solids concentrations were in the range of 60 – 100 mg/L (Gomec 2010). However, Foresti (2002) reported COD removal efficiencies between 65 - 80% of influent COD concentrations for anaerobic reactors operated with 6 – 10 hours HRT and organic loading rates lower than 3 kg COD/m^3 day.

According to Verstraete *et al.* (2009), the maximum COD removal rates by anaerobic systems treating domestic wastewater are within the range of 60 – 70% of influent COD. Two operational problems that affect the performance of UASB systems are insufficient up-flow velocity and uneven distribution of wastewater across the reactor cross section (Moussavi *et al.* 2010), usually caused by inadequate sludge bed expansion when the operational conditions are not properly configured. Other problems associated with UASB reactors are high effluent nutrient (nitrogen and phosphorous) and pathogen concentrations, normally above discharge standards, and also poor odour and inefficient energy recovery (Gomec 2010). Shoener *et al.* (2014) reported a 24.0% average percentage energy recovery from removed COD (standard deviation = 11.4%) for the UASB, corresponding to 12.2kJ/g COD removed.

One observed limitation of the anaerobic process is that there is usually only a partial capture of produced methane due to its solubility and loss in the effluent (Haridas 2010). In practice, lost methane can be as much as 0.1 g COD/L of treated wastewater as dissolved methane in treatment plants effluents (Haridas 2010). Experiments in Columbia on UASB treatment of sewage reported observed

conversion of COD to gas as 0.5 kg CH_4-COD/kg COD removed, with unaccounted COD which may have been lost as dissolved gas in the effluent from the system (Haridas 2010), or alternatively used for synthesizing biomass. In an experiment with a UASB reactor treating domestic wastewater, Banu *et al.* (2007) observed highly variable gas production rates from a 5.9 L bench UASB, which they concluded was due to the fluctuation of organic concentration in the influent. With influent COD concentrations between 800 – 1200 mg/L for 7.3 - 3.3 hours HRT and 800 – 1800 mL/day flow rate, the volume of biogas produced ranged from 1800 mL/day to 7080 mL/day with methane content measured at 62 ± 3%.

Errors in design (for example lack of provision of liquid-gas separation) or operations (for example hydraulic overloading) of anaerobic reactors have led to instability of treatment performance (Foresti 2002), making post treatment of the effluents a necessity. Due to the inherent variation of domestic wastewater characteristics (Mara 2003; Davis 2011), domestic wastewater treatment with either aerobic or anaerobic systems can encounter efficiency problems (Hernandez-Leal *et al.* 2011; Sun *et al.* 2012). Aerobic systems can be operated with some degree of flexibility, therefore high effluent quality is achievable, but usually at high operational costs (Chan *et al.* 2009). While anaerobic systems are usually not limited by operational costs, effluent quality may not always meet regulatory standards (Khan *et al.* 2011). Because the effluent from anaerobic treatment usually contains solubilized and fermented organic matter, aerobic post treatment of anaerobic effluents is considered as a suitable step to ensure effluent discharge standards are satisfied without increasing operational costs (Verstraete and Vandevivere 1999).

Anaerobic–aerobic processes are therefore expected to lead to reduction in operating costs compared with aerobic treatment alone and they should also produce high organic contaminants removal efficiency compared to anaerobic treatment systems (Chan *et al*. 2009). High rate bioreactors, for example UASB and membrane systems, are now considered as technologically sustainable options when operated as anaerobic–aerobic systems (Khan *et al.* 2011). Combinations of high rate reactors in series, for example an up-flow anaerobic sludge bed reactor connected to an anaerobic fluidized bed reactor (UASB-AFB), are easily applicable since the system is based on already standardized units (van Haandel *et al.* 2006). However, high rate anaerobic reactors, for example the UASB, can experience problems during operation, and research has shown that these problems are inherent to the nature of the reactors (Baloch and Akunna 2003; Bassuney *et al.* 2013). For example, due to the fast growth rate of acid producing bacteria compared to methane forming bacteria, accumulation of volatile fatty acids and hydrogen is commonly observed, and the anaerobic process can be inhibited as a result (Langenhoff and Stuckey 2000).

Multi-stage anaerobic digestion processes which have the acid formers and methane formers separated have been developed in order to resolve the process inhibition problem observed in anaerobic reactors (Ahring 2003; Demirel *et al.* 2010). A UASB-septic tank system was evaluated by Luostarinen and Rintala (2005), and they reported high treatment efficiencies. Using synthetic black water and dairy parlour wastewater, their results showed efficient performance for a two-phased up-flow anaerobic sludge blanket (UASB)-septic tank system with a total chemical oxygen

demand (COD_t) removal above 80% and removal of total suspended solids (TSS) above 90%.

Studies that compared single and multi-stage anaerobic systems have observed improved performance and increased process stability by multi-stage systems above the single-stage systems (Demirel *et al.* 2010). In experiments to compare single-stage systems with multi-stage systems, Cohen *et al*. (1982) observed greater overall process stability and maximum COD conversion rates in multi-stage systems. Various configurations of reactors were examined by Azbar *et al.* (2001) and Azbar and Speece (2001), and they observed high treatment performance in multi-stage anaerobic systems compared to single-stage systems.

Therefore treatment of wastewater with concentrations of high particulates, for example unsettled domestic wastewater, may be more efficient in multi-stage anaerobic systems, for example the ABR (Foxon *et al.* 2006; Deng 2006; Foresti *et al.* 2006), where in the first stage efficient suspended solids removal can be achieved and removal of the soluble organics will occur in the subsequent stages. Among the anaerobic reactors reviewed by Shoener *et al.* 2014, the ABR was reported as having the greatest average percentage energy recovery (as methane, hydrogen, or electricity) from degraded COD at 47.5 ± 4.5%, which equates to roughly 110 – 3300 kJ per m^3 of treated wastewater.

2.3 The anaerobic baffled reactor (ABR)

The main characteristic of the anaerobic baffled reactor is the series of vertical baffles which break the reactor volume into several compartments and force the

wastewater to flow through any entrapped biomass (Baloch and Akunna 2003). This system allows for greater contact between wastewater and anaerobic biomass even in the absence of retaining media (Barber and Stuckey 1999). The original ABR design is as schematically represented in Figure 2.1(a), but Figure 2.1(b) is the configuration most commonly referred to as the ABR (Barber and Stuckey 1999).

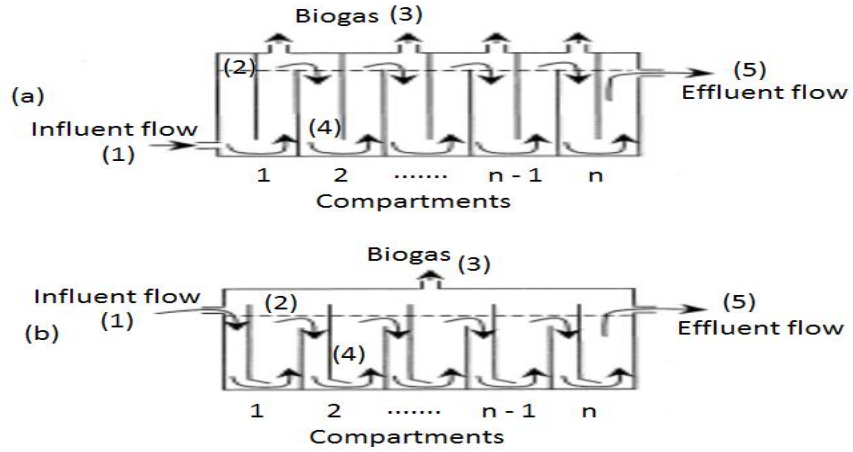


Figure 2.1: Major configurations of the ABR - (a) Original and (b) popular (Barber and Stuckey 1999) where: (1) indicates the location of influent flow; (2) Indicates the structure (compartmentalized or single unit) of the headspace; (3) Indicates the biogas collection point(s); (4) Indicates the location of baffles; (5) Indicates the final effluent flow;

Another characteristic of the ABR is the eventual separation of the anaerobic microorganisms into zones due to its compartmental nature, and therefore the entire treatment process is separated into several phases (Hassan and Dahlan 2013). In effect, each compartment of an ABR can be considered as a separate treatment unit (Barber and Stuckey 1999), thereby allowing for different bacterial populations to dominate each section of the reactor.

2.3.1 ABR performance efficiencies

There are several reviews of the performance of the ABR for the treatment of different types of wastewater, and the principal publications are Barber and Stuckey (1999), Liu *et al.* (2010), Sarathai *et al.* (2010) and Hassan and Dahlan (2013). The general conclusion is that the ABR can be used to treat wastewater from various sources, and recorded COD and TSS removals have corresponded to more than 85% and 90%, respectively (Vossoughi *et al.* 2003). Gopala-Krishna *et al.* (2009) reported greater than 90% COD and BOD removal efficiencies from a laboratory ABR system operated at HRTs between 8 – 10 hours, the equivalent of 1.2 – 1.5 kg COD/m^3 day.

Performance of the ABR is influenced by the hydraulic retention time, the physical configuration and operational condition of the system, along with the capacity of the system to retain active biomass (Hassan and Dahlan 2013). Foxon *et al.* (2007) studied a 3,000 L pilot ABR receiving domestic wastewater at a wastewater treatment works, and measured effluent COD values consistently below 200 mg COD/L at 22 hours HRT, corresponding to a removal rate in the range of 58 - 72% of influent COD. The conclusion of the researchers was that the operating flow rate used was too high to allow complete fermentation of particulate COD.

Gomec (2010) reported that the observed performance of an ABR reactor in Turkey operated at 19^{o}C for 12.8 hours HRT was 67% removal of influent COD (30 – 700 mg/L), and 63% removal of influent COD (30 – 700 mg/L) when operated at 18^{o}C for 9.5 hours HRT. Similarly, for an ABR reactor in Egypt operated at 22 – 28^{o}C with influent COD of 505 – 914 mg/L, there were reported average COD removal rates of 67.5 and 75.6% for 8 and 12 hours HRT respectively (Gomec 2010). Ayaz *et*

al. (2012) reported COD removal in the range of 41 – 50% for an ABR with three compartments treating domestic wastewater operated at ambient temperature (12 – 28ºC) without temperature control. Zhu *et al.* (2015) reported the evaluation of an ABR at ambient temperature (17 – 25ºC) where COD removal efficiencies were between 83 – 94% for a wastewater with influent COD ranging between 500 – 1500 mg/L.

Nachaiyasit and Stuckey (1995) worked with a HRT of 20 hours at three operational temperatures of 35ºC, 25ºC and 15ºC to evaluate the response of anaerobic microorganisms to changes in the environmental conditions. Nachaiyasit and Stuckey (1995) reported organic load removal above 90% when the system was operated at 35ºC and 25ºC; however, the organic load removal dropped to below 80% when the operational condition was changed to 15ºC. Overall, they observed reductions in the growth rates of the microorganisms at 15ºC, and similar results were observed when distillery wastewater was used as the organic load (Nachaiyasit and Stuckey 1997). A critical aspect of the anaerobic baffled reactor is the need for proper establishment of appropriate microbial biomass before high organic load removal and methane production can be achieved (Bodkhe 2009; Liu *et al.* 2010).

Feng *et al.* (2015) reported a start-up period of 65 days for an ABR reactor operated at ambient temperature (22.0°C - 24.8°C) with average influent COD of 444 mg/L. The average effluent COD during the start-up period was 323 mg/L, with a range of 1.66 – 60.05% removal efficiency which improved to 66.4% during the steady state performance period which was achieved after approximately 130 days. The observed average effluent COD during steady state operation was 71 mg/L for influent COD

ranging from 100 – 250 mg/L (Feng *et al.* 2015). Boopathy and Tilche (1991) observed four stages during the operation of a hybrid anaerobic baffled reactor, with the first stage lasting for 40 days with low organic loading rate before suitable biomass was established in the reactor.

Bodkhe (2009) reported a biological acclimatization period of 90 days before COD removal efficiency was steady at 97% for experiments without initial inoculation of biomass. Bodkhe (2009) evaluated the ABR for a period of 375 days at 11 different HRTs ranging from 6 days to 3 hours, with the HRT of 6 hours observed to be the most efficient retention time in terms of organic loading removal and organic loading rates. At a HRT of 6 hours, the treatment efficiency of the system in reduction of total suspended solids (TSS) was 86% and chemical oxygen demand (COD) was 84% similar to what was observed with HRTs ranging from 3 days to 8 hours.

2.3.2 Methane production in the ABR

Reported methane yields from baffled reactors in literature indicate that the nature of substrates and operational conditions are the main factors that influence methane production in the ABR (Barber and Stuckey 1999). Hassan and Dahlan (2013) reported increase in total biogas production corresponding to increase in organic loading, or alternatively decrease in retention time, for various studies on the ABR. For the ABR, reported methane production rates in literature for wastewater with influent COD concentrations less than 1000 mg COD/L and organic loading rates between 0.13 – 4.73 kg COD/m^3day are lower than 1.0 v/v/day (Barber and Stuckey 1999; Hassan and Dahlan 2013). Bodkhe (2009) studied the variation in biogas yield and methane content of biogas at different HRTs for a modified ABR, and at a HRT

of 0.25 day, a yield of 0.34 m^3 CH_4/kg $COD_{removed}$ was observed. The yields for HRTs shorter than 0.25 day were lower than the 0.34 m^3 CH_4/kg $COD_{removed}$ observed value at 0.25 day, corresponding to a drop in organic loading removal efficiency observed at these HRTs.

Also, when comparing the percentage of the total biogas that is methane for different retention times, Bodkhe (2009) observed 68 – 70% methane at 0.75 day, 67% at 0.25 day and 48% at 0.13 day. Sallis and Uyanik (2003), operated two 4 compartment ABR systems under different feeding regimes, normal and split fed, with OLRs of 0.9, 1.5, 2.75, 5.5 and 10.5 (kg COD/m^3 day), where they reported differences in methane production between the two reactors and their individual compartments. For the split fed system, substantial differences in methane percentage in the headspace were not observed for the 0.9, 1.5, 2.75 and 5.5 OLRs, while the 10.5 OLR had percentage methane similar to the normal fed system. The 10.5 OLR was also reported with low methane production (m^3/kg COD removed) for the two reactors, while the highest methane production was observed with the 2.75 OLR for the two systems (Sallis and Uyanik 2003).

Shanmugam and Akunna (2008) observed a decrease in the methane content of biogas from the 1st, 2nd and 3rd compartments with increase in organic loading rate from a bench scale ABR with five equal compartments. They also observed that the 4th and 5th compartments showed constant methane content for all the loadings tested. The substrate availability, which is expected to decrease along the length of the reactor, along with environmental conditions in the compartments will determine the anaerobic consortium and methane production for each compartment (Yu *et al.*

2014). Gopala-Krishna *et al.* (2008) observed compartmentalization of the microbial population in the ABR separating acidogenic and methanogenic activities longitudinally through the reactor. Baloch and Akunna (2003) also reported differences in the characteristics of the sludge bed in the acidogenic and methanogenic zones of the ABR. Observations of low methane yields have been attributed to other influences on the digestion process apart from the diluted nature of domestic wastewater, such as biogas escape and COD removal by sulphate reducing bacteria (Bodkhe 2009). Lettinga *et al*. (1993) observed loss of more than 50% of produced methane with the liquid effluent while experimenting with domestic wastewater.

2.3.3 Design of ABR systems

The ABR possesses characteristics of several established anaerobic treatment reactors, for example the anaerobic contactor reactor, anaerobic filter and the UASB, such as low sophistication of the physical and operational requirements, and low excess sludge production (Liu *et al.* 2010). In its original design, the ABR consisted of a number of equally dimensioned compartments in series, and Bachmann *et al.* (1985: cited in Baloch 2011) considered it suitable primarily for removal of dissolved contaminants. Most reported experiments used reactors with five compartments, or less, but Nachaiyasit and Stuckey (1995) worked with two eight compartment bench reactors and reported organic loading removals above 90%.

Bodkhe (2009) used nine compartments and reported reactor stability and high organic loading removal at a short HRT of 6 hours; this suggests that increasing the compartments, should increase performance stability, probably due to the resulting

increase in retained biomass. However, there are no clearly defined design criteria or guidelines linking organic loading and physical characteristics to treatment efficiency. Foxon and Buckley (2006) suggested simplified design guidelines relating treatment and retention time at steady states, and proposed a design model, Equations 2.1 – 2.7, relating the hydraulic retention time to effluent COD.

$$COD_{t,\ in} = I_{in} + S_{in} + X_{s,\ in} \qquad \text{Equation 2.1}$$

$$\frac{dX_s}{dt} = -f(HRT) \qquad \text{Equation 2.2}$$

$$COD_{t,\ e} = I_{in} + X_{s,\ e} \qquad \text{Equation 2.3}$$

$$X_{s,\ e} = X_{s,\ in} + \int_0^{HRT} f(HRT)dt \qquad \text{Equation 2.4}$$

$$COD_{t,\ e} = I_{in} + X_{s,\ in} + \int_0^{HRT} f(HRT)dt \qquad \text{Equation 2.5}$$

$$\frac{X_{s,\ e}}{X_{s,\ in}} = e^{-k*HRT} \qquad \text{Equation 2.6}$$

$$\int_0^{HRT} f(HRT)dt = X_{s,\ e} - X_{s,\ in} = X_{s,\ in}(e^{-k*HRT} - 1) \qquad \text{Equation 2.7}$$

Where:

$COD_{t,\ in}$ = total influent COD (mg/L).

$COD_{t,\ e}$ = total effluent COD (mg/L).

I_{in} = inert component of the influent COD.

S_{in} = settleable component of the influent COD.

$X_{s,\ in}$ = biodegradable influent COD.

$X_{s,\ e}$ = biodegradable effluent COD.

HRT = retention time (day).

f (HRT) = function that relates HRT to the variables.

k = process rate constant (day^{-1}).

This model, which is in the form of a 'black box' structure of the overall reactor performance, is intended to give a prediction of the effluent characteristics in terms of COD for specific flow and organic loading conditions for a particular design HRT (Foxon and Buckley 2006). The influences of important hydrodynamic characteristics, for example solids retention time, on the performance efficiency of the ABR were not defined in the Foxon and Buckley (2006) design model. The technical challenge in the design of the ABR lies in achieving enhanced bacterial activity and a high degree of mixing so as to ensure a high rate of contact between the microorganisms and the substrate (Barber and Stuckey 1999).

This challenge is addressed by considering operational parameters that have direct impact on the treatment performance, such as the hydraulic retention time, the number of compartments, and the retention of solids (Shanmugam and Akunna 2010). Evaluation of the hydrodynamic characteristics of an ABR using residence time distribution (RTD) experiments is an important step in advancing the design of ABR systems (Sarathai *et al.* 2010). RTD experiments should provide data that can be used to improve calibration of design models, or alternatively validate proposed design models (Dierberg and DeBusk 2005).

Hydrodynamic characteristics

A residence time distribution (RTD) study is an observation of the time distribution for tracers as they flow through a system (Ji *et al.* 2012). This relates the change in tracer concentration over time to the mixing/ dispersion within reactor (Hutnan *et al.* 1999; Levenspiel 1999; Chen *et al.* 2010). A key step in flow experiments is the

identification of an effective theoretical hydraulic retention time (HRT) cut-off, usually twice the length of the design HRT, where it is expected any material remaining in the reactor is stagnant and not part of the flow (Chen *et al.* 2010). The effective cut-off HRT for flow experiments can be up to three times the design HRT (Sallis and Uyanik 2003; Langenhoff and Stuckey 2000), for completely stirred tank reactors with no dead space.

Dyes can be used as indicators of flow patterns in clear fluids, with the advantage that visual observations allow for an enhanced understanding of the flow pattern (Dierberg and DeBusk 2005). Rhodamine WT is commonly the dye used as a water tracer when there is no biomass or adsorption is not considered as a limitation, in order to take advantage of the visible colour impact and the low cost implications (Williams and Nelson 2011). Lithium chloride is the most commonly used compound for tracer studies when biomass is present in the reactor due to its high solubility, and also the low background concentrations of Lithium ions (Li^{+}) in most environments (Dierberg and DeBusk 2005). Alternatively, Barium Chloride and Sodium Fluoride have been used in tracer studies of anaerobic systems, because they are not biodegradable and are rarely absorbed by biomass (Ji *et al.* 2012).

Due to variable mixing and inconsistencies in flow velocities, the actual HRT, which is the measured mean residence time, and the design HRT can be different, leading to performance issues different from what is intended in the design (Levenspiel 1999). The hydraulic retention time (HRT) is the theoretical average time it takes for a unit to move from the inlet of the reactor to the outlet, usually the reactor volume (v) divided by the flow rate (Q). The analysis of RTD data to determine the mean

residence time depends on an RTD function, E(t), Equation 2.8 (Chen *et al.* 2010; Hutnan *et al.* 1999).

$$E(t) = \frac{C_i}{\sum C_i \Delta t_i} \qquad \text{Equation 2.8}$$

Where:

E(t) = RTD function (dimensionless)

C_i = tracer concentration C (mg/L) at time i divided by C_t (mg/L)

C_t = tracer concentration (mg/L) at time of tracer injection

Δt_i = normalized time interval (dimensionless)

(Time interval between sample collections divided by HRT)

The mean residence time for an RTD data set can be determined using Equation 2.9 (Chen *et al.* 2010; Hutnan *et al.* 1999).

$$t = \frac{\int_0^\infty T * E(t) dt}{\int_0^\infty E(t) dt} = \frac{\sum t_i \, C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad \text{Equation 2.9}$$

Where:

t = mean residence time (minutes)

T = actual time since tracer injection (minutes)

E(t) = RTD function (dimensionless)

C_i = tracer concentration C (mg/L) at time i divided by C_t (mg/L)

C_t = tracer concentration (mg/L) at time of tracer injection

t_i = time from tracer injection (minutes)

Δt_i = normalized time interval (dimensionless)

(Time interval between sample collections divided by HRT)

A ratio of the mean residence time to the design hydraulic retention time, Equation 2.10, indicates the fraction of reactor volume that is effective.

$$v_e = \frac{t}{HRT} \qquad \text{Equation 2.10}$$

Where:

v_e = fraction of reactor volume that is effective

t = mean residence time (minutes)

HRT = design hydraulic retention time (minutes)

If v_e < 1, the mean residence time is shorter than the theoretical HRT and dead spaces or flow short circuiting are prominent in the reactor. The fraction of reactor volume that is dead space can be determined using Equation 2.11.

$$v_d = 1 - \frac{t}{HRT} \qquad \text{Equation 2.11}$$

Where:

v_d = fraction of reactor volume that is effective

t = mean residence time (minutes)

HRT = design hydraulic retention time (minutes)

From Equation 2.11, if the reactor volume is not variable, then the dead space is dependent on the mean residence time (*t*). Therefore analysis of the data from the RTD tests should provide indication of the relationship between residence time and

effective volume of the reactor, and consequently lead to identification of an operational configuration that is efficient.

One of the advantages of the ABR is the reported low dead space (< 25%) when compared to other anaerobic reactors, for example anaerobic filters and completely stirred tank reactors (CSTRs), which have observed dead space above 50% (Grobicki and Stuckey 1992). Langenhoff and Stuckey (2000) reported that temperature variations were observed to have only minor influence on reactor hydrodynamics, and they also observed constant dead space, averaging between 25 – 30%, for temperatures of 10, 20 and 35ºC. The reactor dead space is a function of the dead space due to hydraulic channelling and the dead space due to poor mixing as a result of low production of anaerobic biogas (Sarathai *et al.* 2010).

Hydraulic channelling within the reactor, where sections of the reactor become hydraulically and biologically inaccessible and cannot contribute to the effective contact volume, can be caused by high wastewater flow rates (Baloch and Akunna 2003; Sarathai *et al.* 2010; Gopala-Krishna *et al.* 2009). The hydrodynamic (flow and mixing) characteristics can be analysed using any of two non-ideal flow models, dispersed plug flow (DPF) model and tanks in series (TIS) model, with data observed from residence time distribution studies (Sarathai *et al.* 2010).

The dispersed plug flow model

The dispersed plug flow model based on one-dimensional diffusion is the model that is commonly applied to describe the hydrodynamics of treatment reactors, by assuming dispersion occurs only along the direction of flow (Chan *et al.* 2010). The

DPF can be used to evaluate distribution of flow within the reactor, primarily by determining an equivalent dispersion number, which is expected to decrease with decreasing residence time (Grobicki and Stuckey 1992). A direct relationship between the mean velocity and the flow pattern in a reactor can be observed using the dispersion model which provides a measure of mixing in the form of a dispersion number (d), expressed by Equation 2.12.

$$d = \frac{D}{uL} \qquad \text{Equation 2.12}$$

Where:

d = dispersion number of the reactor (dimensionless)

D = axial dispersion coefficient (m^2/s)

L = axial distance of the reactor (m)

u = average flow velocity (m/s)

For d = 0, the flow is considered as an ideal plug flow, and d = 1 indicates an ideal completely mixed flow, while a value of d between the two limits indicates non ideal flow. The dispersion number is also related to the RTD data variance as expressed in Equation 2.13 (Chen *et al.* 2010), and the variance for the RTD data can be determined using Equation 2.14.

$$\frac{\sigma_t^2}{t^2} = 2d - 2d^2\left(1 - e^{-\frac{1}{d}}\right) \qquad \text{Equation 2.13}$$

$$\sigma_t^2 = \frac{\sum t_i^2 C_i \Delta t_i}{\sum C_i \Delta t_i} - t^2 \qquad \text{Equation 2.14}$$

Where:

σ_t^2 = the variance of the RTD data

t = mean residence time (minutes)

d = dispersion number of the reactor (dimensionless)

C_i = tracer concentration C (mg/L) at time i divided by C_t (mg/L)

C_t = tracer concentration (mg/L) at time of tracer injection

t_i = time since tracer injection divided by the HRT (dimensionless)

Analysis of the hydrodynamic characteristics of the ABR based on the dispersion model has shown that the mixing patterns of the ABR can approximate to a completely mixed reactor as the peak flow increases (Sarathai *et al.* 2010). Also, observations show there are potentially no differences in terms of mixing patterns with or without the presence of a sludge bed, indicating that the key influence is from the flow rate and not the sludge bed (Sarathai *et al.* 2010).

Tank in series model

Another model for evaluating flow patterns in treatment reactors is the tank in series (TIS) model, which defines the reactor as an equivalent number of completely stirred tanks (CSTRs) in series (Grobicki and Stuckey 1992). For the TIS model, the number of theoretical stirred tanks (*N*) is expected to increase with increasing peak flow. Sarathai *et al*. (2010) observed that the TIS model can be more suitable than the DPF model to the ABR, especially considering its compartmental configuration. Equation 2.15 can be applied to determine the equivalent number of tanks, N, using the mean residence time (t) and the RTD data variance.

$$N = \frac{t^2}{\sigma_t^2}$$ Equation 2.15

Where:

N = equivalent number of tanks in series for the reactor

σ_t^2 = the variance of the RTD data

t = mean residence time (minutes)

If N ≈ 1, then the reactor is completely mixed and there is substantial back mixing, while if N ≈ ∞, then the reactor flow is plug flow.

Hydraulic efficiency

The TIS model provides a relationship between the RTD data variance and mixing within the reactor in the form of a hydraulic efficiency (λ), which is defined using Equation 2.16.

$$\lambda = \mathrm{v}_e \left(1 - \frac{1}{N}\right)$$ Equation 2.16

Where:

λ = Hydraulic efficiency of the system (dimensionless)

$\mathrm{v_e}$ = effective fraction of volume (dimensionless)

N = tank in series number (dimensionless)

For a hydraulic efficiency that is greater than 0.75, the system is considered to have excellent distribution of inflow and mixing. While hydraulic efficiency values between 0.5 and 0.75 are considered to be indications of good inflow distribution and

mixing, and values less than 0.5 are considered to be indications of poor inflow distribution and mixing (Chen *et al.* 2010).

Backpressure

A possible cause of failure associated with solids in the ABR is sludge accumulation leading to backpressure (Shanmugam and Akunna 2010), which is an indication of resistance to flow due to friction between the influent fluid and media through which it is flowing. Accumulation of sludge in the ABR can be due to two factors, influent feed and build-up due to biomass growth (Foxon *et al.* 2006). If a process point where solids biodegradation is equal to sludge accumulation is maintained then the reactor sludge volumes will be stable and the accumulation of sludge in the compartments will not occur, therefore backpressure or washout of retained biomass will be avoided (Shanmugam and Akunna 2010).

Backpressure is important as pressure variations can lead to flow instabilities or even system failure in high rate gravity systems like the ABR, resulting in overflows, leakages, reversal of flow, or inadequate influent flow distribution. Shanmugam and Akunna (2010) have proposed a model for backpressure profile estimation under various hydraulic conditions, and also demonstrated that increase in backpressure correlated with entrapped biomass depth for any given HRT and baffle position. They observed the lowest backpressure values when the up-flow and down-flow sections of the compartments were of equal dimensions.

Solids retention time (SRT)

Solids retention time (SRT) is the measure of the mean residency of the active biomass inside the reactor and is an indicator of the maturity and complexity of microorganisms responsible for biodegradation in the system (Appels *et al.* 2008). In a continuous reactor like the ABR, solids retention is a critical factor to consider in design, particularly as high wastewater flows can cause process failure through disruption of the biomass settling and high solids washout (Baloch and Akunna 2003). With high flows, an overflow of sludge from one compartment to the next over the baffles can occur leading to accumulation of sludge in the final compartment and a point will be reached when sludge will washout of the reactor (Shanmugam and Akunna 2010).

For wastewater with high particulate solids content, such as domestic wastewater, Boopathy and Sievers (1991) recommended the provision of a settling tank as the initial compartment of the ABR. This set-up was observed to have process stability and satisfactory performance, with the settling chamber ensuring high solids removal even during peak flow periods. Boopathy and Sievers (1991) using a modified baffled reactor to treat waste with 51.7 g/L total solids concentration, reported 60% removal for a two compartment reactor and 74% for a three compartment reactor.

Foxon *et al.* (2007) observed partial degradation of particulate biodegradable organic material in a pilot ABR, with accumulation of dense inert solids at the bottom of the 1st compartment. A scum layer was also formed in the 1st compartment by floating solids that are less dense than water, indicating the importance of having multiple compartments in a treatment reactor. The retention of solids was also enhanced by

minimising the velocity of liquid on the up-flow side of each compartment since solids loss is through carryover of slow-settling solid particles when the up-flow velocity exceeds the particle settling velocity (Foxon *et al.* 2007). A five-year pilot operation of the ABR treating municipal wastewater in South Africa (Foxon *et al.* 2006) showed a rate of sludge accumulation in the reactor that is dependent on the wastewater flow rates, and instances of high flows resulted in noticeable sludge washout.

The characteristics of the inoculum are important to the acclimatization and performance of the reactor, and observations from some experiments (Barber and Stuckey 1999) indicate that the use of non-granular biomass is possibly the main reason the ABR has poor performance at short HRTs when compared with UASB reactors. The non-granular biomass is easily washed out and susceptible to channelling at short HRTs (Baloch 2011), and in order to withstand high loading rates at short HRTs, the inoculum biomass needs to have good settling characteristics, and these advantages are easily achieved with granular biomass. Barber and Stuckey (1999) have expressed the opinion that an ABR does not require granulation for high treatment performance, however regardless of the initial nature of inoculum, biomass granulation has been observed during operations of ABR systems (Boopathy and Tilche 1991; Uyanik *et al.* 2002; Gopala-Krishna *et al.* 2009).

2.3.4 Limitations of the ABR

Zhu *et al.* (2015) have identified the required long start-up periods, up to 90 days, and the absence of a clear definition of the relationship between OLRs, HRTs and

COD removal efficiencies as the key limitations towards the full scale application of the ABR. Low OLRs result in low biomass activity and biogas yields, while high OLRs are usually achieved using short HRTs which can lead to hydraulic inefficiencies and system instability (Zhu *et al.* 2015). Most of the results reported from several experiments show a decrease in process performance at high organic loading rates (OLRs) and low HRTs (Grobicki and Stuckey 1991; Bodkhe 2009). Baloch (2011) reported a decrease in the performance efficiencies of the ABR was observed at short HRTs and OLRs. Shanmugam and Akunna (2008) observed low efficiency by the ABR in comparison with a UASB reactor, particularly at high OLRs.

The anaerobic digestion process has been extensively researched, and while system design is critical for achieving high performance efficiency, operational controls and strategies are also important (Moussavi *et al.* 2010). According to Barber and Stuckey (1999), the ABR performance is closely related to the nature of flow and degree of mixing, which in turn can be indirect measures of contact between substrate and biomass. Zhu *et al.* (2015) suggest that advancing the understanding of the relationships among the compartments in ABR and the nature of the microbial communities in the ABR compartments should minimize the operational problems associated with the system.

Yu *et al.* (2014) highlighted problems in the ABR associated with scum build-up and clogging, and they recommended the use of media inside the reactor to prevent floatation of biomass, encourage formation of a biofilm and rapid development of granular sludge. Feng *et al.* (2008) also recognized mixing and encouragement of

high rate contact between biomass and organic loading as key limitations of the process, and they recommended the use of hollow-sphere bamboo carriers as a media for biofilm development. The biomass developed in the attached form is expected to decrease clogging and sludge washout, and also reduce biomass disintegration as a result of mixing, usually observed with short HRTs (Feng *et al.* 2008)

The operation of the ABR at ambient temperature also need to be researched (Zhu *et al.* 2015; Ayaz *et al.* 2012), especially in terms of process pathways and stability of the microbial community, for example sludge disintegration. This should provide guidance towards identification of appropriate inoculation and start-up of ABR systems at ambient temperature, and also prediction and minimization of operational problems (Zhu *et al.* 2015). Accumulation of intermediate fatty acids is also possible at low temperatures because the production of fatty acids occurs at a high rate relative to methanogenesis (Nozhevnikova *et al.* 1994). Nachaiyasit (1995) observed increased acid production at the rear of an ABR operated at 25^{o}C compared to another ABR operated at 37^{o}C.

Identifying the operational conditions and configurations of the ABR that are the optimum in performance efficiencies should be a future research focus (Zhu *et al.* 2015). For the ABR to be developed as an anaerobic-aerobic system, the COD removal efficiency of the anaerobic stage should be optimized (Zhu *et al.* 2015). Processes that will enhance the recovery of nutrients and pathogens (Gomec 2010) and energy in the ABR also need to be evaluated (Zhu *et al.* 2015), in order to enhance the energy balance of the system and enable comparisons with other favoured anaerobic systems, such as the UASB. Partial recovery of organic carbon

energy content during domestic wastewater treatment with anaerobic digestion systems is also considered to be a major obstacle to the adoption of the systems as conventional treatment alternatives (Verstraete *et al.* 2009). Overall analysis of the ABR for treatment of wastewater leads to the conclusion that the operational areas for consideration are the process stability, system hydraulics, fate of solids, loss of methane and final effluent quality (Hassan and Dahlan 2013).

2.4 Anaerobic digestion processes

Anaerobic digestion is a complex process which is as a result of a self-regulating mixed culture of various microorganisms, and it currently plays an important role in wastewater treatment (Sanders 2001; Visvanathan and Abeynayaka 2012). Anaerobic degradation of organic matter in wastewater normally involves the following stages (Griffin 2012; Sanders 2001): hydrolysis (liquefaction), acidogenesis (acid formation), acetogenesis (acetate formation) and methanogenesis (methane formation), as shown schematically in Figure 2.2.

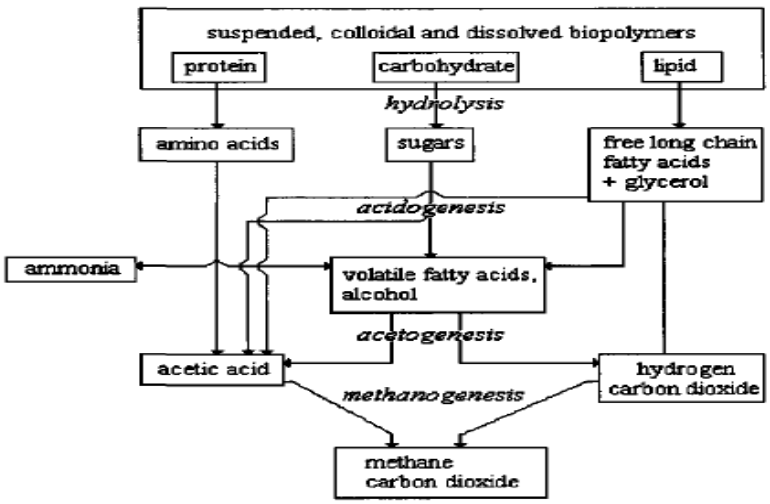


Figure 2.2: Stages and pathways for anaerobic digestion of wastewater (Sanders 2001).

The biological decomposition of organic wastes usually generates gases, as a final step, known as biogas which can be composed of several substances such as methane, hydrogen, water vapour, hydrogen sulphide and carbon dioxide (Abbasi *et al.* 2012; Verstraete *et al.* 2009; Mara and Horan 2003).

2.4.1 Hydrolysis

The utilization of waste during anaerobic digestion is usually governed by a rate limiting step which is the slowest step in the process, which usually tends to be hydrolysis (Vavilin *et al.* 2008). Hydrolysis is the conversion of the complex biodegradable organic matter into more readily soluble biodegradable matter which can then serve as necessary carbon source for the completion of the anaerobic process (Esposito *et al.* 2012; Foresti 2002; Vavilin *et al.* 2008). Increase in the concentration of soluble organics through hydrolysis of particulates leads to high efficiency of the anaerobic digestion process (Yuan *et al.* 2011).

Where the concentrations of particulate organics in the wastewater being treated are high, because of the nature of hydrolysis as a limiting step in the anaerobic process, low efficiency of organic matter degradation is usually observed (Aldin 2010). The factors known to influence hydrolysis and anaerobic digestion include substrate characteristics, reactor configuration (Weichgrebe and Rosenwinkel 2013; Vavilin *et al.* 2008), operational parameters (SRT, OLR and HRT), the type of microorganisms present in the biomass, and environmental factors, like temperature and pH (Vavilin *et al.* 2008; Sanders 2001). In wastewater treatment, the effluent quality and methane yield from any anaerobic system is therefore sensitive to environmental temperature

as the microorganisms and enzymes responsible for the process are directly influenced (Batstone 2000; Vavilin *et al.* 1996).

2.4.2 Acidogenesis and Acetogenesis

After hydrolysis, the next step in anaerobic digestion is the acid phases which are referred to as acidogenesis and acetogenesis (Aldin 2010). Under acidogenesis, the products of hydrolysis (dissolved sugars, long-chain fatty acids and amino acids) are converted to short-chain (volatile) fatty acids (mainly acetic, butyric, valeric and propionic acids), alcohols, hydrogen and carbon dioxide (Appels *et al.* 2008). Acidogenesis occurs when the smaller molecules resulting from hydrolysis penetrate into the cells of fermentative bacteria and are converted into several simpler compounds, which are then excreted by the cells (Aldin 2010). A large and diverse group of fermentative bacteria is considered responsible for the acid phase, mainly like the *Bacteriodaceae* and species belonging to the *Clostridia* genus (Gallert and Winter 2005).

Acetogenesis is the conversion of the short chain acids (propionic, butyric and lactic acids), into acetic acid, carbon dioxide and hydrogen. These steps are often the fastest steps in the anaerobic process, due to two factors which influence the reaction rates (Vavilin *et al.* 2008); the first factor is the availability of soluble molecules of degradable compounds as suitable substrates (Gallert and Winter 2005). The second factor is the utilization of these soluble molecules as sources of energy and growth by microorganisms. Because of the fast rate of the acid phase compared to the other stages in anaerobic digestion, anaerobic reactors can become subjected to sudden pH

drops due to accumulation of the acids, especially when they are overloaded or disturbed by toxic compounds (Bassuney *et al.* 2013).

2.4.3 Methanogenesis

Methanogenic organisms consume acetic acid, hydrogen and some of the carbon dioxide produced during acetogenesis to produce methane, using three possible biochemical pathways (Abbasi *et al.* 2012). The acetoclastic pathway is the major pathway that leads to production of methane in a single step process and is usually the source of approximately 70% of methane produced (Tomei *et al.* 2009). The second pathway, hydrogenotrophic methanogenesis, involves methane from dissolved hydrogen and carbon dioxide and is usually the source of approximately 30% of produced methane in the anaerobic process. In a situation where single carbon compounds, for example methanol, are predominantly available, then it is possible for a third pathway, the methylotrophic pathway, to be observed (Abbasi *et al.* 2012). Demirel *et al.* (2010) reviewed the production of methane from various substrates, and some of the observed yields are presented in Table 2.3.

Table 2.3: Methane yields for selected substrates **(**Demirel *et al.* 2010)

Substrate type	**Methane yield**
Liquid swine waste	0.36 m^3 CH4/kg VS_{added}
Brewery wastewater	0.28 – 0.035 m^3 CH_4/kg $COD_{removed}$
Hog + poultry waste	0.130 ± 0.020 m^3CH_4/kg $VS_{removed}$
Waste activated sludge	0.5 – 0.6 m^3/kg $VS_{removed}$
Barley waste	0.363 m^3 CH_4/kg VS
Dairy manure	0.125 – 0.166 m^3 CH_4/kg VS
Food waste (single phase reactor at HRT of 10 – 28 days)	0.348 – 0.435 m^3 CH_4/kg VS
Sewage sludge + OFMSW	0.024 m^3/kg VSS_{added}
Fruit and vegetable waste	0.320 m^3 CH_4/kg COD_{added}
Food waste (two phase laboratory UASB)	0.21 m^3 CH_4/kg VS_{added}
Food waste (two phase UASB)	0.25 m^3 CH_4/kg VS
OFMSW – organic fraction of municipal solid wastes UASB – up flow anaerobic sludge bed reactor VS – volatile solids VSS – volatile suspended solids COD – chemical oxygen demand	

Zhang (2010) cited Sato *et al*. (2001), Speece (2001) and Rittmann and McCarty (2000) with reported gas production from primary and waste activated sludge samples in mL/g VS as 612 and 380; 362 and 281; and 375 and 275 respectively, while Neves *et al.* (2006) reported the methane yields from the anaerobic co-digestion of five coffee wastes and sewage sludge in the range of 0.24 – 0.28 m^3/kg VS. Observations from anaerobic digestion of grease trap sludge containing fats, oils and grease (FOGs) showed high methane potentials (845 – 928 mL/g VS_{added}) in laboratory and pilot plants (Davidsson *et al.* 2008). According to Arthur and Blanc (2013) there are a number of studies which have proven that co-digestion of FOGs with other substrates increases biogas production and degradation, and they reported a 30% increase in biogas production from anaerobic digestion in wastewater treatment plants with the addition of FOGs collected from food service industry.

2.4.3.1 pH range for methane production

Due to the high sensitivity of methanogens to environmental changes, any change in pH can affect their activity (Lettinga 1995). Methanogens operate within a narrow pH range, 6.5 – 7.5, and maintaining the pH throughout an anaerobic reactor is vital to the success of the anaerobic process (Appels *et al.* 2008). Any substantial accumulation of VFA within the reactor will inhibit the activity of methanogenic bacteria because the pH will fall outside the ideal range. According to Vavilin *et al.* (2008), intermediate compounds in anaerobic digestion can be possible inhibitors of the process. de Baere *et al.* (1985: cited in Veeken *et al.* 2000) had proposed that 30 g/L is the maximum concentration of organic acids sustainable in anaerobic digestion. Brummeler *et al.* (1991: cited in Veeken *et al.* 2000) observed that a VFA concentration of 33 g/L causes inhibition of the process but only if the pH is below 5.5.

Increase of alkalinity, bicarbonate compounds, in the liquid phase, along with a constant concentration of carbon dioxide in the gas phase, can cause an increase in pH in the system (Appels *et al.* 2008). During the anaerobic digestion process there will be consumption of alkalinity at the acid phase, and during methanogenesis the consumed alkalinity will be recovered (Olvera and Lopez-Lopez 2012), and therefore alkalinity should remain unchanged. Any wastewater with a high protein concentration can develop high alkalinity due to the high release of carbon dioxide, amino groups and ammonia production (Olvera and Lopez-Lopez 2012).

A buffering system based on the alkaline effects of bicarbonate compounds and carbon dioxide usually counteracts the effects of acids during the digestion process (Martin *et al.* 2010; Hansson *et al.* 2013). Equilibrium between carbon dioxide and the bicarbonate compounds in a digester usually indicates a stable alkalinity and pH, and this helps maintain the pH levels within the narrow range as well as buffering the effect caused by VFA (Appels *et al.* 2008). A stable VFA-bicarbonate ratio, at least 0.7, is also an indication of a stable digestion process (Appels *et al.* 2008), and a ratio lower than 0.4 indicates low risk of process acidification (Jimenez *et al.* 2003).

2.4.3.2 Other factors that influence methane production

Apart from temperature, pH and nature of substrate, there are physical and chemical properties that will influence methane production. The important physical properties are mixing, organic loading rate and retention time (Hassan and Dahlan 2013). With adequate mixing in the reactor, uniform distribution of substrates and microorganisms should be maintained, and also thermal stratification across the height of the reactor should be eliminated. The organic loading rate influences the production of methane based on the ratio of bacteria to substrates, with a high OLR capable of causing low system efficiency while a low OLR will be an inefficient design due to low substrate to biomass ratio (Lettinga *et al.* 2001).

For retention times there is either the hydraulic retention time which gives the mean residence of influent wastewater in the reactor or the solid retention time which gives the mean residence of bacteria and biomass in the reactor. The HRT influences the effective volume of the reactor, and a long HRT will result in large reactor sizes and consequently high initial costs for the system (Hassan and Dahlan 2013). Also, if

there is heating requirements, then the operational requirements will be increased with long HRTs. Short HRTs can reduce efficiency and cause washout of bacteria thereby reducing the SRT (Shanmugam and Akunna 2008), and a short SRT will mean less time for the necessary biochemical reactions to take place. The relationship of retention time to biogas production obtained from laboratory experiments is described in Figure 2.3 (Appels *et al*. 2008).

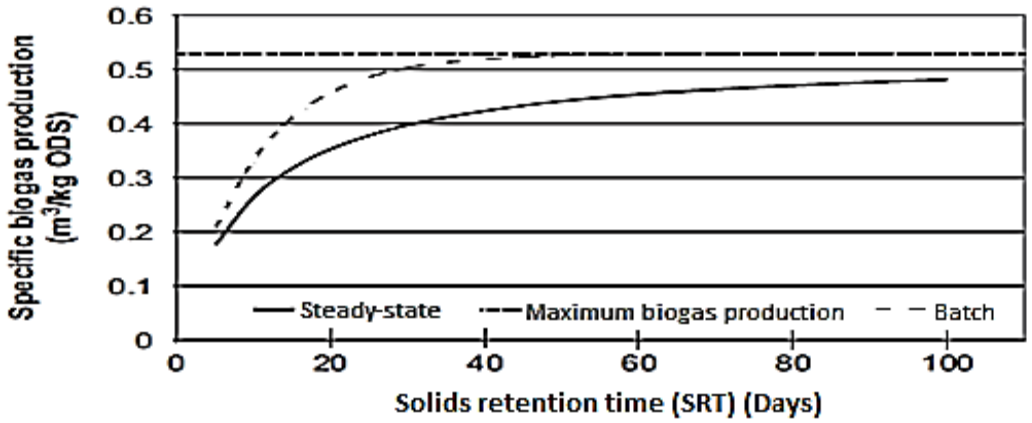


Figure 2.3: Relationship of biogas production to SRT in terms of volume of biogas produced per kg of organic dry solids of the sludge (ODS) (Appels *et al.* 2008).

The chemical factors that are important are availability of nutrients and the presence of toxic compounds and inhibitors (Ahring 2003). Nutrients are categorised either as macronutrients (such as carbon, nitrogen, sulphur, iron and calcium) which are necessary for growth of microorganisms, or as micronutrients (such as zinc, nickel and copper) capable of influencing activity of bacteria (Baloch 2011). The presence or high concentrations of certain substances can have toxic or inhibitory effects on anaerobic processes, for example a high concentration of metal cations such as sodium, calcium and magnesium, can be harmful to bacteria (Appels *et al.* 2008).

Even though enzymes and co-enzymes depend on the presence of trace concentrations of these metal cations for their activation and processes, large concentrations of these cations can disrupt the chemical structure and functions of the enzymes and therefore cause inhibition to the biological processes (Appels *et al.* 2008). Ammonia from organic compounds such as protein or urea, will be toxic to bacteria at concentrations higher than 1500 mg/L if the pH levels are higher than 7.4, however biomass acclimatization to high concentrations of ammonia have been reported (Yenigun and Demirel 2013). Knowledge on the pathways for ammonia inhibition of anaerobic microorganisms, particularly methanogens, is limited; however there are indications that three possible pathways are responsible. The first is the change in intracellular pH of methanogens that occurs due to high concentrations of ammonia, the second is the increase in energy requirements for anaerobic chemical reactions, and the third is the inhibition of enzyme activities (Rajagopal *et al.* 2013).

2.4.4 Anaerobic digestion process models

There are various diverse reasons behind the developments of models for anaerobic treatment of domestic wastewater, and the most common reasons are operational analysis (optimization, stability and process predictions), technology development and design (Batstone 2006). With greater focus on operational considerations in order to meet strict effluent standards, and for process optimization, models are gaining popularity. The Anaerobic digestion model (ADM1) was developed, with operational considerations as motivation (Batstone *et al*. 2002), as a standardised model. Most developed models have similar basic structures based on the four phases of anaerobic digestion (hydrolysis, acidogenesis, acetogenesis, and methanogenesis),

with simple steady state conversion of known substrates to known products (Dewil *et al.* 2011).

As anaerobic digestion systems normally consists of reactors with a liquid volume and a gas headspace, they can be represented as completely mixed tanks, Figure 2.4, with single input and output streams, and constant liquid volumes so that the influent flow is equivalent to the effluent flow over any giving time period (Batstone *et al.* 2002).

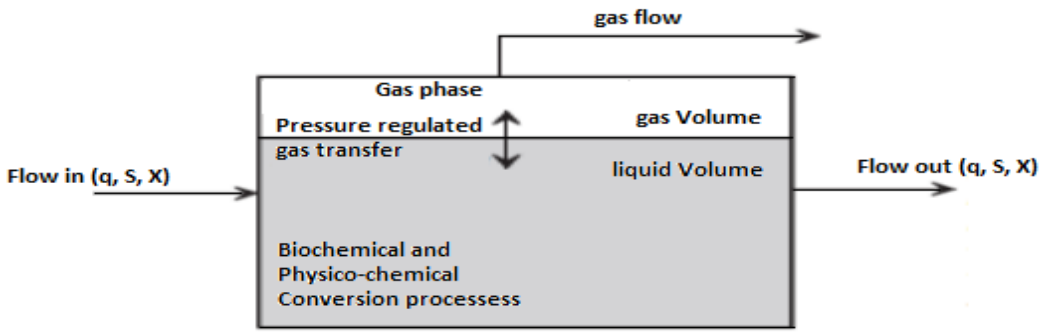


Figure 2.4: Schematic of a typical single tank reactor; where q = flow (m^3/day); S = concentrations of soluble components; X = concentrations of particulate components (Batstone *et al.* 2002)

Three basic kinetic models are commonly applied; these are Monod, First Order and Contois (Hassan and Dahlan 2013). The Monod and the First Order models are more commonly applied to predict steady state performance of anaerobic digestion, with the First Order model being considered the simplest.

2.4.4.1 Hydrolysis models

Hydrolysis rates and process models based on the kinetics of anaerobic digestion can provide an understanding of hydrolysis behaviour and ensure accurate design and

operation of anaerobic treatment of wastewater (Luo *et al.* 2012). Eastman and Ferguson (1981) developed a model for sludge digestion based on experiments with a completely stirred tank reactor (CSTR) and primary sewage sludge as substrate. Equation 2.17 defines the first order model for the rate of hydrolysis (R_h) that is generally recommended based on the Eastman and Ferguson (1981) model (Vavilin *et al.* 2008).

$$R_h = \frac{dP}{dt} = -k_h P \qquad \text{Equation 2.17}$$

Where:

R_h = rate of hydrolysis (mg/L.day^{-1})

k_h = first order hydrolysis rate constant (day^{-1})

P = concentration of degradable particulate matter (mg/L)

t = time (day)

When considering the products of particulate matter hydrolysis, the expression for rate of hydrolysis (R_h) can be presented as Equation 2.18.

$$R_h = \frac{dS}{dt} = \propto k_h P \qquad \text{Equation 2.18}$$

Where:

R_h = rate of hydrolysis (mg/L.day^{-1})

S = concentration of products (mg/L)

α = coefficient of degradable particulates conversion to products

k_h = first order hydrolysis rate constant (day^{-1})

P = concentration of degradable particulate matter (mg/L)

t = time (day)

Other models for hydrolysis have been proposed in literature, with some examples shown in Table 2.4 as described by Aldin (2010) and Morgenroth *et al.* (2002).

Table 2.4: Hydrolysis rate models (Aldin 2010; Morgenroth *et al.* 2002)

Model	**Name**
k_hP	Chemical first order
k_hPB	Biological first order
$k_hPB^{0.5}$	Half order biomass kinetics
k_hPB^A	A order biomass kinetics
$k_hPB/(K_s + P)$	Michaelis-Menten equation
$\alpha_{max}PB/[Y(K_s + P)]$	Monod equation
$k_hPB/(K_sB + P)$	Contois model
$k_hPB/[(K_s + P)(K_B + B)]$	Two phase model
$[v^2_{max} + k_h(P_o - P)]^{0.5}$	Step diffusion equation
k_hP_{surf}	Surface based kinetics model
Where: A = exponent in any A order biomass kinetic equation, B = concentration of biomass or enzyme (mol/L), K_B = saturation constant for biomass or enzyme (mol/L), k_h = hydrolysis rate constant ($hour^{-1}$) K_s = saturation constant for the substrate (mol/L), P = substrate concentration (mol/L), P_o = initial substrate concentration (mol/L), P_{surf} = surface area of the organic solid (cm^2), v_{max} = maximum hydrolysis rate (mol/L.hour), α_{max} = maximum specific growth rate ($hour^{-1}$)	

Most of the models proposed in literature are considered to have a major limitation, which is they are usually based on specific experimental conditions, for example very high or very low substrates to microorganism ratio (Aldin 2010). In a comparison of hydrolysis kinetic models, Vavilin *et al.* (1996) concluded that their experimental data fits all the tested hydrolysis models comparatively well and

therefore the application of first-order kinetics, which is the simplest way to describe the hydrolysis rate, is acceptable.

Batch systems

The first order relationship can be used to model hydrolysis in a batch process in the form of Equation 2.19 (Vavilin *et al.* 1996).

$$P = P_o e^{-k_h t} \qquad \text{Equation 2.19}$$

Where:

P = current degradable particulate matter concentrations (mg/L)

P_o = initial degradable particulate matter concentrations (mg/L)

k_h = hydrolysis rate constant (day^{-1})

t = time (day)

To obtain a linear plot of the hydrolysis process, Equation 2.19 can be converted to Equation 2.20 (Dyar and Notari 1998) and this is expected to provide a negative slope that corresponds to the hydrolysis rate.

$$Ln(P) = Ln(P_o) - k_h * t \qquad \text{Equation 2.20}$$

Where:

P = current degradable particulate matter concentrations (mg/L)

P_o = initial degradable particulate matter concentrations (mg/L)

k_h = hydrolysis rate constant (day^{-1})

t = time (day)

A linear plot of the substrate biological reduction data over time can lead to a determination of the hydrolysis rate and the hydrolysis rate constant (Dyar and Notari 1998), but the non-linear least squares fit method is a more accurate method for evaluating the hydrolysis rates and biological reduction from batch and continuous experiments. However, the linear plot, Equation 2.20, can indicate possible deviations from the first order kinetics that hydrolysis is expected to fit. Taking into consideration the non-biodegradable component of the substrates, Equation 2.19 then becomes Equation 2.21.

$$P = P_o(1 - f_h) + f_h P_o e^{-k_h t} \qquad \text{Equation 2.21}$$

Where:

P = concentration of total substrate (mg/L)

P_o = initial concentration of total substrate (mg/L)

f_h = biodegradable fraction of substrate

k_h = hydrolysis rate constant (day^{-1})

t = time (day)

For simplicity of calculations, the linearized version of Equation 2.21 is given as Equation 2.22.

$$\ln\left[\frac{(P - P_o(1-f_h))}{P_o f_h}\right] = -k_h t \qquad \text{Equation 2.22}$$

Similarly when the products of hydrolysis are considered, Equation 2.23 can be applied (Veeken and Hamelers, 1999).

$$S = S_o + \propto P_o(1 - e^{-k_h t}) \qquad \text{Equation 2.23}$$

Where:

S = concentration of products (mg/L)

S_o = initial concentration of products (mg/L)

α = coefficient of degradable particulates conversion to products

k_h = hydrolysis rate constant (day^{-1})

P_o = initial concentration of degradable particulate matter (mg/L)

t = time (days)

Luo *et al.* (2012) observed enhanced WAS hydrolysis at 50ºC and with conversions rates of the substrates to products corresponding to Equation 2.23, Equation 2.24.

$$S = S_o + 0.266P_o(1 - e^{-0.442t}) \qquad \text{Equation 2.24}$$

Based on the components of Equation 2.24, the hydrolysis rate constant for the system is 0.442 day^{-1} and the conversion coefficient α is 0.266. It is possible to determine k_h and α for process conditions based on experimental data and the proposed models using regression analysis (Dyar and Notari 1998).

Continuous flow systems

For continuous flow in completely mixed reactors, Vavilin *et al.* (2008) and Sanders (2001) proposed a steady state first order kinetics expression for hydrolysis, Equation 2.25 for degradation of the particulates and Equation 2.26 for the products from hydrolysis.

$$P = \frac{P_o}{(1+HRT*k_h)} \qquad \text{Equation 2.25}$$

$$S = \frac{\propto*HRT*k_h}{1+HRT*k_h} \qquad \text{Equation 2.26}$$

Where:

P = concentration of total substrate (mg/L)

P_o = initial concentration of total substrate (mg/L)

S = concentration of products (mg/L)

k_h = hydrolysis rate constant (day^{-1})

α = coefficient of degradable particulates conversion to products

HRT = hydraulic retention time (day)

The first order relationship in Equation 2.25 can be transformed with consideration for the non-biodegradable solids and expressed as Equation 2.27.

$$P = \frac{P_o f_h}{(1+HRT*k_h)} + P_o(1-f_h) \qquad \text{Equation 2.27}$$

Where:

P = concentration of substrate (mg/L)

P_o = initial concentration of substrate (mg/L)

f_h = biodegradable fraction of substrate

k_h = hydrolysis rate constant (day^{-1})

HRT = hydraulic retention time (day)

The linearized version of Equation 2.27 is given as Equation 2.28.

$$HRT = P_o f_h \frac{HRT}{(P_o - P)} - \frac{1}{k_h} \qquad \text{Equation 2.28}$$

Researchers have also considered the effect of VFA inhibition to hydrolysis and proposed a rate correction factor on hydrolysis rates to account for influence of VFA concentrations (Vavilin *et al.* 2008). After testing the first-order and Monod models on observed experimental data without getting a significant fit, Llabres-Luengo and Mata-Alvarez (1988) proposed a model with a hydrolysis rate proportional to the substrate and biomass concentrations, and inversely proportional to the VFA concentration. However, according to Veeken and Hamelers (1999), hydrolysis rate constants determined based on first-order relationships have indicated statistically insignificant effects by VFA concentrations, but influence by pH was observed.

2.4.4.2 Hydrolysis rates

The hydrolysis rates reported in literature are based on specific experimental conditions (Feng *et al.* 2009). Secondary sludge has observed degradation rates half the rates reported for primary sludge and the performance of the anaerobic process will be influenced accordingly based on the nature of the substrate used as feedstock (Appels *et al.* 2008; Mottet *et al.* 2010). Values of rate coefficients for different

substrates according to Aldin (2010) are summarized as primary sludge (0.0096 – 1.94 day^{-1}), sewage sludge (0.005 – 0.2 day^{-1}) and for most types of sludge (0.08 – 2.0 day^{-1}). Other researchers, specifically Eastman and Ferguson (1981), Batstone *et al.* (2002) and Siegrist *et al*. (2002) have observed hydrolysis rates for primary sludge between 0.2 - 0.5 day^{-1} at mesophilic conditions, Castillo *et al.* (1999) also reported similar rates for particulate COD in wastewater.

According to Aldin (2010) the constants have been observed between 0.0096 – 0.17 day^{-1} for lipids and proteins, and 0.21 – 1.94 day^{-1} for carbohydrates in primary sludge. Veeken and Hamelers (1999), operating at 30ºC determined the first-order hydrolysis rate constants for several bio-wastes and obtained values in the range of 0.076 – 0.264 day^{-1}. In another set of experiments, the first-order hydrolysis rate constant ranging from 0.06 - 0.24 (day^{-1}) was obtained (Veeken and Hamelers 2000). At 30ºC, Brummeler *et al.* (1991: cited in Veeken *et al.* 2000) obtained rate constants that are two to five times smaller (0.038 - 0.048 day^{-1}) for bio-waste. Kassab *et al.* (2013) calculated hydrolysis rate constants based on first order kinetics as 0.006 day^{-1} with $R^2 = 0.877$ for seeded domestic wastewater sludge and 0.004 day^{-1} with $R^2 = 0.873$ for unseeded domestic wastewater sludge. These constants are low when compared with those calculated by Mahmoud (2002) for settleable solids from domestic wastewater at 35°C (0.23 day^{-1}).

The wide range of the values reported in literature is due to different experimental conditions and biomass-to-substrate ratios. The low rate constants calculated by Kassab *et al.* (2013) represent low anaerobic biodegradability and may be attributed to the excessive use of detergents in the study area. Some researchers, Nielsen

(2005), Lee Ferguson and Brownawell (2003) and Jimenez-Gonzalez *et al.* (2001), have reported poor anaerobic degradation due to detergents, mainly as a result of process inhibition (Mensah and Foster 2003; Hernandez-Leal *et al.* 2011).

2.4.4.3 Methanogenesis models

With a known composition of organic matter, the theoretical methane yield potential can be calculated from the Buswell's equation as expressed in Equation 2.29 if all the material is converted to biogas (Angelidaki and Sanders 2004).

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right)CO_2$$

Equation 2.29

The methane yield can be calculated if the composition of the substrate is known based on the mass of volatile substrate and oxygen demand. If based on oxygen demand, then a gram of oxygen demand removed could theoretically produce 350 mL of methane for all substrates (Angelidaki and Sanders 2004). The theoretical yield gives a rough potential for biogas production from any substrate, but factors such as the use of some of the substrate for synthesizing bacterial biomass are not considered in the calculations (Labatut *et al.* 2011). Also, there is often a part of the substrate that is inaccessible due to limited surface areas, and there can also be limitations or inhibitions by other factors such as temperature, pH or nutrients (Vavilin *et al.* 2008).

For an identified organic substrate, the volume of methane produced per mass of organic substrate added, the specific methane produced, can be determined with

Equation 2.30 using data from biochemical methane potential experiments (Hansson *et al.* 2013).

$$B_s = \frac{B}{X_{added}} \qquad \text{Equation 2.30}$$

Where:

B_s = specific methane produced (mL/gram of substrate added)

X_{added} = organic substrate added (gram)

B = methane produced (mL)

If part of the substrate that is not reduced in the digestion process is considered, Equation 2.31 defines the ultimate methane produced which is the actual volume of methane produced per mass of substrate removed.

$$B_u = \frac{B}{X_{removed}} \qquad \text{Equation 2.31}$$

Where:

B_u = ultimate methane produced (mL/gram of substrate removed)

$X_{removed}$ = organic substrate removed (gram)

B = methane produced (mL)

Alternatively, the specific methane yield can be determined using Equations 2.19 and 2.23 for batch systems, and Equation 2.26 for continuous systems, with methane as the products of the digestion of the substrates.

2.4.5 Relationship between temperature and anaerobic digestion

The overall effect of temperature on the hydrolysis and digestion of particulate wastes is based on its effect on enzyme kinetics, reaction rates and pathways, rates of bacterial growth and decay, and solubility of the substrate (Bergamo *et al.* 2009; Appels *et al.* 2008; Yuan *et al.* 2011). A stable temperature is important for the microorganisms because once adapted, they can only tolerate very small changes in environmental conditions and major increases or drops in temperature will affect the performance of anaerobic microorganisms (Gao *et al.* 2011; Lu 2006).

Microorganisms can be grouped into three categories based on temperature (Gao *et al.* 2011), psycrophiles (temperatures below 20ºC), mesophiles (temperatures 25 - 40ºC) and thermophiles (temperatures higher than 45ºC). Figure 2.5, from Madigan *et al.* (2012), shows the relationships between temperature and growth rate for different categories of microorganisms.

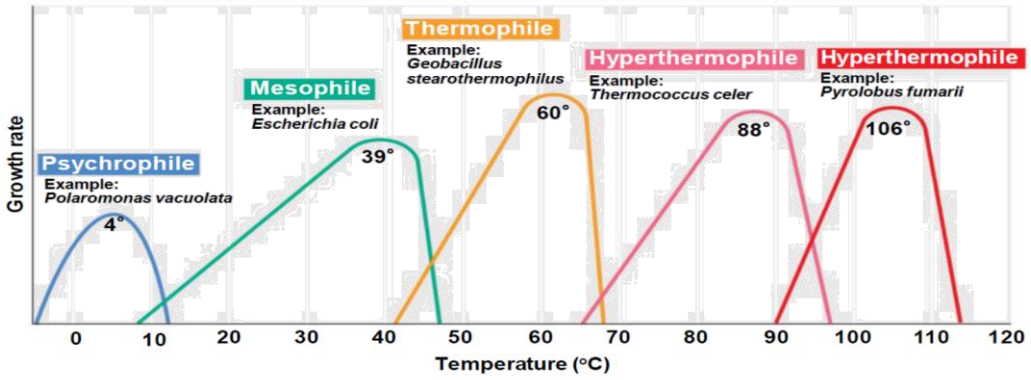


Figure 2.5: Relationship between bacterial growth rate and temperature (Madigan *et al.* 2012).

Fluctuations in environmental temperature are harmful to the microorganisms in the digestion process, especially the methanogenic bacteria (Gao *et al.* 2011). Bacteria that are mesophilic can withstand variations in temperature over a 30ºC range

without major changes to their activity, but a stable temperature is important for adaptation and sustained efficiency in treatment systems (Donoso-Bravo *et al.* 2013). The various temperature ranges for biological processes have advantages and disadvantages but in terms of operational stability, the mesophilic range is generally favoured (van Lier *et al.* 2001). For processes in the mesophilic range, less operational energy is required to maintain these temperatures and there is usually high process stability due to the diversity of the organisms.

Apart from its influence on the microorganisms, any major change in temperature usually causes a change of the physical and chemical properties of wastewater (Mrowiec and Suschka 2006; Bergamo *et al.* 2009), for example as temperature levels drop below 20°C the solubility of gases increases, causing a high concentrations of gases like methane, hydrogen sulphide and hydrogen in the effluent of low temperature reactors. Figure 2.6 presents saturation dissolved methane concentrations by Watanabe *et al.* (2014) at monitored temperatures based on the percentage of biogas that is methane.

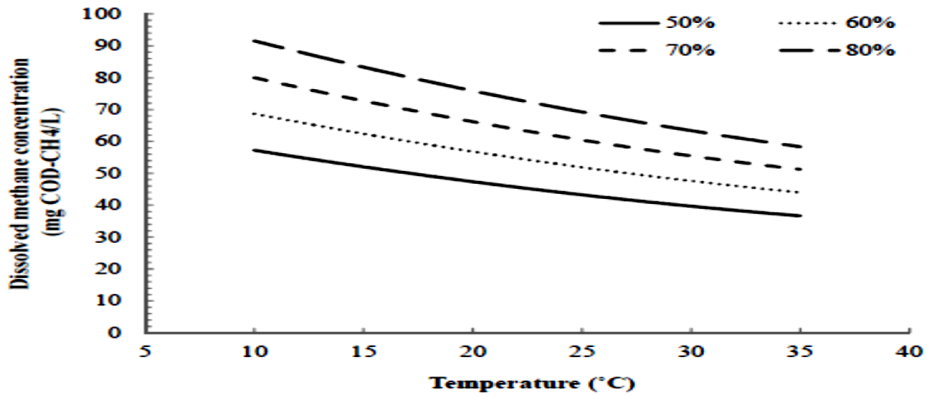


Figure 2.6: Reported dissolved methane concentrations in mg COD/L based on temperature in terms of percentage of biogas that is methane (Watanabe *et al.* 2014).

From Figure 2.6, the concentration of methane that remains dissolved has been observed to increase with decreasing temperature. Viscosity of liquids is also influenced by temperature; therefore different energy requirements for mixing will exist depending on the operational temperature (Lettinga *et al.* 2001). In general it is expected that at ambient temperature, for every 10°C increase in temperature there is a doubling of chemical reaction rates (Sanders 2001). This relationship is usually expressed by Equations 2.32 and 2.33, the Arrhenius equation, and has proven reliable for determination of the relationship of reaction rates to temperature changes (Dyar and Notari 1998).

$$k = A * e^{(-\frac{E_a}{RT})} \qquad \text{Equation 2.32}$$

$$\ln k = -\frac{E_a}{RT} + lnA \qquad \text{Equation 2.33}$$

Where:

k = kinetic rate constant (day^{-1})

E_a = activation energy (kJ/mol)

A = pre-exponential factor (day^{-1})

R = gas constant (kJ/mol.Kelvin)

T = absolute temperature (Kelvin)

The activation energy is considered to be the least amount of energy needed for the anaerobic reaction (Peleg *et al.* 2012). Feng *et al.* (2009) observed a linear relationship between the ln k and temperature for the biodegradation of waste activated sludge, where for a temperature influenced rate change that obeys the Arrhenius equation, a plot of ln k versus T^{-1} gives a straight line where the slope and

the intercept can be used to determine E_a and A. Reported activation energy values in literature for anaerobic hydrolysis are between 15 to 70 kJ/mol (Sanders 2001; Dyar and Notari 1998); however the pre-exponential factor values reported have not shown any trend (Feng *et al.* 2009; Dyar and Notari 1998).

2.5 Summary of key outcomes from the literature review

Despite high treatment efficiencies, conventional aerobic wastewater treatment systems are not sustainable wastewater treatment options due to the high energy requirements when compared to anaerobic systems (Chan *et al.* 2009). Sustainability of a wastewater system can be evaluated with various tools, such as energy audits, energy quality analysis, or through life cycle assessment (LCA) methodologies (Remy *et al.* 2011). These methods are based on selected energy efficiency criteria based on defined benchmarks, for example the average energy consumption per volume of wastewater treated (kWh/m^3) or per mass of organic loading removed (kWh/gram) through the treatment system (Remy *et al.* 2011). Conventional aerobic treatment systems, such as activated sludge plants, have been reported as having high energy benchmarks for aeration and pumping (Water environment federation 2009). Normally, up to 50% of the total energy consumption of an activated sludge treatment system is used in the aeration and pumping process (Lazarova *et al.* 2012)

The pragmatic approach towards energy sustainability in wastewater treatment should be based on the need to minimize energy consumption and maximize energy recovery from the wastewater stream (Mo and Zhang 2013). High rate anaerobic digestion (AD) systems are widely accepted as energy efficient alternatives to conventional aerobic technologies, but they have been observed to fail in meeting

effluent discharge standards (Gomec 2010). Combined processes, in the form of anaerobic-aerobic systems, offer the advantages of the two biological processes without the disadvantages, where the initial anaerobic units offer energy recovery, which can be utilized by the aerobic process to ensure high effluent quality (Chan *et al.* 2009). High rate anaerobic reactors are currently considered as viable anaerobic-aerobic systems with the potential for short (< 48 hours) retention periods and low land and energy requirements (Demirel *et al.* 2010). The next developmental step is to enhance each stage of the anaerobic-aerobic system by ensuring an ideal system sizing in order to improve COD removal rates.

Due to the reported low energy recovery rates, below 50% of organic carbon energy, by systems treating domestic wastewater (Verstraete *et al.* 2009), operation of domestic wastewater treatment systems is expected to become energy efficient if heating is avoided and the treatment processes occur at ambient temperature (Foresti 2002). At low temperatures, the compartmentalisation of anaerobic reactors might enhance the hydrolysis of less readily degradable substrates due to the low pH that can be obtained in the front of the reactor (Schiener *et al.* 1998). The ABR is a high rate anaerobic system suitable for low energy domestic wastewater treatment due to its reported high energy efficiency (Shoener *et al.* 2014) and its capability to be developed as an integrated bioreactor with anaerobic and aerobic zones using its compartmental nature. Unfortunately, there are very few design guidelines available in literature relating the physical configuration and operational conditions to influent loading and treatment efficiency, therefore the design of ABR systems is still generally based on the experience of the practitioner (Section 2.3.3).

Contact between the organic substrates and biomass is an important consideration in design of the ABR, and there are no design details relating the retention of biomass, number of compartments, HRT, OLR and performance efficiency. Bodkhe (2009) adopted nine compartments and reported 85% organic loading removal efficiency with 6 hour HRT, while Nachaiyasit and Stuckey (1995) adopted eight compartments with a reported 90% removal efficiency. However, most researchers adopted an ABR design with a maximum of five compartments, therefore analysis of the anaerobic digestion process kinetics in the ABR, with a view toward identification of conditions for efficient operation at ambient temperature is required. Particular emphasis is placed on the biological reduction of solids and methane production at low temperatures ($\leq 25^{o}C$), and the proposal of a suitable process model for a multi-stage system. Laboratory experiments were therefore designed with focus on the biological reduction of domestic wastewater sludge and methane productions at ambient temperature, and the influences of temperature and operational hydrodynamics on removal of organic contaminants and system efficiency.

Chapter Three – Experimental plan and methodology

3.0 Introduction

The anaerobic-aerobic treatment system adopted is a biological process, and the selection of an energy efficient configuration of the system requires the determination of operational conditions that are suitable for biological removal of contaminants from domestic wastewater. Therefore, the evaluation of the relationship between operational conditions and anaerobic reduction processes is the focus of the experiments in this study. A post-positivist research paradigm (Burns 2000), where the research interest is observed objectively and the observations compared to existing scientific knowledge to determine aspects that are repeatable or falsifiable, was adopted as the basis for an experimental plan. Experiments to evaluate biological reduction processes can be based on the observation of the substrates or products of the biological reaction in the system (Angelidaki and Sanders 2004; Veeken and Hamelers 1999; Miron *et al*. 2000; Angelidaki *et al*. 2009). Examples of substrates are the concentrations of total solids, volatile solids and COD/BOD/DOC. While the products are primarily process intermediates such as acid changes (pH and volatile fatty acids) and end products such as biogas production.

Two main experimental approaches can be applied, these are the batch assays, biochemical methane potential (BMP) tests, as described in Angelidaki *et al.* (2009) and Veeken and Hamelers (1999) and the continuous reactor experiments as described in Miron *et al*. (2000). For the batch assays, the selected substrate is incubated at a specific temperature with or without an inoculation of biomass suitable for the biological process. For the continuous reactor experiments, selected

parameters are monitored at steady state in a CSTR and specific temperatures and hydraulic retention times (HRT).

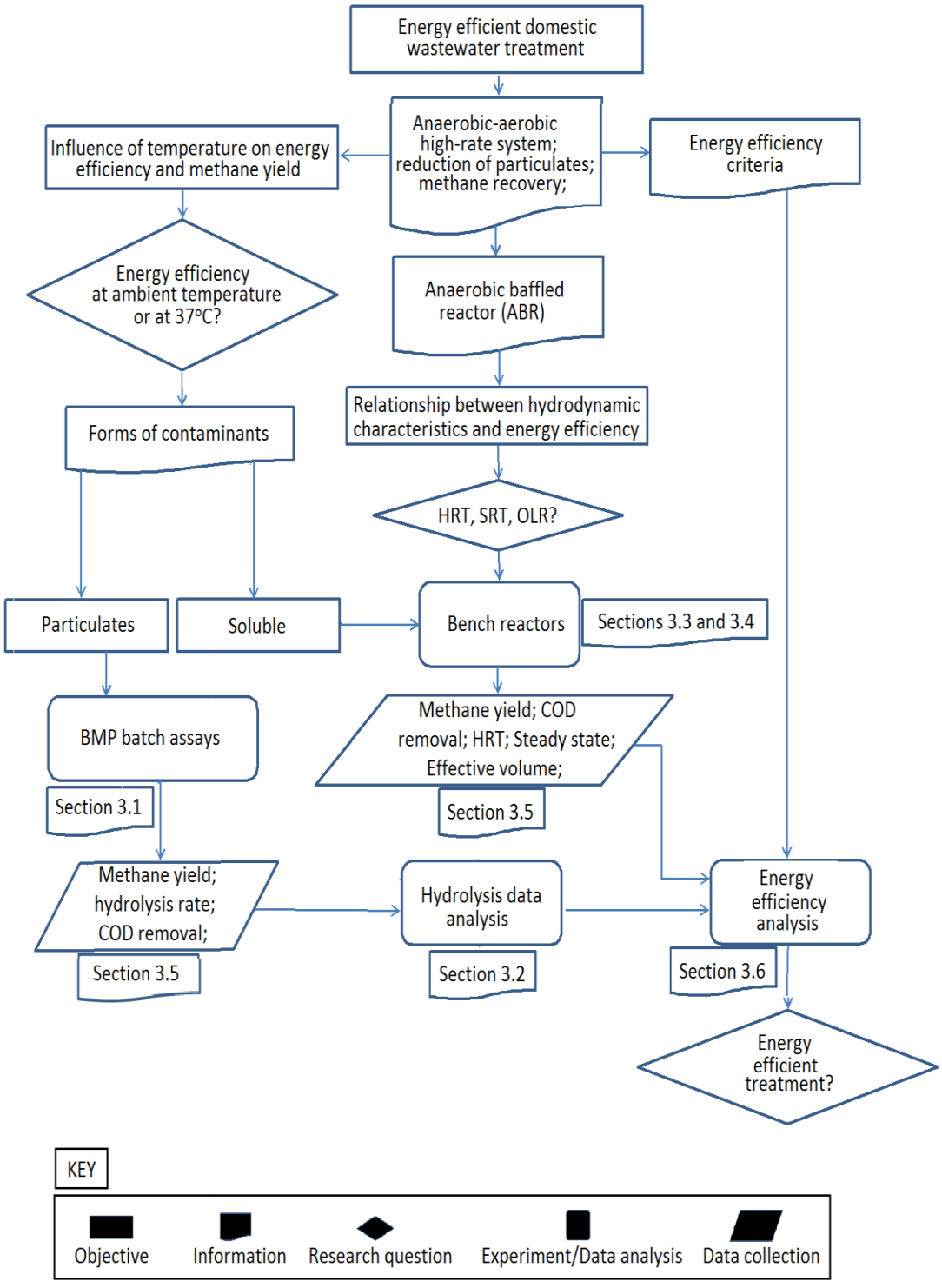


Figure 3.1: A schematic representation of the experimental plan

The experimental plan adopted for this study is schematically presented in Figure 3.1 with two main experimental groups, biochemical methane potential (BMP) batch assays and continuous reactor experiment with ABR bench models. The data from these experimental groups were then used in an energy analysis using identified energy efficiency criteria. The methodology adopted for each experimental group was aimed at developing an understanding of the relationship of temperature with anaerobic digestion processes in the anaerobic baffled reactor (ABR). The BMP assay experiment, methodology described in Section 3.1, focused on the evaluation of the influence of temperature on anaerobic reduction and hydrolysis of domestic wastewater sludge, and also the characteristics of methane production. The data obtained from the reduction and hydrolysis of domestic wastewater sludge was then analysed using non-linear regression as described in Section 3.2 to evaluate the data fit to the hydrolysis models presented in Section 2.4.4.1.

The ABR bench experiments, methodologies described in Sections 3.3 and 3.4, focused on the evaluation of the influence of temperature on the organic loading removal, methane production and hydrodynamic properties of a model ABR. Sample collection and parameter analysis methodologies used in the two experimental groups are described in Section 3.5. The data obtained from the experiments were then analysed using energy efficiency evaluation criteria presented in Sections 3.6, towards proposing a model system for low energy treatment of domestic wastewater.

3.1 Biochemical methane potential (BMP) assays

For this study, in order to evaluate the biological reduction of domestic wastewater sludge and corresponding methane production at ambient temperature, the BMP assays were performed at an ambient temperature of 25ºC and compared against a mesophilic temperature of 37ºC.

Substrates

Two solid substrates were adopted for the BMP assays, primary sludge from the primary settling tank of Meigle wastewater treatment plant, Scotland and secondary sludge from the clarifier of Ardler wastewater treatment plant, Scotland. Both treatment plants have a catchment that consists of only small residential areas, and are located within a radius of 10 km from Abertay University, Dundee. Initial characteristics of the sludge samples are provided in Table 3.1.

Table 3.1: Characteristics of solid substrates

Parameter	Primary sludge	Secondary sludge
Total solids (g/L)	28.96	32.11
Volatile solids (g/L)	19.43	21.05
Volatile fatty acids (mg/L)	359.30	240.10
pH	5.98	6.22

Inoculum

Anaerobic biomass, sourced from the anaerobic digester of Hatton wastewater treatment plant in Arbroath, Scotland, was used as microbial inoculum for all the assays with solid substrates. The characteristics of the sludge were: TS = 18.38 g/L and VS = 9.06 g/L.

Nutrient medium

A nutrient medium was added as a source of micronutrients and trace metals necessary for growth of microorganisms; this was a solution containing several minerals without any significant amount of organic carbon dissolved in distilled water (Angelidaki and Sanders 2004). The composition of the nutrient medium was: 75 mg/L Ammonium Bicarbonate (NH_4HCO_3), 400 mg/L Potassium Dihydrogen Phosphate (KH_2PO_4), 5.0 mg/L Magnesium Sulphate ($MgSO_4$), 5.0 mg/L Iron (III) Chloride ($FeCl_3$), 5.0 mg/L Calcium Chloride ($CaCl_2$), 5.0 mg/L Potassium Chloride (KCl), 1.0 mg/L Cobalt (II) Chloride ($CoCl_2$), 1.0 mg/L Nickel Chloride ($NiCl_2$) and 2,000 mg/L Sodium Bicarbonate ($NaHCO_3$).

Preparation of the BMP batch assays

500 mL bottles, sealed with thick rubber septum and aluminium caps, were used for the assays according to the details provided in Table 3.2, where each mixture was incubated in duplicate bottles, except for the assays without inoculation where only single bottles were used.

Table 3.2: 350 mL BMP assays for domestic wastewater sludge

ID	Temp. (°C)	Substrate volume (mL)	Inoculum volume (mL)	Nutrient solution volume (mL)
PS 37°C	37	Primary sludge 150	Anaerobic biomass 100	100
PS 25°C	25	Primary sludge 150	Anaerobic biomass 100	100
PS nol 37°C	37	Primary sludge 150	-	200
PS nol 25°C	25	Primary sludge 150	-	200
SS 37°C	37	Secondary sludge 150	Anaerobic biomass 100	100
SS 25°C	25	Secondary sludge 150	Anaerobic biomass 100	100
SS nol 37°C	37	Secondary sludge 150	-	200
SS nol 25°C	25	Secondary sludge 150	-	200
Blank	37	-	Anaerobic biomass 100	250
Blank	25	-	Anaerobic biomass 100	250

4000 mL of the nutrient solution was prepared and divided into three containers, to account for the three different assay conditions. 1500 mL for the blanks, 1000 mL for the assays with inoculation and 1500 mL for the assays without inoculation. For the assays with inoculation, 1000 mL of the anaerobic biomass inoculum was measured using graduated cylinders, in order to have 100 mL anaerobic biomass per bottle. The inoculum was mixed with the 1000 mL nutrient solution, and thereafter the 2000 mL mixture was divided into two 1000 mL volumes for the two substrates. 750 mL of the primary sludge was measured and carefully mixed with the 1000 mL inoculum + nutrient solution mixture, and similarly, 750 mL of the secondary sludge was mixed second 1000 mL inoculum + nutrient solution mixture.

For the blank assays, the 1500 mL nutrient solution was mixed with 600 mL of the anaerobic biomass inoculum. For the assays without inoculation, the nutrient solution was divided into two 600 mL volumes, and 450 mL of the primary sludge was mixed into the first container, while 450 mL of the secondary sludge was mixed into the second container. The pH values of the final mixtures were adjusted by carefully adding a few drops of a 10M Sodium Hydroxide (NaOH) solution to each mixture until the pH reading was between 7.51 and 7.88. Then into carefully labelled bottles, 350 mL of the mixtures were measured allowing for a headspace of 210 mL in order to avoid pressure build-up in the bottles once methane production has started. The bottles were capped and the headspace was flushed with pure Nitrogen gas for 2 min to induce anaerobic (oxygen free) conditions, and then placed in incubators. The ratios of the mass of volatile solids in the substrates to the mass of volatile solids in the inoculum in the inoculated assays were 3.21:1 for the primary sludge assays and 3.47:1 for the secondary sludge assays.

3.2 Analysis of solid substrates reduction data

Regression analysis of observed solids reduction data during the BMP assays with domestic wastewater sludge, Section 3.1, was carried out for Equation 2.21 (Section 2.4.1.1) using non-linear least squares fit method with the Matlab curve fitting toolkit (Matlab R2013a student version, MathWorks, Cambridge, UK). Statistical analysis of the data fit to the model tested was carried out using R^2, the sum of squares due to errors (SSE) and root mean squared error (RMSE). R^2 indicates the square of the correlation between the predicted model values to the initial observed values (Palmer and O'Connell 2009), while RMSE is the root-mean-square error, which is a measure of the differences between values predicted by the model and the values observed

(Willmott *et al.* 1985). SSE is the sum of squares due to error, which measures the total deviation of the predicted values to the observed values.

3.3 ABR bench experiments

The bench reactors design was based on an initial reactor design from Baloch *et al.* (2008) and Shanmugam and Akunna (2008), comprising five equal compartments in series with a total effective treatment volume of 8.75 litres (1.75 litres for each compartment). Each compartment was divided into equal up-comer and down-comer volumes using baffles as represented in Figure 3.2. Modifications were adopted in order to overcome operation and performance issues highlighted in Chapter Two, Section 2.3. In order to influence solid retention and enhance hydrolysis, the model design adopted provided for an additional 1st compartment that is larger than the subsequent compartments as recommended by Boopathy and Sievers (1991), and therefore the effective treatment volume was increased to 17.5 litres. Appendix E presents the design details of the bench reactors.

In order to have a reactor unit that can fit on the available laboratory work space, the 1st compartment was placed behind the other compartments during fabrication, as shown in Figure 3.2 (b), and a 12 mm diameter tube was used as connection.

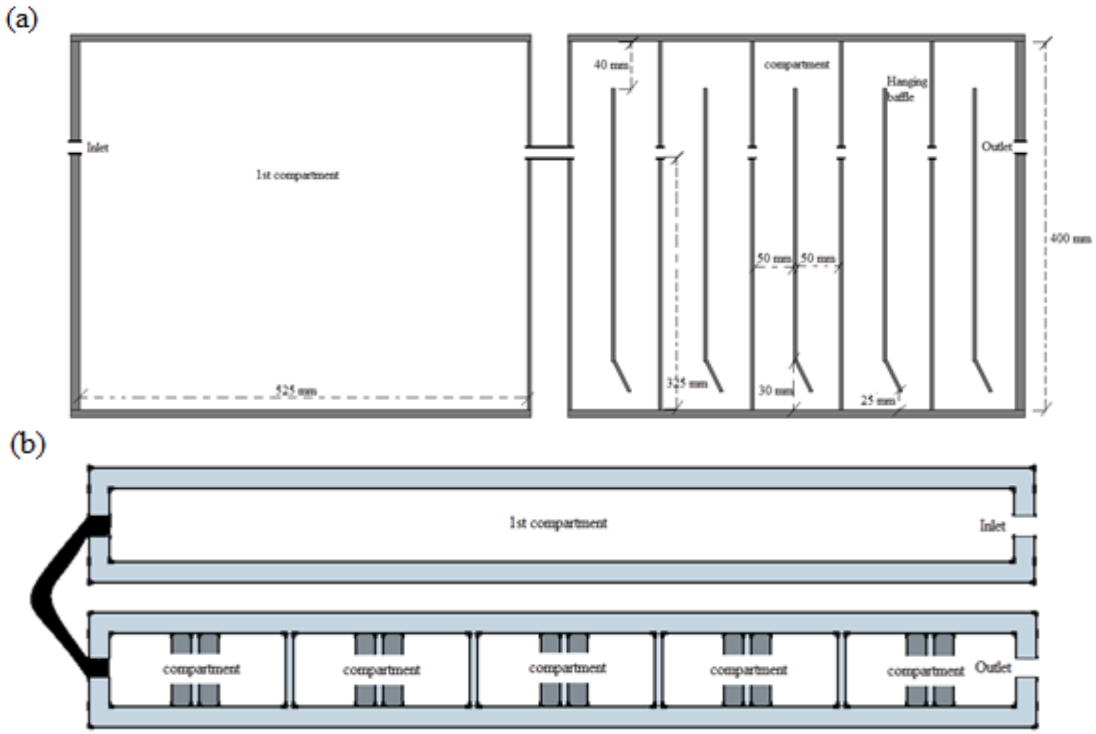


Figure 3.2: A schematic representation of the bench reactors design (not drawn to scale), with (a) – cross section of the compartments; and (b) – bench layout;

Materials used for the models fabrication were 10 mm thick acrylic sheets and glue, where the sheets were formed to sections and then glued together to ensure water and air tightness. 12mm diameter holes were threaded at various locations to provide for sample collection from the six compartments and also to provide for biogas outlets from the top of the compartments. The 6 sampling points for liquid samples are located in the centre of the up-comer sections, 20 mm below the outlet of each compartment.

Laboratory bench set-up

The laboratory bench set-up adopted is as indicated in Figure 3.3, with the two bench reactors receiving the same feed at a set organic loading rate using Masterflex variable speed peristaltic pumps (Cole-Parmer UK).

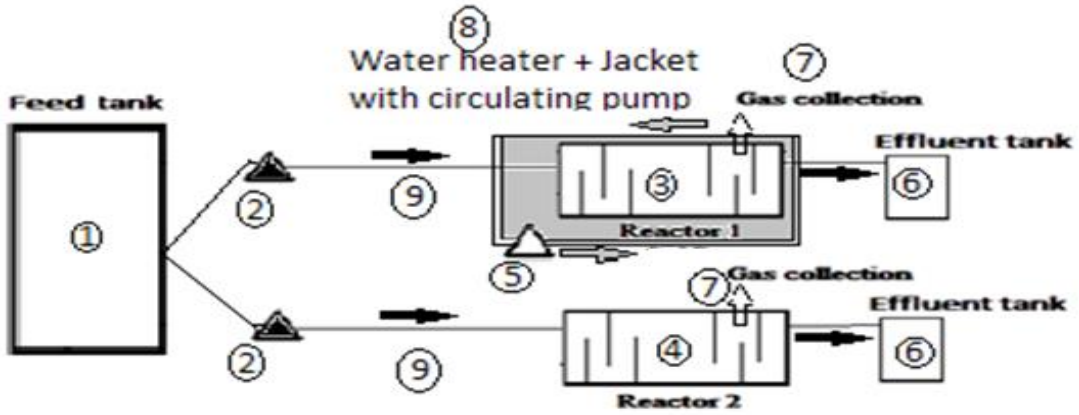


Figure 3.3: laboratory bench set-up. The major items in the set-up include: (1) a feed mixing tank, (2) variable peristaltic pumps; (3) Reactor 1 set at 37±3ºC; (4) Reactor 2 at ambient temperature; (6) Effluent collection tanks; (5) Water jacket circulator pump; (7) Gas collection and measurement; and (8) Water heater and jacket for temperature control.

Reactor 1 (R1) was operated at a temperature of 37 ± 3ºC, while Reactor 2 (R2) was operated at ambient temperature (mean = 22ºC, range = 17 – 25ºC) without temperature control.

Inoculation of the bench reactors with anaerobic biomass

For each of the two bench reactors, 40% of the volumes (3.5 L) of the 2nd – 6th compartments were inoculated with anaerobically digested sludge sourced from Hatton wastewater treatment plant, while the 1st compartments were not inoculated. The inoculum was initially conditioned and degassed by incubating at 37ºC prior to inoculation. The characteristics of the inoculum were: pH = 7.02, VFA = 90 mg/L, TS = 25.21 g/L and VS = 14.29 g/L.

Organic loading

Loading for the two reactors was from the same feed tank and start-up was with an organic loading rate (OLR) of 1.25 kg COD/m^3.day, which was subsequently

adjusted in order to evaluate the bench reactors at the hydraulic retention times identified in Table 3.3.

Table 3.3: Applied HRT and OLR in the bench reactors

S. No.	HRT (hours)	OLR (kg COD/m^3.day)	Flow rate (m^3/day)
1	48	1.25	0.009
2	36	1.67	0.011
3	24	2.50	0.017
4	12	5.00	0.034
5	6	10.00	0.068

To set the operating conditions to a specific HRT and OLR, the settings of the peristaltic pumps were gradually adjusted, in order to avoid turbulence, by changing the peristaltic pump speed until the desired flow rate was achieved.

Operation

The two ABR bench models were operated simultaneously during the period of March to October of 2013. After the initial inoculation and start-up, the system was operated for 45 days before analysis was performed due to the reported start-up periods in literature (Barber and Stuckey 1999; Bodkhe 2009; Nachaiyasit and Stuckey 1997). Steady state was determined by monitoring variation of pH values in the compartments of the two bench reactors after operation for five times the duration of the design HRT. If the variation of the pH in each compartment remained within a range of ±0.2 over a three days period, the system was considered to have achieved steady state performance.

Ghaniyari-Benis *et al.* (2009) considered stable VFA and COD values, with a 5% range for variations, as indication of steady state performance. Similarly, Martin *et*

al. (2010) adopted a period 6 – 7 times the operational HRT and stable values for parameters with a 5% range for variation of measurements over 5 consecutive days as the conditions for steady state performance. Bodkhe (2009) considered steady state performance was achieved when five consecutive measurements for parameters were consistently similar, while Zhu *et al.* (2008) considered steady state performance was achieved when consistent measurements were obtained from four different samples.

Other researchers, Yu *et al.* (2014) and Feng *et al.* (2008), considered stable pH values with less than 5% variation as indications of steady state performance. Kayranli and Ugurlu (2011) considered the first two months of their experiment as an acclimatization period before steady state performance was achieved, while Fernandez *et al.* (2008) and Akhbari *et al.* (2011) considered only a period of five times the operational HRT as necessary for achieving steady state performance. For this study, once stable pH values were observed, the biomass was assumed to have acclimatized to the organic loading rate. Samples were then collected from each compartment and analysed for the concentrations of VFA, COD and solids using methods described in Section 3.5.

Operation with incremental changes of HRT – Reactor 3 (R3)

As an alternative to acclimatization of the biomass for determination of loading rates adjustments, another bench experiment was performed using incremental adjustments of OLR and HRT after a fixed 10 days operational period at 37ºC. This experiment is presented as Reactor 3 (R3) in Section 5.2, with a similar start-up period to Reactors 1 and 2, where anaerobic biomass was inoculated into the last five

compartments of the reactor before introduction of the synthetic feed. The HRT and OLR were adjusted after every ten days of operation, and the organic loading removal efficiencies were monitored for the HRTs defined in Table 3.3.

Feed

The feed used for this study is based on a synthetic feed with characteristics from Shanmugam and Akunna (2008), Gopala-Krishna *et al.* (2009) and Ghaniyari-Benis *et al.* (2009). The characteristics of the influent feed were: mean COD = 2479.50 mg/L (range = 2150.00 – 2727.00 mg/L); mean VFA = 98.50 mg/L (range = 64.00 – 148.00 mg/L); and mean pH = 7.42 (range = 6.91 – 7.86). Appendix A provides justifications behind the choice of pure cane molasses as a carbon source and the composition of the feed adopted for this research.

The components of the feed were: 2.8 g/L pure cane molasses, 75 mg/L Ammonium bicarbonate (NH_4HCO_3), 400 mg/L Potassium dihydrogen phosphate (KH_2PO_4), 1 mg/L Magnesium sulphate (Epsom salts) ($MgSO_4$), 1 mg/L Iron (III) chloride (Ferric chloride) ($FeCl_3$), 1 mg/L Calcium chloride ($CaCl_2$), 1 mg/L Potassium chloride (KCl), 0.2 mg/L Cobalt (II) chloride ($CoCl_2$), 0.2 mg/L Nickel chloride ($NiCl_2$) and 2 g/L Sodium bicarbonate ($NaHCO_3$). The feed was prepared daily for the long retention times (48, 36 and 24 hours), and twice daily for the short retention times (12 and 6 hours).

3.4 Determination of hydrodynamic characteristics

The hydrodynamic characteristics of the ABR bench models were evaluated at ambient temperature in order to compare against what has been reported in literature,

so as to properly analyse the energy efficiency of the selected system. Section 2.3.3 identified the hydrodynamic properties that are important in the design of the anaerobic baffled reactor. The characteristics evaluated were the mean residence time, flow dispersion, volume that is dead space, equivalent tank in series number and hydraulic efficiency using data obtained from residence time distribution (RTD) studies using flow experiment with tracer. The retention of solids at 37ºC and ambient temperature were also evaluated.

Preparation of working solution of tracer dye

For this research, in a clean reactor state, a red Rhodamine WT dye, 25 g/L liquid solution supplied by Cole-Parmer UK, was used as the tracer. The required tracer concentration was prepared by diluting concentrated tracer solutions with distilled water. To get the working concentration, the dilution procedure consisted of serial dilutions of the initial concentrated dye solution. The volume of distilled water required to achieve the dilution to the working concentration was computed using Equation 3.1 (Levenspiel 1999).

$$C_n = C_i\left(\frac{V_d}{V_w+V_d}\right); \qquad V_w = V_d\left(\frac{C_i}{C_n}-1\right) \qquad \text{Equation 3.1}$$

Where:

V_w = volume of the added diluent (L)

V_d = volume of the concentrated tracer solution (L)

C_i = initial tracer concentration (mg/L)

C_n = new concentration after dilution (mg/L)

All working solutions were retained in airtight bottles and were stored in a dark cabinet to avoid degradation by UV light. To avoid concentration stratification, all final solutions were agitated until each was thoroughly mixed before they were used.

Calibration of DR 5000 spectrophotometer for tracer detection

A calibration curve was prepared for the Rhodamine WT solution used in the tracer study by measuring the light absorbance of standard dilutions of the original tracer solution. Using Equation 3.1, standard dilutions of the tracer were prepared in concentrations of 1000 mg/L, 750 mg/L, 500 mg/L, 250 mg/L and 125 mg/L. A blank solution was also prepared using only distilled water. A wavelength of 550 nm was examined during the calibration, this is the absorbance wavelength identified from recommendations in literature (Tai and Rathbun 1988; Dierberg and DeBusk 2005; Williams and Nelson 2011) for evaluation of red tracer concentrations by spectrophotometer. At 550 nm, the curve fit (R^2) after the calibration was 0.9973 for the five points, and absorbance of the 1000 mg/L standard solution was 1.30 Abs.

After the calibration at 550 nm wavelength was stored, a blank sample was measured, to determine the potential error in the method, and an average reading of 8.04 mg/L was obtained. The method was evaluated with a different set of standard solutions, and the variation between the estimated tracer concentration and the measured tracer concentration with the spectrophotometer were all within a range of ± 8.00 mg/L. Therefore, the measurement error margin for the detection method was adopted as ± 8.00 mg/L for samples with concentrations lower than 1000 mg/L.

Flow experiment with tracer

There are two methods for injecting the tracer into the system (Dierberg and DeBusk 2005), either continuously or as a pulse. In the continuous method, the tracer is injected at a constant dosage until the concentration at the system outlet reaches a steady level. In a pulse test, a controlled amount of tracer is instantaneously added to the system at the inlet of the system and samples are collected at the outlet over time as the tracer passes through the system. For this study, the flow tests were carried out using a modified pulse method where a 100 mL solution with a known mass of the tracer, Table 3.4, was injected at the inlet at the beginning of each test.

The flow tests were carried out with retention times and influent velocities designed to achieve continuous and intermittent flows conditions using peristaltic pumps and programmable timers. Three tests (tests no. 1, 6 and 7 in Table 3.4) were conducted under continuous flow conditions, therefore displacement and mixing was occurring in the reactor for the entire test duration. To maintain the corresponding residence times of 60, 180 and 240 min, the influent flow rates (and velocities) were adjusted accordingly. The flow velocity was determined based on the length of flow in the reactor around the baffles (3000 mm) and the duration of flow achieved with the pumping, Table 3.4.

Table 3.4: RTD tests

Test	Flow condition	HRT (min)	Flow duration (min)	Flow rate (m^3/min)	Flow velocity (m/min)	Tracer injected (mg)
1	Continuous	60.00	60.00	1.46E-04	0.050	1000.00
2	Intermittent	180.00	90.00	9.72E-05	0.033	800.00
3	Intermittent	180.00	60.00	1.46E-04	0.050	800.00
4	Intermittent	180.00	45.00	1.94E-04	0.067	400.00
5	Intermittent	180.00	60.00	1.46E-04	0.050	800.00
6	Continuous	180.00	180.00	4.86E-05	0.017	1200.00
7	Continuous	240.00	240.00	3.65E-05	0.013	1200.00
8	Intermittent	360.00	45.00	1.94E-04	0.067	200.00

For the intermittent tests, the timers were programed to interrupt the power supply to the peristaltic pumps at fixed time intervals in order to achieve the test HRT while satisfying the flow rate and velocity. For each test, the tracer dilution was introduced into the section of the reactor with baffles at the start, and then using a programmed peristaltic pump the water in the reactor was displaced and samples were collected at the reactor outlet at $1/12^{th}$ of HRT time intervals using clean vials. The fraction of red dye tracer present within each test reactor at each sampling interval was measured with the DR 5000 spectrophotometer using the programmed calibration method.

Determination of SRT

The determination of the solids retention time (SRT) was based on initial values of total solids in the reactor and daily measurements of total solids influents and effluents. SRT can be estimated using a ratio of the total solids in the system to total solids leaving the system (Zakkour *et al.* 2001) as described in Equation 3.2.

$$SRT = \frac{\frac{Kg}{day} of\ solids\ retained\ in\ system}{\frac{Kg}{day} of\ solids\ leaving\ system} \qquad \text{Equation 3.2}$$

The method for determining the total solids concentration is as in standard methods (American Public Health Association 1998) which are described in Section 3.5.

3.5 General sampling and analysis

Collection of liquid samples from the bench reactors, Section 3.3, was carried out using clean 25 mL plastic vials through the sampling points provided. Gas samples were collected from the headspaces of the compartments in the bench reactors using a 100 µL Hamilton SampleLock syringe and needle supplied by Sigma-Aldrich, UK. Solid and liquid samples were collected from the BMP assays, Section 3.1, through the septum cap using Plastipak® 2 mL disposable plastic hypodermic syringes and 21-guage needles supplied by Fisher Scientific, UK. While gas samples were collected through the septum cap using a 100 µL Hamilton SampleLock syringe.

Depending on the number of parameters to evaluate, solid and liquid samples were collected from the BMP bottles, Section 3.1, for each assay condition in volumes ranging from 2 – 10 mL in order not to deplete the volumes inside the assay bottles before the experimental period elapsed. The samples were mixed to make composite samples for each assay condition before analyses for COD, Volatile fatty acids (VFA), pH, total and volatile solids.

Determination of solids concentrations

Total solids concentrations were determined by drying the samples in an oven at 105^{o}C over 24 hours, while the volatile solids concentrations were determined by

igniting the dried samples in a furnace at 550^{o}C for two hours. Four measurements were made for each sample, these are:

1. Actual volume of sample (V mL)
2. Weight of empty crucible (W_1 mg)
3. Weight of crucible with dried sample (W_2 mg)
4. Weight of crucible after igniting sample in furnace (W_3 mg)

The concentration of total solids in a sample can then be determined using Equation 3.3, while the concentration of volatile solids can be determined using Equation 3.4 (American Public Health Association 1998).

$$TS\left(\frac{mg}{L}\right) = \frac{(W_2 - W_1)x1000}{V} \qquad \text{Equation 3.3}$$

$$VS\left(\frac{mg}{L}\right) = \frac{(W_2 - W_3)x1000}{V} \qquad \text{Equation 3.4}$$

The measurements were performed in duplicate for each sample, and the average TS and VS was adopted.

Determination of pH

The pH of the samples was determined using a SenSION3 pH probe and meter (Hach Company, Loveland Colorado U.S.A). This method determines the pH of a solution based on the negative logarithmic value of the concentration of hydrogen ions in the solution (Heirholtzer 2013). The pH meter is programmed with a pH slope determined through measurements of standard pH solutions, and the pH of any

subsequent solution is determined based on direct comparison with the standard pH slope. Calibration of the pH probe was carried out before evaluation of the samples using standard 4.00, 7.00 and 10.00 pH buffer solutions supplied with the pH probe. According to Heirholtzer (2013), the accuracy (closeness of agreement between a test result and a reference value) of this method is ±0.2% for the pH probe and meter as reported by the manufacturer (Hach Company).

Determination of VFA concentrations

Total VFA was measured and expressed as Acetic acid by spectrophotometry using the Ferric hydroxamate method for determination of carboxylic esters (Hierholtzer *et al.* 2012), also known as the Montgomery method. In this procedure, aqueous sample (0.5 mL) was taken into a dry test-tube, and 1.5 mL 99% ethylene glycol reagent and 0.2 mL of a 19.5 N sulphuric acid solution were added and mixed with the sample. The mixture was heated for 3 minutes in a water bath, and then allowed to cool to ambient temperature. After cooling, 0.5 mL of 10% hydroxylamine hydrochloride solution, 2 mL of 4.5 N NaOH solution and 10 mL of 10% ferric chloride solution were added. The process was performed in triplicates for each sample, and the average of the three measurements was adopted as the VFA concentration for the sample. This method has a reported precision (closeness of agreement between results from several independent tests under standard conditions) of 4.1 (Heirholtzer 2013).

Calibration of DR 5000 spectrophotometer for VFA measurements

The DR 5000 Hach Lange spectrophotometer is automatically calibrated for the Montgomery method, which is identified as Method 8196 Esterification method in

the spectrophotometer user operational manual (Hach Company 2005). The method has been calibrated to determine VFA concentrations expressed as Acetic acid (mg/L) within the range of 27 – 2800 mg/L, by measuring the absorbance of light at a wavelength of 495 nm. A check for accuracy or adjustment of the calibration can be made using samples of standard concentration of volatile acids, with a 500 mg/L recommended by the spectrophotometer manufacturer. If the reading of the DR 5000 is not accurate, the calibration is adjusted by recording the concentration of the standard into the spectrophotometer as the actual reading. This adjustment was achieved by preparing a standard solution of known concentration using instructions provided in the DR 5000 user manual, and then measuring the VFA concentration using the Esterification method. This procedure was performed regularly before the samples collected from the BMP and bench reactor experiments were measured.

Determination of COD concentrations

Analysis for COD in mg/L was carried out using a DR 5000 spectrophotometer (Hach Lange, Salford Manchester, UK). The concentrations of COD (mg/L) in collected samples were determined using colorimetric determination with the Hach-Lange DR 5000 spectrophotometer Method 8000 (Hach Company 2005). The spectrophotometer was calibrated by the manufacturer for the method with the use of specifically prepared Hach-Lange COD cuvettes for ranges of COD concentrations relating the amount of green chromic ions to concentrations of COD (mg/L). The DR 5000 spectrophotometer was calibrated for COD measurements by the manufacturer, Hach Lange, to work with cuvettes predefined for specific ranges of COD values in mg/L, based on the absorbance of light at a wavelength of 620 nm. Standard solutions, supplied by Hach Lange, and also blank samples prepared in the laboratory

were adopted for testing of the accuracy and adjustment of the spectrophotometer calibration using methods described in the user manual (Hach Company 2005).

The COD cuvettes supplied for the method needed to be shaken a few times in order to mix the strong oxidising agent (potassium dichromate) in the cuvette properly, and then a volume of each sample was pipetted into a specific cuvette. The cuvettes were then placed in a test tube heater at 150ºC for two hours, during which organic compounds react with the dichromate ion (Heirholtzer 2013), and produce green chromic ion (Cr^{3+}). After the two hours, the cuvettes were carefully removed from the heater and shaken a few times and allowed to cool to ambient temperature before analysing with the spectrophotometer. Only one COD measurement was obtained for each sample, and Heirholtzer (2013) reported the accuracy and precision of this method of analysis as 6.5 and 2.7%, respectively.

Gas Chromatography

The methane gas concentrations were determined through gas chromatography (GC) with a Hewlett-Packard 5890 Series II gas chromatograph with dual thermal conductivity detector and an Alltech Heliflex® AT-Alumina stainless steel capillary column. The output of the GC was monitored and integrated by a desktop personal computer (PC) using Clarity Lite© chromatography station operated in a Windows XP© environment. Before introducing a sample, the GC injector was allowed to reach a temperature of 120ºC; the oven was allowed to reach a temperature of 50°C and the detector was allowed to reach a temperature of 150°C. Once the samples were injected into the GC, helium was used as a carrier gas at a flow rate of 7.0 mL/min, and the measurements from the GC were monitored, processed and stored

by the PC. Heirholtzer (2013) reported the accuracy and precision of the GC and the gas concentration analysis method as 12 and 1%, respectively.

To calibrate the GC and PC for methane measurements, standard methane gas samples (≥ 99.9% Analytical standard supplied by Sigma-Aldrich, UK) are utilized, by taking different volumes (10.0, 20.0, 40.0, 60.0, and 80.0 µL) in the Hamilton SampleLock syringes, making the samples to have predefined methane percentages (10.0, 20.0, 40.0, 60.0, and 80.0%). A retention time, 1.2 minutes, is defined in the PC indicating the estimated time before methane is detected by the GC after injection, and the time span, 0.8 minutes, for the detection curve. This is a critical step, as the PC will only interpret signals within this time period as methane. The standard samples prepared are then injected into the GC and the measurements recorded by the PC and stored as data files. Use of a 100% methane sample was avoided in order to ensure there will be no oversaturation of the GC detector, as this can introduce error in the calibration if the limit of detection is exceeded. The recorded measurements in the PC are then defined according to their corresponding percentages of methane and stored in the PC as the standards for methane detection.

Determination of methane production and concentrations

The production of methane in the BMP assays was monitored using 50 mL disposable plastic syringes and needles supplied by Fisher Scientific, UK. The bottles were monitored for gas build-up by frequent observation of the flexibility of the septum, and once the septum becomes inflexible the gas content of the bottle was measured. Initially, 100 µL gas sample was collected from each BMP assay bottle using a Hamilton SampleLock syringe, and each sample was transferred to the gas

chromatograph, where the percentage of methane in the sample was measured. After gas samples for GC analysis have been collected, the volume of gas in the headspace of the bottle was determined by releasing the gas using the 50 mL disposable syringe and needle under a ventilation hood. The plunger of the syringe was allowed to rise due to the high pressure in the bottles until equilibrium was achieved between the bottles and atmospheric pressure. The gas collected in the syringe was flushed through the ventilation hood.

The total volume of gas released into the syringe from each bottle was recorded as the volume of gas released with reference to the ambient temperature and pressure at the time of the release. To determine the corresponding total volume of methane produced at standard temperature and pressure, Equations 3.5 – 3.7 were applied.

$$CH_4\ in\ headspace\ (mL) = \frac{\%CH_4 * V_{headspace} * T_{standard} * P_{room}}{100 * T_{room} * P_{standard}} \quad \text{Equation 3.5}$$

$$CH_4\ released\ (mL) = \frac{\%CH_4 * V_{released} * T_{standard} * P_{room}}{100 * T_{room} * P_{standard}} \quad \text{Equation 3.6}$$

$$CH_4\ cummulative\ (mL) = CH_4\ released\ (mL) + CH_4\ in\ headspace\ (mL) \quad \text{Equation 3.7}$$

Where:

% CH_4 = Percentage of methane measured by the GC

$V_{headspace}$ = Volume of the headspace in the assay bottle (mL)

$V_{released}$ = Volume of methane released from the assay bottle (mL)

$T_{standard}$ = Standard temperature as defined in STP (K)

P_{room} = Atmospheric pressure (hPa)

T_{room} = Ambient temperature (K)

$P_{standard}$ = Standard atmospheric pressure (hPa)

Wet-tip gas metres

The production of methane in the bench reactors was monitored using wet-tip gas metres (Wet-Tip Gas Meter Company, Nashville, Tennessee, USA). These are transparent acrylic boxes, each with an inverted double-chambered plastic tipping container placed on a pivoting point, designed to work based on the liquid displacement principle (Saady and Maase 2015). A magnetic counter placed close to the inverted container is designed to record each tipping of the chamber caused by a predetermined volume of gas filling one of two chambers. Each box was filled with water and sealed, except for the air inlet and outlet pipes, Figure 3.4, which were connected to the bench reactors and gas discharge system respectively.

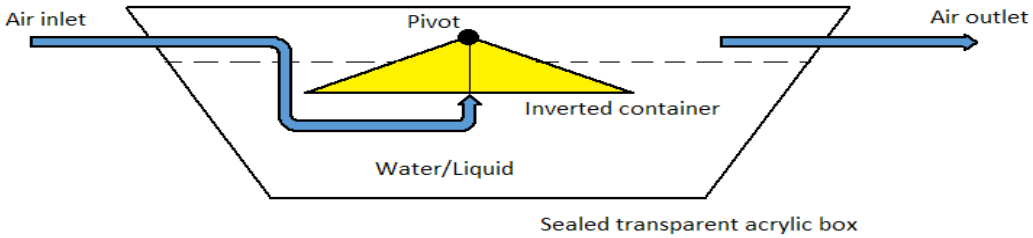


Figure 3.4: Wet-tip gas meter

When the first chamber became filled with the calibrated gas volume, the buoyancy of the gas should cause a tipping; the gas should then be released into the liquid and allowed to escape through the outlet, and the process is repeated with each tipping recorded by the counter. The total volume of gas produced for any given time period can be determined by multiplying the total number of tipping by the calibrated gas volume per tip. The wet tip metre is reported to be accurate for gas production volumes ≥ 100 mL/day, and the units were calibrated to tip for every 50 mL of gas,

and Equation 3.6 is adopted to convert the measured gas volume to corresponding volume at standard pressure and temperature.

Water displacement gas measurements

A water displacement system was also applied in the evaluation of the gas production from the bench reactors. In this method, up to 100 mL of a 400 mL laboratory beaker was filled with water, after which a 100 mL plastic test tube was also filled with water and inverted and submerged below the water level in the beaker, and held in place vertically using a clamp. A pipe was then connected from the bench reactor to the test tube, Figure 3.5, and any biogas in the headspace should have moved into the test tube and the water in the test tube should have been transferred into the beaker due to pressure. The measured drop in water level in the test tube should therefore provide an equivalent measure of biogas produced corresponding to the ambient temperature and pressure (Raposo *et al.* 2011).

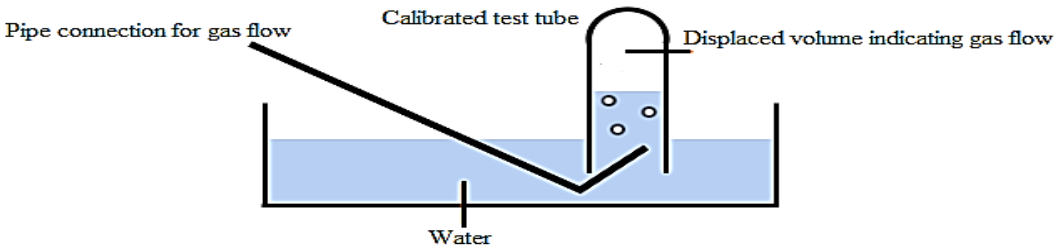


Figure 3.5: Water displacement system for gas production measurement

For the percentage of methane in the headspaces of the compartments of the bench reactors, 100 µL samples were collected using a Hamilton SampleLock syringe and transferred to the gas chromatograph.

3.6 Energy efficiency analysis

Based on reviewed literature (Lubken *et al.* 2007; Mo and Zhang 2012; Barry 2007; Ko *et al.* 2000; Merlin and Lissolo 2010; Chae and Kang 2013; Lazarova *et al.* 2012), the steps for a comprehensive evaluation of the energy in wastewater treatment alternatives are:

1. Definition of alternatives
2. Definition of evaluation criteria
3. Selection of system models
4. Establishing system boundaries
5. Inventory analysis and benchmarking
6. Cost and impacts analysis

The alternative systems that are being considered need to be clearly defined, in terms of their operational and performance capacities, and the associated impacts on energy. With the alternatives clearly identified, the next step is the application of the evaluation criteria in order to determine the alternative with the best energy efficiency.

Evaluation criteria

There are several benchmarks in literature that are applicable as evaluation criteria, and criteria defined by Melin and Lissolo (2010) are modified and adopted for this study, namely:

- **Functional efficiency index (FEI)**: This is defined as the total energy consumed (kWh/kg$COD_{removed}$), and the ideal FEI for an energy efficient

system should be a very low number, where the COD removed is high while the energy consumed is low. A high FEI represents a system with very low energy efficiency.

- **Effluent quality index (EQI)**: This is a weighted sum of effluent pollutant loads, and a low value indicates high removal of pollutant loads in the system, which is desirable.
- **Energy yield ratio (EYR)**: This is defined as the ratio of total energy in the system to the energy consumed by the system, and a high value EYR indicates low energy consumption and therefore high energy efficiency.
- **Environmental loading ratio (ELR)**: The ELR is a ratio of all non-renewable energy consumed or produced to the renewable energy consumed or produced. A low value ELR indicates a system that is more dependent on renewables sources of energy, and therefore presents a low environmental impact.
- **Sustainability index (SI)**: This is a ratio of the EYR to the ELR, and a high SI indicates a sustainable system which consumes low energy and is largely dependent on renewable energy sources. The sustainability index indicates the stress inducing capability of the system to the environment.

System models and boundaries

The selected alternatives are then developed as system models which will serve as the basis for an inventory analysis to determine the corresponding data for all processes within the system boundaries in terms of inputs (energy, staff, reagents, maintenance and waste management) and outputs (effluent COD, BOD and solids; and energy recovered).

Inventory and benchmarking

All processes related to the wastewater treatment within the system boundary, for example the use of electricity (for operational processes), consumption of chemicals and additional fuels, the transport and disposal of sludge, pumping and heating, are identified and evaluated for their corresponding energy characteristics. A major aspect of wastewater treatment is the energy consumption during heating or cooling of the wastewater to operational temperatures. This energy is defined as the thermal energy in the treatment process, and it can be either energy loss especially for heating or energy gain through heat capture. The thermal energy in the treatment system can be estimated with Equation 3.8 (Chae and Kang 2013).

$$E_{thermal} = \rho C_p \delta T \qquad \text{Equation 3.8}$$

Where:

$E_{thermal}$ = thermal energy (kcal/m^3),

ρ = density of the wastewater (kg/m^3),

C_p = specific heat of the wastewater (kcal/kg.^{o}C) and

δT = temperature that can be extracted (^{o}C).

The available energy for heating and cooling depends on the coefficient of performance (COP) for cooling and heating which is a property of the heat pump (Chae and Kang 2013), and is defined by Equations 3.9 and 3.10.

$$E_{cool} = \frac{E_{thermal} * COP_c}{(COP_c + 1)} * 0.001163 \qquad \text{Equation 3.9}$$

$$E_{heat} = \frac{E_{thermal} * COP_h}{(COP_h - 1)} * 0.001163 \qquad \text{Equation 3.10}$$

Where:

E_{cool} = available energy for cooling (kWh/m^3),

0.001163 = conversion factor for kcal to kWh,

COP_c = coefficient of performance for cooling (unit less),

E_{heat} = available energy for heating (kWh/m^3), and

COP_h = coefficient of performance for heating (unit less).

The electrical energy input is estimated by considering the electrical load of pumps and motors (kW) and their corresponding operational times in hours (h) with respect to the total amount of wastewater treated, as expressed by Equation 3.11 (Singh *et al.* 2012).

$$E_{power} = \frac{PT}{Q} \qquad \text{Equation 3.11}$$

Where:

E_{power} = electrical energy (kWh/m^3),

Q = total flow of wastewater (m^3/day),

P = rated power of the electrical motor (kW), and

T = operation hours in a day (h/day).

The rated power of the pumps or motors is the amount of energy input (kW) and is a function of the amount of power required to drive the pump or motor. The manual (human) energy required for activities such as operating valves and switches and collection of sludge is calculated using Equation 3.12 (Singh *et al.* 2012).

$$E_{manual} = \frac{\sum_{i=0}^{i=n} \sum_{j=0}^{j=m} E_{ij} N_{ij} T_{ij}}{Q} \qquad \text{Equation 3.12}$$

Where:

E_{manual} = manual energy in kWh/m^3,

n = number of nature of activities

m = number of gender (male, female),

E = human power equivalent (kW),

N = number of persons engaged in an activity, and

T = total time devoted in the activity (h/day).

Mechanical energy ($E_{mechanical}$) is energy derived from other fuel sources in kWh/m^3, and this is calculated using Equation 3.13 (Singh *et al.* 2012).

$$E_{mechanical} = \frac{cD}{Q} \qquad \text{Equation 3.13}$$

Where:

$E_{mechanical}$ = Energy derived from fuel sources in kWh/m^3

Q = Wastewater flow rate (m^3/day)

c = unit energy value of the fuel in kWh/L

D = amount of fuel consumed in L/day

Chemical energy ($E_{chemical}$) is energy released or absorbed during a chemical reaction in kWh/m^3, and this is calculated using Equation 3.14 (Singh *et al.* 2012).

$$E_{chemical} = \frac{n[\sum \Delta H_p - \sum \Delta H_t]}{Q} * 0.000278 \qquad \text{Equation 3.14}$$

Where:

$E_{chemical}$ = Energy related to chemical reactions in kWh/m^3,

n = number of moles (mol/day),

0.000278 = conversion factor from kJ to kWh,

ΔH_p = enthalpy (heat) of formation of products (kJ/mol)

ΔH_t = enthalpy (heat) of formation of reactants (kJ/mol)

Q = Wastewater flow rate (m^3/day)

If the process temperature is higher than the ambient temperature, heat losses can occur, and this can be determined using Equation 3.15 based on the operational time of the system in hours.

$$E_{Loses} = \frac{\upsilon.A.\Delta T.Time}{Q} \qquad \text{Equation 3.15}$$

Where:

E_{Loses} = heat losses (kWh/m^3)

υ = overall coefficient of heat transfer (kW/m^2.K)

Time = operational time of the system (hours/day)

Q = wastewater flowrate (m^3/day)

A = cross sectional area for heat loss (m^2)

ΔT = temperature difference (K)

Energy function

If the treatment system is considered as a continuous reactor in steady state, the resulting net energy consumption (E_{net}) of the system is represented by Equation 3.16, a modified version of the model proposed by Lubken *et al.* (2007).

$$E_{net} = E_{methane} + E_{cool} - E_{heat} - E_{power} - E_{manual} - E_{chemical} - E_{mechanical} - E_{Loses}$$

Equation 3.16

For a system to be energy positive, the methane production and heat capture need to be high, while the energy consumed in heating, providing power to machinery and other activities needs to be low. $E_{methane}$ is defined as the equivalent kWh/m^3 of methane produced (m^3), Equation 3.17, per unit volume of wastewater (m^3) treated (Abbasi *et al.* 2012).

$$E_{methane} = 5.815 * Methane\ produced\ (m^3/m^3)$$ Equation 3.17

Chapter Four – Anaerobic reduction of domestic wastewater sludge

4.0 Introduction

Anaerobic digestion is influenced by the environmental conditions of the process (Section 2.4), primarily temperature, pH and characteristics of the substrate (Vavilin *et al.* 2008). To improve the understanding of the potential for efficient low temperature domestic wastewater treatment and energy recovery using the ABR, it is necessary to study the mechanisms of anaerobic reduction of domestic wastewater sludge (DWS). The objective of this chapter is to present an evaluation of the anaerobic digestion of domestic wastewater sludge, and the corresponding methane production, at ambient temperature compared against anaerobic digestion at 37^{o}C. Furthermore, observed anaerobic reduction of DWS at ambient temperature and at 37^{o}C would be compared with an existing hydrolysis model, Equation 2.21 (Section 2.4.4.1), in order to evaluate the potential for modelling and prediction of domestic wastewater treatment at ambient temperature. Also, methane production at ambient temperature would be compared against methane production at 37^{o}C in order to evaluate the potential for energy recovery from the anaerobic reduction of domestic wastewater sludge at ambient temperature.

4.1 Reduction of DWS in batch reactors

The conversion of DWS to intermediate compounds (VFA) and methane gas was monitored for a period of 40 days using BMP assays based on the methodology described in Section 3.1. The results indicate that biological reduction of the substrates were substantial in the initial 10 days of the experiment, similar to reports

by Mahmoud *et al.* (2004). Table 4.1 presents the summary of the reduction by mass for the conditions tested in terms of the fractions of the substrates removed during the assay.

Table 4.1: Reduction of solid substrates during BMP assays

Conditions			Fraction reduced			
			Total solids		Volatile solids	
Substrate	**Inoculation**	**Temp (°C)**	**Observed**	**Modelled**	**Observed**	**Modelled**
Primary sludge	With	25	0.46	0.47	0.40	0.40
		37	0.58	0.58	0.55	0.56
	Without	25	0.30	0.30	0.24	0.32
		37	0.27	0.26	0.36	0.35
Secondary sludge	With	25	0.27	0.27	0.23	0.22
		37	0.30	0.34	0.33	0.38
	Without	25	0.25	0.21	0.15	0.30
		37	0.24	0.21	0.39	0.39
The modelled data were obtained using non-linear regression analysis of the observed data using Equation 2.21 as the process model.						

The observed reduction of volatile solids of the substrates, Table 4.1, showed low reduction at ambient temperature compared to 37°C. For the primary sludge (PS) assay with inoculation of anaerobic biomass, over 55% of the volatile solids were reduced at 37°C, while only 40% reduction was observed at 25°C (Table 4.1). For the secondary sludge (SS) assay with inoculation of anaerobic biomass, over 33% of the volatile solids were reduced at 37°C, while only 22% reduction was observed at 25°C. Similar results were also observed for the reduction of total solids in the assays with inoculation of anaerobic biomass, Table 4.1, where 58% and 47% of the total solids of the primary sludge (PS) assay with inoculation were reduced at 37°C and 25°C, respectively. While the secondary sludge (SS) assay with inoculation had a 34% and 27% total solids reduction at 37°C and 25°C respectively (Table 4.1). Figures 4.1 – 4.4 present remaining fractions of volatile solids (Figures 4.1 and 4.2) and total solids (Figures 4.3 and 4.4) of the substrates against time (days), and the

results indicate that the biological reduction of the solid substrates for all the experimental conditions tested exhibited a similar trend.

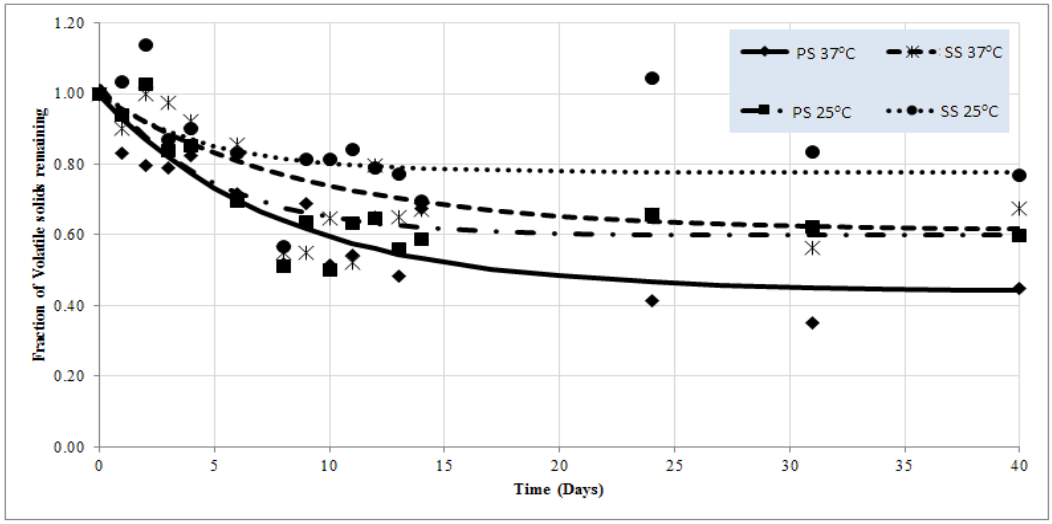


Figure 4.1: Reduction in volatile solids from the BMP assay of domestic wastewater sludge with inoculation of anaerobic biomass.

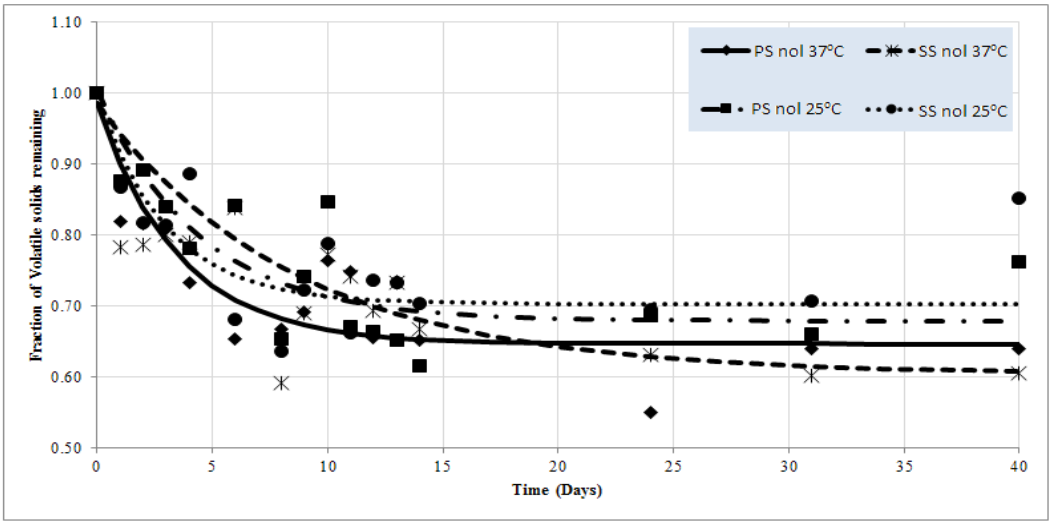


Figure 4.2: Reduction in volatile solids from the BMP assay of domestic wastewater sludge without inoculation of anaerobic biomass.

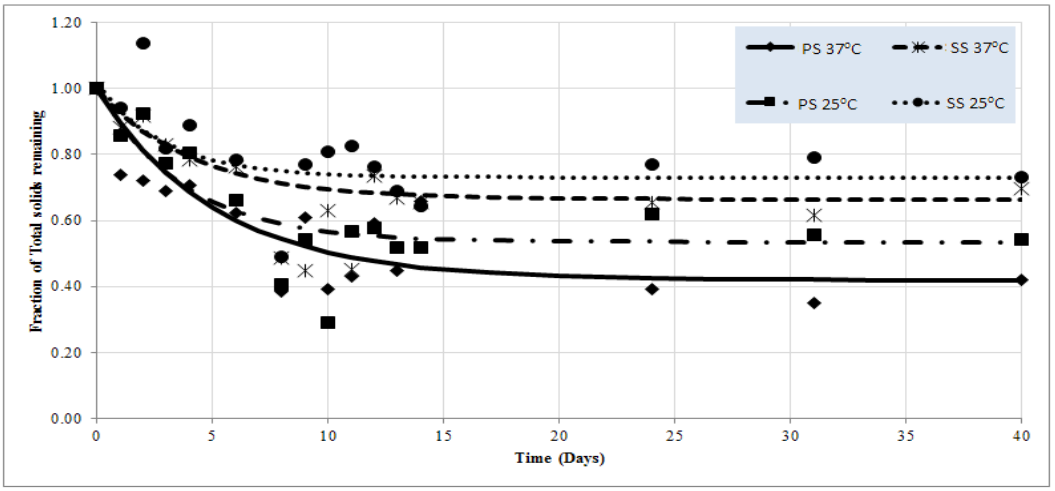


Figure 4.3: Reduction in total solids from the BMP assay of domestic wastewater sludge with inoculation of anaerobic biomass.

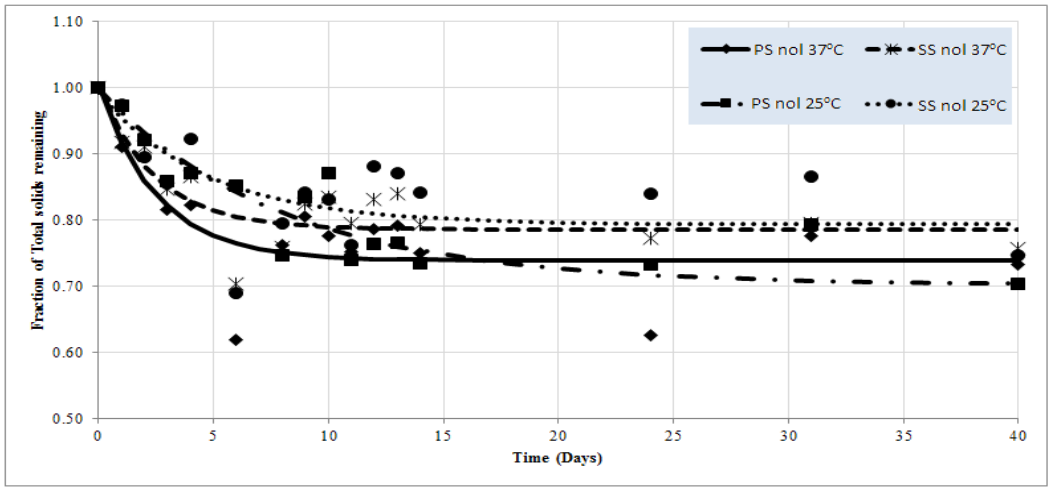


Figure 4.4: Reduction in total solids from the BMP assay of domestic wastewater sludge without inoculation of anaerobic biomass.

From Figures 4.1 – 4.4, the 'best fit lines' were based on regression analysis of the solids reduction data fitted to Equation 2.21, details of this analysis are presented in Section 4.2. Some of the data points presented in Figures 4.1 – 4.4 were higher than 1.0, which is impossible for fractions of an initial mass. These values indicate experimental errors, potentially as a result of the sampling method adopted, where needles and syringes were used. The American Public Health Association (1998) encourages caution on the use of pipettes to collect solid samples, and highlighted

some of the potential problems with accuracy and errors to be expected such as solids adhering to the pipette. Unfortunately, several publications reviewed (Angelidaki *et al.* 2009; Raposo *et al.* 2011; de Vrieze *et al.* 2015; Hansen *et al.* 2004; Owen *et al.* 1979) did not provide specific details of methods for collection of solid samples from closed batch assay experiments such as the BMP assays.

For BMP assays where the bottles are expected to remain sealed, there are two alternatives for solid sample collection; the most reliable method is through the use of a multi-bottle system with wasting of a bottle after a defined period in order to obtain a data point (Hernandez-Leal *et al.* 2011). This method provides the opportunity to have sufficient quantities of samples for parameter analysis, and therefore errors and problems of data accuracy are minimized. A major disadvantage of this method is the need for several bottles for each assay condition; this can become a logistical problem especially where incubator space is limited. In the BMP assay experiment (Figures 4.1 – 4.4), a maximum of 18 data points were obtained for each assay condition, a multi-bottle system will therefore require 18 bottles for each condition at the initial stage. The second method requires the collection of samples through the septum, without disturbing the anaerobic environment in the bottle, and this can be accomplished by passing needles through the septum (Young *et al.* 2013).

Needles are produced in various sizes, gauged based on the size of the diameter, for example a 21-gauge needle will have an internal diameter of 0.51 mm (Gill and Prausnitz 2007). In a study on the biodegradability of municipal sludge, Young *et al.* (2013) used a 10-gauge (2.69 mm internal diameter) needle for solid sample collection. Use of small diameter needles, such as the 21-gauge, minimizes damage

to the septum caused by large diameter needles, but creates problems with accuracy of sampling solids. According to Aldin (2010), 66.5% of the particles in primary sludge are categorized with diameters greater than 100 μm (micrometre), while for waste activated sludge only 49% of the particles are greater than 100 μm. This is because the large particles in domestic wastewater are normally removed during the primary sedimentation, and therefore form the primary sludge (Morgenroth *et al.* 2002).

For raw influent wastewater, Morgenroth *et al.* (2002) reported that only 43% of the particles are greater than 100 μm. Feng *et al.* (2015) reported average particles size greater than 100 μm for an ABR reactor treating domestic wastewater after 130 days start-up period, and average size greater than 200 μm after the initial 50 days of operation with acclimatized biomass. Therefore using a needle that does not have an adequate internal diameter could create inaccuracies in the samples collected. Use of needles in medical and clinical studies has been observed to cause breakdown of blood cells during the sample collection process (Bowen *et al.* 2010), therefore the use of needles to collect samples from the BMP bottles may also cause breakdown of the substrate particles and influence the hydrolysis process and the results observed.

4.2 Relationship between anaerobic reduction of DWS and hydrolysis model

To associate the observed biological reduction of the substrates to kinetic models defined in literature, the solids reduction data obtained were tested using nonlinear least squares regression. The data was compared to Equation 2.21 (Section 2.4.4.1), with the results obtained presented in Appendix B, and summarized in Table 4.2. The coefficient of determination (R^2) values obtained are indications of the usefulness of

the model in predicting the process as a function of time (Palmer and O'Connell 2009). This means if R^2 is a value close to 1.0, then Equation 2.21 is useful in predicting the hydrolysis of the substrate and the length of time is important in determining the reduction in the substrate. Alternatively, if the R^2 value is close to 0.0, then Equation 2.21 is not useful in predicting the hydrolysis of the substrate, and the length of time is not important in determining the reduction in the substrate.

From Table 4.2, the R^2 values indicate a good correlation between the model, digestion time and the reduction of the total solids of the primary sludge assay with inoculation at 25ºC, with an R^2 value of 0.8249 based on 14 data points. While the reduction of the total solids of the secondary sludge assay with inoculation at 25ºC had an R^2 value of 0.4869 based on 15 data points (Table 4.2), an indication of a poor correlation between the model and the observed data. Also, the model fit indicates that time is not an important factor in the prediction of the hydrolysis of total solids of the secondary sludge at 25ºC.

Table 4.2: Hydrolysis rate constants (k_h) obtained with Equation 2.21 from data for anaerobic reduction of DWS

Parameter	Substrate	Inoculation	Temp. (°C)	Fraction reduced (observed)	Fraction reduced (modelled)	Data points	k_h (d^{-1})	Bounds (±)	R^2	RMSE	SSE
Total solids	Primary sludge	With	25	0.46	0.47	14	0.2688	0.1962	0.8249	0.0739	0.0601
			37	0.58	0.58	13	0.1921	0.1619	0.7323	0.1042	0.1086
		Without	25	0.30	0.30	13	0.1249	0.0891	0.8671	0.0382	0.0146
			37	0.27	0.26	15	0.3924	0.4070	0.6632	0.0616	0.0456
	Secondary sludge	With	25	0.27	0.27	15	0.3372	0.5271	0.4869	0.0935	0.1050
			37	0.30	0.34	12	0.2365	0.1405	0.9138	0.0384	0.0133
		Without	25	0.25	0.21	12	0.2099	0.2673	0.7394	0.0437	0.0172
			37	0.24	0.21	14	0.4087	0.3683	0.7405	0.0426	0.0200
Volatile solids	Primary sludge	With	25	0.40	0.40	13	0.2089	0.0971	0.9325	0.0411	0.0169
			37	0.55	0.56	13	0.1278	0.0964	0.8103	0.8100	0.0656
		Without	25	0.24	0.32	15	0.2257	0.1990	0.7126	0.0643	0.0497
			37	0.36	0.35	14	0.2828	0.2021	0.7981	0.0537	0.0317
	Secondary sludge	With	25	0.23	0.22	12	0.2194	0.2094	0.7490	0.0420	0.0158
			37	0.33	0.38	11	0.1098	0.1036	0.8301	0.6570	0.0345
		Without	25	0.15	0.30	14	0.3320	0.2870	0.7315	0.0551	0.0334
			37	0.39	0.39	11	0.1157	0.0588	0.9224	0.0364	0.0106

SSE = sum of squares due to errors
RMSE = root mean squared error
Temp. = Temperature of BMP assay condition
R^2 = coefficient of determination

Based on the R^2 values obtained, Table 4.2, Equation 2.21 can be a reliable model in the prediction of the reduction of primary and secondary sludge in batch systems with temperature and inoculation conditions similar to the BMP assays in this study (Section 4.1). However, the R^2 values for the total solids of inoculated secondary sludge at 25ºC and the total solids of primary sludge without inoculation at 37ºC were below 0.7000, Table 4.2, indicating a poor model fit compared to the other assays with R^2 values above 0.7000. From Table 4.2, the number of data points is an indication of the number of outlying data points (due to errors in measurements) that were removed before a data fit was achieved to Equation 2.21. Initially, there were 18 data points, and for the assays at least three outlying points had to be removed (Table 4.2). Some of the secondary sludge assays had at least 6 outlying data points removed before a fit to the model was observed, but primary sludge assays had no more than five data points removed. These observed outlying data points are probably due to errors as a result of the sampling process, Section 3.5, and the statistical error analysis (RSME and SSE in Table 4.2) provides additional details on the distribution of the observed data points relative to the model with respect to time (Willmott *et al.* 1985).

The highest RSME value in Table 4.2 was for the volatile solids of the primary sludge assay with inoculation at 37ºC, observed with RSME = 0.81, and also for the volatile solids of the secondary sludge assay with inoculation at 37ºC, observed with RSME = 0.6570. The differences between the predicted model values and the observed values for the other assays were small, as reflected by the small RSME values (Table 4.2), indicating that most of the observed values are close to the predicted model values. The SSE values provide another basis for comparison of the

deviation of the predicted values from the observed values, and low SSE values in Table 4.2 indicate that the model, based on the hydrolysis rate and time, is predicting values that are close to the observed values. The hydrolysis rates constants (k_h) obtained, Table 4.2, are within the range of values observed in literature for primary sludge and secondary sludge, Section 2.4.4.2, and summarized in Table 4.3.

Table 4.3: Summary of k_h values from literature and this study

Study	Substrate	kh (day-1)	Conditions
Aldin (2010)	Sewage sludge	0.0050 – 0.2000	Varying
Aldin (2010)	Sludge	0.0800 – 2.0000	Varying
Aldin (2010)	Primary sludge - lipids and proteins	0.0096 – 0.1700	Varying
Aldin (2010)	Primary sludge - carbohydrates	0.2100 – 1.9400	Varying
Kassab et al. (2013)	Domestic wastewater - sludge	0.0060	25°C - seeded
Kassab et al. (2013)	Domestic wastewater - sludge	0.0040	25°C - unseeded
Luo et al. (2012)	Secondary sludge	0.4420	50°C
Mahmoud (2002)	Domestic wastewater - Settleable solids	0.2300	35°C
This study	Primary sludge - volatile solids	0.2089 ± 0.0971	25°C - seeded
This study	Primary sludge - volatile solids	0.1278 ± 0.0964	37°C - seeded
This study	Secondary sludge - volatile solids	0.2194 ± 0.2094	25°C - seeded
This study	Secondary sludge - volatile solids	0.1098 ± 0.1036	37°C - seeded
This study	Primary sludge - volatile solids	0.2257 ± 0.1990	25°C - unseeded
This study	Primary sludge - volatile solids	0.2828 ± 0.2021	37°C - unseeded
This study	Secondary sludge - volatile solids	0.3320 ± 0.2870	25°C - unseeded
This study	Secondary sludge - volatile solids	0.1157 ± 0.0588	37°C - unseeded

From Table 4.3, the k_h values presented by Aldin (2010) was for a wide range of experiments, while Kassab *et al.* (2013) reported the potential influence of high

concentrations of detergents in their substrate as the reason for the low rate constants. The k_h values from this study are close to the values reported by Mahmoud (2002) and Lou *et al.* (2012), and the values also fall within the ranges reported by Aldin (2010). The summary presented in Table 4.3 indicates wide ranges of values for the hydrolysis rates, and this could be attributed to the differences in the nature and characteristics of the substrates and the experimental conditions. The relationships between the hydrolysis rates and temperature were also evaluated, and Table 4.4 presents the relationship of the natural log of mean k_h with the inverse of temperature.

Table 4.4: Relationship of ln k_h (mean values) with temperature (T^{-1}) for the domestic wastewater sludge BMP assays.

Parameter	Condition	Temperature (K^-)		Trend
		37°C (0.0032)	25°C (0.0034)	
Total Solids	PS inoculum	-1.6497	-1.3138	Increasing
	PS no inoculum	-0.9355	-2.0802	Decreasing
	SS inoculum	-1.4418	-1.0871	Increasing
	SS no inoculum	-0.8948	-1.5611	Decreasing
Volatile Solids	PS inoculum	-2.0573	-1.5659	Increasing
	PS no inoculum	-1.2630	-1.4885	Decreasing
	SS inoculum	-2.2091	-1.5169	Increasing
	SS no inoculum	-2.1568	-1.1026	Increasing

Feng *et al.* (2009) observed a decrease in the rate of reaction with decrease in temperature, compatible with the Arrhenius relationship, which should correspond to a decrease in the ln k_h when the inverse of temperature (T^{-1}) increases. Generally, the ln k_h values relating to the BMP assays in this study, Table 4.4, increased with an increase in the inverse of temperature, corresponding to an increase in reaction rate with decrease in temperature, indicating a lack of compatibility with the Arrhenius relationship. However, the observed variation of ln k_h with temperature for the

primary sludge assay without inoculation and the total solids of the secondary sludge assay without inoculation show a decrease in ln k_h for a corresponding increase in the inverse of temperature, indicating a compatibility with the Arrhenius relationship.

There is a possibility that other factors, for example surface area of the substrate and biomass to substrate ratio, influenced the hydrolysis rates determined, but these variables were not monitored in the BMP batch experiment. It is not necessary that the reductions in solids mass observed correspond to progress in the anaerobic digestion process, and therefore evaluation of the corresponding concentrations of intermediate products (VFA) is necessary in order to ascertain anaerobic biodegradation of the substrates.

4.3 Intermediate products

The stages of anaerobic digestion are: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Section 2.4), and the acid producing phase can start once some soluble substrates are available from the initial hydrolysis phase. This process was observed in the BMP assays with solid substrates, Section 3.1, with the rise of VFA during the initial phase of the experiment even while hydrolysis was still on-going. The observed VFA concentrations and pH values against time (days) for the batch BMP assays are presented in Figures 4.5 - 4.8.

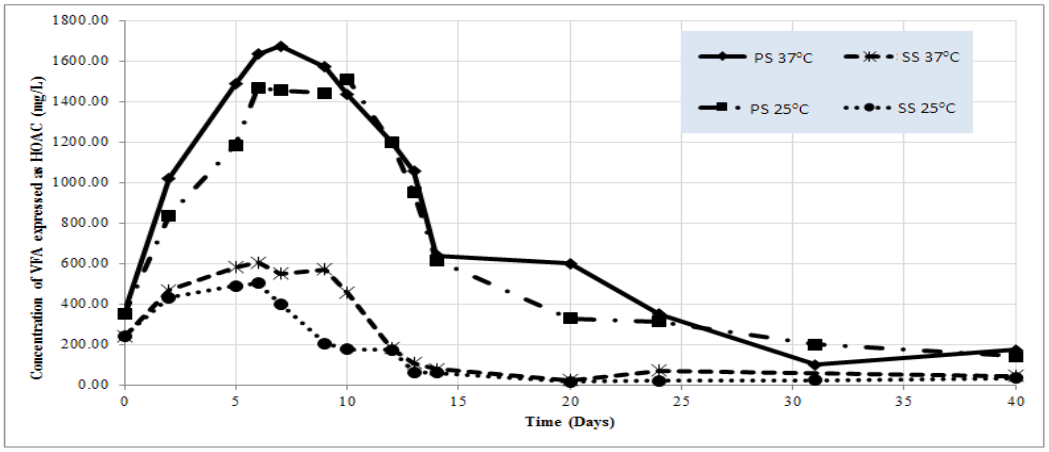


Figure 4.5: Volatile fatty acids concentrations (mg/L) from the BMP assay of domestic wastewater sludge with inoculation of anaerobic biomass.

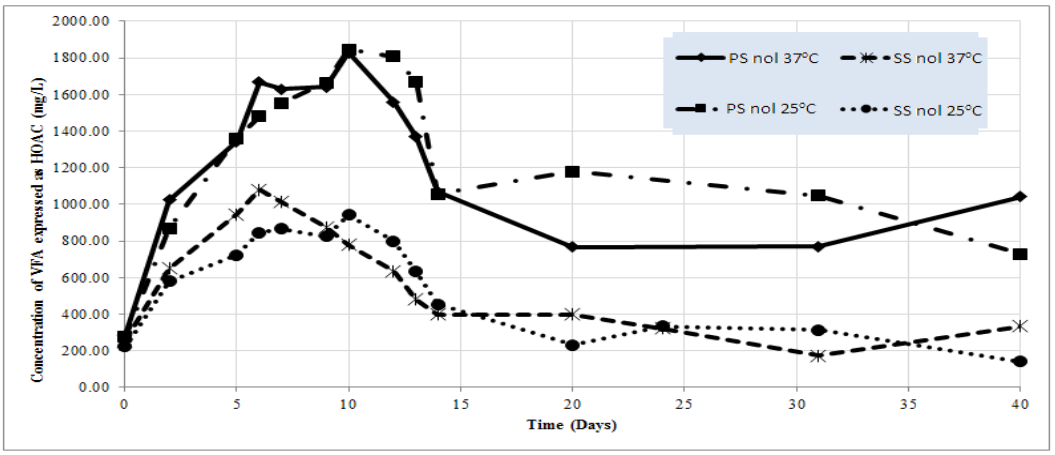


Figure 4.6: Volatile fatty acids concentrations (mg/L) from the BMP assay of domestic wastewater sludge without inoculation of anaerobic biomass.

The measurements of VFA concentrations taken during the BMP assay with solid substrates show three stages in the process (Figures 4.5 and 4.6). The first stage was a period during which a continuous increase in intermediate compounds concentrations was observed along with a decrease in pH values, lasting for up to 10 days for all the batches, probably as a consequence of the high hydrolysis rates at the beginning of the experiment (Figures 4.1 – 4.4). This stage was followed by a stage where the acids were depleted over a short period of time, approximately between 4 – 8 days. The third stage is identified by a relatively stable concentration of VFA until the endo of the experiment (Figures 4.5 and 4.6). Figures 4.7 and 4.8 present observed pH values against time (days) for the experimental conditions.

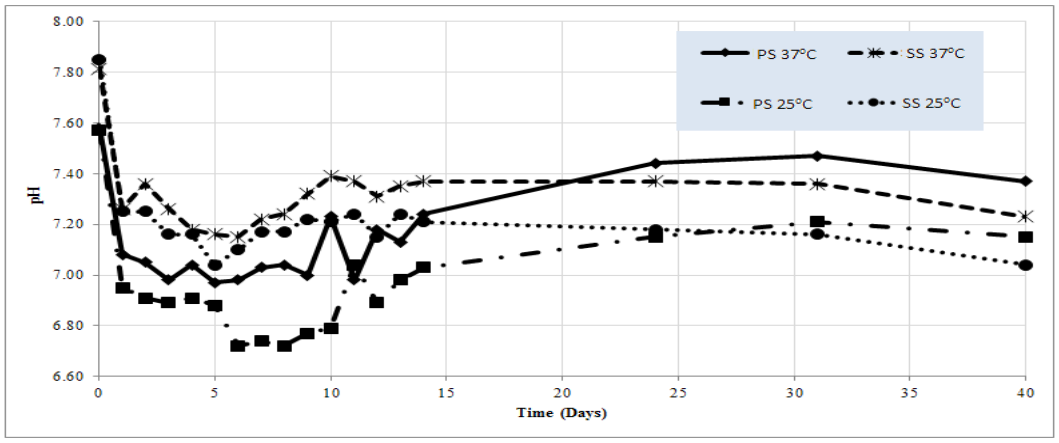


Figure 4.7: Observed pH values from the BMP assay of domestic wastewater sludge with inoculation of anaerobic biomass.

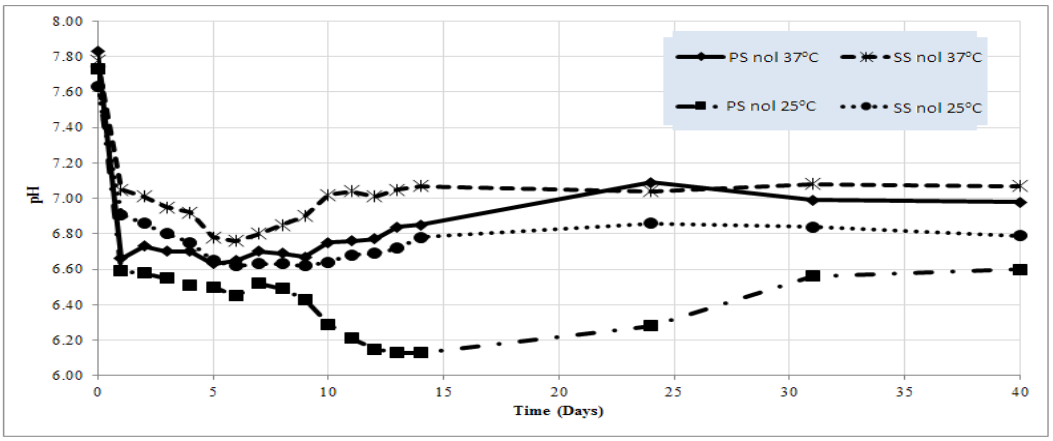


Figure 4.8: Observed pH values from the BMP assay of domestic wastewater sludge without inoculation of anaerobic biomass.

High concentrations of volatile fatty acids can affect the final phases of the anaerobic digestion, while low concentrations of acids will result in low biogas production (Section 2.4.3.1). Siegert and Banks (2005: cited in Appels *et al.* 2008) reported inhibition of the fermentation of glucose and the production of biogas when VFA concentrations were above 4 g/L and 8 g/L, respectively, while Angelidaki *et al.* (2005) reported stable operation of full scale biogas plants when VFA concentrations were below 1.5 g/L. After the initial 10 days of the experiment, the observed stability in pH values and the decrease in VFA concentrations were probably due to the corresponding low reduction of the substrates during the experimental period,

Figures 4.1 – 4.4. Alternatively, the decrease in the VFA concentrations could be as a result of established methanogenesis and therefore the conversion of the VFA to methane.

4.4 Methane production

The pH values observed in this study were all higher than 5.0 for all the assays, Figures 4.7 and 4.8, which is the threshold for pH inhibition of methane production, Section 2.4. Figures 4.9 and 4.10 present the cumulative methane produced (mL/g VS added) against time (days) during the assays of primary and secondary sludge.

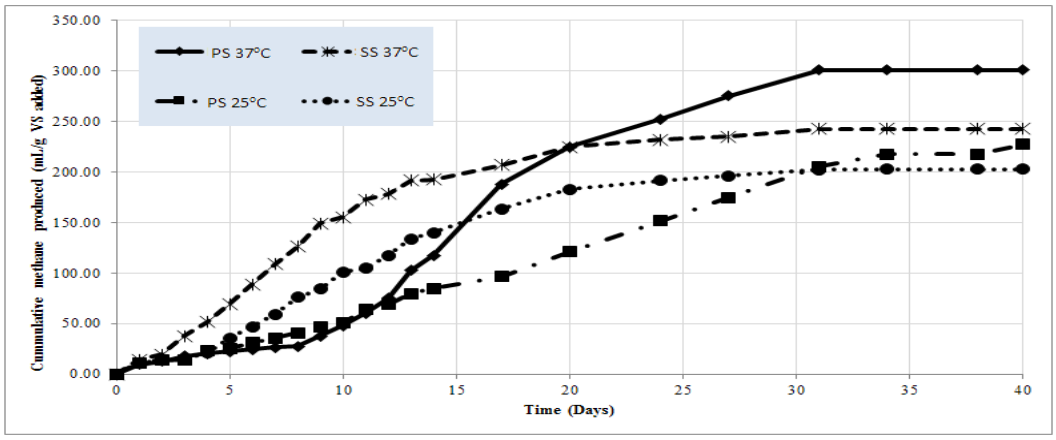


Figure 4.9: Cumulative methane produced (mL/g VS added) from the BMP assay of domestic wastewater sludge with inoculation of anaerobic biomass.

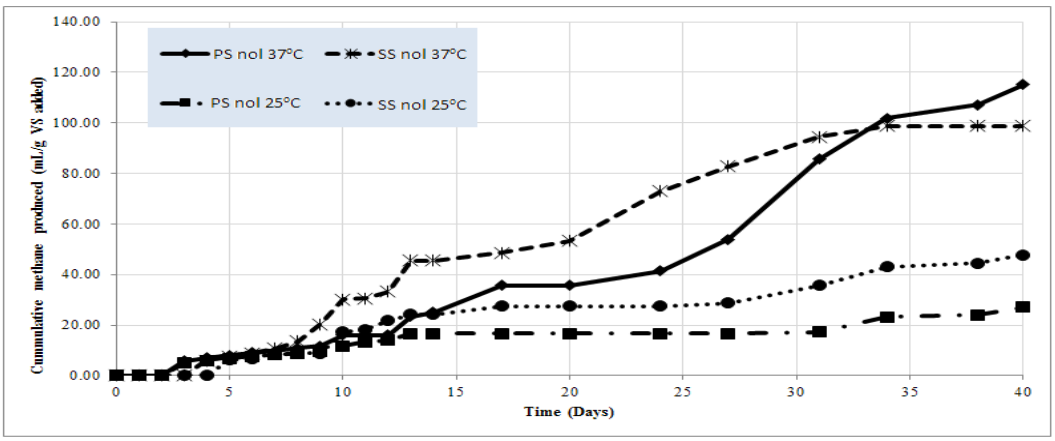


Figure 4.10: Cumulative methane produced (mL/g VS added) from the BMP assay of domestic wastewater sludge without inoculation of anaerobic biomass.

During the initial 10 days of the BMP experiment, no major changes were observed in the methane production for all the assays, except the secondary sludge assays with inoculation of biomass where the methane production recorded were observed to increase steadily from the first experimental day. The secondary sludge assay with inoculation of anaerobic biomass was also observed to have a steady rate of methane production over the first 15 and 20 days of the experiment at 37ºC and at ambient temperature, respectively, contrary to the other assays where different phases of methane production were observed. For a 10-day period, day 10 – 20, the rate of methane production observed for the primary sludge assay with inoculation at 37ºC was high compared to what was observed for the other assays. Similarly, the primary sludge assay without inoculation of anaerobic biomass at 37ºC was observed to have a high rate of methane production between days 25 - 35 of the experiment, a 10 days period similar to the days 10 – 20 for the primary sludge with inoculation. Table 4.5 presents the corresponding methane production from the 40 days BMP assays with domestic wastewater sludge. The methane produced from the reduction of volatile solids in domestic wastewater sludge indicates higher cumulative methane production at 37ºC than at 25ºC (Table 4.5).

Table 4.5: Observed methane productions from BMP assays of domestic wastewater sludge

Substrate	Inoculation	Temp. (°C)	Volume (mL)	Initial volatile solids (g/L)	Fraction of VS reduced (observed)	Fraction of VS reduced (modelled)	Cumulative methane produced (mL)	Methane produced (mL/g VS added)	Methane produced (mL/g VS reduced)
Primary sludge	With	25	350.00	10.03	0.40	0.40	797.01	227.04	565.19
		37	350.00	10.03	0.55	0.56	1056.21	300.87	541.17
	Without	25	350.00	11.10	0.24	0.32	105.08	27.05	84.25
		37	350.00	11.10	0.36	0.35	447.55	115.20	325.89
Secondary sludge	With	25	350.00	9.45	0.23	0.22	671.05	202.89	911.18
		37	350.00	9.45	0.33	0.38	801.88	242.44	631.09
	Without	25	350.00	12.05	0.15	0.30	201.10	47.68	160.54
		37	350.00	12.05	0.39	0.39	416.69	98.80	251.86
Methane produced (mL/g VS reduced) was calculated based on modelled fraction of VS reduced;									

The methane produced per initial mass of VS (mL/g VS added) for both substrates increased with increase in temperature (Table 4.5), in agreement with the Arrhenius relationship. However the methane produced per mass of VS reduced (mL/g VS removed) decreased with increase in temperature, except in the case of the assays without inoculation where an increase was observed (Table 4.5). Also, the secondary sludge with inoculation was observed to have higher methane production per gram of substrate removed than the primary sludge with inoculation, despite the low degradation of volatile solids in the former compared to the latter. From Table 2.3, Section 2.4.3, the methane yield from waste activated sludge was reported as between 0.5 – 0.6 m^3/kg VS removed (Demirel *et al.* 2010), and this range is lower than that observed in this study, 0.63 m^3/kg VS removed at 37ºC and 0.91 m^3/kg VS removed at 25ºC (Table 4.5). For the primary sludge, Table 4.5, the methane yields obtained were 0.54 m^3/kg VS removed at 37ºC and 0.57 m^3/kg VS removed at 25ºC, Table 4.5, and these values fall within the range of 0.116 – 2.063 m^3/kg VS removed, average = 0.68, reported by de Mes *et al.* (2003) for domestic wastewater sludge.

In the BMP assays without inoculation of biomass, the methane production was very low, in all cases less than half the production from the corresponding BMP assay with inoculation. This confirms that the methane producing organisms were unable to adequately establish without inoculation, despite the long experimental run time. From Figure 4.6, concentrations of volatile fatty acids were observed to decline in the assays without inoculation after the first 10 days of the experiment. This decline corresponds to recorded increase in methane production, Figure 4.10, but the decline in VFA concentrations ceased after the 15th day of the experiment even though high concentrations of VFA remained in the assays (greater than 1000 mg/L for the PS at

25ºC, 400 mg/L for SS at 37ºC, 200 mg/L for SS at 25ºC, and 700 mg/L for the PS at 37ºC after day 20). The pH values in the system were within the same range for all the assays, Figures 4.7 and 4.8, indicating there was no inhibition of methanogenesis.

For the assays with inoculation of anaerobic biomass, observed VFA concentrations after the 15th day of the experiment were approximately 600 mg/L for the primary sludge assays and less than 100 mg/L for the secondary sludge assays, Figure 4.5. The high VFA concentrations in the assays without inoculation of anaerobic biomass are potentially the reason why the measured methane productions in these assays were lower than the methane production in the corresponding assays with inoculation.

4.5 Summary of key outcomes from anaerobic reduction of DWS

The potential for anaerobic reduction of domestic wastewater sludge at ambient temperature was evaluated and compared against anaerobic reduction at 37ºC, and generally the secondary sludge (SS) showed more resistance towards biological reduction than the primary sludge (Table 4.1). The results also revealed higher biological reduction at 37°C than at 25°C for the volatile solids of the primary sludge (Table 4.1). For the primary sludge (PS) assay with inoculation of anaerobic biomass, over 55% of the volatile solids were reduced at 37ºC, while only 40% reduction was observed at 25ºC. For the secondary sludge (SS) assay with inoculation of anaerobic biomass, over 33% of the volatile solids were reduced at 37ºC, while only 22% reduction was observed at 25ºC. Generally, a good correlation was observed between Equation 2.21 and the data from the BMP assays of domestic wastewater sludge based on the R^2 values obtained (Table 4.2).

R^2 values calculated based on the data from the reduction of the domestic wastewater sludge (Table 4.2), indicated a good correlation of the hydrolysis model (Equation 2.21), digestion time and the reduction of the total solids of the primary sludge assay with inoculation at 25ºC. Whereas, the R^2 values for the digestion of the secondary sludge assay with inoculation at 25ºC indicated a poor correlation between Equation 2.21 and the recorded data (Table 4.2), indicating that digestion time is not an important factor in the prediction of the hydrolysis of total solids of the secondary sludge at 25ºC. The hydrolysis rate constants (k_h) from this study are similar to values reported by other researchers (Mahmoud 2002; Lou *et al.* 2012; Aldin 2010), Table 4.3, and the observed variation of ln k_h for the BMP assays with the inverse of temperature indicates a lack of compatibility with the Arrhenius relationship (Table 4.4). However, compatibility with the Arrhenius relationship, indicated by a decrease in ln k_h corresponding to an increase in the inverse of temperature, was observed for the primary sludge assay without inoculation and the total solids of the secondary sludge assay without inoculation (Table 4.4).

The lack of compatibility with the Arrhenius relationship observed in the BMP assays may be due to factors (for example the available surface area and the composition of the sludge) that were not evaluated in this study. In the initial days of the experiment, the intermediate acids concentrations in the assays without inoculation were similar to the concentrations in the assays with inoculation (Figures 4.5 and 4.6), and the concentrations of volatile fatty acids were observed to decline after the first 10 days of the experiment. However, this decline ceased after the 15th day of the experiment in the assays without inoculation even though high

concentrations of VFA remained in the assays (greater than 1000 mg/L for the PS at 25ºC, 400 mg/L for SS at 37ºC, 200 mg/L for SS at 25ºC, and 700 mg/L for the PS at 37ºC after day 20). Methane production was first observed after an 8-day lag period from the primary sludge assays with inoculation, Figure 4.9, while half of the cumulative methane produced from the secondary sludge assays was detected in the first ten days of the experiment.

The methane yields obtained for the primary sludge BMP assays, Table 4.5, were within the range reported by de Mes *et al.* (2003) for domestic wastewater sludge. Higher cumulative methane production was observed at 37ºC than at ambient temperature (Table 4.5), and higher methane production (mL/g VS removed) was observed for the secondary sludge assays than that observed for the primary sludge assays. The secondary sludge is expected to have smaller particle sizes than the primary sludge (Morgenroth *et al.* 2002), therefore, the secondary sludge should be more accessible to the microorganisms during hydrolysis, and this may account for the higher methane production from secondary sludge compared to primary sludge. However the methane produced (mL/g VS removed) decreased with increase in temperature, except in the case of the assays without inoculation where an increase was observed (Table 4.5). In the BMP assays without inoculation of biomass, the methane production was very low, in all cases less than half the production from the corresponding BMP assays with inoculation. In the summary of the results presented in Table 4.1, the observed conversion of organic material from particulates to soluble compounds was not higher than 60% by mass, so there is a potential to improve the process.

The biological reduction rates observed, and the corresponding methane recovery rates, should be useful in predictive simulations of treatment options for wastewater with high concentrations of solids such as domestic wastewater. Errors in the data from the reduction of the DWS were identified in Figures 4.1 – 4.4, potentially as a result of sampling with needles and syringes, and this resulted in the need to discard several data points in order to achieve a fit of the data to Equation 2.21. However, the first order hydrolysis model, Equation 2.21, and the Arrhenius relationship can be very useful in reactor design, as they correlate conversion rates with environmental temperature. Temperature and the concentrations of acids appear to influence the rate of hydrolysis of the domestic wastewater solids, and this will have a corresponding influence on the retention and biodegradation of solids in the ABR. The high VFA concentrations in the assays without inoculation of anaerobic biomass are potentially the reason why the measured methane productions in these assays were lower than the methane production in the corresponding assays with inoculation. Presumably the methane producing organisms were unable to adequately establish, and this is important with respect to the recovery of a treatment system after biomass loss during operation, for example after removal of sludge.

Chapter Five – Performance efficiency of ABR bench models

5.0 Introduction

Shanmugam and Akunna (2008) reported differences in methane yields between the compartments of the ABR, attributed to the phase separation that is eventually achieved in the reactor due to the development of different microbial populations in each compartment. An evaluation of the influences of temperature on the kinetics of phase separation, hydrodynamics and methane production in the ABR will enhance the potential for future application of the ABR as an efficient low energy domestic wastewater treatment option. Reactor performance is influenced by the retention of biomass and rates of biodegradation, as well as the nature of flow and degree of mixing (Barber and Stuckey 1999). Also the dead space in the reactor volume, consequently the effective treatment volume, is a critical factor to consider in reactor design (Sarathai *et al.* 2010).

Low process temperature, combined with high flow rates and short retention times, can cause process failure in the ABR. For example high levels of solids washout or backpressure due to potentially low biodegradation rates of influent particulate compounds can lead to accumulation of solids in the reactor (Shanmugam and Akunna 2010), and decrease in the effective treatment volume. The objective of this chapter is to present an evaluation of the influences of temperature on the operating characteristics of the ABR, based on observed data from ABR bench experiments, methodologies presented in Sections 3.3 – 3.5. The influences of temperature on hydrodynamic characteristics, phase separation and methane production in ABR

bench models were therefore examined by comparing a bench ABR operated at a mesophilic temperature of 37ºC (R1) to a bench ABR operated at ambient temperature (R2).

5.1 Hydrodynamic characteristics

Langenhoff and Stuckey (2000) reported that temperature does not have any substantial influence on the hydrodynamics of the ABR, therefore a comparison of flow control (either intermittent or continuous) was carried out in order to fully investigate the potential for low energy operations. Continuous flow conditions should be achieved with flow balancing, a feature that enhances process stability by eliminating variation of influent flow and loadings, and consequently improved energy balance in the system (Caldwell 2009). Alternatively, variation of influent flow and concentration, represented as intermittent flow, is the natural pattern of domestic wastewater generation (Davis 2011), and therefore the potential for energy saving should be considered if the system can be operated with minimum flow control.

The presence of a sludge bed is considered to have negligible influence on the hydrodynamic characteristics of the ABR (Sarathai *et al.* 2010); therefore the residence time distribution (RTD) experiment was carried out before inoculation of the two bench reactors using methodologies described in Section 3.4. The results obtained from the RTD experiment are presented in Appendix C, and the analysis of the results using Equations 2.8 – 2.16, Section 2.3.3, is presented in Table 5.1.

Table 5.1: Results of RTD tests on ABR bench models at ambient temperature

Test nos.	Flow	HRT (min)	Mean velocity (m/min)	t (min)	e	V_d	d	N	λ	C_r
1	Continuous	60.000	0.050	51.287	0.855	0.145	0.057	9.339	0.763	0.892
2	Intermittent	180.000	0.033	137.531	0.764	0.236	0.104	5.346	0.621	0.923
3	Intermittent	180.000	0.050	165.250	0.918	0.082	0.085	6.429	0.775	0.930
4	Intermittent	180.000	0.067	166.456	0.925	0.075	0.053	9.915	0.831	0.865
5	Intermittent	180.000	0.050	176.414	0.980	0.020	0.099	5.595	0.805	0.914
6	Continuous	180.000	0.017	148.970	0.828	0.172	0.070	7.718	0.720	0.854
7	Continuous	240.000	0.013	207.282	0.864	0.136	0.076	7.092	0.742	0.880
8	Intermittent	360.000	0.067	327.472	0.910	0.090	0.047	11.173	0.828	0.704

HRT - Theoretical hydraulic retention time (minutes);
t - Mean hydraulic retention time (minutes);
e - Effective fraction of reactor volume;
V_d - Fraction of volume that is considered as dead space;
d - Dispersion number;
N - Tank in series number;
λ - Hydraulic efficiency;
C_r - Fraction of total tracer detected;

Grobicki and Stuckey (1992) observed a dispersion number d = 0.0752 which is an intermediate flow; and suggested that d = 0.2 can be considered as being close to ideal completely mixed flow. From Table 5.1, the dispersion numbers obtained for the experiment are: (1) = 0.057; (2) = 0.104; (3) = 0.085; (4) = 0.053; (5) = 0.099; (6) = 0.070; (7) = 0.076 and (8) = 0.047. Based on the observed dispersion numbers, Table 5.1, the flows in the bench reactors were neither ideal plug flows nor completely mixed flows, but rather intermediate flows were observed in all the test conditions. For the three continuous flow tests (nos. 1, 6 and 7 from Table 5.1), similar effective reactor volumes were observed (0.855, 0.828 and 0.864), and subsequently similar dead space volumes. This indicates that with continuous displacement inside the reactor, the hydrodynamic characteristics appear to be independent of the HRT and influent velocities.

Substantial differences were observed in the hydrodynamic characteristics of continuous flow conditions compared to intermittent flow conditions (Table 5.1). For example, at a HRT of 180 minutes in tests nos. 2 to 6 (Table 5.1), high mean influent velocities were applied for the intermittent flow conditions compared to the continuous flow conditions. The mean hydraulic retention times observed for the intermittent flow tests (nos. 3, 4 and 5) were high (165.2, 166.5 and 176.4 minutes, respectively), while for the continuous flow test (nos. 6) the mean HRT observed was 149.0 minutes (Table 5.1). For the intermittent flow test nos. 2, the influent velocity was lower than that of the other intermittent flow tests, nos. 3 to 5, and the observed mean HRT was lower than the mean HRTs for the tests and the continuous flow test nos. 6 (Table 5.1). The effective reactor volume in test nos. 2 was also lower than the effective volumes in tests nos. 3 to 6, suggesting that the flow condition and influent

velocity are important in determining the effective volume in the reactor. From Table 5.1, tests nos. 4 and 8, having the same influent velocity but with different HRT, appear to show similar characteristics with only slight variation. However when compared to other intermittent flow tests (nos. 2, 3 and 5 in Table 5.1) with different influent velocities, the differences in the characteristics appear to be high. This indicates that the influent velocity is potentially more important in determining hydrodynamic characteristics than HRT if the anaerobic baffled reactor is operated under intermittent flow conditions.

5.2 Acclimatization of biomass in bench reactors

The bench experiment to evaluate the organic loading removal efficiency of the ABR was initiated with introduction of anaerobic biomass and a synthetic feed (Section 3.3) into two bench reactors (R1 and R2). The 1st compartments of the ABR bench models were not inoculated with anaerobic biomass, but the final five compartments of the bench reactors were inoculated with anaerobic digested sludge (Section 3.3). The time for the reactors to acclimatize to the initial organic loading rate of 1.25 kg COD/m^3 was expected to be long based on reported acclimatization periods in literature, Section 2.3.1. Monitored pH in the effluents of the compartments of the two bench reactors, presented in Appendix D, indicated that achieving stable pH values at 48 hours HRT required a longer time period for R2 compared to R1, Figure 5.1.

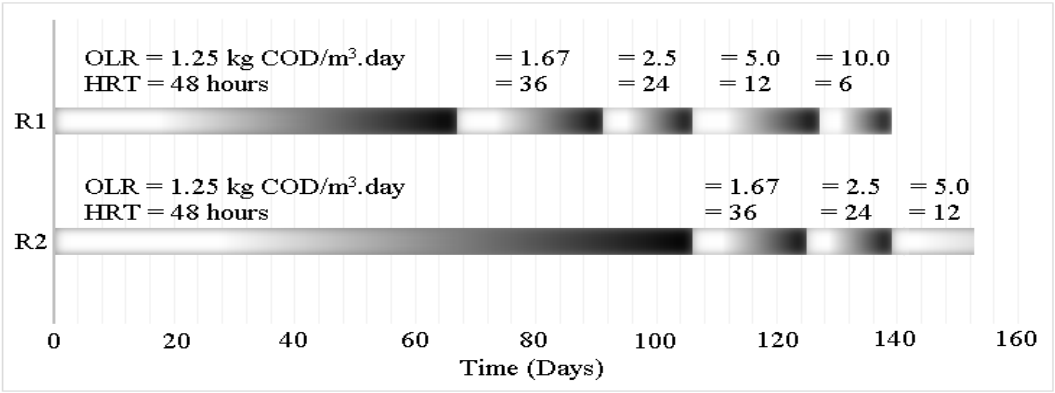


Figure 5.1: Days before stable pH values were observed at evaluated HRTs (hours) and OLRs (kg COD/m^3) for ABR bench models R1 and R2.

The first segment of the horizontal bars in Figure 5.1 indicates the time taken for stable pH values to be observed at 48 hours HRT and 1.25 kg COD/m^3.day ORL, which was approximately 70 days for R1 and 110 days for R2. The operational conditions were adjusted to the next HRT and OLR (Table 3.3), for each bench reactor after stable pH values were observed at 48 hours HRT, and consequently the bench reactors were each evaluated at other HRTs (36, 24, 12 and 6 hours) and OLRs (1.67, 2.5, 5.0 and 10 kg COD/m^3.day). The bar lengths indicate the period after adjusting the operational conditions until stable pH values were observed at the corresponding HRT and OLR for each bench reactor.

R1 required approximately 20, 15, 25, and 15 days before stable pH values were observed at 36, 24, 12, and 6 hours HRT (Figure 5.1), respectively, while R2 required approximately 25 and 20 days before stable pH values were observed at 36 and 24 hours HRT, respectively. Stable pH values were not observed in R2 at 12 hours HRT before the experiment was terminated due to a decline in the ambient temperature in the laboratory. Figure 5.2 shows the effluent COD values for the two reactors at experimental Day 67 during the period stable pH values were observed in R1 at 48 hours HRT.

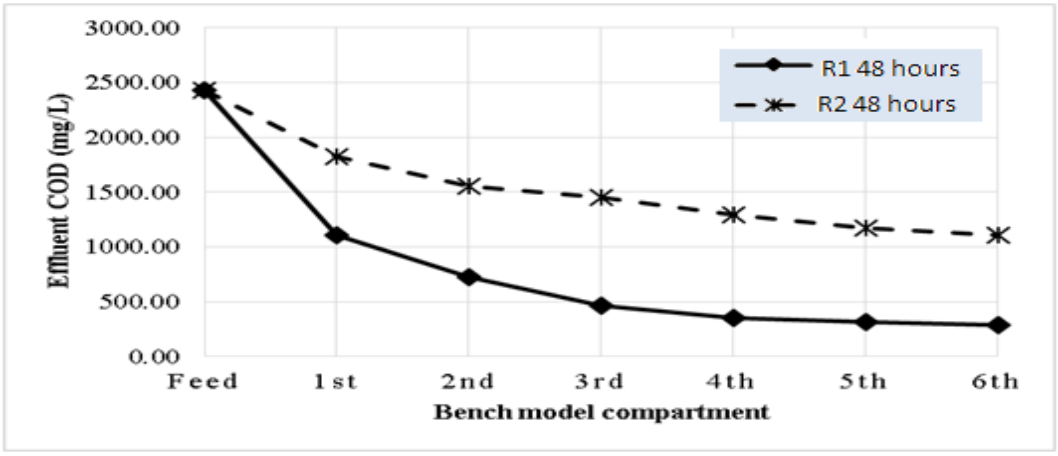


Figure 5.2: Effluent COD (mg/L) for each compartment in R1 and R2 after 67 days of operation at 48 hours HRT with 1.25 kg COD/m^3.day OLR.

From Figure 5.2, the removal of COD loading by R1 was 88% of the influent feed on day 67, while for R2 it was 55%. By Day 105 of the ABR bench experiment, a 73% influent COD removal was observed for R2, which was less than the 88% for R1 at day 67. This indicates that R1 showed quicker acclimatization than R2, and also eventually high COD removal was achieved in R1 compared to R2. The difference in the results of the 1st compartments from the two reactors, which were initially not inoculated with any biomass, may be due to the temperature differences because both compartments were receiving the same feed at the same flow rates (2479.50 mg COD/L).

From Figure 5.2, a 54% influent COD removal was observed for the 1st compartment of R1, against a 25% influent COD removal for the 1st compartment of R2. The COD removal in the 1st compartments appear critical to the overall performance of the reactors; if the 1st compartments are disregarded, the observed COD removal from the last five compartments of R1 at day 67 was 34% of the influent COD, while R2 had 30% removal of the influent COD in the last five compartments (Figure 5.2).

5.3 Organic loading removal

The final effluent chemical oxygen demand (COD) concentrations from the bench reactors are presented in Figure 5.3 for the evaluated HRTs, (Table 3.3). For R2, the effluent COD concentrations at the evaluated HRTs were not considerably different, contrary to what was obtained for R1 (Figure 5.3) where substantial differences in final effluent COD concentrations were observed for the HRTs evaluated.

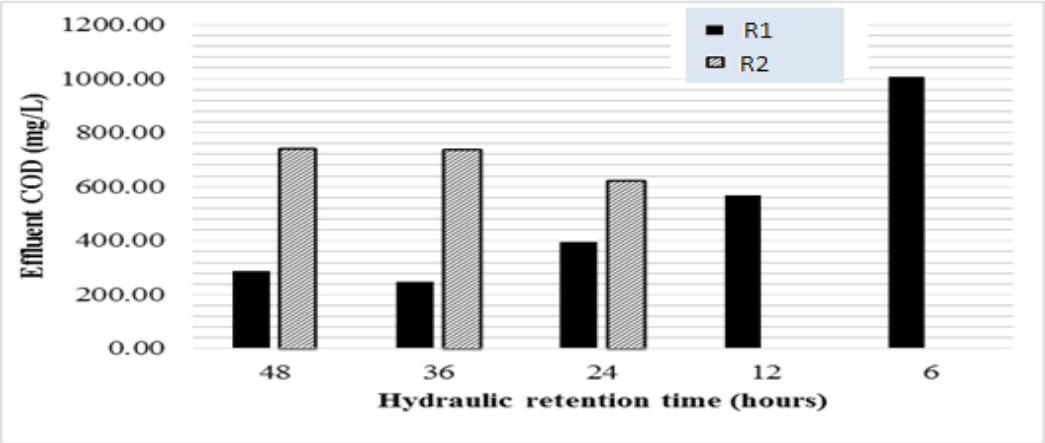


Figure 5.3: Final effluent COD (mg/L) corresponding to evaluated HRTs for R1 and R2 with average influent COD = 2479.50 mg/L.

From Figure 5.3, the final effluent with the lowest COD concentration (mg/L) was obtained for R1 at 36 hrs HRT with effluent COD of 248 mg/L, Table 5.2, which corresponds to 90% reduction from the influent feed (mean = 2479.50 mg/L). Table 5.2 presents the effluent COD (mg/L) and COD removal efficiencies (%) for R1 and R2, where the highest removal rate observed for the operational conditions examined was 90% of the influent COD at 36 hours HRT for R1.

Table 5.2: Observed effluent COD (mg/L) and percentage removal of COD (%) for the ABR bench models based on average influent COD = 2479.50 mg/L.

Reactor	Temp. (°C)	HRT (Hours)	Effluent COD (mg/L)	COD removal (%)
R1	37	48.00	287.00	88.43
		36.00	248.00	90.00
		24.00	396.00	84.03
		12.00	570.00	77.01
		6.00	1008.00	59.35
R2	Ambient	48.00	740.00	70.16
		36.00	735.00	70.36
		24.00	620.00	74.99

From Table 5.2, 88.43, 90.00, 84.03, 77.01 and 59.35% of the influent COD (mean = 2479.50 mg/L) were removed at 48, 36, 24, 12 and 6 hour HRTs, respectively, in R1, while 70.16, 70.36 and 74.99% of the influent COD were removed in R2 at 48, 36 and 24 hour HRTs, respectively. The lowest observed effluent COD for R2 was at 24 hours HRT, which was about 600 mg/L, a value that is similar to that observed at 12 hours HRT for R1 (Table 5.2). Figure 5.4 presents the observed volatile fatty acids concentrations in the effluents from the compartments of the bench reactors for the evaluated HRTs (Table 3.3).

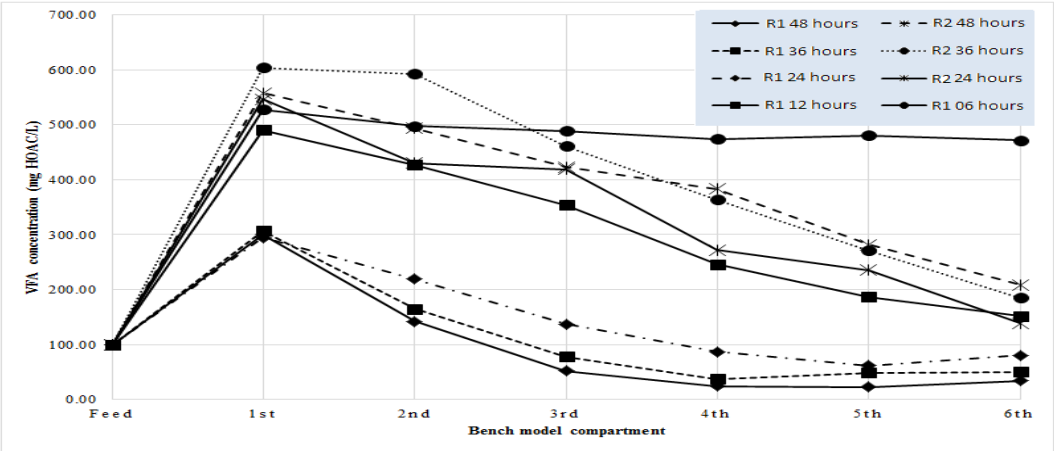


Figure 5.4: Volatile fatty acids concentrations (mg HOAC/L) in the effluents of compartments of R1 and R2 for different hydraulic retention times.

From Figure 5.4, the VFA profiles shown were for 48, 36, 24, 12 and 6 hours HRT which correspond to 1.25, 1.67, 2.5, 5 and 10 kg COD/m^3.day OLR, respectively. From Figure 5.4, the observed average effluent VFA concentration at 12 hours HRT for R1 was approximately double the effluent VFA concentrations at 48, 36 and 24 hours for the same reactor. For R1 at 6 hours HRT (OLR = 10 kg COD/m^3.day), there was virtually no change in VFA concentrations along the length of the reactor (Figure 5.4), probably because the methane producing biomass was yet to acclimatize to the synthetic feed at the time of measurements. The effluent VFA concentrations for R2 were higher than effluent VFA concentrations observed for corresponding HRTs for R1, Figure 5.4, and they were also higher than the observed effluent VFA concentration at 12 hours HRT for R1 which was 152 mg HOAC/L.

For the VFA profiles for the two reactors, Figure 5.4, the 1st compartments are again the critical sections of the reactor operation, where R1 was able to maintain approximately 300 mg/L VFA concentrations for all the evaluated HRTs except 12 and 6 hours HRT, while R2 maintained VFA concentrations above 500 mg/L in its 1st compartment for all HRTs. For R1, once the biomass acclimatized, the readily biodegradable substrates were mostly removed before the 4th compartment (Figure 5.4), indicating that four compartments may be adequate to achieve high COD removal at 37^{o}C. While for R2, there appears to be no substantial change in VFA concentrations between consecutive compartments, and the change was relatively constant between the compartments. This suggests that zones of microbial dominance were developed in R1, while in R2 there were no definite zones of

dominance established. Figure 5.5 presents pH values at steady state for the effluents from the compartments of the bench reactors.

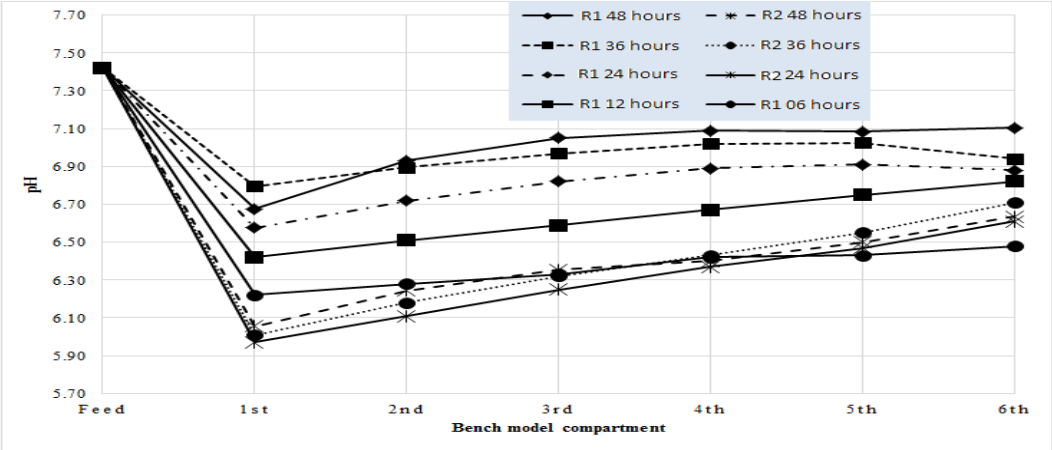


Figure 5.5: pH values for the corresponding HRTs in the effluents of the compartments of R1 and R2.

For the HRTs evaluated, the pH values recorded were between 5.90 and 7.30 (Figure 5.5), indicating a pH range suitable for methane producing organisms. However, for R2 there are considerable VFA concentrations in the final effluents of the reactor, presented as effluent from the 6th compartment in Figure 5.4, indicating inefficient depletion of the VFA concentrations by methanogens. The critical aspect of the operations at ambient temperature, R2, is the savings in energy consumption by avoiding heating requirements. However the results presented in Figures 5.3 – 5.5 indicate a loss in treatment performance in R2 when compared to a reactor operated at a stable mesophilic temperature (R1). Alternatively, with a long hydraulic retention time at ambient temperature, substantial percentages of the organic load may be removed. According to Ghaniyari-Benis *et al.* (2012), reducing the HRT of a system leads to a reduction in the contact time between the organic substrate and anaerobic microorganisms, and therefore, the concentrations of total VFA should decrease

when the HRT is increased, and decreased concentrations of VFA will be observed in the effluents of the system.

Ghaniyari-Benis *et al.* (2009) reported increase in total VFA concentrations corresponding to increase in OLRs and decrease in HRTs, with accumulation of intermediate products of the anaerobic process when short HRTS were adopted. Zhu *et al.* (2008) observed increase in total VFA concentrations with increase in influent COD in a four compartment ABR, however variation in composition of VFA concentrations was not observed with variation of OLRs. Propionic and butyric acids were the main acids in the first compartment, greater than 60% of total VFA concentration, while acetic acid was the main acid in the second compartment (Zhu *et al.* 2008), and the total VFA concentrations steadily declined from the first compartment until the fourth compartment. Acetate constituted more than 60% of total VFA concentrations in the first two chambers of a nine chambered ABR (Bodkhe 2009), and the concentrations were observed to decline in the last chambers of the reactor, a similar trend was also observed for propionic and butyric acids.

According to Yaun *et al.* (2011), the change in microbial populations as a result of changes in operational conditions, for example temperature, can lead to shifts in the anaerobic digestion pathway and consequently the nature of the intermediate products. During sludge digestion experiments under thermophilic conditions with a continuous flow reactor, Aitken *et al.* (2005) observed that the most abundant VFA in the effluent was propionate. In experiments with an anaerobic membrane bioreactor, Yuzir *et al.* (2011) reported detection of propionate with a HRT of 1 day, however propionate was not detected when HRTs longer than 1 day were examined (3, 7 and

17 day). Yuzir *et al.* (2011) observed an increase in butyric acid as a percentage of VFA concentrations after a decrease in pH, where potentially the low pH conditions induced a stressful environment for the microorganisms causing a change in pathway and led to increase in butyric acid production. Butyric acid is not consumed by methanogens, however, available butyric acids become converted to acetic acids during acetogenesis when acetic acid concentrations are low (Grobicki and Stuckey 1991).

This indicates there may be a difference in the composition of the VFA concentrations at ambient temperature compared to the composition at 37ºC. Consequently the low removal rates observed at ambient temperature may be due to the predominance of a different form of intermediate acid which is not readily converted to methane compared to the acetic acid. Therefore, apart from the observed low substrates reduction in Section 4.1 at ambient temperature, another limiting factor for efficient ambient temperature anaerobic digestion may be the transformation of the substrates to acetic acid.

Operation with incremental changes to HRT at 37ºC

Operation with incremental changes to HRT at ambient temperature was not carried out due to the observed difficulty of achieving steady state in R2 over periods in excess of 10 days, Section 5.2. The operation of R3 was primarily in order to simulate the impact of a steady accumulation of retained solids on the volume of the reactors, consequently reducing the effective hydraulic retention time. Figure 5.6 presents the recorded effluent COD concentrations for R3 after ten days of operation at the corresponding HRTs.

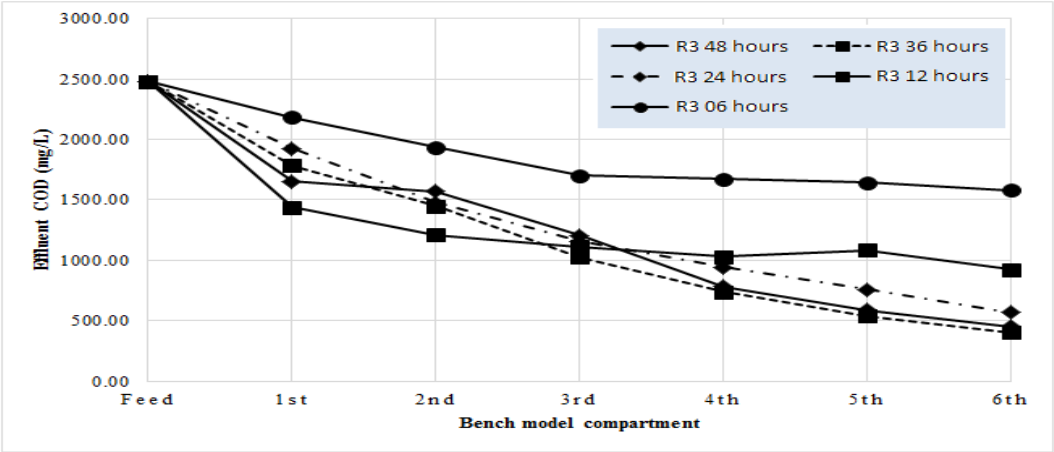


Figure 5.6: Effluent COD (mg/L) from compartments of R3 with incremental changes in HRT at 37ºC.

The observed final effluent COD, the 6th compartment in Figure 5.6, at the monitored HRTs were higher than the results presented in Figure 5.3 for R1, where steady state performance was achieved before changes to the HRT. For the two reactors operated at 37ºC (R1 and R3), high COD removals were observed for the HRTs evaluated in R1 (Table 5.2), where biomass acclimatization was encouraged, compared to R3 where acclimatization was not considered (Figure 5.6). From Table 5.2, R1 recorded the highest COD removals for each of the evaluated HRTs, while R2 had COD removals lower than R3 (Figure 5.6) for all the HRTs. Figure 5.7 presents the corresponding VFA profiles obtained for R3, indicating high concentrations of acids in the effluents compared to that observed in Figure 5.4 for R1. While Figure 5.8 presents the corresponding pH values for R3, with pH values below 5.60 for 6 hours HRT and between 5.80 – 6.90 for 48, 36, 24 and 12 hours HRT, again generally low compared to corresponding HRTs and compartments for R1 (Figure 5.5).

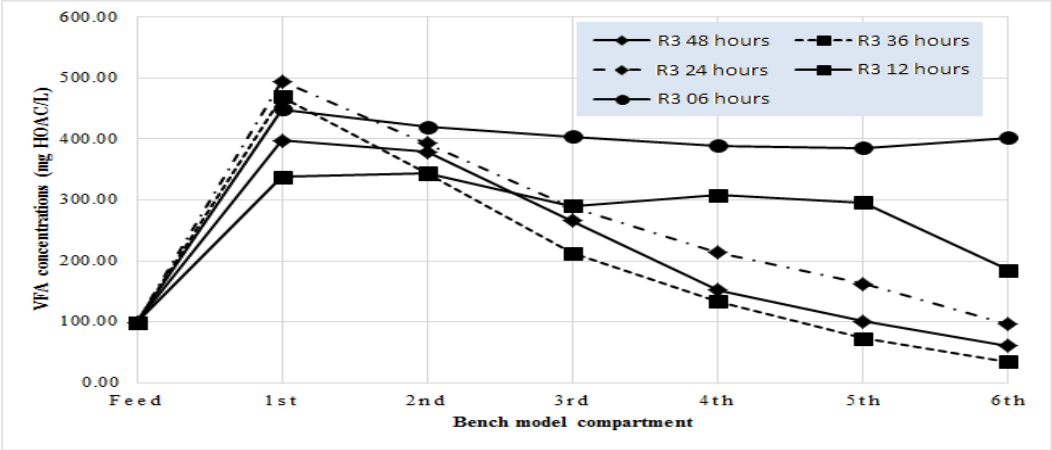


Figure 5.7: Volatile fatty acids concentrations (mg HOAC/L) in the effluents of compartments of R3 operated with incremental changes in HRT at 37°C.

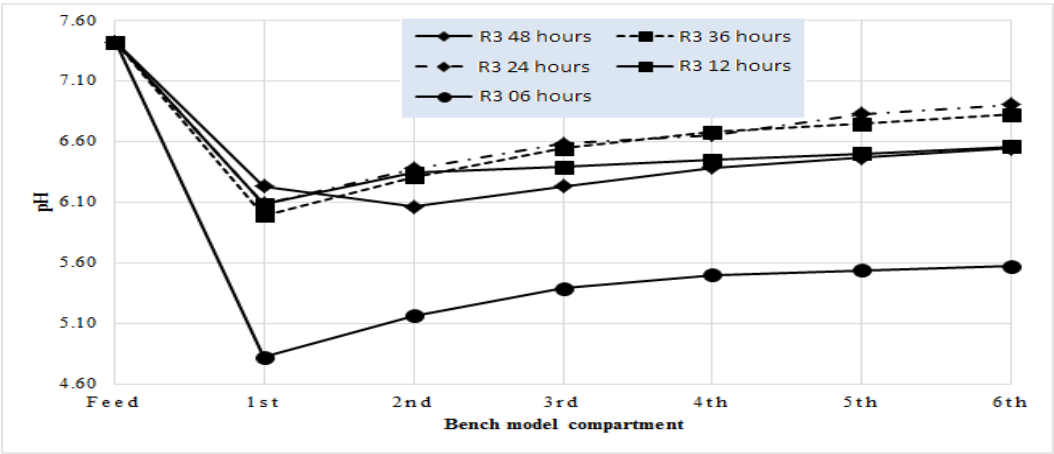


Figure 5.8: Observed pH in the effluents of compartments of R3 with incremental changes at 37°C

The results presented in Figures 5.3 – 5.8 indicate that for efficient operation and performance, the dynamic characteristics of the ABR in terms of acclimatization of the biomass need to be considered, especially at start-up and before steady state is attained. Operation of the bench reactor with incremental changes to HRT (R3), Figures 5.6 – 5.8, produced high effluent concentrations of VFA and COD compared to the effluent concentrations of VFA and COD observed in R1 (Figures 5.3 and 5.4). R3 simulated the potential impact of accumulation of solids and reduction of the effective volume on the system efficiencies, represented by changing the HRT. The

results in Figures 5.3 and 5.6 indicate that performance efficiencies will be low for systems where the effective volume is decreased by accumulating solids compared to systems where the effective volume is not influenced by additional retained solids. Table 5.3 presents a summary of reported COD removal efficiencies of ABR systems.

Table 5.3: Summary of reported COD removals (%) in ABR systems.

Source	Temp. (°C)	HRT (Hours)	COD removal (%)
Ayaz *et al.* (2012)	12 – 28	12.1	41 – 50
Bodkhe (2009)	35	6	84
Feng *et al.* (2015)	22.0 - 24.8	10	66.4
Foxon *et al.* (2007)	-	22	58 - 72
Gomec (2010)	19	12.8	67
Gomec (2010)	18	9.5	63
Gomec (2010)	22 – 28	8 and 12	67.5 and 75.6
Gopala-Krishna *et al.* (2009)	23 - 31	8 – 10	≥ 90
Nachaiyasit and Stuckey (1995)	35 and 25	20	90
Nachaiyasit and Stuckey (1995)	15	20	80
This study – R1	37	6 - 48	59.35 - 90.00
This study – R2	17 – 25	24 - 48	70.16 - 74.99
This study – R3	37	6 - 48	36.28 - 83.80

From Table 5.3, apart from the reported COD removals below 50% by Ayaz *et al.* (2012) and the 36.3% COD removal at 6 hours HRT for R3 in this study, the reported COD removal efficiencies were generally between 58 and 90%. This range is similar to the reported range of COD removal efficiencies for the treatment of domestic wastewater at ambient temperature using the up-flow anaerobic sludge blanket (UASB) reactor (Table 2.2).

5.4 Retention of total solids

To evaluate the potential influence of temperature on the fate of solids in the ABR, analysis of total solids in the effluents of the compartments of the bench reactors were carried out using methodologies described in Section 3.4. Effluent total solids (TS) from the bench reactors were monitored at steady state for the HRTs evaluated, with a mean influent total solids of 3.87 g/L (3.5 – 4.12 g/L) in the synthetic feed. The total solids in the effluents from each compartment of R1 and R2 are presented in Figure 5.9.

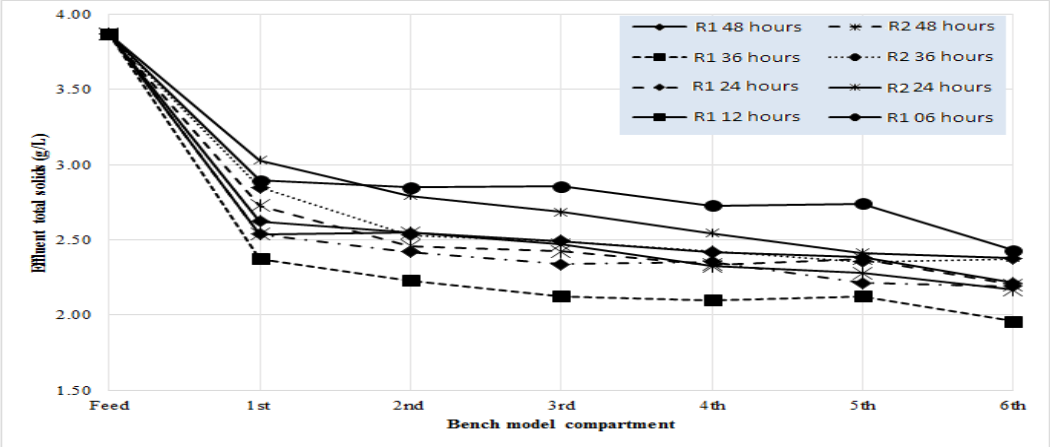


Figure 5.9: Effluent total solids concentrations (g/L) from the compartments of R1 and R2 for the corresponding HRTs.

From Figure 5.9, the effluent TS for R1 and R2 from the final compartment at all hydraulic retention times examined was not greater than 2.5 g/L. This indicates that there is generally a 1.3 g/L difference between the influent and the effluent, indicating possible solid retention or biological reduction within the reactors of approximately 1.3 g/L. Table 5.4 presents the estimated solids retention time (SRT) for R1 and R2 for evaluated HRTs based on Equation 3.2 presented in Section 3.4, where the sludge retained is based on the initial inoculum introduced to the reactors

which had a total solids concentration of 25.21 g/L (85.71 grams in each reactor). Changes in concentrations of retained total solids in the compartments due to the biodegradation of the solids within the reactors and also increase in biomass due to microbial population growths were not considered for the estimated SRTs presented in Table 5.4, and the sludge inside the reactors were considered to have remained constant for the duration of the experiment.

Table 5.4: Estimates of solids retention time based on influent and effluent total solids

Reactor	HRT (hrs)	Influent total solids (g/day)	Effluent total solids (g/day)	Total solids retained (%)	SRT (days)
R1	48	32.90	18.82	42.80	6.30
	36	43.86	22.21	49.36	5.83
	24	65.79	37.23	43.41	4.07
	12	131.58	73.78	43.93	2.95
	6	263.16	165.24	37.21	2.11
R2	48	32.90	18.70	43.16	6.34
	36	43.86	26.90	38.67	4.82
	24	65.79	40.46	38.50	3.74

The percentage of influent total solids retained, Table 5.4, shows that high total solids retention was observed at a HRT of 36 hours for R1. The other operational HRTs fall into two categories, with 48, 24 and 12 hours HRT for R1 and 48 hours for R2 showing similar retention of solids despite the different operational conditions, while 24 and 36 hours HRT for R2 along with 6 hours HRT for R1 form a second group with similar retention percentages. This analysis did not consider the effect of biodegradation or biomass growth inside the reactors, which may be the factor causing the retention percentages to appear within similar ranges for the two categories identified (Table 5.4). From Figure 5.9, there is high effluent total solids with short retention times; this indicates that the flow rate is important in terms of

retention of solids. It is possible that the direct correlation between SRT and HRT that can be observed from the values in Table 5.4 is valid, in agreement with the opinion of other researchers (Appels *et al.* 2008).

5.5 Methane production

Monitored methane gas percentages in the headspace of R1 and R2 at 48 hours HRT, Figure 5.10, indicate a difference in the percentages of methane between the various compartments of the two bench reactors. From Figure 5.10, for the 1st compartment, R2 did not contain any substantial percentage of methane in the headspace, but for the other compartments there are noticeable percentages of methane in the headspaces. For R1, the average percentages of methane in the headspace of the first three compartments were measured at above 50% (Figure 5.10), while for the 4th, 5th and 6th compartments, the average methane percentages were 28, 40 and 20%, respectively.

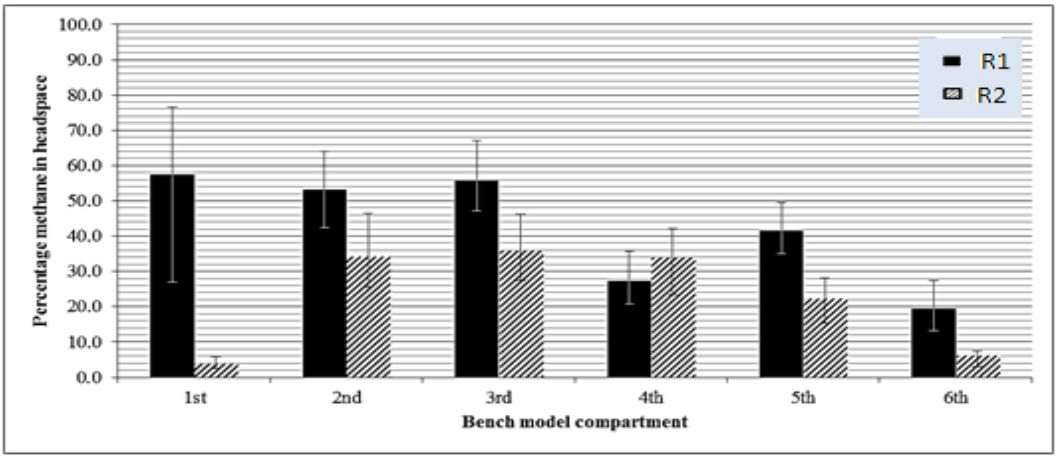


Figure 5.10: Average percentage methane in headspace of the compartments of R1 and R2 at 48 hours HRT, where the error bars indicate the range of values recorded for each compartment.

Fluctuations in temperature have been observed to affect the activity of methane-forming microorganisms (Bergamo *et al*. 2009), and this may explain the low percentages of methane in the headspaces of the compartments of R2 when compared to R1. Also there is the low COD removal rates observed in the compartments of R2, Figure 5.2, where the changes in COD for successive compartments in R2 were not substantial. The removal of monitored VFA concentrations in R1 was higher than that observed in R2, Figure 5.4, indicating that R2 appeared to have difficulty converting the acids to methane, and therefore low percentages of methane were observed for R2 compared to R1. However the measured pH values in R1 and R2, Figure 5.5, were higher than the threshold for pH inhibition of methanogenesis, Section 2.4.3, therefore methane production was expected to have occurred in all the compartments.

From Figure 5.5, the pH values of the 1st, 2nd and 3rd compartments in R2 fall outside the ideal range (6.5 – 7.5) for methane production, and there were reductions in COD and VFA concentrations in these compartments (Figures 5.3 and 5.4). The 2nd and 3rd compartments of R2 were observed to have considerable percentages of methane in the headspace (Figure 5.10), approximately 35%, but this is still low compared to 55% for the same compartments in R1. Attempts to measure the rate of gas production (and consequently methane production) directly for each compartment during the operating period using a wet-tip gas meter and a liquid displacement system provided incomplete data where no gas flows were observed. Using the theoretical methane production of 350 mL per gram COD removed, Section 2.4.4.3, the estimated methane productions from the compartments of R1 and R2 at 48 hours HRT are presented in Table 5.5.

Table 5.5: Estimated methane production from the compartments of R1 and R2 at 48 hours HRT (OLR = 1.25 kg/m^3 day)

Reactor	Compartment	COD removed (mg/L)	Estimated methane (mL/L)	COD removed (g/day)	Estimated rate (mL/day)
R1	1st	1370.50	479.68	11.99	4196.85
	2nd	384.00	134.40	3.36	1176.00
	3rd	263.00	92.05	2.30	805.35
	4th	111.00	38.85	0.97	339.85
	5th	38.00	13.30	0.33	116.55
	6th	26.00	9.10	0.23	79.80
R2	1st	653.50	228.73	5.72	2001.30
	2nd	269.00	94.15	2.35	823.90
	3rd	109.00	38.15	0.95	333.90
	4th	152.00	53.20	1.33	465.50
	5th	126.00	44.10	1.10	385.70
	6th	66.00	23.10	0.58	202.30

The estimated production rates of methane, Table 5.5, suggest that biogas escape may have occurred from the bench reactors due to leakages, and therefore the consequent failure to measure the production rates. A major oversight in the experimental methodology was the lack of examination of the bench reactors for air leakages during the operational period, which should have provided critical information about the efficiency of the bench reactors. If leakage of air had been proven, then the lack of observed gas production would have been explained as a failure in making the bench reactors airtight. However, since leakage cannot be established conclusively, other reasons must be considered as possible explanations for the absence of methane flow into the measurements systems adopted. Another alternative to consider is the loss of biogas as a result of solubility in water, since no consideration was given to the loss of biogas through absorption into the water in the gas meters (Walker *et al.* 2009). The solubility of methane in water increases with

decrease in temperature (Watanabe *et al.* 2014), Section 2.4.5, and the gas meters were filled with tap water and not subjected to any temperature control. Therefore, the potential that biogas was lost in the water displacement systems should also be considered.

Also, with the continuous flow conditions in the bench reactors, produced biogas may have escaped with the effluent, before sufficient pressure builds-up in the headspace, instead of flowing through the gas outlets (Figure 3.3). The possibility also exists that all the three potential problems highlighted above, leakages, loss with effluent flow and solubility in the gas meter fluid, were actively contributing to the lack of detection of methane flow from the bench reactors. Ayaz *et al.* (2012) observed very low methane production from the ABR system they evaluated at 12 - 28ºC, and they concluded the possibility of methane loss with the effluents or instability in the system due to uneven distribution of the retained biomass in the reactor. Ayaz *et al.* (2012) observed biomass accumulation in one compartment of the reactor, potentially due to high flow rates and transport of biomass across the compartments. However, transport and accumulation of biomass to one compartment was not observed in this study in any of the bench reactors evaluated. Bodkhe (2009) reported a methane yield of 0.34 m^3 CH_4/kg $COD_{removed}$ from an ABR system at 35ºC, a value close to the theoretical value (0.35 m^3 CH_4/kg $COD_{removed}$) adopted for estimation of methane production in Table 5.5. Gopala-Krishna *et al.* (2009) reported methane yields between 0.25 to 0.30 m^3 CH_4/kg $COD_{removed}$ from an ABR system at 30ºC, while Zhu *et al.* (2015) reported 0.18 m^3 CH_4/kg $COD_{removed}$ at 30ºC.

5.6 Summary of key outcomes from bench experiments

The dispersion numbers obtained from the RTD experiment in the ABR bench model with intermittent and continuous flow conditions, Table 5.1, indicated that the mixing in the bench reactors were intermediate. Also, similar effective reactor volumes were observed for the three continuous flow tests, indicating that the hydrodynamic characteristics appear to be independent of the HRT and influent velocities with continuous displacement inside the reactor. While, high effective reactor volumes were observed for intermittent flows compared to continuous flows, and the influence of the influent velocity appears critical to the reactor hydrodynamics for intermittent flow conditions where high influent velocities were correlated to high effective volumes. Presumably, the influent velocity is more important a factor in determining reactor hydrodynamic characteristics, compared to the HRT, if the anaerobic baffled reactor is operated under intermittent flow conditions. Furthermore, bench experiments were performed with continuous operation of ABR systems at 37^{o}C (R1) and ambient temperature (R2) with 48, 36, 24, 12 and 6 hours HRTs, which corresponded to 1.25, 1.67, 2.5, 5 and 10 kg COD/m^3.day OLR, respectively.

In terms of acclimatization of the retained biomass in the reactors, Figure 5.1, R1 showed pseudo steady state characteristics after approximately 60 days of continuous operation with 48 hours HRT, while R2 reached steady state after approximately 100 days of continuous operation. Furthermore, stable pH values were not observed in R2 at 12 hours HRT before the experiment was terminated due to a decline in the ambient temperature in the laboratory. After 67 days of continuous operation, R2 was observed to remove only 25% of influent COD load in the 1st compartment, lower

than the 54% influent COD load removal in the 1st compartment of R1. This indicates that R1 showed quicker acclimatization than R2, and also eventually high COD removal was achieved in R1 compared to R2, potentially as a result of difficulty in achieving adequate biomass in the 1st compartment of R2 for conversion of the substrate to methane. However, if the 1st compartments are disregarded, the observed COD removal from the last five compartments of R1 at day 67 was 34% of the influent COD, while R2 had 30% removal of the influent COD in the last five compartments (Figure 5.2).

After 67 days of operation at 48 hours HRT, the removal of COD loading was 88% of the influent feed by R1, and 55% by R2, and subsequently, 73% by R2 after 105 days of the ABR bench experiment. 88.43, 90.00, 84.03, 77.01 and 59.35% of the influent COD (mean = 2479.50 mg/L) were removed at 48, 36, 24, 12 and 6 hour HRTs, respectively, in R1, while 70.16, 70.36 and 74.99% of the influent COD were removed in R2 at 48, 36 and 24 hour HRTs, respectively. A VFA concentration of approximately 300 mg/L was maintained in the 1st compartment of R1 for all the evaluated HRTs except 12 and 6 hours HRT, Figure 5.4, while VFA concentrations in the 1st compartment of R2 were consistently above 500 mg/L for all HRTs. The volatile fatty acids were mostly removed before the 4th compartment of R1 (Figure 5.4), indicating that four compartments may be adequate to achieve high COD removal at 37ºC, while there appears to be no substantial change in VFA concentrations between consecutive compartments in R2. This suggests that zones of microbial dominance were developed in R1, while in R2 there were no definite zones of dominance established.

Total COD removed, generally between 58 and 90% of influent COD, by the ABR in this study were similar to reported COD removals for the treatment of domestic wastewater at ambient temperature using the up-flow anaerobic sludge blanket (UASB) reactor, Table 2.2, apart from the 36.3% COD removal at 6 hours HRT for R3. High effluent concentrations of VFA and COD were observed in R3, operated with incremental changes to HRT (Figures 5.6 – 58), compared to the effluent concentrations of VFA and COD observed in R1 (Figures 5.3 and 5.4) where biomass acclimatization was encouraged. Therefore, performance efficiencies are expected to be low in systems where the effective volume, and consequently the HRT, is decreasing (R3) compared to systems where the effective volume and consequently the HRT, is relatively stable. Furthermore, estimated SRTs based on observed influent and effluent total solids indicated that SRT and HRT have a direct correlation (Table 5.4), in accordance with reports by Appels *et al.* (2008), and therefore, periodic removal of sludge should be part of the operational requirements of the system in order to maintain the effective treatment volume.

Methane production rates were not measured during the experiment, due to potential biogas escape from the bench reactors due to leakages, or alternatively, due to loss of biogas with effluent flow and solubility in the gas meter fluid. R2 appeared to have difficulty converting the available volatile fatty acids to methane, and also the changes in COD for successive compartments of R2 were not substantial, Figure 5.2, even though the pH values in R2, Figure 5.5, were generally higher than the threshold for pH inhibition of methanogenesis. However, the pH values in the 1st, 2nd and 3rd compartments of R2, were outside the ideal range (6.5 – 7.5) for methane production, and the 2nd and 3rd compartments of R2 were observed to have

considerable percentages of methane in the headspace (Figure 5.10), approximately 35%, which was lower than the 55% observed for the same compartments in R1. The advantage of treating domestic wastewater at ambient temperature, R2, is the savings in energy consumption by avoiding heating requirements, however, the results, Figures 5.3 – 5.5, indicate a low treatment performance in a reactor operated at ambient temperature (R2) compared to a reactor operated at a stable mesophilic temperature (R1).

Heating provides temperature control and stable temperatures, while operating without heating at ambient temperature would cause fluctuations in the systems temperature, and anaerobic digestion pathway has been observed to change as a results of fluctuations in temperature (Bergamo *et al*. 2009). Experiments by Aitken *et al.* (2005) and Yuzir *et al.* (2011) reported detection of propionate as the most abundant acid in the effluent and when short HRTs were adopted, respectively, also increase butyric acid concentrations were reported after decrease in pH. Consequently the low removal rates observed at ambient temperature may be due to the predominance of a different form of intermediate acid which is not readily converted to methane, and therefore a limiting factor for efficient ambient temperature anaerobic digestion may be the transformation of the organic contaminants to acetic acid. Alternatively, performance efficiency may be achieved at ambient temperature with a long hydraulic retention time; increasing the HRT of a system should lead to an increase in the contact time between the organic substrate and anaerobic microorganisms (Ghaniyari-Benis *et al.* 2012).

Yuzir *et al.* (2011) did not detect high concentrations of propionate with HRTs longer than 1 day, Indicating additional treatment capacity may be required before the COD removal at ambient temperature will become equivalent to what was observed at 37ºC. The next chapter of this thesis presents an energy analysis based on the results presented so far, with a view to identifying the operational condition with the best energy efficiency, in terms of operational temperatures and hydraulic retention time, and also the identification of a suitable set of design criteria for energy efficient treatment of domestic wastewater.

Chapter Six - Energy efficiency analysis and recommended design criteria

6.0 Introduction

The proposed integrated anaerobic-aerobic system, schematically represented in Figure 6.1, needs to be evaluated as a potential energy efficient system. The integrated anaerobic-aerobic reactor is expected to have three sections (Figure 6.1), where the first section is a compartment for retention of influent domestic wastewater solids. The second section is a baffled anaerobic section where methane can be recovered, and the third section is an aerobic section in order to ensure the final effluent quality from the system can satisfy target effluent discharge standards.

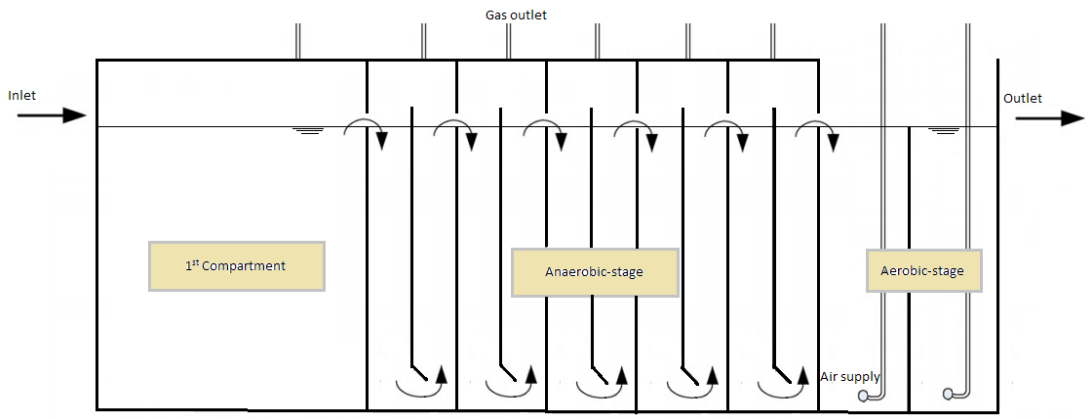


Figure 6.1: Schematic representation of the proposed integrated anaerobic-aerobic reactor

The objective of this chapter is to present a comparison of the energy efficiencies of the evaluated anaerobic stage of the anaerobic-aerobic system, represented by the ABR bench models R1 and R2, in order to determine the operational condition with the best energy efficiency. The experimental results presented in Chapters Four and Five and parameters in literature are the basis for the evaluation of the energy

efficiencies of the ABR bench models. The evaluation is based on determination of net energy in each bench reactor for the evaluated HRTs, using Equation 3.16 and the methodology described in Section 3.6.

6.1 Energy efficiency analysis

The two bench reactors were evaluated using the defined energy efficiency criteria (Section 3.6), for the hydraulic retention times where steady state performance was observed (48, 36, 24, 12 and 6 hours for the model reactor operated at 37ºC, and 48, 36 and 24 hours for the reactor operated at ambient temperature). Inputs to the system are mainly in the form of energy as electricity for operational processes such as pumping, mixing and heating and manual human activities such as residual waste management. Outputs from the system are in the form of effluent COD and solids, and possible energy recovered in the form of methane and heat.

The energy and resources used in the construction of the systems were considered to be primarily influenced by the design volume, and therefore the hydraulic retention times were considered to represent the differences in volumes. For this analysis, $E_{mechanical}$ and $E_{Chemical}$ were disregarded since the bench reactors only relied on pumps powered with electricity supply for the experimental period and no chemicals were used as part of the treatment process. The other terms of Equation 3.16 were determined using parameters presented in Table 6.1 as required by Equations 3.8 – 3.15 of Section 3.6.

Table 6.1: Parameters for energy efficiency analysis

Parameter	Units	Value	Ref.
Energy equivalent for Methane	kWh/m^3	5.815	Abbasi *et al.* 2012
Coefficient of performance for cooling COP_c	-	4.48	Chae and Kang (2013)
Coefficient of performance for heating COP_h	-	5.35	"
Specific heat of wastewater C_p	kJ/kg.K	4.186	Davis (2011)
Density of wastewater ρ	kg/m^3	1000	"
Wastewater flow Q	m^3	1	-
Temperature difference from datum δT	Kelvin	Ambient = 0 37°C = 12	-
Rated power of peristaltic pumps P	kW	0.037	Cole-parmer UK
Number of activities n	-	1	-
gender involved in activities	-	1	-
Persons engaged in activity	-	1	-
Human power equivalent	kW	0.075	Sousa (2008)
Duration of manual activities	h/day	8	-
Coefficient of heat transfer v	$W/m^2.K$	1	-
Cross sectional area for heat losses	m^2	1	-

Tables 6.2 and 6.3 provide the observed COD removal, effluent solids and the corresponding energy values calculated from the two bench reactors during the experimental period for the hydraulic retention times monitored. The effluent COD indicates only the soluble COD without consideration for the particulate compounds which were considered recoverable as sludge. Also, since methane production measurements were incomplete, no methane values were included in the analysis.

Table 6.2: Results from ABR bench experiments

	HRT (days)	Soluble eff (mg/L)	Particulate eff (mg/L)	COD effluent (mg/L)	COD removed (mg/L)	COD removed (%)	Methane (mL/L)
R1	2	287.00	2210.00	287.00	2213.00	88.52	-
	1.5	248.00	1960.00	248.00	2252.00	90.08	-
	1	396.00	2190.00	396.00	2104.00	84.16	-
	0.5	570.00	2170.00	570.00	1930.00	77.20	-
	0.25	1008.00	2430.00	1008.00	1492.00	59.68	-
R2	2	740.00	2200.00	740.00	1760.00	70.40	-
	1.5	735.00	2370.00	735.00	1765.00	70.60	-
	1	620.00	2380.00	620.00	1880.00	75.20	-

Table 6.3: Residuals and related energy values for the bench reactors

	Residuals				Energy						
	HRT (days)	Effluent COD (mg/L)	Temp. (°C)	Sludge (mg/L)	E_{cool} (kWh/m^3)	E_{heat} (kWh/m^3)	$E_{methane}$ (kWh/m^3)	E_{power} (kWh/m^3)	E_{Manual} (kWh/m^3)	E_{loses} (kWh/m^3)	E_{net} (kWh/m^3)
R1	2	287.00	37.00	2210.00	0.01	0.03	0.00	1.78	0.60	0.004	-2.40
	1.5	248.00	37.00	1960.00	0.01	0.03	0.00	1.33	0.60	0.004	-1.96
	1	396.00	37.00	2190.00	0.01	0.03	0.00	0.89	0.60	0.004	-1.51
	0.5	570.00	37.00	2170.00	0.01	0.03	0.00	0.44	0.60	0.004	-1.07
	0.25	1008.00	37.00	2430.00	0.01	0.03	0.00	0.22	0.60	0.004	-0.85
R2	2	740.00	25.00	2200.00	0.00	0.01	0.00	1.78	0.60	0.000	-2.38
	1.5	735.00	25.00	2370.00	0.00	0.01	0.00	1.33	0.60	0.000	-1.94
	1	620.00	25.00	2380.00	0.00	0.01	0.00	0.89	0.60	0.000	-1.49

The corresponding energy analysis for the two bench reactors is presented in Table 6.4, with the systems net energy at the monitored hydraulic retention times and temperatures, and the respective energy efficiency evaluation criteria.

Table 6.4: Energy efficiency analysis results for the bench reactors

	HRT (days)	E_{net} (kWh/m^3)	FEI	EQI	EYR	ELR	SI
R1	2	-2.40	1.07	2.50	0.00	3.00	0.00
	1.5	-1.96	0.86	2.21	0.00	2.26	0.00
	1	-1.51	0.71	2.59	0.01	1.52	0.34
	0.5	-1.07	0.54	2.74	0.01	0.78	0.94
	0.25	-0.85	0.55	3.44	0.01	0.41	2.25
R2	2	-2.38	1.35	2.94	0.00	2.97	0.00
	1.5	-1.94	1.09	3.11	0.00	2.21	0.00
	1	-1.49	0.79	3.00	0.00	1.47	0.00
Shaded cells indicate the cells with the best values for the corresponding criterion/column.							

From Table 6.4, the best FEI value, which is also the lowest, is associated with 12 hours HRT (0.5 days) at 37ºC. For the effluent quality index, EQI, the lowest value obtained is for 36 hours (1.5 days) at 37ºC. If the values of the other criteria, EYR, ELR and SI, are considered, then the best operational condition is 6 hours HRT (0.25 days) at 37ºC, which also has the best net energy value and the second best FEI value, but does not have a good EQI value as a result of high effluent COD concentrations. The operational condition with the second best energy efficiency from the results obtained is 12 hours HRT (0.5 days) at 37ºC, which has the second best values for all the criteria except for the functional efficiency index (FEI) where it has the best value and the effluent quality index (EQI) where it has the fourth ranked value, higher than 6 hours HRT at 37ºC.

The absence of methane production observations may have led to a skewed outcome for the energy analysis. When consideration is given to potential methane production using the theoretical 350 mL methane production per gram of COD removed presented in Section 2.4.3, estimated methane production rates (mL/L) based on COD removal in the bench reactors can then be included in the energy analysis. Accordingly, the energy analysis provides a different outcome, Tables 6.5 and 6.6, for the energy calculations and criteria evaluations.

Table 6.5: Estimated methane production and energy for the bench reactors

	HRT (days)	**COD removed (mg/L)**	**Methane (mL/L)**	**$E_{methane}$ (kWh/m^3)**	**E_{net} (kWh/m^3)**
R1	2	2213.00	774.55	4.50	2.10
	1.5	2252.00	788.20	4.58	2.62
	1	2104.00	736.40	4.28	2.77
	0.5	1930.00	675.50	3.93	2.86
	0.25	1492.00	522.20	3.04	2.19
R2	2	1760.00	616.00	3.58	1.20
	1.5	1765.00	617.75	3.59	1.65
	1	1880.00	658.00	3.83	2.33

Table 6.6: Energy efficiency analysis results for the bench reactors based on estimated methane production

	HRT (days)	E_{net} (kWh/m^3)	FEI	EQI	EYR	ELR	SI
R1	2	2.10	1.07	2.50	1.87	0.35	5.30
	1.5	2.62	0.86	2.21	2.33	0.26	8.91
	1	2.77	0.71	2.59	2.82	0.19	15.04
	0.5	2.86	0.54	2.74	3.65	0.10	35.11
	0.25	2.19	0.55	3.44	3.55	0.07	51.98
R2	2	1.20	1.35	2.94	1.50	0.43	3.53
	1.5	1.65	1.09	3.11	1.85	0.32	5.86
	1	2.33	0.79	3.00	2.56	0.20	12.85
Shaded cells indicate the cells with the best values for the corresponding criterion/column.							

If the selection is based only the net energy, then 12 hours HRT (0.5 day) at 37ºC should be selected, and this operational condition also presents the best energy yield ratio (EYR) value, and is ranked second in the environmental loading ratio (ELR) and sustainability index (SI) columns. The 12 hours HRT (0.5 day) at 37ºC does appear to be the best alternative, especially as it provides the best functional efficiency index (FEI), and it also has the fourth ranked EQI. This operational condition should be recommended above the other operational conditions which tend to have poor ranks in some criteria. The 24 hours HRT (1.00 day) at 37ºC does not have a value that is ranked lower than fourth in all the criteria, and is therefore considered as the second option after the 12 hours HRT at 37ºC. Adoption of a unit coefficient of heat transfer and unit surface area for the energy efficiency analysis in this study, Table 6.1, provided a simple basis for comparing the two bench reactors while avoiding the complexity of modelling the heat losses and heating efficiencies of systems with variable effective volumes.

Variable heat transfer and surface area will potentially cause substantial differences in the values obtained for heating and cooling energies and the energy losses due to heat losses (E_{cool}, E_{heat} and E_{loses}), and subsequently the net energy (E_{net}) and the energy efficiency criteria. The FEI (kWh/kg$COD_{removed,}$), EYR, ELR and SI values obtained (Tables 6.4 and 6.6) will be directly affected by changes to the heating efficiencies of the systems. The HRT, representing the effective volume of the bench reactors, is an influential factor with respect to the energy efficiency criteria, Tables 6.4 and 6.6, and therefore a variable effective volume will be substantially influential on the outcome of the energy efficiency analysis. Furthermore, a reduction of HRTs should lead to increase in the effluent loads (Ghaniyari-Benis *et al.* 2009), and

subsequently the EQI should also depreciate, however, decrease in HRT and corresponding increase in OLR has been reported as leading to increase in total biogas production (Hassan and Dahlan 2013).

Other researchers reported decrease in methane yields corresponding to increase in OLRs (Bodkhe 2009; Shanmugam and Akunna 2008), and also loss of biogas (Lettinga *et al.* 1993; Ayaz *et al.* 2012). Sallis and Uyanik (2003) reported methane production rates of 0.163 – 0.190, 0.227 – 0.289, 0.291 – 0.374, 0.221 – 0.327 and 0.099 – 0.144 (m^3/kg_{COD}) corresponding to 0.9, 1.5, 2.75, 5.5 and 10.5 (kg COD/m^3day) OLRs, respectively, for an ABR operated with a fixed HRT and increasing influent COD concentrations. From the data reported by Sallis and Uyanik (2003), the OLR with the highest range of methane yield was the 2.75 kg COD/m^3day OLR, which was neither the highest nor the lowest OLR evaluated, and therefore the potential gain in biogas production as a result of increasing OLR may not be adequate to offset the depreciation in performance efficiency. The energy efficiency analysis should identify an operational condition, in terms of OLR, HRT and temperature, where the performance efficiency and energy recovery are at an equilibrium, and therefore an efficient design can be adopted. Table 6.7 provides the summary of the selected operational conditions from both the energy analysis without methane measurements and the analysis with estimated methane productions.

Table 6.7: Selected operational conditions

Analysis conditions	**Rank**	condition
Without methane measurements	Best	6 hours HRT at 37^{o}C
Without methane measurements	Second	12 hours HRT at 37^{o}C
With methane estimates	Best	12 hours HRT at 37^{o}C
With methane estimates	Second	24 hours HRT at 37^{o}C

The selected operational conditions presented in Table 6.7 indicate the adoption of 12 hours HRT at 37^{o}C should be the preferred system operational condition for the anaerobic stage of the anaerobic-aerobic domestic wastewater treatment system.

6.2 Recommended design criteria

With the selected operational condition in terms of temperature and hydraulic retention time as 12 hours HRT at 37^{o}C, the number of compartments necessary for an effective anaerobic stage should be at least five, as adopted by most researchers (Section 2.3.3). Design parameters for the ABR recommended by Foxon and Buckley (2006) were modified to accommodate experimental observations and findings in literature, and are presented in Table 6.8.

Table 6.8: Recommended design parameters for the anaerobic stage

Parameter	Notation	Units	Range or Equation
Wastewater flow rate	Q	m^3/hour	-
Hydraulic retention time	HRT	hour	12
Effective volume	V_w	m^3	$Q * HRT$
Volume of 1st compartment	V_1	m^3	(0.3 to 0.5) * V_w
Peak up-flow velocity	υ_p	m/hour	0.54
Design up-flow velocity	υ_d	m/hour	$\frac{\upsilon_p}{1.8} = 0.3$
Up-flow area	A_u	m^2	Q/υ_d
Up-flow to down-flow area ratio	$R_{u:D}$	-	1 : 1
Number of compartments	N	-	≥ 5
Compartment width to length ratio	$C_{w:L}$	-	3 - 4
Compartment area	A_c	m^2	$A_u * 2$
Reactor depth	r_D	m	1 - 3
Reactor width	r_w	m	$\sqrt{\frac{V_w * C_{w:L}}{N * r_D}}$
Reactor length	r_L	m	$\frac{N * r_w}{C_{w:L}}$
Hanging baffle clearance	d_h	m	0.15 - 0.20

The adoption of a 1:1 ratio for the up-flow to down-flow area is based on the recommendations of Shanmugam and Akunna (2008) which is expected to ensure hydraulic stability in the system. Consideration can be provided in the 1st compartment to accommodate estimated sludge accumulation by provision of adequate reactor space for sludge digestion where mixing will occur and sludge storage between periods of sludge removal or for the duration of the design sludge retention time. Mara (2003) reported sludge accumulation rates for anaerobic ponds

in warm climates ranging from 0.1 to 0.4 m^3/person per year. Therefore a sludge accumulation volume can be determined by multiplying the sludge accumulation rate with the retention time based on recommended SRTs for anaerobic systems and the number of persons served by the system. From Figure 2.3, as reported by Appels *et al.* (2008), a design SRT of 40 days is recommended for domestic wastewater treatment systems.

Aerobic stage

For the aerobic stage, Figure 6.1, the provision of adequate volume and aeration to ensure reducing the effluent loads from the anaerobic stage is the key consideration. To provide for fully aerobic zones where all the effluent substrate from the anaerobic stage is removed from the wastewater stream and converted to biomass, summarized model formulas are defined in Equations 6.1 and 6.2 (Davis 2011), and typical values for aerobic process parameters at 20ºC are presented in Table 6.9.

$$\left(\frac{F}{M}\right) ratio = \frac{S_{in}}{X} \qquad \text{Equation 6.1}$$

$$\theta_{min} = \frac{Y * \theta_c (S_{in} - S)}{X(1 + k_d * \theta_c)} \qquad \text{Equation 6.2}$$

Where:

$\left(\frac{F}{M}\right) ratio$ = ratio of available substrate to microbial biomass

S_{in} = influent concentration of soluble substrate (mg/L)

X = concentration of microorganisms/biomass produced (mg/L)

Θ_{min} = minimum retention time to avoid substrate washout (day)

Y = solids yield (mgVSS/mgCOD)

Θ_c = solid retention time (day)

S = concentration of soluble substrate (mg/L)

k_d = decay coefficient (mg/mg.day)

Table 6.9: Typical values for aerobic parameters at 20°C (Davis 2011)

Parameter	Typical	Range	Units
k_d	0.10	0.0 – 0.30	d^{-1}
Y	0.6	0.4 – 0.8	mg.VSS/mg.BOD_5
F/M ratio	-	0.04 – 2.0	mg/mg.d
SRT (Θ_c)	-	3 - 15	days

The minimum retention required to avoid washout in the aerobic stage can therefore be determined for the soluble effluents of the anaerobic stage using Equations 6.14 and 6.15, once the parameters described in Table 6.9 are specified. For example, if k_d is set at 0.15 d^{-1}, Y at 0.65 mg/mg.COD, F/M at 0.2 mg/mg.day and SRT at 5 days, then the minimum retention times required for the observed effluent COD of the anaerobic stage at the monitored HRTs and temperatures can be determined, Table 6.10, based on a set target of 50 mg/L COD as final effluent from the system.

Table 6.10: Minimum retention required for aerobic stage

	Anaerobic stage HRT (days)	Anaerobic effluent COD (mg/L)	Sludge (mg/L)	Aerobic stage HRT (days)	System HRT (days)
R1	2.00	287.00	1435.00	0.307	2.307
	1.50	248.00	1240.00	0.297	1.797
	1.00	396.00	1980.00	0.325	1.325
	0.50	570.00	2850.00	0.339	0.839
	0.25	1008.00	5040.00	0.353	0.603
R2	2.00	740.00	3700.00	0.346	2.346
	1.50	735.00	3675.00	0.346	1.846
	1.00	620.00	3100.00	0.341	1.341

From Table 6.10, the selected operational condition of 12 hours (0.5 days) HRT at 37ºC for the anaerobic stage has a corresponding minimum retention time of 0.339 days for the aerobic stage, making the total required retention time for the anaerobic-aerobic system to achieve efficient organic loading removal as 0.839 days. The proposed design criteria provided a fixed HRT, however, it is obvious that the OLR applied on the system is also an important factor to consider in the operation of the system, especially in terms of biogas production. There is no identified optimum OLR to recommend for the anaerobic stage, however, a range of 1.5 – 5.5 OLR (kg COD/m^3day), with an average 2.75 OLR should be the recommended applied OLR for the ABR. The recommended operational condition with the best energy efficiency rating from this study, 12 hour HRT, corresponds to 5.0 OLR, however steady state was observed only for up to 2.5 OLR at ambient temperature and according to the data from Sallis and Uyanik (2003) methane production is expected to peak at 2.75 OLR, therefore the 1.5 – 5.5 OLR range is proposed.

However, because of the variable nature of domestic wastewater flow and organic load concentration, the proposed design criteria cannot be based on the organic loading rate because that will require a flexible effective treatment volume. The nature of the ABR suggests that flexibility can be adopted as part of operational control, for example the effective treatment volume can be adjusted in line with the variable nature of domestic wastewater flow in such a way as to ensure that the OLR and HRT are maintained at the recommended values of 2.75 kg COD/m^3day and 12 hours, respectively. Specifically, when the wastewater flow is high for locations with high influent COD concentrations, for example Jordan and South Africa, the effective volume of the treatment system should be increased by providing additional

capacity for the anaerobic stage in order not to exceed 5.5 OLR. However, for locations with very low influent COD concentrations, for example in European cities, maintaining the specified OLR may be difficult unless very low HRTs are adopted, for example between 4 – 6 hours.

6.3 Proposed dynamic model for the anaerobic stage

Due to the limitations of the experimental scope with the bench reactors in terms of the ability to perform a comprehensive evaluation of potential operational conditions (HRT, OLR, SRT and temperature), a preliminary process model of the anaerobic stage of the system is presented as the first step in future process optimization research of the system. The compartmental nature of the system was represented as a series of blocks, each considered as an independent reactor and assumed to be homogeneous completely mixed tank reactors at steady-state. This assumption provides for a steady state where the populations of microorganisms and substrate concentrations in each block are considered to remain constant, i.e. the growth rate of the microorganisms is approximately the same as the rates of decay.

The model is developed using mass balance equations applied for each tank reactor which relate the consumption and accumulation of specified state variables within the system and the influent and effluent flows across the system (Ghaniyari-Benis *et al.* 2010). At steady state, the concentrations of the variables in the system remain constant, while the influent and effluent mass for each variable will be influenced by the flow rate adopted. The reduced mass of a variable, or in some cases produced, can be determined based on reaction and conversion rates for each variable, and if mixing is highly efficient, then the effluent mass concentration will be equal to the

mass concentration in the reactor. These principles lead to Equation 6.3 as a general description of mass balance across each tank reactor (Batstone 2006; Veeken *et al*. 2000; García-Diéguez *et al.* 2013).

$$Q_{in}C_{in} \pm Vr_c - Q_{out}C_{sys} - Q_wC_r = \frac{V*\partial C_{sys}}{\partial t} \qquad \text{Equation 6.3}$$

Where:

V = volume of reactor (L)

Q = the flow rate in, or out (L/day)

Q_w = flow rate of wasted biomass/sludge (L/day),

C_{in} = concentration of variable in influent (mg/L)

C_r = concentration of variable retained as sludge/biomass (mg/L)

r_c= change (consumption or production) of variable (mg/L.day)

C_{sys} = concentration of variable in reactor (mg/L)

If Equation 6.3 is divided by the volume of the reactor, it then transforms to Equation 6.4, which relates the process to the retention time (Θ) adopted.

$$\frac{\partial C_{sys}}{\partial t} = \frac{C_{in}}{\theta} \pm r_c - \frac{C_{sys}}{\theta} - \frac{q\,C_r}{\theta} \qquad \text{Equation 6.4}$$

Where:

q = the ratio of the wasted flow (Q_w) to the flow rate (Q)

An important aspect of Equation 6.4 is the change in the variable due to consumption or production, which is usually based on biological process models, while the other terms are based on the physical configuration of the system (volume, retention time

and flow rate). Some basic assumptions necessary for reactions that occur in the tank reactors are:

- There is immediate dilution of the influent and the reactor is homogenous with only a single dominating phase.
- The mass balances for biomass are affected by the biomass washout in the effluent and biomass death/decay.
- The biomass mean cell residence time, solids retention time (SRT) θ_c (d) is considered to be equivalent to the retention time (Θ).
- Temperature influences on process parameters are considered to be defined by the Arrhenius relationship.

For the anaerobic processes, biodegradable organic matter is assumed to be converted to methane and carbon dioxide through three steps which are the solid, liquid and gas phases, as shown in Figure 6.2. The digestion process adopted starts with the first step where the particulate substrate (solid) is converted to soluble organic matter (liquid), based on the relevant reaction rates. Then the soluble substrates are converted to volatile fatty acids, which are eventually converted to biogas.

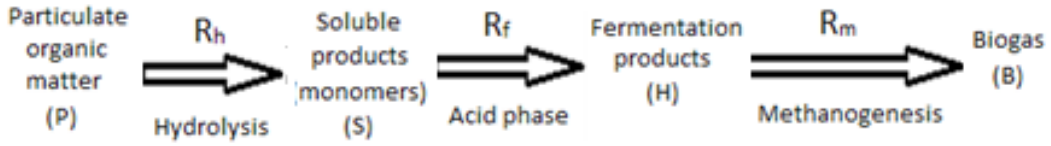


Figure 6.2: Anaerobic processes for reduction of biodegradable organic matter in domestic wastewater (Veeken *et al*. 2000) where: R_h is the rate of hydrolysis, R_f is the rate of fermentation, and R_m is the rate of methanogenesis.

The anaerobic stage model equations are summarised in Equations 6.5 – 6.10, which define the rates of substrate utilization and methane production. Equations 6.5 – 6.9 provide reaction rates for five identified state variables for the anaerobic stage model (Dochain and Vanrolleghem 2001; Donoso-Bravo *et al.* 2013; Noykova *et al.* 2002; Veeken *et al.* 2000). Equation 6.10 provides a model for methane production rates based on consumption of acetic acid.

$$\frac{\partial P}{\partial t} = \frac{P_o}{\theta} - \propto k_h P - \frac{P}{\theta} \qquad \text{Equation 6.5}$$

$$\frac{\partial S}{\partial t} = \frac{S_o}{\theta} + \propto k_h P - \frac{\mu_f * S * F}{Y_{f1} * (K_{sf} + S)} - \frac{S}{\theta} \qquad \text{Equation 6.6}$$

$$\frac{\partial F}{\partial t} = \frac{F_o}{\theta} + \frac{\mu_f * S * F}{K_{sf} + S} - k_{df} F - \frac{F}{\theta} \qquad \text{Equation 6.7}$$

$$\frac{\partial H}{\partial t} = \frac{H_o}{\theta} + \frac{Y_{f2} * \mu_f * S * F}{(K_{sf} + S)} - \frac{\mu_m * H * M}{Y_m * (K_{sm} + H)} - \frac{H}{\theta} \qquad \text{Equation 6.8}$$

$$\frac{\partial M}{\partial t} = \frac{M_o}{\theta} + \frac{\mu_m * H * M}{K_{sm} + H} - k_{dm} M - \frac{M}{\theta} \qquad \text{Equation 6.9}$$

$$Methane\ Produced = B_u * \Delta H_{Acetic\ acid\ as\ COD} = B_u * \frac{\mu_m * H * M}{(K_{sm} + H)} \qquad \text{Equation 6.10}$$

Where:

P = concentration of particulate substrate in the system (mg/L)

P_o = influent concentration of particulate organic substrate (mg/L)

Θ = hydraulic retention time (day)

α = coefficient of degradable particulates conversion to soluble substrates

k_h = the first order hydrolysis rate constant (day^{-1})

S = concentration of soluble substrate in the system (mg/L)

S_o = influent concentration of soluble organic substrate (mg/L)

F = concentration of fermentation organisms in the system (mg/L)

Y_{fl} = yield coefficient for fermentation organisms

(mg/mg substrate)

μ_f = specific growth rate for fermentation organisms (mg/mg.day)

K_{sf} = half saturation constant for fermentation (mg/L)

F_o = influent concentration of fermentation organisms (mg/L)

k_{df} = decay coefficient for fermentation organisms (mg/mg.day)

H = concentration of intermediate acids in the system (mg/L)

H_o = influent concentration of intermediate acids (mg/L)

Y_{f2} = yield coefficient for intermediate acids (mg/mg substrate)

μ_m = specific growth rate for methanogens (mg/mg.day)

M = concentration of methanogens in the system (mg/L)

Y_m = yield coefficient for methanogens (mg/mg substrate)

K_{sm} = half saturation constant for methanogenesis (mg/L)

M_o = influent concentration of methanogens (mg/L)

k_{dm} = decay coefficient for methanogens (mg/mg.day)

B_u = the methane yield (mL/mg acids removed)

Typical values for anaerobic process parameters for mesophilic digestion are presented in Table 6.11.

Table 6.11: Typical values for anaerobic parameters (Davis 2011)

Parameter	Typical	Range	Units
Y fermentation	0.10	0.06 – 0.12	g.VSS/g.COD
Y methanogenesis	0.04	0.02 – 0.06	g.VSS/g.COD
Y combined	0.08	0.05 – 0.10	g.VSS/g.COD
k_d fermentation	0.04	0.02 – 0.06	g/g.d
k_d methanogenesis	0.02	0.01 – 0.04	g/g.d
k_d combined	0.03	0.02 – 0.04	g/g.d
µ combined 35°C	0.35	0.30 – 0.38	g/g.d
µ combined 30°C	0.25	0.22 – 0.28	g/g.d
µ combined 25°C	0.20	0.18 – 0.24	g/g.d
K_s combined 35°C	160	60 – 200	mg/L
K_s combined 30°C	360	300 – 500	mg/L
K_s combined 25°C	900	800 – 1100	mg/L

The retention of solids during the treatment process is primarily a factor of the reactor hydrodynamics, which is governed by the flow velocity and settling velocity of the particulate substances based on observations presented in Section 5.4. Therefore Equation 6.5 needs to be modified with consideration for the effect of a sludge accumulation term which is based on an accumulation rate defined by Fleming (2002: cited in Cesur and Albertson 2005). At steady state, where the anaerobic state variables are constant, the sludge accumulation rate is defined by Equation 6.11.

$$Sludge\ rate = P_r * Q_w = P * A * Vel_s \qquad \text{Equation 6.11}$$

Where:

P_r = concentration (mass/volume) of retained particulate substances

Q_w = flow rate (volume/time)

P = mass concentration of particulate substances in the system

A = cross sectional area normal to the settling flow

Vel_s = settling velocity

Dividing both sides with the reactor volume (V), as was the case in Equation 6.3 will yield Equation 6.12.

$$\frac{q*P_r}{\theta} = \frac{P*A*Vel_s}{V} \qquad \text{Equation 6.12}$$

Where:

q = ratio of the retained flow (Q_w) to the wastewater flow (Q)

Θ = retention time

When the cross sectional area (A) is replaced by the relationship expressed in Equation 6.13, then Equation 6.12 becomes Equation 6.14 (with consideration for the relationship between volume, HRT and flow rate).

$$A = \frac{Q}{Vel_{flow}} \qquad \text{Equation 6.13}$$

$$\frac{q*P_r}{\theta} = \frac{P*Vel_s}{\theta*Vel_{flow}} = \frac{P*q_v}{\theta} \qquad \text{Equation 6.14}$$

Where:

Q = influent wastewater flow rate

Vel_{flow} = flow velocity

q_v = ratio of settling velocity to flow velocity

Equation 6.14 relates the sludge accumulation in the reactor to the concentration of particulates, the settling velocity, the flow velocity and the hydraulic retention time, and when Equation 6.5 is modified with consideration for Equation 6.14, the model equation for particulate substrates becomes Equation 6.15.

$$\frac{\partial P}{\partial t} = \frac{P_o}{\theta} - \propto k_h P - \frac{P}{\theta} - \frac{P * q_v}{\theta}$$ Equation 6.15

A deficiency of kinetic parameters is the major obstacle against the evaluation of the proposed model, especially for operation at ambient temperature and for high rate systems with phase separation like the ABR. As reported by Donoso-Bravo *et al.* (2013), there are very few anaerobic digestion studies based on domestic wastewater in published literature, and as a result developing reliable system models and parameter calibration for domestic wastewater treatment is currently very difficult. The anaerobic model can be the basis for process optimization once the model parameters are properly defined.

6.4 Summary of energy analysis results and design criteria

The two bench reactors were evaluated using the defined energy efficiency criteria without consideration for methane capture, and the operational condition with the best energy efficiency in terms of temperature and HRT was determined to be 6 hours HRT (0.25 days) at 37^{o}C. Using theoretical 350 mL methane production per gram of COD removed, estimated methane production rates (mL/L) based on COD removal in the bench reactors were considered in the energy analysis. The operational condition with the best energy efficiency with consideration for potential methane production was 12 hours HRT (0.5 day) at 37^{o}C. 12 hours HRT at 37^{o}C was therefore adopted as the operational condition for the anaerobic stage of the anaerobic-aerobic domestic wastewater treatment system. The energy efficiency analysis can be applied when there is a need to identify the OLR, HRT and temperature, with an equilibrium of performance and energy efficiency, however, the

analysis methodology applied in this study was simplified with respect to the complexity of heating, mixing and energy losses in wastewater treatment facilities.

Therefore, the poor ranking of the ambient temperature alternatives by the energy analysis method did not consider inefficiencies in the temperature control system and potential recovery of heat at 37ºC, but the poor performance efficiencies of ambient temperature operation were part of the analysis (Tables 6.2 and 6.3). The energy analysis methodology adopted was based on methods applied by Lubken *et al.* (2007) and Merlin and Lissolo (2010) on full scale anaerobic digesters and wastewater treatment plants, respectively. However, the analysis was simplified in order to facilitate a comparison of the two bench reactors which had identical surface areas and were built using the same materials. A full-scale wastewater treatment facility is usually operated differently from a laboratory-scale reactor (Shoener *et al.* 2014), especially in terms of operational control, mixing and management of energy losses, therefore the energy analysis applied in this study cannot be considered as a process that can be easily adopted for a full scale system.

When evaluating full scale treatment systems, the heating requirements and energy losses will be influenced by the size and surface area of the process units, the material used to construct the system, and the operational efficiency, for example any energy saving strategy adopted by the operators (Lubken *et al.* 2007). Furthermore, in full scale treatment systems, mixing may not be efficient, due to the large treatment volume compared to the small volume of a bench reactor, and therefore differences in thermal and process conditions will exist (Abbasi *et al.* 2012). Consequently, the losses in efficiencies will be variable across the different sections

of the treatment process, and the data collection should be designed in such a way as to cover the complete range of efficiencies in mixing and heating (Fenu *et al.* 2010). The coefficient of heat transfer and surface area are influenced by the total volume and dimensions of the system, and the HRT represented the effective volume of the bench reactors in the energy efficiency analysis (Tables 6.4 and 6.6). A decrease in HRT should lead to increase in the effluent loads and poor energy efficiency, however, decrease in HRT and corresponding increase in OLR has been reported as leading to increase in total biogas production (Hassan and Dahlan 2013), but the potential gain in biogas production as a result of increasing OLR may not be adequate to offset the depreciation in performance efficiency.

Design criteria were proposed with considerations for number of compartments (≥ 5), up-flow to down-flow area ratio (1:1) and the minimum retention time in the aerobic stage to avoid washout of organic loading from the system. A recommendation was provided for the design of the 1st compartment of the system, responsible for retention of influent domestic wastewater solids, with consideration for sludge accumulation between periods of sludge removal from the system. To provide for fully aerobic zones where all the effluent substrate from the anaerobic stage is removed from the wastewater stream and converted to biomass, the minimum retention required to avoid washout was determined based on a set target of 50 mg/L COD as final effluent from the system (Section 6.2). The anaerobic operational condition of 12 hours (0.5 days) HRT at 37^{o}C requires a minimum retention time of 0.339 days for the aerobic stage, making the total required retention time for the anaerobic-aerobic system to achieve efficient organic loading removal as 0.839 days (Table 6.10).

Due to the limitations of the experimental scope with the bench reactors in terms of the ability to perform a comprehensive evaluation of potential operational conditions and extensive data collection, a preliminary process model of the system is presented as the first step in future process optimization research of the system (Section 6.3). For an ABR represented as a series of independent completely mixed tank reactors at steady-state, organic contaminants are assumed to be eventually converted to methane through three steps. The model processes are three steps, where the first step is the conversion of particulate substrates to soluble substrates, Figure 6.2, and the second step is the conversion of the soluble substrates to volatile fatty acids, which are eventually converted to biogas in the final step. The dynamic process model also included a consideration for the accumulation of retained solids in the system related to influent velocity, and the potential effect on effective volume and performance efficiency. The anaerobic model can be the basis for process optimization once the model parameters are properly defined based on extensive experimental and data analysis.

Chapter Seven - General conclusions and recommendations for future work

7.0 Summary of key research outcomes

Despite high treatment efficiencies, conventional aerobic wastewater treatment systems are not sustainable options due to high energy requirements, therefore, this study aimed to develop and characterise an efficient low-energy domestic wastewater treatment system. Energy efficiency in domestic wastewater treatment can be achieved when energy consumption is minimized and energy recovery is maximised. Domestic wastewater treatment systems are expected to become energy efficient if heating is avoided and the treatment processes occur at ambient temperature. The ABR is a high rate anaerobic system suitable for low energy domestic wastewater treatment due to its reported high energy efficiency and its capability to be developed as an integrated bioreactor with anaerobic and aerobic zones using its compartmental nature. Contact between the organic substrates and biomass is an important consideration in the design and performance efficiency of the ABR, however, there are few design guidelines available in literature relating the physical configuration and operational conditions to influent loading and treatment efficiency (Section 2.3).

Anaerobic processes were evaluated with a focus on domestic wastewater constituents in order to identify an energy efficient operational condition for domestic wastewater treatment. Laboratory experiments focused on the reduction of domestic wastewater sludge and methane productions at ambient temperature, and the influences of temperature and operational hydrodynamics on removal of organic contaminants. Anaerobic reduction of domestic wastewater sludge at ambient

temperature was compared with anaerobic reduction at 37ºC using BMP assays, and resistance towards reduction was observed in the assays with secondary sludge (SS) more than in the assays with primary sludge (Table 4.1). The results also revealed higher biological reduction at 37°C than at 25°C for the volatile solids of the primary sludge (Table 4.1), with a good correlation observed between Equation 2.21, digestion time and the sludge reduction data from the BMP assays based on the R^2 values obtained (Table 4.2).

However, the R^2 values for the digestion of the secondary sludge assay with inoculation at 25ºC indicated that digestion time is not an important factor in the prediction of the hydrolysis of total solids of the secondary sludge at 25ºC. A lack of compatibility with the Arrhenius relationship, increase reaction rate with increase in temperature, was observed for the BMP assays (Table 4.4), with the exception of the primary sludge assay without inoculation and the total solids of the secondary sludge assay without inoculation. However, this lack of compatibility with the Arrhenius relationship may be due to factors not evaluated in this study (for example surface area and composition of the sludge). Higher methane production was observed at 37ºC than at ambient temperature (Table 4.5), and higher methane production (mL/g VS removed) was observed for the secondary sludge assays than that observed for the primary sludge assays, potentially due to the smaller particle sizes in the secondary sludge than that in the primary sludge.

RTD experiment with intermittent and continuous flow conditions indicated intermediate mixing in the ABR bench model, Table 5.1, with high effective reactor volumes observed for intermittent flows compared to continuous flows. The RTD

experiment also identified the influent velocity as an important factor in determining reactor hydrodynamic characteristics if the anaerobic baffled reactor is operated with intermittent/variable flow conditions. Furthermore, bench experiments were performed with continuous operation of ABR systems at 37°C (R1) and ambient temperature (R2) with 48, 36, 24, 12 and 6 hours HRTs, which corresponded to 1.25, 1.67, 2.5, 5 and 10 kg COD/m^3.day OLR, respectively. R1 showed pseudo steady state characteristics and acclimatization of biomass after approximately 60 days of continuous operation with 48 hours HRT, Figure 5.1, while R2 reached steady state after approximately 100 days of continuous operation.

At pseudo steady state with a 48-hour HRT, R2 was observed to remove only 25% of influent COD load in the 1st compartment, lower than the 54% influent COD load removal in the 1st compartment of R1. However, if the 1st compartments are disregarded from the total COD removal, the observed COD removal with a 48-hour HRT from the last five compartments of R1 and R2 were 34% and 30%, respectively (Figure 5.2). 88.43, 90.00, 84.03, 77.01 and 59.35% of the influent COD (mean = 2479.50 mg/L) were removed at 48, 36, 24, 12 and 6 hour HRTs, respectively, in R1, while 70.16, 70.36 and 74.99% of the influent COD were removed in R2 at 48, 36 and 24 hour HRTs, respectively. In R1, the volatile fatty acids were mostly removed before the 4th compartment (Figure 5.4), while there appears to be no substantial change in VFA concentrations between consecutive compartments in R2. An average of approximately 300 mg/L VFA concentration was maintained in the 1st compartment of R1 for all the evaluated HRTs except 12 and 6 hours HRT, Figure 5.4, while VFA concentrations in the 1st compartment of R2 were consistently above 500 mg/L for all HRTs.

Operation with incremental changes to HRT at 37ºC (R3), indicated higher effluent concentrations of VFA and COD, Figures 5.6 – 58, than that observed in R1, Figures 5.3 and 5.4, where biomass acclimatization was encouraged. Furthermore, estimated SRTs based on observed influent and effluent total solids indicated that SRT and HRT have a direct correlation (Table 5.4), therefore, periodic removal of sludge should be part of the operational requirements of the system in order to maintain the effective treatment volume. Methane production rates were not measured during the experiment, due to potential biogas escape from the bench reactors due to leakages, and also potentially due to loss of biogas with effluent flow and solubility in the gas meter fluid. Changes in COD concentrations for successive compartments of R2 were not substantial, and the reactor also appeared to have difficulty converting the available volatile fatty acids to methane (Figure 5.2).

The two bench reactors were evaluated using the defined energy efficiency criteria, with estimated methane production rates (mL/L) based on COD removal and theoretical 350 mL methane production per gram of COD removed. The operational condition with the best energy efficiency was 12 hours HRT (0.5 day) at 37ºC, which was adopted as the operational condition for the anaerobic stage of the anaerobic-aerobic domestic wastewater treatment system. The analysis was simplified in order to facilitate a comparison of the bench reactors which had identical surface areas and were built using the same materials, however there was no consideration for potential variations or inefficiencies in temperature control, mixing and heat recovery. Mixing may not be efficient in treatment systems, and therefore differences in thermal and process conditions will exist across the treatment process, and consequently, losses

and efficiencies will be variable across the different sections of the treatment process. Recommended design criteria were presented in terms of the number of compartments (≥ 5), up-flow to down-flow area ratio (1:1), consideration for sludge accumulation in the 1st compartment and the minimum retention time in the aerobic stage (0.339 days).

7.1 Conclusions

The batch BMP assays, continuous bench reactor experiments and energy efficiency analysis presented in this study have provided a basis for defining the characteristics of an energy efficient system for the treatment of domestic wastewater. 12 hours HRT at 37ºC has been proposed as an energy efficient operational condition for the anaerobic stage of an integrated anaerobic-aerobic system for domestic wastewater treatment. However, poor methane recovery in the anaerobic stage may be a potential limit to the adoption of the proposed anaerobic-aerobic system as an alternative to current conventional treatment systems. Identified process challenges at ambient temperature are the high concentrations of organic loads in the effluent, and the low conversion of substrates to final products of the anaerobic process. The advantage of treating domestic wastewater at ambient temperature is the savings in energy consumption by avoiding heating requirements, however, low treatment performance was observed at ambient temperature compared to the performance observed at a stable mesophilic temperature. Changes in COD concentrations for successive compartments at ambient temperature were not substantial, and available volatile fatty acids were not substantially removed.

Performance efficiency may be achieved at ambient temperature with a hydraulic retention time ≥ 24 hours, mainly because increasing the HRT should lead to an increase in the contact time between organic substrates and anaerobic microorganisms. Furthermore, estimated SRTs based on observed influent and effluent total solids indicated that SRT and HRT have a direct correlation, potentially there could be increase in retention of influent solids with increase in HRT. An increase in HRT should lead to a decrease in the effluent loads, however, the corresponding decrease in OLR could lead to a decrease in total biogas production, and the potential low biogas production may depreciate the energy efficiency of the system. There are also economic challenges, defined by the required large volumes to accommodate the long retention periods at ambient temperature, however, the required effective volume will not be consistent due to the variable nature of domestic wastewater flow and organic load concentration.

Therefore, an operational criterion based on the organic loading rate is also proposed, along with the design criteria, where a range of 1.5 – 5.5 OLR (kg COD/m^3day), with an average 2.75 OLR should be the recommended applied OLR for the ABR. This OLR range can be maintained by adoption of a flexible effective treatment volume based on the nature of the ABR, for example by either increasing or decreasing the effective treatment volume so that the OLR and HRT are maintained at the recommended values of 2.75 kg COD/m^3day and 12 hours, respectively.

7.2 Recommendations for future wok

Future research on anaerobic treatment of domestic wastewater at ambient temperature should focus on identified process limitations that are stopping the

achievement of energy and performance efficiency. The possibility of achieving energy and performance efficiency for domestic wastewater treatment at ambient temperature with anaerobic systems is limited by several challenges, mainly low reduction of particulate substrates, low influent solids retention and low recovery of methane. The requirements for sludge management processes, and consequently energy consumption, will be reduced if the percentage of biological reduction of domestic wastewater sludge at ambient temperature can be improved.

Determination of the size of the 1st compartment relative to the other compartments is also very important for system efficiency and hydrodynamics at ambient temperature. An advantage of the reactor operated at 37ºC over the ambient temperature reactor was the observed COD removal in the 1st compartment and the stability of VFA concentrations in ranges that could not inhibit methanogens. The 1st compartment was also responsible for the difference in recorded solids washout from the bench reactors, where the 1st compartment of the ambient temperature reactor was observed to have high concentrations of effluent solids compared to the 1st compartment in the 37ºC reactor.

High solubility of methane in low temperatures has been reported in literature, and this may adversely affect methane release for ambient temperature wastewater treatment systems. Processes targeted at overcoming this problem have been proposed in literature, for example the use of a physical media to separate the dissolved gases before the final effluent from the system (Feng *et al.* 2008; Zhu *et al.* 2015). Smith *et al.* (2012) identified the use of degassing membranes, down-flow hanging sponge (DHS) systems and post-treatment aeration as processes for

capturing dissolved methane from the effluents of anaerobic systems, however, the methane that can be recovered has not yet been properly determined (Smith *et al.* 2012).

The experimental approach adopted for this study limited the potential to evaluate the ABR for energy and performance efficiency in ambient temperature treatment of domestic wastewater. The bench experiments might have provided an opportunity for comprehensive data collection and analysis, especially for the rates of biogas production, if a simple laboratory set-up had been adopted using a set of conical flasks in series similar to bioreactors adopted by Yuan *et al.* (2015) and Colussi *et al.* (2014). Yuan *et al.* (2015) adopted 500 mL conical flasks to simulate activated sludge reactors, while Colussi *et al.* (2014) adopted two coupled 5 L glass flasks to simulate an anaerobic sequencing batch reactor, with the hydrolysis and acidogenic stages in the first flask and the methanogenesis stage in the second flask.

The sample collection methodology adopted for the BMP assays resulted in experimental errors in the data which might be indications of disturbance of the solid substrates by the needles and syringes used for sample collection. The disturbance could have been disruption of the physical structure of the substrate or variation in the composition of the solid substrates in the samples because of the small size of the internal diameter of the needles. Reviewed literature on BMP assay methodologies identified a lack of details relating to accurate methods that should be adopted for collection of solid samples. Funding and laboratory bench spaces are usually limited for research, therefore an alternative method for monitoring solid substrates

reduction in BMP assays that does not require the use of multiple bottles or needles and syringes for sample collection should be researched.

The energy efficiency analysis method adopted might be simplistic, especially in terms of the required rigorous approach and comprehensive data analysis for a holistic assessment of sustainability of treatment systems (Merlin and Lissolo 2010). Full scale treatment facilities will have differences in terms of effective treatment volume, mixing and heat capture, and also there should be temporal and geographical variations in OLRs, removal of COD, nature of biomass, methane production and recovery. In order to adapt the energy efficiency analysis method to a full scale treatment facility, extensive data acquisition will be required to account for temporal variations in loadings and process parameters, and also variations in the operation of the treatment units in the facility (Fenu *et al.* 2010). The energy efficiency analysis method therefore could be modified with the addition of a data quality analysis as an initial step so that the natural variations in treatment facilities will be readily adapted into the analysis.

Future development of ambient treatment of domestic wastewater will require improvements in process prediction, design and control, which will require extensive experiments and data collection. However experiments and data collection are usually limited by inadequate funding, and therefore, a dynamic model has been proposed for the anaerobic stage of the process, so that the optimum energy efficient operational condition can be identified without expensive experimentation. The proposed dynamic model considers the key variables to be the concentrations of the particulate and soluble substrates, the available microorganisms for acedogenesis and

methanogenesis, and the concentrations of the intermediate acids. Consideration was also given to the retention of the particulates in the system in order to accommodate wastewater characteristics with potentially high solid loads such as domestic wastewater.

In the proposed dynamic process model, the variation in wastewater composition over time was not incorporated, and also the influent loading was not defined according to the various components of wastewater, for example soluble COD and suspended solids. Also, temperature and hydraulic retention time were not considered as variables in the dynamic process model, while in real systems these will definitely be variables and should have important impacts on the range of parameter values. The potential impacts of these additional variables to the model suggest the need for variable parameters over time and also between the compartments of the system. The proposed dynamic system model, with the adoption of variable parameters for each compartment to account for phase separation, should improve the prediction of potential deficiencies in the system and be a useful tool in system design and operational control. Process parameters related to high rate anaerobic systems with phase separation, for example the ABR, need to be estimated for ambient temperature domestic wastewater treatment, in order to make further development of the process model possible.

References

Abbasi, T., Tauseef, S. M. and Abbasi, S. A. 2012. Anaerobic digestion for global warming control and energy generation - an overview. *Renewable and Sustainable Energy Reviews.* 16 (5): pp.3228 – 3242.

Ahring, B. K. 2003. Perspectives for Anaerobic Digestion. *Biomethanation I - Advances in Biochemical Engineering/ Biotechnology*. 81 (Mar): pp.1 – 30.

Aitken, M. D., Walters, G. W., Crunk, P. L., Willis, J. L., Farrell, J. B., Schafer, P. L., Arnett, C. and Turner, B. G. 2005. Laboratory evaluation of thermophilic-anaerobic digestion to produce Class A biosolids. 1. Stabilization performance of a continuous-flow reactor at low residence time. *Water environment research*. 77(7): pp3019-3027.

Akhbari, A., Zinatizadeh, A. A. L., Mohammadi, P., Irandoust, M., and Mansouri, Y. 2011. Process modelling and analysis of biological nutrients removal in an integrated RBC-AS system using response surface methodology. *Chemical engineering journal*. 168(1): pp.269 - 279.

Aldin, S. 2010. *The effect of particle size on hydrolysis and modelling of anaerobic digestion*. [PhD dissertation]. University of Western Ontario, Ontario, Canada. Available from: http://ir.lib.uwo.ca/cgi/viewcontent.cgi?article=1167&context=etd.

Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, L., Guwy, A., and van Lier, J. B. 2009. Defining the bio-methane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water science and technology*. *59*(5): pp.927 - 934.

Angelidaki, I. and Sanders, W. 2004. Assessment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Biotechnology.* 3 (2): pp.117 - 129.

Angelidaki, I., Boe, K. and Ellegaard, L. 2005. Effect of operating conditions and reactor configuration on efficiency of full-scale biogas plants. *Water Science and Technology*. *52*(1-2): pp189-194.

American Public Health Association. 1998. *Standard methods for the examination of water and wastewater*. 20th ed. Washington, DC: American Public Health Association (APHA) – American Water Works Association (AWWA) – Water Environment Federation (WEF).

Appels, L., Baeyens, J., Degre`ve, J., and Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science*. 34 (6): pp.755 – 781

Arthur, S. and Blanc, J. 2013. *Management and recovery of FOG (fats, oils and greases)*. [Online]. CREW project CD2013/6. Available from: crew.ac.uk/publications.

Avallone, E. A., Baumeister III, T. and Sadegh, A. M. 2007. *Mark's standard handbook for mechanical engineers.* 11th ed. New York: McGraw-Hill Book Co.

Ayaz, S. Ç., Akça, L., Aktaş, Ö. Fındık, N., and Öztürk, İ. 2012. Pilot-scale anaerobic treatment of domestic wastewater in up-flow anaerobic sludge bed and anaerobic baffled reactors at ambient temperatures. *Desalination and water treatment*. 46(1-3): pp.60 - 67.

Azbar, N. and Speece, R. E. 2001. Two-phase, two-stage, and single-stage anaerobic process comparison. *Journal of Environmental Engineering*. 127 (3): pp.240 – 248.

Azbar, N., Ursillo, P. and Speece, R. E. 2001. Effect of process configuration and substrate complexity on the performance of anaerobic processes. *Water Research*. 35 (3): pp.817 – 829.

Bachmann, A., Beard, V.L. and McCarty, P.L. 1985. Performance characteristics of the anaerobic baffled reactor. *Water Research.* 19 (1): pp.99 – 106.

Balkema, A. J., Preisig, H. A., Otterpohl, R., and Lambert, F. J. 2002. Indicators for the sustainability assessment of wastewater treatment systems. *Urban Water*. *4* (2): pp.153 - 161.

Baloch, M. I. 2011. Methanogenic granular sludge as a seed in an anaerobic baffled reactor. *Water and Environment Journal. 25* (2): pp.171 - 180.

Baloch, M.I. and Akunna, J.C. 2003. Effect of rapid hydraulic shock loads on the performance of granular bed baffled reactor. *Environmental Technology.* 24 (3): pp.361 - 368.

Baloch, M.I., Akunna, J.C., Kierans, M. and Collier, P.J. 2008. Structural analysis of anaerobic granules in a phase separated reactor by electron microscopy. *Bio-resource Technology.* 99 (5): pp.922 – 929.

Banu, J. R., Kaliappan, S. and Yeom, I. T. 2007. Treatment of domestic wastewater using upflow anaerobic sludge blanket reactor. *International Journal of Environmental Science and Technology.* 4 (3): pp.363 - 370.

Barber, W. P. and Stuckey, D. C. 1999. The use of the anaerobic baffled reactor (ABR) for wastewater treatment: a review. *Water Research*. 33 (7): pp.1559 – 1578.

Barry, J. A. 2007. *WATERGY: Energy and water efficiency in municipal water supply and wastewater treatment – cost - effective savings of water and energy.* [Online]. The Alliance to Save Energy. Available from: **http://www.gwp.org/Global/ToolBox/References/WATERGY.%20Water%20Efficiency%20in%20Municipal%20Water%20Supply%20and%20Wastewater%20Treatment%20%28The%20Alliance%20to%20Save%20Energy,%202007%29.pdf.**

Bassuney, D. M., Ibrahim, W. A., and Moustafa, M. A. E. 2013. Performance of an Anaerobic Baffled Reactor (ABR) during start-up period. *International Journal of Chemical, Environmental and Biological Sciences.* 1 (4): pp.571 – 575.

Batstone, D. 2000. *High rate anaerobic treatment of complex wastewater*. [PhD dissertation]. University of Queensland, Australia. Available from: http://www.researchgate.net/publication/37616650_High_Rate_Anaerobic_Treatment_of_Complex_Wastewater.

Batstone, D. J. 2006. Mathematical modelling of anaerobic reactors treating domestic wastewater: rational criteria for model use. *Reviews in Environmental Science and Biotechnology*. 5 (1): pp.57 – 71.

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhnyi, S. V., Pavlostathis, S.G., Rozzi, A., Sanders, W. T. M., Siegrist, H. and Vavilin, V. A. 2002. Anaerobic Digestion Model No1 (ADM1). *Water Science and Technology*. 45 (10): pp.65 – 73.

Bergamo, C. M., Monaco R. D., Ratusznei S. M., Rodrigues J. A. D., Zaiat M. and Foresti E. 2009. Effects of temperature at different organic loading levels on the performance of a fluidized-bed anaerobic sequencing batch bioreactor. *Chemical Engineering and Processing: Process Intensification*. 48 (3): pp.789 – 796.

Bodkhe, S.Y. 2009. A modified anaerobic baffled reactor for municipal wastewater treatment. *Journal of Environmental Management*. 90 (8): pp.2488 – 2493.

Boopathy, R. and Sievers D. M. 1991. Performance of a modified anaerobic baffled reactor to treat swine waste. *Transactions of the ASAE (USA)*. 34 (6): pp.2573 - 2578.

Boopathy, R. and Tilche A. 1991. Anaerobic digestion of high strength molasses wastewater using a hybrid anaerobic baffled reactor. *Water research*. 25 (7): pp.785 - 790.

Bowen, R. A., Hortin, G. L., Csako, G., Otañez, O. H., and Remaley, A. T. 2010. Impact of blood collection devices on clinical chemistry assays. *Clinical biochemistry*. 43(1): pp.4 - 25.

Brissaud, F. 2007. Low technology systems for wastewater treatment: perspectives. *Water science and technology*. 55(7): pp.1 - 9.

Brummeler, E. T., Horbach, H. C. J. M., and Koster, I. W. 1991. Dry anaerobic batch digestion of the organic fraction of municipal solid waste. *Journal of Chemical Technology and Biotechnology*. *50* (2): pp.191 - 209.

Burns, R. B. 2000. *Introduction to Research Methods*. 4th ed. London: Sage Publications.

Burubai, W., Akor, A. J., and Lilly, M. T. 2007. Performance evaluation of septic system for high water-table areas. *American-Eurasian journal of scientific research*. 2(2): pp.112 - 116.

Caldwell, P. 2009. Energy efficient sewage treatment - can energy positive sewage treatment works become the standard design? In: *Proceedings of the 3rd European Water and Wastewater Management Conference.* Birmingham, UK *22nd - 23rd September*. Wakefield: AquaEnviro. Available from: http://www.hydrok.co.uk/downloads/CaldwellPaper.pdf.

Castillo, A., Llabres, P. and Mata-Alvarez, J. 1999. A kinetic study of a combined anaerobic–aerobic system for treatment of domestic sewage. *Water Research*. 33 (7): pp.1742 – 1747.

Cesur, D. and Albertson, M. L. 2005. *Modification of anaerobic digestion model No. 1 for accumulation and biomass recycling*. [Online]. Colorado State University Hydrology days. Available from: http://hydrologydays.colostate.edu/Proceedings_2005.htm.

Chae K. J. and Kang J. 2013. Estimating the energy independence of a municipal wastewater treatment plant incorporating green energy resources. *Energy Conversion and Management*. 75 (Nov): pp.664 – 672.

Chan, Y. J., Chong, M. F., Law, C. L. and Hassell, D. G. 2009. A review on anaerobic–aerobic treatment of industrial and municipal wastewater. *Chemical Engineering Journal*. *155* (1): pp.1 - 18.

Chen, X. G., Zheng, P., Guo, Y. J., Mahmood, Q., Tang, C. J., and Ding, S. 2010. Flow patterns of super-high-rate anaerobic bioreactor. *Bioresource Technology*. *101* (20): pp.7731 - 7735.

Cohen, A., Breure, A. M., Van Andel, J. G. and Van Deursen, A. 1982. Influence of phase separation on the anaerobic digestion of glucose – II: stability and kinetic responses to shock loadings. *Water Research*. 16 (4): pp.449 – 455.

Crawford, G. and Sandino, J. 2010. *Energy efficiency in wastewater treatment in North America: a compendium of best practices and case studies of novel approaches*. [Online]. Alexandria, VA, USA: The water environment research foundation. Available from: https://www.nyserda.ny.gov/-/media/Files/EERP/Commercial/Sector/Municipal-Water-Wastewater-Facilities/north-american-drinking-water-utilities.pdf.

Daelman, M. R. J., Van Voorthuizen, E. M., Van Dongen, U., Volcke, E. I. P. and Van Loosdrecht, M. C. M. 2012. Methane emission during municipal wastewater treatment. *Water Research*. 46 (11): pp.3657 - 3670.

Davidsson, A., Lövstedt, C., la Cour Jansen, J., Gruvberger, C. and Aspegren, H. 2008. Co-digestion of grease trap sludge and sewage sludge. *Waste Management*. 28 (6): pp.986 – 992.

Davis, M. L. 2011. *Water and wastewater engineering – design principles and practice*. New York: McGraw-Hil Co.

de Baere, L., Verdonck, O. and Verstraete, W. 1985. High rate dry anaerobic composting process for the organic fraction of solid wastes. In: Scott, C. D. ed. *Proceedings of the 7th symposium on biotechnology for fuels and chemicals*. Gatlinburg, Tennessee, USA. May 14 - 17**.** London: John Wiley. 1986. Pp.321 – 330.

de Mes, T.Z.D., Stams, J.H., Reith, J.H., and Zeeman, G. 2003. Methane production by anaerobic digestion of wastewater and solid wastes. In: Reith JH, Wijffels RH, Barten H. eds. *Bio-methane and bio-hydrogen*: *status and perspectives of biological methane and hydrogen production*. Dutch Biological Hydrogen Foundation, Petten. The Netherlands, pp.58 – 102

de Vrieze, J., Raport, L., Willems, B., Verbrugge, S., Volcke, E., Meers, E, and Boon, N. 2015. Inoculum selection influences the biochemical methane potential of agro-industrial substrates. *Microbial biotechnology*.

Demirel, B., Scherer, P., Yenigun, O. and Onay, T. 2010. Production of methane and hydrogen from biomass through conventional and high-rate anaerobic digestion processes. *Critical Reviews in Environmental Science and Technology*. *40* (2): pp.116 - 146.

Deng, H. 2006. *Combined anaerobic digestion (CAD) of sewage sludge and other urban solid wastes*. [PhD dissertation]. Loughborough University, UK.

Dewil, R., Lauwers, J., Appels, L., Gins, G., Degrève, J., and Van Impe, J. 2011. Anaerobic digestion of biomass and waste: current trends in mathematical modelling. In: *Preprints of the 18th IFAC world congress.* Milano, Italy. August 28th -September 2th.

Dierberg, F. E. and DeBusk, T. A. 2005. An evaluation of two tracers in surface flow wetlands: Rhodamine-WT and lithium. *Wetlands*. 25 (1): pp.8 – 25.

Dochain, D. and Vanrolleghem, P. 2001. *Dynamical modelling and estimation in wastewater treatment processes*. London: IWA publishing.

Donoso-Bravo, A., Bandara, W. M. K. R. T. W., Satoh, H., and Ruiz-Filippi, G. 2013. Explicit temperature-based model for anaerobic digestion: application in domestic wastewater treatment in a UASB reactor. *Bioresource technology*. 133 (Feb): pp.437 – 442.

Dyar S. C., and Notari R. E. 1998. Hydrolysis kinetics and stability predictions for a mixture of R and S *temocillin* isomers. *International Journal of Pharmaceutics.* 173 (1): pp.221 – 232.

Eastman, J. A. and Ferguson, J. F. 1981. Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. Journal *Water Pollution Control Federation*. 53 (3): pp.352 – 366.

Elmitwalli, T. A., Shalabi, M., Wendland, C., and Otterpohl, R. 2007. Grey water treatment in UASB reactor at ambient temperature. *Water science and technology*. 55(7): pp.173 - 180.

Esposito, G., Frunzo, L., Giordano, A., Liotta, F., Panico, A. and Pirozzi, F. 2012. Anaerobic co-digestion of organic wastes. *Reviews in Environmental Science and Biotechnology*. *11* (4): pp.325 - 341.

Feng, H., Hu, L., Mahmood, Q., Qiu, C., Fang, C., and Shen, D. 2008. Anaerobic domestic wastewater treatment with bamboo carrier anaerobic baffled reactor. *International Biodeterioration and Biodegradation*. 62(3): pp.232 - 238.

Feng, H. J., Hu, L. F., Shan, D., Fang, C. R., and Shen, D. S. 2008. Effects of temperature and hydraulic residence time (HRT) on treatment of dilute wastewater in a carrier anaerobic baffled reactor. *Biomedical and environmental sciences: BES*. 21(6): pp.460.

Feng, J., Wang, Y., Ji, X., Yuan, D., and Li, H. 2015. Performance and bio-particle growth of anaerobic baffled reactor (ABR) fed with low-strength domestic sewage. *Frontiers of environmental science and engineering*. 9(2): pp.352 - 364.

Feng, L., Yan Y., and Chen Y. 2009. Kinetic analysis of waste activated sludge hydrolysis and short-chain fatty acids production at pH 10. *Journal of Environmental Sciences*. *21* (5): pp.589 - 594.

Fernández, N., Montalvo, S., Borja, R., Guerrero, L., Sánchez, E., Cortés, I., and Raposo, F. 2008. Performance evaluation of an anaerobic fluidized bed reactor with natural zeolite as support material when treating high-strength distillery wastewater. *Renewable Energy*. 33(11): pp.2458 - 2466.

Fleming, J. G. 2002. *Novel simulation of anaerobic digestion using computational fluid dynamics*. [PhD dissertation]. University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. Available online at: http://repository.lib.ncsu.edu/ir/handle/1840.16/4158.

Foresti, E. 2002. Anaerobic treatment of domestic sewage: established technologies and perspectives. *Water Science and Technology.* 45 (10): pp.181 – 186.

Foresti, E., Zaiat, M. and Vallero, M. 2006. Anaerobic processes as the core technology for sustainable domestic wastewater treatment: consolidated applications, new trends, perspectives, and challenges. *Reviews in Environmental Science and Biotechnology*. 5 (1): pp.3 – 19.

Foxon, K.M. and Buckley, C.A. 2006. *Guidelines for the implementation of anaerobic baffled reactors for on-site or decentralised sanitation.* [Online]. Pollution research group, school of chemical engineering, University of KwaZulu-Natal, Durban, South Africa. Available from: http://www.sswm.info/sites/default/files/reference_attachments/FOXON%202006%20Guidelines%20for%20the%20implementation%20of%20anaerobic%20baffled%20reactors%20for%20on-site%20or%20decentralised%20saniation.pdf.

Foxon, K. M., Buckley, C. A., Brouckaert, C. J., Dama, P., Mtembu, Z., Rodda, N., Smith, M., Pillay, S., Arjun, N., Lalbahadur, T. and Bux, F. 2006. *The evaluation of the anaerobic baffled reactor for sanitation in dense peri-urban settlements*. [Online]. Water research commission WRC Report No 1248/01/06 South Africa. Available from: http://www.wrc.org.za/Knowledge%20Hub%20Documents/Research%20Reports/1248-1-06.pdf.

Foxon, K. M., Pillay, S., Lalbahadur, T., Rodda, N., Holder, F. and Buckley, C. A. 2007. The anaerobic baffled reactor (ABR): an appropriate technology for on-site sanitation. *Water S. A.* 30 (5): pp.44 – 50.

Gallert, C. and Winter, J. 2005. Bacterial metabolism in wastewater treatment systems. In: Jordening, H. J. and Winter, J. eds. *Environmental biotechnology - concepts and applications*. Weinheim: Wiley-VCH. 2005: pp.1 - 48.

Gao, W. J., Leung, K. T., Qin, W. S. and Liao, B. Q. 2011. Effects of temperature and temperature shock on the performance and microbial community structure of a submerged anaerobic membrane bioreactor. Bioresource Technology. 102 (19): pp.8733 – 8740.

García-Diéguez, C., Bernard, O. and Roca, E. 2013. Reducing the anaerobic digestion model No. 1 for its application to an industrial wastewater treatment plant treating winery effluent wastewater. *Bioresource Technology.* 132 (Jan): pp.244 – 253.

Ghaniyari-Benis, S., Borja, R., Monemian, S. A., and Goodarzi, V. 2009. Anaerobic treatment of synthetic medium-strength wastewater using a multistage biofilm reactor. *Bioresource technology*. *100* (5): pp.1740 - 1745.

Ghaniyari-Benis, S., Martín, A., and Borja, R. 2010. Kinetic modelling and performance prediction of a hybrid anaerobic baffled reactor treating synthetic wastewater at mesophilic temperature. *Process Biochemistry*. *45* (10): pp.1616 - 1623.

Ghaniyari-Benis, S., Martín, A., Borja, R., Martín, M. A., and Hedayat, N. 2012. Comparison of two mathematical models for correlating the organic matter removal efficiency with hydraulic retention time in a hybrid anaerobic baffled reactor treating molasses. *Bioprocess and biosystems engineering*. 35(3): pp.389 - 397.

Gill, H. S., and Prausnitz, M. R. 2007. Does needle size matter? *Journal of diabetes science and technology*. 1(5): pp725 - 729.

Goldstein, R., and Smith, W. 2002. *Water and Sustainability (volume 4): U.S. electricity consumption for water supply and treatment – the next half century*. [Online]. Palo Alto, California, USA: Electric Power Research Institute. Available from: http://www.rivernetwork.org/sites/default/files/Water%20and%20Sustainability%20(Volume%204)-%20EPRI.pdf.

Gomec, C. Y. 2010. High-rate anaerobic treatment of domestic wastewater at ambient operating temperatures: a review on benefits and drawbacks. *Journal of environmental science and health part A*. 45(10): pp.1169 - 1184.

Gopala-Krishna, G. V. T., Kumar, P., and Kumar, P. 2008. Treatment of low strength complex wastewater using an anaerobic baffled reactor (ABR).*Bioresource technology*. *99* (17): pp.8193 - 8200.

Gopala-Krishna, G. V. T., Kumar, P., and Kumar, P. 2009. Treatment of low-strength soluble wastewater using an anaerobic baffled reactor (ABR). *Journal of environmental management*. *90* (1): pp.166 - 176.

Griffin, L. P. 2012. *Anaerobic digestion of organic wastes: the impact of operating conditions on hydrolysis efficiency and microbial community composition*. [Master's thesis]. Colorado State University, USA. Available online at: http://erefdn.org/images/uploads/Griffin_Laura.pdf.

Grobicki, A. and Stuckey, D.C. 1991. Performance of the anaerobic baffled reactor under steady-state and shock loading conditions. *Biotechnology and bioengineering*. *37* (4): pp.344 - 355.

Grobicki, A. and Stuckey, D.C. 1992. Hydrodynamic characteristics of the anaerobic baffled reactor. *Water Research*. *26* (3): pp.371 - 378.

Hach Company. 2005. *DR5000 Spectrophotometer procedures manual: Catalog number DOC082.98.00670*. 2nd ed. Germany.

Hansen, T. L., Schmidt, J. E., Angelidaki, I., Marca, E., la Cour Jansen, J., Mosbæk, H., and Christensen, T. H. 2004. Method for determination of methane potentials of solid organic waste. *Waste management*. 24(4): pp.393 - 400.

Hansson, M., Laurell, J., Nordberg, Å., Rasmuson, Å., Liu, J., Nistor, M., Strömberg, S. and Costa, J. 2013. *In-situ methane enrichment of raw biogas in the anaerobic digestion process*. [Online]. Malmo, Sweden: Swedish Gas Technology Centre SGC Rapport 280. Available from: http://www.sgc.se/ckfinder/userfiles/files/SGC280.pdf.

Haridas A. 2010. *Anaerobic reactor development for complex organic wastewater*. [PhD dissertation]. Cochin University of Science and Technology, Kerala, India. Available online at: http://dyuthi.cusat.ac.in/jspui/bitstream/purl/2358/1/Dyuthi-T0630.pdf.

Hassan S. R., and Dahlan, I. 2013. Anaerobic wastewater treatment using anaerobic baffled bioreactor: a review. *Central European Journal of Engineering*. *3* (3): pp.389 - 399.

Hegazy, T. A., Abdel-Magied, M. A., Al-Asmar, A., and Ibrahim, M. S. 2011. Environmental studies of domestic wastewater treatment using integrated anaerobic/aerobic system. *Journal of American science*. 7(3): pp.1 - 5.

Heidrich, E. S., Curtis, T. P., and Dolfing, J. 2011. Determination of the internal chemical energy of wastewater. *Environmental science and technology*. 45(2): pp.827 - 832.

Helmer, R. and Hespanol, I. eds. 1997. *Water pollution control - a guide to the use of water quality management*. London: E. and FN. Spon.

Hernández-Leal, L., Temmink, H., Zeeman, G. and Buisman, C. J. N. 2011. Characterization and anaerobic biodegradability of grey water. *Desalination*. *270* (1): pp.111 - 115.

Hernández-Sancho, F., Molinos-Senante, M., and Sala-Garrido, R. 2011. Energy efficiency in Spanish wastewater treatment plants: a non-radial DEA approach. *Science of the Total Environment*. *409* (14): pp.2693 - 2699.

Hierholtzer, A. 2013. *Investigating factors affecting the anaerobic digestion of seaweed: modelling and experimental approaches*. [PhD dissertation]. Abertay University, Dundee, United Kingdom. Available online at: https://repository.abertay.ac.uk/jspui/bitstream/handle/10373/2025/Heirholtzer_InvestigatingFactorsAffectingthe%20AnaerobicDigestionofSeweed_Redacted_2013.pdf;jsessionid=3C1A64153FB6FB04DE0D340EA9D038EB?sequence=2.

Hierholtzer, A., Chatellard, L., Kierans, M., Akunna, J. C., and Collier, P. J. 2013. The impact and mode of action of phenolic compounds extracted from brown seaweed on mixed anaerobic microbial cultures. *Journal of applied microbiology*. *114* (4): pp.964 - 973.

Hu, Z., Houweling, D., and Dold, P. 2012. Biological nutrient removal in municipal wastewater treatment: new directions in sustainability. *Journal of Environmental Engineering*. *138* (3): pp.307 - 317.

Huggins, T., Fallgren, P. H., Jin, S., and Ren, Z. J. 2013. Energy and performance comparison of microbial fuel cell and conventional aeration treating of wastewater. *Microbial and Biochemical Technology. 6*:2.

Hutnan, M., Drtil, M., Mrafkova, L., Derco, J., and Buday, J. 1999. Comparison of start-up and anaerobic wastewater treatment in UASB, hybrid and baffled reactor. *Bioprocess Engineering*. *21* (5): pp. 439 - 445.

Ji, J. Y., Zheng, K., Xing, Y. J., and Zheng, P. 2012. Hydraulic characteristics and their effects on working performance of compartmentalized anaerobic reactor. *Bioresource Technology*. *116 (Apr)*: pp.47 - 52.

Jiménez, A. M., Borja, R., and Martın, A. 2003. Aerobic–anaerobic biodegradation of beet molasses alcoholic fermentation wastewater. *Process biochemistry*. 38(9): pp.1275 - 1284.

Jimenez-Gonzalez, A., Salazar-Gonzlez, M., Gutirrez-Rojas, M., and Monroy, O. 2001. Anaerobic digestion of a nonionic surfactant: inhibition effect and biodegradation. *Water Science and Technology*. *44* (4): 175-181.

Jonasson, M. 2007. *Energy benchmark for wastewater treatment process — a comparison between Sweden and Austria*. [PhD dissertation]. Lund University, Lund, Sweden. Available online at: https://www.iea.lth.se/publications/MS-Theses/Full%20document/5247_full_document.pdf.

Kassab, G., Halalsheh, M., Abu-Ghunmi, L. and Shatanawi, K. 2013. Characterization and anaerobic biodegradation of single house wastewater. *Jordan Journal of Civil Engineering*. 7 (2): pp.202 - 210.

Kassab, G., Halalsheh, M., Klapwijk, A., Fayyad, M., and Van Lier, J. B. 2010. Sequential anaerobic–aerobic treatment for domestic wastewater– a review. *Bio-resource technology*. 101(10): pp.3299 - 3310.

Kayranli, B., and Ugurlu, A. 2011. Effects of temperature and biomass concentration on the performance of anaerobic sequencing batch reactor treating low strength wastewater. *Desalination*. 278(1): pp.77 - 83.

Khan, A. A., Gaur, R. Z., Tyagi, V. K., Khursheed, A., Lew, B., Mehrotra, I., and Kazmi, A. A. 2011. Sustainable options of post treatment of UASB effluent treating sewage: a review. *Resources, Conservation and Recycling*. *55* (12): pp.1232 - 1251.

Klein, G., Krebs, M., Hall, V., O'Brien, T. and Blevins, B. 2005. *California's water–energy relationship*. [Online]. California Energy Commission. Available from: http://www.energy.ca.gov/2005publications/CEC-700-2005-011/CEC-700-2005-011-SF.PDF.

Ko, J., Martin, J. and Day, J. W. 2000. Embodied energy and emergy analysis of wastewater treatment using wetlands. [Online]. In: Brown, M. T., Brandt-Williams, S., Tilley, D. and U1giati, S. eds. Emergy synthesis: theory and applications of the Emergy methodology. *Proceedings from the First Biennial Emergy Analysis Research Conference*. University of Florida, Gainesville, Florida, September, 1999.

Kothari, R., Singh, D. P., Tyagi, V. V., and Tyagi, S. K. 2012. Fermentative hydrogen production–An alternative clean energy source. *Renewable and sustainable energy reviews*. 16(4): pp.2337 - 2346.

Krozer, Y., Hophmayer-Tokich, S., van Meerendonk, H., Tijsma, S., and Vos, E. 2010. Innovations in the water chain–experiences in The Netherlands. *Journal of cleaner production*. 18(5): pp.439 - 446.

Labatut, R. A., Angenent, L. T., and Scott, N. R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. *Bio-resource technology*. 102(3): pp.2255 - 2264.

Langenhoff, A. A. and Stuckey, D. C. 2000. Treatment of dilute wastewater using an anaerobic baffled reactor: effect of low temperature. *Water Research*. *34* (15): pp.3867 - 3875.

Lazarova, V., Choo, K. H., and Cornel, P. 2012. *Water-energy interactions in water reuse*. London: IWA publishing.

Lee Ferguson, P. and Brownawell, B. J. 2003. Degradation of nonylphenol ethoxylates in estuarine sediment under aerobic and anaerobic conditions. *Environmental Toxicology and Chemistry*. *22* (6): pp.1189 - 1199.

Lettinga, G. 1995. Anaerobic digestion and wastewater treatment systems. *Antonie van leeuwenhoek*. *67* (1) pp.3 - 28.

Lettinga, G., De Man, A., Van der Last, A. R. M., Wiegant, V. D. W., Van Knippenberg, K., Frijns, J., and Van Buuren, J. C. L. 1993. Anaerobic treatment of domestic sewage and wastewater. *Water Science and Technology*. *27* (9): pp.67 - 73.

Lettinga G., Rebac, S. and Zeeman, G. 2001. Challenge of psychrophilic anaerobic wastewater treatment. *Trends in Biotechnology*. 19 (9): pp.363 - 370.

Levenspiel, O. 1999. *Chemical Reaction Engineering*. 3rd ed. New York: John Wiley and Sons Inc.

Liu, R., Tian, Q., and Chen, J. 2010. The developments of anaerobic baffled reactor for wastewater treatment: A review. *African Journal of Biotechnology*. *9* (11): pp.1535 - 1542.

Llabres-Luengo, P., and Mata-Alvarez, J. 1988. The hydrolytic step in a dry digestion system. *Biological wastes*. *23* (1): pp.25 - 37.

Lu, Jingquan. 2006. *Optimization of anaerobic digestion of sewage sludge using thermophilic anaerobic pre-treatment*. [PhD dissertation]. Technology University of Denmark, Lyngby, Denmark. Available online at: http://orbit.dtu.dk/fedora/objects/orbit:82233/datastreams/file_4692519/content.

Lübken, M., Wichern, M., Schlattmann, M., Gronauer, A. and Horn, H. 2007. Modelling the energy balance of an anaerobic digester fed with cattle manure and renewable energy crops. *Water Research*. *41* (18): pp.4085 - 4096.

Lundie, S., Peters, G. M., and Beavis, P. C. 2004. Life cycle assessment for sustainable metropolitan water systems planning. *Environmental Science and Technology*. *38* (13): pp.3465 - 3473.

Luo, K., Yang, Q., Li, X., Yang, G., Liu, Y., Wang, D., Zheng, W. and Zeng, G. 2012. Hydrolysis kinetics in anaerobic digestion of waste activated sludge enhanced by α-amylase. *Biochemical Engineering Journal*. *62 (Jan)*: pp.17 - 21.

Luostarinen, S.A. and Rintala, J.A. 2005. Anaerobic on-site treatment of black water and dairy parlour wastewater in UASB-septic tanks at low temperatures. *Water research*. *39* (2): pp.436 - 448.

Madigan, M. T., Martinko, J. M., Stahl, D. A. and Clark, D. P. 2012. *Brock biology of microorganisms*. 13th ed. New Jersey: Pearson Education, Inc.

Mahmoud, N. 2002. *Anaerobic pre-treatment of sewage under low temperature (15°C) conditions in an integrated UASB-digester system*. [PhD dissertation]. Wageningen University, Wageningen, the Netherlands. Available online at: http://www.ircwash.org/resources/anaerobic-pre-treatment-sewage-under-low-temperature-15-c-conditions-integrated-uasb.

Mahmoud N.1., Zeeman G., Gijzen H. and Lettinga G. 2004. Anaerobic stabilisation and conversion of biopolymers in primary sludge — effect of temperature and sludge retention time. *Water Research.* 38 (4): pp.983 - 991.

Mara, D. 2003. *Domestic wastewater treatment in developing countries*. London: Earthscan.

Mara, D. and Horan, N. ed. 2003. *Handbook of water and wastewater microbiology*. London: Academic press.

Martín, M. A., De la Rubia, M. A., Martín, A., Borja, R., Montalvo, S. and Sánchez, E. 2010. Kinetic evaluation of the psychrophylic anaerobic digestion of synthetic domestic sewage using an upflow filter. *Bioresource Technology*. *101* (1): pp.131 - 137.

McCarty, P. L., Bae, J., and Kim, J. 2011. Domestic wastewater treatment as a net energy producer–can this be achieved? *Environmental science and Technology*. *45* (17): pp.7100 - 7106.

McKeown, R. M., Hughes, D., Collins, G., Mahony, T., and O'Flaherty, V. 2012. Low - temperature anaerobic digestion for wastewater treatment. *Current Opinion in Biotechnology*. *23* (3): pp.444 - 451.

Mensah, K.A. and Forster, C. F. 2003. An examination of the effects of detergents on anaerobic digestion. *Bioresource Technology*. *90* (2): pp.133 - 138.

Merlin, G. and Lissolo, T. 2010. Energy and Emergy analysis to evaluate sustainability of small wastewater treatment plants: application to a constructed wetland and a sequencing batch reactor. *Journal of Water Resource and Protection.* 2: pp.997 – 1009.

Mgana, S. 2003. *Towards sustainable and robust on-site domestic wastewater treatment for all citizens* [PhD dissertation]. Wageningen Universiteit, the Netherlands.

Miron, Y., Zeeman, G., Van Lier, J. B. and Lettinga, G. 2000. The role of sludge retention time in the hydrolysis and acidification of lipids, carbohydrates and proteins during digestion of primary sludge in CSTR systems. *Water research*. *34* (5): pp.1705-1713.

Morgenroth, E., Kommedal, R. and Harremos, P. 2002. Processes and modelling of hydrolysis of particulate organic matter in aerobic wastewater treatment-a review. *Water Science and Technology*. *45* (6): pp.25 - 40.

Mo, W. and Zhang, Q. 2012. Can municipal wastewater treatment systems be carbon neutral? *Journal of environmental management*. 112 (Sept.): pp.360 - 367.

Mo, W. and Zhang, Q. 2013. Energy – nutrients – water nexus: integrated resource recovery in municipal wastewater treatment plants. *Journal of environmental management.* 127 (Jun): pp.255 – 267.

Mottet, A., François, E., Latrille, E., Steyer, J. P., Déléris, S., Vedrenne, F., and Carrère, H. 2010. Estimating anaerobic biodegradability indicators for waste activated sludge. *Chemical engineering journal*. 160(2): pp.488 - 496.

Moussavi, G., Kazembeigi, F., and Farzadkia, M. 2010. Performance of a pilot scale up-flow septic tank for on-site decentralized treatment of residential wastewater. *Process safety and environmental protection*. 88(1): pp.47 - 52.

Mrowiec, B. and Suschka, J. 2006. *Anaerobic wastewater treatment processes*. [Online]. University of Bielsko-Biala, Poland. Available from: http: //www2.lwr.kth.se/forskningsprojekt/Polishproject/rep16/MrowiecSuschka.pdf.

Nachaiyasit, S. 1995. *The effect of process parameters on reactor performance in an anaerobic baffled reactor*. [PhD dissertation]. Imperial College, London, U.K.

Nachaiyasit, S. and Stuckey, D.C. 1995. Microbial response to environmental changes in an anaerobic baffled reactor (ABR). *Antonie van Leeuwenhoek*. 67 (1): pp.111 - 123.

Nachaiyasit, S. and Stuckey, D.C. 1997. Effect of low temperatures on the performance of an anaerobic baffled reactor (ABR). *Journal of Chemical Technology and Biotechnology*. *69* (2): pp.276 - 284.

Neves, L., Oliveira, R. and Alves, M. M. 2006. Anaerobic co-digestion of coffee waste and sewage sludge. *Waste Management*. *26* (2): pp.176 - 181.

Nielsen, S. 2005. Mineralisation of hazardous organic compounds in a sludge reed bed and sludge storage. *Water Science and Technology*. *51* (9): pp.109-117.

Noykova, N., Muller, T. G., Gyllenberg, M. and Timmer, J. 2002. Quantitative analyses of anaerobic wastewater treatment processes: identifiability and parameter estimation. *Biotechnology and Bioengineering*. 78 (1): pp. 89 – 103.

Nozhevnikova, A. N., Kotsyurbenko O. R. and Simankova M. V. 1994. Acetogenesis at low temperature. In: Drake, H. L. ed. *Acetogenesis*. New York: Springer. 1994, pp. 416 - 444.

Olvera, J. D. R. and Lopez-Lopez, A. 2012. Biogas production from anaerobic treatment of agro - industrial wastewater. In: Kumar, S. ed. *Biogas*. InTech. Available from: http://www.intechopen.com/books/biogas/biogas-production-from-anaerobic-treatment-of-agro-industrial-wastewater.

Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., and McCarty, P. L. 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water research*. 13(6): pp.485 - 492.

Palmer, P. B., and O'Connell, D. G. 2009. Regression analysis for prediction: understanding the process. *Cardiopulmonary physical therapy journal*. 20(3): pp.23.

Peleg, M., Normand, M. D., and Corradini, M. G. 2012. The Arrhenius equation revisited. *Critical reviews in food science and nutrition*. 52(9): pp.830 - 851.

Rajagopal, R., Massé, D. I., and Singh, G. 2013. A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bio-resource technology*. 143: pp.632 - 641.

Raposo, F., De la Rubia, M. A., Fernández-Cegrí, V., and Borja, R. 2011. Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. *Renewable and sustainable energy reviews*. 16(1): pp.861 - 877.

Remy, C., Lesjean, B. and Hartmann, A. 2011. *Using life cycle assessment for a comprehensive evaluation of energy demand in wastewater treatment*. [Online]. Berlin, Germany: Berlin Centre of competence for water. Available from: http://www.kompetenz-wasser.de/fileadmin/user_upload/pdf/presse/pressespiegel/KA06_2011_Remy_LCA_en.pdf.

Rittmann, B. and McCarty, P. 2000. *Environmental biotechnology: principals and applications*. New York: McGraw-Hill.

Rojas, J., and Zhelev, T. 2012. Energy efficiency optimisation of wastewater treatment: study of ATAD. *Computers and chemical engineering*. 38: pp.52 - 63.

Saady, N. M. C., and Massé, D. I. 2015. Impact of organic loading rate on psychrophilic anaerobic digestion of solid dairy manure. *Energies*. 8(3): pp.1990 - 2007.

Sallis, P. J., and Uyanik, S. 2003. Granule development in a split-feed anaerobic baffled reactor. *Bio-resource technology*. 89(3): pp.255 - 265.

Sanders, W.T.M. 2001. *Anaerobic hydrolysis during digestion of complex substrate*. [PhD dissertation]. Wageningen University, the Netherlands. Available from: http://www.researchgate.net/publication/40218308_Anaerobic_hydrolysis_during_digestion_of_complex_substrates.

Sarathai, Y., Koottatep, T. and Morel, A. 2010. Hydraulic characteristics of an anaerobic baffled reactor as onsite wastewater treatment system. *Journal of Environmental Sciences*. *22* (9): pp.1319 - 1326.

Sato, K., Ochi, S. and Mizuochi, M. 2001. Up-to date modification of the anaerobic sludge digestion process introducing a separate sludge digestion mode. *Water science and technology*. 44: pp.143 – 147.

Schiener, P., Nachaiyasit, S. and Stuckey, D. C. 1998. Production of soluble microbial products (SMP) in an anaerobic baffled reactor: composition, biodegradability, and the effect of process parameters. *Environmental Technology*. *19* (4): pp.391 - 399.

Seghezzo, L., Zeeman, G., Van Lier, J.B., Hamelers, H.V.M. and Lettinga, G. 1998. A review: the anaerobic treatment of sewage in UASB and EGSB reactors. *Bioresource Technology*. *65* (3): pp.175 - 190.

Shanmugam, A. S. and Akunna, J. C. 2008. Comparing the performance of UASB and GRABBR treating low strength wastewaters. *Water Science and Technology*. 58 (1): pp.225 – 232.

Shanmugam A.S. and Akunna J.C. 2010. Modelling head losses in granular bed anaerobic baffled reactors at high flows during start-up. *Water Research*. 44 (18): pp.5474 – 5480.

Shoener, B. D., Bradley, I. M., Cusick, R. D. and Guest, J. S. 2014. Energy positive domestic wastewater treatment: the roles of anaerobic and phototrophic technologies. *Environmental Science: Processes and Impacts*. *16* (6): pp.1204 - 1222.

Siegert, I. and Banks, C. 2005. The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochemistry*. *40*(11): pp3412-3418.

Siegrist, H., Vogt, D., Garcia-Heras, J. L. and Gujer, W. 2002. Mathematical model for meso-and thermophilic anaerobic sewage sludge digestion. *Environmental science and technology*. *36* (5): pp.1113 - 1123.

Singh, D., Singh, V. and Agnihotri. A. K. 2013. Study of textile effluent in and around Ludhiana district in Punjab, India. *International Journal of Environmental Sciences. 3* (4): pp.1271 - 1278.

Singh, P., Carliell-Marquet, C. and Kansal, A. 2012. Energy pattern analysis of a wastewater treatment plant. *Applied Water Science*. *2* (3): pp.221 - 226.

Sousa, L. de. 20 July 2008. *What is a human being worth in terms of energy?* [Online]. The oil drum Europe. Available from: http://www.theoildrum.com/node/4315

Speece, R. 2001. *Anaerobic biotechnology for industrial wastewaters*. Nashville, Tennessee, USA: Archae Press.

Stillwell, A.S., Hoppock, D.C. and Webber, M. E. 2010. Energy recovery from wastewater treatment plants in the United States: a case study of the energy-water nexus. *Sustainability*. *2* (4): pp.945 - 962.

Sun, L., Wan, S., Yu, Z., Wang, Y. and Wang, S. 2012. Anaerobic biological treatment of high strength cassava starch wastewater in a new type up-flow multistage anaerobic reactor. *Bioresource Technology*. *104 (Jan)*: pp.280 - 288.

Tai, D. Y. and Rathbun, R. E. 1988. Photolysts of rhodamine-WT dye. *Chemosphere*. *17* (3): pp.559 - 573.

Tandukar, M., Ohashi, A., and Harada, H. 2007. Performance comparison of a pilot-scale UASB and DHS system and activated sludge process for the treatment of municipal wastewater. *Water research*. 41(12): pp.2697 - 2705.

Tchobanoglous, G., Burton, F. L. and Stensel, H. D. eds. 2003. *Meltcalf and Eddy Inc.'s wastewater engineering: treatment, disposal and reuse*. 4th ed. New York: McGraw-Hill, Inc.

Tilley, E., Lüthi, C., Morel, A., Zurbrügg, C. and Schertenlieb, R. 2008. *Compendium of Sanitation Systems and Technologies.* [Online]. Eawag aquatic research. Available from: http://www.eawag.ch/organisation/abteilungen/sandec/publikationen/compendium.

Tomei, M. C., Braguglia, C. M., Cento, G., and Mininni, G. 2009. Modelling of anaerobic digestion of sludge. *Critical reviews in environmental science and technology*. 39(12): pp.1003 - 1051.

Uyanik, S., Sallis, P. J. and Anderson, G. K. 2002. The effect of polymer addition on granulation in an anaerobic baffled reactor (ABR) - part I: process performance. *Water Research*. *36* (4): pp.933 - 943.

van Haandel, A., Kato, M. T., Cavalcanti, P. F. F. and Florencio, L. 2006. Anaerobic reactor design concepts for the treatment of domestic wastewater. *Reviews in Environmental Science and Biotechnology*. *5* (1): pp.21 - 38.

van Lier, J.B., Tilche, A., Ahring, B.K., Acarie, H., Moletta, R., Dohanyos, M., HulshoffPol, L.W., Lens, P. and Verstraete, W. 2001. New perspectives in anaerobic digestion. *Water Science and Technology*. 43 (1): pp.1 – 18.

Vavilin, V.A., Fernandez, B., Palatsi, J. and Flotats, X. 2008. Hydrolysis kinetics in anaerobic degradation of particulate organic material: an overview. *Waste management*. *28* (6): pp.939 - 951.

Vavilin, V.A., Rytov, S.V., and Lokshina, L.Y. 1996. A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresource Technology*. *56* (2): pp.229 - 237.

Veeken A., and Hamelers, B. 1999. Effect of temperature on the hydrolysis rate of selected bio-waste components. *Bioresource Technology*. 69 (3): pp.249 - 254.

Veeken, A., and Hamelers, B. 2000. Effect of substrate-seed mixing and leachate recirculation on solid state digestion of bio-waste. *Water Science and Technology.* 41 (3): pp.255 – 262.

Veeken, A., Kalyuzhnyi, S., Scharff, H. and Hamelers, B. 2000. Effect of pH and VFA on hydrolysis of organic solid waste. *Journal of environmental engineering*. 126 (12): pp.1076-1081.

Verstraete, W., and Vandevivere, P. 1999. New and broader applications of anaerobic digestion. *Critical reviews in environmental science and technology*. 29(2): pp.151.

Verstraete, W., Van de Caveye, P. and Diamantis, V. 2009. Maximum use of resources present in domestic used water. *Bioresource Technology*. 100 (23): pp.5537 - 5545.

Visvanathan, C. and Abeynayaka, A. 2012. Developments and future potentials of anaerobic membrane bioreactors (AnMBRs). *Membrane Water Treatment*. 3 (1): pp.1 - 23.

Vossoughi, M., Shakeri, M., and Alemzadeh, I. 2003. Performance of anaerobic baffled reactor treating synthetic wastewater influenced by decreasing COD/SO_4 ratios. *Chemical Engineering and Processing: Process Intensification*. 42 (10): pp.811 - 816.

Vuono, D., Henkel, J., Benecke, J., Cath, T. Y., Reid, T., Johnson, L., and Drewes, J. E. 2013. Flexible hybrid membrane treatment systems for tailored nutrient management: a new paradigm in urban wastewater treatment. *Journal of membrane science*. 446: pp.34 - 41.

Walker, M., Zhang, Y., Heaven, S., and Banks, C. 2009. Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes. *Bio-resource technology*. 100(24): pp.6339 - 6346.

Weiss, P., Eveborn, D., Kärrman, E., and Gustafsson, J. P. 2008. Environmental systems analysis of four on-site wastewater treatment options. *Resources, conservation and recycling*. 52(10): pp.1153 - 1161.

Willmott, C. J., Ackleson, S. G., Davis, R. E., Feddema, J. J., Klink, K. M., Legates, D. R., and Rowe, C. M. 1985. Statistics for the evaluation and comparison of models. *Journal of geophysical research*. 90: pp.8995 - 9005.

Watanabe, R., Qiao, W., Norton, M., Wakahara, S. and Li, Y. 2014. Recent developments in municipal wastewater treatment using anaerobic membrane bioreactor: a review. *J. Water Sustainability*. 4 (2): pp.101 – 122.

Water Environment Federation. 2009. *Energy conservation in water and wastewater treatment facilities - water environment federation WEF manual of practice No. 32*. New York: McGraw-Hill Inc.

Weichgrebe S. A. D. and Rosenwinkel K. H. 2013. Determination of the hydrolysis constant using Anaerobic Batch Tests. Paper presented at: *13th IWA world congress on anaerobic digestion*. Santiago de Compostela, Spain. June 25-28.

Wett, B., Buchauer, K. and Fimml, C. 2007. Energy self-sufficiency as a feasible concept for wastewater treatment systems. Paper presented at: *IWA Leading Edge Technology Conference*. Singapore. September, 21 - 24.

World Health Organisation. 2014. *Progress on drinking water and sanitation– 2014 update.* [Online]. Geneva, Switzerland: World health organisation (WHO) Press. Available from: http://www.who.int/water_sanitation_health/publications/2014/jmp-report/en/.

Wiesmann, U., Choi, I. S. and Dombrowski, E. 2007. *Fundamentals of biological wastewater treatment*. Weinheim: JohnWiley and Sons.

Williams, C. F. and Nelson, S. D. 2011. Comparison of Rhodamine-WT and bromide as a tracer for elucidating internal wetland flow dynamics. *Ecological Engineering*. *37* (10): pp.1492 - 1498.

Yenigun, O. and Demirel, B. 2013. Ammonia inhibition in anaerobic digestion: a review. *Process Biochemistry*. *48* (5): pp.901 - 911.

Yoshida, N., Yano, K., Morita, T., McNiven, S. J., Nakamura, H., and Karube, I. 2000. A mediator-type biosensor as a new approach to biochemical oxygen demand estimation. *Analyst*. 125(12): pp.2280 - 2284.

Young, B., Delatolla, R., and Kennedy, K. 2013. Biodegradability and mesophilic co-digestion of municipal sludge and scum. *Bioprocess and bio-systems engineering*. 36(11): pp.1703 - 1714.

Yu, Y., Lu, X., and Wu, Y. 2014. Performance of an anaerobic baffled filter reactor in the treatment of algae-laden water and the contribution of granular sludge. *Water*. 6(1): pp.122 - 138.

Yuan, Q., Sparling, R. and Oleszkiewicz, J.A. 2011. VFA generation from waste activated sludge: effect of temperature and mixing. *Chemosphere*. *82* (4): pp.603 - 607.

Yuzir, A., Chelliapan, S. and Sallis, P. J. 2011. Influence of step increases in hydraulic retention time on (RS)-MCPP degradation using an anaerobic membrane bioreactor. *Bioresource technology*. *102*(20): 9456-9461.

Zakkour, P. D., Gaterell, M. R., Griffin, P., Gochin, R. J. and Lester, J. N. 2001. Anaerobic treatment of domestic wastewater in temperate climates: treatment plant modelling with economic considerations. *Water Research*. *35* (17): pp.4137 - 4149.

Zhang, H. J. 2010. *Sludge treatment to increase biogas production*. [Master's thesis]. Royal Institute of Technology, Stockholm, Sweden. Available online at: http://www.sjostadsverket.se/download/18.79cc091012c369366d9800017089/1350483757500/LWR_EX_10_20.pdf.

Zhu, G. F., Li, J. Z., Wu, P., Jin, H. Z., and Wang, Z. 2008. The performance and phase separated characteristics of an anaerobic baffled reactor treating soybean protein processing wastewater. *Bio-resource technology*. 99(17): pp8027 - 8033.

Zhu, G., Zou, R., Jha, A. K., Huang, X., Liu, L., and Liu, C. 2015. Recent developments and future perspectives of anaerobic baffled bioreactor for wastewater treatment and energy recovery. *Critical reviews in environmental science and technology*. 45(12): pp.1243 - 1276.

Bibliography

Aitken, M. D., Sobsey, M. D., Shehee, M., Blauth, K. E., Hill, V. R., Farrell, J. B., Nappier, S. R., Walters, G. W., Crunk, P. L. and Abel, N. V. 2005. Laboratory evaluation of thermophilic-anaerobic digestion to produce Class A biosolids - 2 Inactivation of pathogens and indicator organisms in a continuous flow reactor followed by batch treatment. *Water Environment Research*. 77 (7): pp.3028 - 3036.

Bachmann, A., Beard, V. L. and McCarty, P. L. 1983. Comparison of fixed film reactors with a modified sludge blanket reactor. In: Wu, Y.C. and Smith, E.D. eds. *Fixed film biological processes for wastewater treatment.* Park Ridge, NJ: Noyes Data Corp. 1983 .pp. 384 – 402.

Banister, S. S. and Pretorius, W. A. 1998. Optimization of primary sludge acidogenic fermentation for biological nutrient removal. *Water S. A.* 24 (1): pp.35 - 41.

Boopathy, R., Larsen V. F. and Senior E. 1988. Performance of anaerobic baffled reactor (ABR) in treating distillery waste-water from a Scotch whisky factory. *Biomass*. 16 (2): pp.133 - 143.

Cheremisinoff N. P. 2002. *Handbook of water and wastewater treatment technologies*. Boston: Butterworth-Heinemann.

Downing, L., Johnson, T., Corominas, Ll. and McNicol, S. 2014. Modelling the cost implications of energy production and reduction. Paper presented at: *4th IWA/WEF Wastewater Treatment Modelling Seminar*. Spa, Belgium, March 30th – April 2nd.

Faisal, M. and Unno, H. 2001. Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. *Biochemical Engineering Journal*. *9* (1): pp.25 - 31.

Henze, M., van Loosdrecht, M. C. M., Ekama, G. A. and Brdjanovic, D. eds. 2008. *Biological wastewater treatment: principles modelling and design.* London: IWA publishing.

Nielsen, B. and Petersen, G. 2000. Thermophilic anaerobic digestion and pasteurisation - practical experience from Danish wastewater treatment plants. *Water science and technology*. *42* (9): pp.65 - 72.

Paula, D. R. and Foresti, E., 2009. Sulfide toxicity kinetics of a UASB reactor. *Brazilian Journal of Chemical Engineering*. 26 (4): pp.669 - 675.

Rashed, I. G. A., Akunna, J., El-Halwany, M. M. and Atiaa, A. F. F. A. 2010. Improvement in the efficiency of hydrolysis of anaerobic digestion in sewage sludge by the use of enzymes. *Desalination and Water Treatment*. *21* (1-3): pp.280 – 285.

Sayed, S. K. I. 1987. *Anaerobic treatment of slaughterhouse wastewater using the UASB process*. [PhD dissertation]. Wageningen University, Wageningen, the Netherlands. Available from: http://www.dart-europe.eu/full.php?id=877024.

Scottish Environmental Protection Agency. 2014. *Regulation of sewage discharges to surface waters - WAT-RM-03*. [Online]. Scottish environmental protection agency SEPA. Available from: http://www.sepa.org.uk/water/water_regulation/guidance/pollution_control.aspx.

Spellman, F. R. 2003. *Handbook of water and wastewater treatment plant operations*. Florida: Lewis Publishers.

Suto, P., Gray, D. M. D., Larsen, E. and Hake, J. 2006. Innovative anaerobic digestion investigation of fats, oils, and grease. In: *Proceedings of the Water Environment Federation, Residuals and Biosolids Management* 2: pp.858 - 879.

United Nations Environment Programme. 2014. *Sludge treatment, reuse and disposal*. [Online]. United Nations Environment Programme. Available from: http://www.unep.or.jp/ietc/publications/freshwater/sb_summary/index.asp.

Appendix A - Synthesizing domestic wastewater for laboratory experiments

Various compositions of domestic wastewater have been reported in literature (Mara 2003; Burubai *et al.* 2007; Hegazy *et al.* 2011 and Mgana, 2003). Mgana (2003) measured centralized municipal wastewater in various locations in Tanzania, with average COD (564 mg/L) and BOD (360 mg/L), while Hegazy *et al.* (2011) observed the influent to a municipal wastewater treatment plant with average temp (25.7^{o}C), BOD (185 mg/L), COD (446 mg/L) and pH (7.0). Burubai *et al.* (2007) recorded mean values of domestic wastewater with BOD (603 - 665 mg/L) and COD (734 – 765 mg/L), while Hernandez-Leal *et al.* (2011) reported COD concentrations in municipal wastewater ranging from 171 – 4770 mg/L.

Several researches have adopted different methods for the synthesizing of laboratory wastewater. Gopala-Krishna *et al.* (2009) used a feed of 500 mg/L COD composed of 450 mg sucrose COD/L and 50 mg peptone COD/L. Additional buffer and trace elements used in the feed were: (in mg/L) $NaHCO_3$ (326); NH_4Cl (175); $(NH_4)_2HPO_4$ (40); KH_2PO_4 (7.2); $CoC1_2 \cdot 6H_2O$ (1.2); $Na_2Mo_4 \cdot 2H_2O$ (1.0); $FeC1_3$ (5.0); $CuSO_4 \cdot 5H_2O$ (5.0); $MgSO_4 \cdot 7H_2O$ (39.0); $MnSO_4 \cdot 4H_2O$ (13.9); $CaCl_2 \cdot 2H_2O$ (36.8). Yoshida *et al.* (2000) adopted a synthetic wastewater composed of 1 g of glucose, 1 g of peptones and 1 g of mono-potassium phosphate dissolved in 1 litre of water, and sodium hydroxide to adjust pH to 7.0 +/- 1.0.

Shanmugam and Akunna (2008) used a synthetic feed mixture of $C_6H_{12}O_6$ (2,500mg/L), NH_4HCO_3 (500mg/L), H_2PO_4 (200mg/L), $NaHCO_3$ (1,250mg/L), $MgSO_4$ (2.5mg/L), $FeCl_3$ (2.5mg/L), $CaCl_2$ (2.5mg/L), KCl (2.5mg/L), $CoCl_2$ (0.5 mg/L) and $NiCl_2$ (0.5mg/L). The feed used by Vossoughi *et al.* (2003) contained molasses as a carbon source, urea (0.007 g/g COD) as nitrogen source and K_2HPO_4 (0.0006 g/g COD) as phosphate source. The COD concentration varied between 1800 and 3000 mg/l, while sulphate concentration was varied from 0.0 (control), 150, 180, 350, 500 mg/l by using sodium sulphate.

Ghaniyari-Benis *et al.* (2009) used a feed with molasses as a carbon source and diluting with tap water to achieve the COD concentration required. The main features of the molasses obtained by diluting 1 g of raw molasses into 1 L of distilled water were: pH(7.6); COD (1124 mg/L); BOD (411 mg/L); Kjeldahl nitrogen (16.64 mg/L); total phosphate (0 mg/L); Ca_2^+ (59.2 mg/L); K^+ (3.1 mg/L); alkalinity (196 mg/L); total sugars (47.4%); free sugars, (18.7%); non-fermentable sugars (6%) and total dissolved solids (38%).

The feed composition for this research was developed with pure cane molasses, from Holland and Barretts, as the source of carbon due to the nature of molasses as waste products from sugar processing. Molasses is made of many chemical compounds, but the main content is usually sugar (sucrose) ($C_{12}H_{22}O_{11}$). Some of the minerals found in molasses are potassium, sodium, calcium and magnesium (Jimenez *et al.* 2003). After collection of the materials for the synthetic feed, tests were conducted on the feed to establish its consistency and stability.

For the consistency test, several dilutions of the feed were made, and each tested for parameters (COD, pH, and VFA). For the stability, the feed was diluted and stored in ambient conditions over several days, and two parameters (pH and COD) were observed for indications of variations. Results of the consistency and stability tests indicate changes in terms of pH and COD over a seven day period after storage in ambient conditions. The pH was steadily declining over time, between 5.8 – 5.55, indicating the need for alkalinity supplement to be used in the feed. The result of the COD analysis for the consistency indicated a stable COD concentration, with a variation between 940 - 1100 mg COD per gram of molasses.

Appendix B – Batch BMP assays data and data fit to Equation 2.21

$P = P_o * f_h * e^{(-kh*t)} + P_o (1 - f_h)$ (95% confidence bounds);

Volatile solids

PS 37ºC 13 data points
f_h = 0.5541 (0.3621, 0.7461);
kh = 0.1278 (0.0315, 0.2242);
$1 - f_h$ = 0.4407 (0.2917, 0.5898);
SSE: 0.0656 R-square: 0.8103 Adjusted R-square: 0.7724 RMSE: 0.0810

SS 37ºC 11 data points
f_h = 0.3827 (0.2370, 0.5285);
kh = 0.1098 (0.0062, 0.2134);
$1 - f_h$ = 0.6111 (0.4925, 0.7297);
SSE: 0.0345 R-square: 0.8301 Adjusted R-square: 0.7877 RMSE: 0.0657

PS nol 37ºC 14 data points
f_h = 0.3388 (0.2232, 0.4544);
kh = 0.2828 (0.0807, 0.4849);
$1 - f_h$ = 0.6465 (0.5941, 0.6989);
SSE: 0.0317 R-square: 0.7981 Adjusted R-square: 0.7613 RMSE: 0.0537

SS nol 37ºC 11 data points
f_h = 0.3808 (0.2904, 0.4712);
kh = 0.1157 (0.0569, 0.1744);
$1 - f_h$ = 0.6040 (0.5412, 0.6669);
SSE: 0.0106 R-square: 0.9224 Adjusted R-square: 0.9030 RMSE: 0.0364

PS 25ºC 13 data points
f_h = 0.4229 (0.3424, 0.5034);
kh = 0.2089 (0.1118, 0.3059);
$1 - f_h$ = 0.5982 (0.5510, 0.6453);
SSE: 0.0169 R-square: 0.9325 Adjusted R-square: 0.9190 RMSE: 0.0411

SS 25ºC 12 data points
f_h = 0.2233 (0.1258, 0.3209);
kh = 0.2194 (0.0100, 0.4288);
$1 - f_h$ = 0.7773 (0.7241, 0.8306);
SSE: 0.0158 R-square: 0.7490 Adjusted R-square: 0.6932 RMSE: 0.0420

PS nol 25ºC 15 data points
f_h = 0.3247 (0.1931, 0.4562);
kh = 0.2257 (0.0268, 0.4247);
$1 - f_h$ = 0.6789 (0.6107, 0.7470);
SSE: 0.0497 R-square: 0.7126 Adjusted R-square: 0.6647 RMSE: 0.0643

SS nol 25ºC 14 data points
f_h = 0.2941 (0.1733, 0.4149);
kh = 0.3320 (0.0450, 0.6190);
$1 - f_h$ = 0.7030 (0.6521, 0.7540);
SSE: 0.0334 R-square: 0.7315 Adjusted R-square: 0.6827 RMSE: 0.0551

Total solids

PS 37ºC 13 data points
f_h = 0.5783 (0.3319, 0.8246);
kh = 0.1921 (0.0302, 0.3539);
1 – f_h = 0.4189 (0.2916, 0.5463);
SSE: 0.1086 R-square: 0.7323 Adjusted R-square: 0.6788 RMSE: 0.1042

SS 37ºC 12 data points
f_h = 0.3299 (0.2527, 0.4072);
kh = 0.2365 (0.0960, 0.3770);
1 – f_h = 0.6641 (0.6165, 0.7117);
SSE: 0.0133 R-square: 0.9138 Adjusted R-square: 0.8947 RMSE: 0.0384

PS nol 37ºC 15 data points
f_h = 0.2662 (0.1412, 0.3913);
kh = 0.3924 (0.0000, 0.7994);
1 – f_h = 0.7389 (0.6878, 0.7900);
SSE: 0.0456 R-square: 0.6632 Adjusted R-square: 0.6071 RMSE: 0.0616

SS nol 37ºC 14 data points
f_h = 0.2165 (0.1279, 0.3050);
kh = 0.4087 (0.0404, 0.7770);
1 – f_h = 0.7860 (0.7487, 0.8232);
SSE: 0.0200 R-square: 0.7405 Adjusted R-square: 0.6933 RMSE: 0.0426

PS 25ºC 14 data points
f_h = 0.4704 (0.3248, 0.6160);
kh = 0.2688 (0.0726, 0.4651);
1 – f_h = 0.5347 (0.4617, 0.6076);
SSE: 0.0601 R-square: 0.8249 Adjusted R-square: 0.7931 RMSE: 0.0739

SS 25ºC 15 data points
f_h = 0.2817 (0.0955, 0.4679);
kh = 0.3372 (0.0000, 0.8643);
1 – f_h = 0.7308 (0.6516, 0.8101);
SSE: 0.1050 R-square: 0.4869 Adjusted R-square: 0.4014 RMSE: 0.0935

PS nol 25ºC 13 data points
f_h = 0.2954 (0.2036, 0.3872);
kh = 0.1249 (0.0358, 0.2139);
1 – f_h = 0.7021 (0.6177, 0.7866);
SSE: 0.0146 R-square: 0.8671 Adjusted R-square: 0.8406 RMSE: 0.0382

SS nol 25ºC 12 data points
f_h = 0.1983 (0.1090, 0.2877);
kh = 0.2099 (0.0000, 0.4772);
1 – f_h = 0.7934 (0.7225, 0.8644);
SSE: 0.0172 R-square: 0.7394 Adjusted R-square: 0.6815 RMSE: 0.0437

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Cumulative methane per batch (mL)																							
Blank 37oC 1	0.00	23.44	32.62	40.48	47.81	53.07	58.68	63.01	77.85	68.09	95.01	89.08	103.19	108.91	122.07	96.72	54.31	54.59	57.04	56.70	63.42	60.12	62.84
Blank 37oC 2	0.00	23.86	31.25	38.49	43.71	51.44	55.31	62.53	70.57	71.30	99.93	87.91	94.73	109.80	102.88	99.87	54.34	58.40	62.10	53.55	69.03	67.69	72.36
PS 37oC 1	0.00	56.24	74.98	100.19	113.00	119.86	130.85	145.77	159.35	190.02	250.23	288.72	347.10	459.58	503.09	714.11	805.77	905.24	984.53	1071.56	1083.25	1058.55	1054.20
PS 37oC 2	0.00	56.81	76.02	103.95	120.90	139.59	154.47	165.54	183.11	215.27	279.57	310.88	376.95	480.87	545.17	802.34	878.81	979.59	1066.14	1150.37	1161.62	1132.99	1131.75
SS 37oC 1	0.00	68.00	95.54	164.60	216.52	276.33	345.60	416.05	484.02	557.22	606.49	664.62	685.74	748.80	752.48	785.58	808.82	833.66	843.32	869.02	873.20	840.61	824.85
SS 37oC 2	0.00	73.13	97.01	164.42	216.07	286.42	354.73	428.43	498.03	569.76	616.92	653.59	691.98	737.85	744.83	781.23	787.23	814.75	830.50	844.99	848.02	735.83	818.24
PS nol 37oC 1	0.00	0.00	0.00	22.35	27.50	31.55	35.08	37.76	41.92	45.29	62.34	52.18	61.50	90.83	97.16	138.44	122.07	160.87	209.13	332.38	395.99	416.71	447.55
SS nol 37oC 1	0.00	0.00	0.00	0.00	24.92	31.55	37.34	45.42	57.12	85.78	127.12	128.74	139.95	191.79	54.91	204.49	224.61	307.87	348.96	398.00	416.69	394.53	398.58
Blank 25oC 1	0.00	0.00	0.00	23.73	27.58	29.53	32.93	34.47	37.02	39.39	55.06	51.58	52.09	62.80	46.02	57.76	18.74	20.81	22.37	22.40	25.31	23.82	24.61
Blank 25oC 2	0.00	0.00	0.00	21.58	26.69	28.39	30.98	34.23	36.32	39.93	38.91	54.74	62.19	58.56	56.20	55.99	15.30	16.77	17.08	18.16	17.71	18.04	18.41
PS 25oC 1	0.00	38.49	46.94	68.44	109.04	111.84	134.51	159.93	185.24	203.07	228.02	303.72	299.90	332.48	344.35	384.58	425.45	520.88	597.68	703.98	747.47	744.46	781.40
PS 25oC 2	0.00	39.97	50.45	75.97	109.44	123.77	146.80	160.02	175.55	204.71	218.92	254.22	296.43	345.64	353.96	405.44	457.15	579.47	669.09	776.49	822.82	822.91	855.63
SS 25oC 1	0.00	35.35	46.77	70.42	92.74	144.30	184.79	234.46	293.45	328.24	375.01	408.95	447.14	500.83	516.77	601.59	622.11	653.90	670.26	695.54	697.97	657.69	653.94
SS 25oC 2	0.00	36.62	46.06	76.84	97.93	149.77	187.60	225.31	281.60	307.92	387.51	389.48	442.20	503.89	509.62	594.90	620.43	650.10	666.85	684.45	687.16	652.76	648.24
PS nol 25oC 1	0.00	0.00	0.00	18.88	22.10	26.74	29.57	31.64	34.73	37.22	45.12	51.72	54.03	64.79	42.75	60.66	27.81	35.92	48.17	67.21	89.72	93.61	105.08
SS nol 25oC 1	0.00	0.00	0.00	0.00	0.00	25.76	28.32	35.21	37.34	35.63	72.67	76.87	91.39	102.07	79.52	116.12	82.60	99.67	121.45	150.49	181.89	187.56	201.10
Average cummulative methane per assay (mL)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC	0.00	23.65	31.94	39.49	45.76	52.26	56.99	62.77	74.21	69.69	97.47	88.50	98.96	109.36	112.47	98.30	54.33	56.49	59.57	55.13	66.22	63.90	67.60
PS 37oC	0.00	56.52	75.50	102.07	116.95	129.72	142.66	155.66	171.23	202.65	264.90	299.80	362.02	470.22	524.13	758.23	842.29	942.42	1025.34	1110.97	1122.44	1095.77	1092.98
SS 37oC	0.00	70.57	96.27	164.51	216.30	281.37	350.17	422.24	491.02	563.49	611.70	659.11	688.86	743.32	748.66	783.40	798.02	824.20	836.91	857.00	860.61	788.22	821.55
PS nol 37oC	0.00	0.00	0.00	22.35	27.50	31.55	35.08	37.76	41.92	45.29	62.34	52.18	61.50	90.83	97.16	138.44	122.07	160.87	209.13	332.38	395.99	416.71	447.55
SS nol 37oC	0.00	0.00	0.00	0.00	24.92	31.55	37.34	45.42	57.12	85.78	127.12	128.74	139.95	191.79	54.91	204.49	224.61	307.87	348.96	398.00	416.69	394.53	398.58
Blank 25oC	0.00	0.00	0.00	22.66	27.13	28.96	31.96	34.35	36.67	39.66	46.99	53.16	57.14	60.68	51.11	56.87	17.02	18.79	19.73	20.28	21.51	20.93	21.51
PS 25oC	0.00	39.23	48.69	72.20	109.24	117.80	140.65	159.98	180.39	203.89	223.47	278.97	298.16	339.06	349.16	395.01	441.30	550.18	633.38	740.24	785.14	783.69	818.52
SS 25oC	0.00	35.98	46.41	73.63	95.34	147.03	186.20	229.88	287.52	318.08	381.26	399.22	444.67	502.36	513.20	598.25	621.27	652.00	668.56	689.99	692.57	655.22	651.09
PS nol 25oC	0.00	0.00	0.00	18.88	22.10	26.74	29.57	31.64	34.73	37.22	45.12	51.72	54.03	64.79	42.75	60.66	27.81	35.92	48.17	67.21	89.72	93.61	105.08
SS nol 25oC	0.00	0.00	0.00	0.00	0.00	25.76	28.32	35.21	37.34	35.63	72.67	76.87	91.39	102.07	79.52	116.12	82.60	99.67	121.45	150.49	181.89	187.56	201.10
Subtracting methane production by blanks from average values per assay (mL)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
PS 37oC	0.00	32.87	43.56	62.58	71.19	77.46	85.67	92.88	97.02	132.95	167.43	211.30	263.06	360.87	411.66	659.93	787.96	885.92	965.76	1055.84	1056.21	1056.21	1056.21
SS 37oC	0.00	46.92	64.34	125.02	170.54	229.12	293.17	359.46	416.81	493.80	514.23	570.61	589.90	633.97	636.18	685.11	743.70	767.71	777.34	801.88	801.88	801.88	801.88
PS nol 37oC	0.00	0.00	0.00	22.35	27.50	31.55	35.08	37.76	41.92	45.29	62.34	62.34	62.34	90.83	97.16	138.44	138.44	160.87	209.13	332.38	395.99	416.71	447.55
SS nol 37oC	0.00	0.00	0.00	0.00	24.92	31.55	37.34	45.42	57.12	85.78	127.12	128.74	139.95	191.79	191.79	204.49	224.61	307.87	348.96	398.00	416.69	416.69	416.69
PS 25oC	0.00	39.23	48.69	49.55	82.11	88.84	108.69	125.63	143.72	164.23	176.48	225.81	241.02	278.38	298.05	338.13	424.28	531.39	613.66	719.96	763.63	763.63	797.01
SS 25oC	0.00	35.98	46.41	50.97	68.21	118.07	154.24	195.53	250.85	278.42	334.27	346.06	387.53	441.68	462.09	541.37	604.25	633.21	648.83	669.71	671.05	671.05	671.05
PS nol 25oC	0.00	0.00	0.00	18.88	22.10	26.74	29.57	31.64	34.73	37.22	45.12	51.72	54.03	64.79	64.79	64.79	64.79	64.79	64.79	67.21	89.72	93.61	105.08
SS nol 25oC	0.00	0.00	0.00	0.00	0.00	25.76	28.32	35.21	37.34	37.34	72.67	76.87	91.39	102.07	102.07	116.12	116.12	116.12	121.45	150.49	181.89	187.56	201.10
Cummulative methane per assay (mL/g VS added)																							
PS 37oC	0.00	9.37	12.41	17.84	20.29	22.08	24.41	26.47	27.65	37.89	47.72	60.22	74.97	102.85	117.32	188.08	224.57	252.49	275.24	300.91	301.02	301.02	301.02
SS 37oC	0.00	14.18	19.45	37.80	51.56	69.27	88.64	108.68	126.02	149.30	155.48	172.52	178.35	191.68	192.35	207.14	224.85	232.11	235.02	242.44	242.44	242.44	242.44
PS nol 37oC	0.00	0.00	0.00	5.75	7.08	8.12	9.03	9.72	10.79	11.66	16.05	16.05	16.05	23.38	25.01	35.63	35.63	41.41	53.83	85.55	101.93	107.26	115.20
SS nol 37oC	0.00	0.00	0.00	0.00	5.91	7.48	8.85	10.77	13.54	20.34	30.14	30.53	33.18	45.47	45.47	48.49	53.26	73.00	82.74	94.37	98.80	98.80	98.80
PS 25oC	0.00	11.18	13.88	14.12	23.40	25.32	30.98	35.80	40.96	46.81	50.30	64.36	68.69	79.34	84.94	96.37	120.92	151.44	174.89	205.19	217.63	217.63	227.15
SS 25oC	0.00	10.88	14.03	15.41	20.62	35.70	46.63	59.12	75.84	84.18	101.07	104.63	117.17	133.54	139.71	163.68	182.69	191.45	196.17	202.48	202.89	202.89	202.89
PS nol 25oC	0.00	0.00	0.00	4.86	5.69	6.88	7.61	8.14	8.94	9.58	11.61	13.31	13.91	16.68	16.68	16.68	16.68	16.68	16.68	17.30	23.09	24.09	27.05
SS nol 25oC	0.00	0.00	0.00	0.00	0.00	6.11	6.71	8.35	8.85	8.85	17.23	18.23	21.67	24.20	24.20	27.53	27.53	27.53	28.80	35.68	43.13	44.47	47.68

Total solids (g/L)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC 1	13.40	13.50	12.50	11.75	13.25	12.50	12.55	3.20	12.05	12.80	13.05	12.75	12.15	12.35	11.60			12.50		13.40			12.30
Blank 37oC 2	12.80	12.75	12.00	10.15	11.35	12.60	12.70	1.35	10.00	12.60	11.80	11.90	11.95	12.65	11.20			11.30		12.50			11.60
Blank 37oC 3	11.10																	11.35		12.80			11.55
Blank 37oC 4	11.80																	11.20		12.80			11.05
PS 37oC 1	24.60	21.75	20.60	19.15	19.55	14.75	20.55	4.30	19.35	20.95	19.40	18.25	20.50	17.95	20.55			16.10		17.20			16.70
PS 37oC 2	23.40	21.75	20.75	18.90	21.60	13.10	19.25	2.85	11.70	18.70	14.65	16.50	17.45	17.55	17.65			16.55		16.90			16.40
PS 37oC 3	23.90																	16.45		17.05			16.10
PS 37oC 4	24.00																	15.55		16.80			16.95
SS 37oC 1	23.30	24.00	22.65	20.85	21.55	19.75	22.45	4.25	20.80	21.85	21.20	20.00	21.40	20.85	19.55			19.65		20.10			20.35
SS 37oC 2	25.40	23.60	24.05	21.10	22.00	20.30	21.25	4.10	13.05	14.40	18.90	15.60	20.50	20.35	19.10			18.95		20.20			19.90
SS 37oC 3	25.00																	20.05		20.50			20.10
SS 37oC 4	23.80																	19.30		20.45			19.90
PS nol 37oC 1	16.50	15.50	15.10	13.60	14.00	1.75	9.80	2.80	12.80	14.10	13.60	13.40	13.20	13.10	12.40			10.75		12.85			12.05
PS nol 37oC 2	16.60	14.65	14.55	13.40	13.25	2.90	10.70	5.40	12.40	12.55	12.10	11.50	12.85	13.10	12.45			9.95		12.85			12.20
SS nol 37oC 1	18.80	16.80	16.90	15.60	15.65	3.55	12.70	5.00	14.65	16.90	16.10	15.15	15.20	15.35	14.60			13.70		14.65			14.30
SS nol 37oC 2	17.90	16.85	16.50	15.50	16.10	3.35	13.10	2.40	13.20	13.35	14.50	14.00	15.30	15.45	14.50			14.65		14.55			13.45
Blank 25oC 1	13.40	12.05	11.05	11.50	12.30	11.95	12.00	2.25	11.10	12.05	12.00	11.85	12.50	12.15	11.95			12.05		11.55			11.60
Blank 25oC 2	12.80	12.80	11.45	11.15	12.15	12.55	12.75	1.15	10.35	11.20	11.10	10.75	12.20	13.05	12.10			11.00		11.75			11.30
Blank 25oC 3	11.10																	12.25		11.95			11.90
Blank 25oC 4	11.80																	12.15		11.80			11.75
PS 25oC 1	24.60	22.15	22.15	20.20	21.00	21.55	20.55	2.45	18.30	19.30	18.45	18.10	18.90	18.70	18.25			19.15		18.90			18.10
PS 25oC 2	23.40	22.80	21.95	20.55	22.25	20.90	19.70	6.55	12.65	16.65	11.45	17.75	19.30	18.60	17.90			18.95		18.55			17.60
PS 25oC 3	23.90																	19.15		18.00			18.35
PS 25oC 4	24.00																	19.25		17.70			17.90
SS 25oC 1	23.30	23.75	25.05	21.95	23.25	22.50	22.00	2.65	21.55	22.95	22.10	21.75	21.35	21.15	21.65			21.40		20.50			21.05
SS 25oC 2	25.40	23.85	24.95	20.50	22.75	22.60	21.75	1.65	11.75	18.95	20.60	20.80	21.85	20.70	18.00			21.80		21.95			20.45
SS 25oC 3	25.00																	20.95		21.65			20.45
SS 25oC 4	23.80																	20.60		21.25			19.95
PS nol 25oC 1	16.50	16.00	15.40	14.50	13.45	3.35	18.20	7.60	12.90	14.20	14.80	13.20	12.75	12.95	12.00			12.30		13.15			12.30
PS nol 25oC 2	16.60	16.20	15.10	13.95	15.35	2.55	10.00	2.95	11.80	13.40	14.00	11.30	12.55	12.40	12.30			11.95		13.10			11.00
SS nol 25oC 1	18.80	17.55	16.70	15.55	17.30	1.95	12.60	4.80	15.35	16.35	16.25	15.75	16.45	16.05	15.85			15.75		15.90			13.65
SS nol 25oC 2	17.90	18.20	16.15	15.85	16.55	7.35	12.70	2.85	13.85	14.55	14.25	12.20	15.90	15.90	15.05			15.10		15.90			13.75
Average Total solids per assay (g/L)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC	12.27	13.13	12.25	10.95	12.30	12.55	12.62	2.28	11.03	12.70	12.42	12.32	12.05	12.50	11.40			11.59		12.88			11.62
PS 37oC	23.98	21.75	20.67	19.03	20.58	13.93	19.90	3.58	15.53	19.83	17.02	17.37	18.98	17.75	19.10			16.16		16.99			16.54
SS 37oC	24.38	23.80	23.35	20.97	21.78	20.02	21.85	4.17	16.93	18.12	20.05	17.80	20.95	20.60	19.33			19.49		20.31			20.06
PS nol 37oC	16.55	15.08	14.82	13.50	13.63	2.32	10.25	4.10	12.60	13.33	12.85	12.45	13.02	13.10	12.42			10.35		12.85			12.13
SS nol 37oC	18.35	16.82	16.70	15.55	15.87	3.45	12.90	3.70	13.93	15.12	15.30	14.57	15.25	15.40	14.55			14.17		14.60			13.88
Blank 25oC	12.27	12.42	11.25	11.32	12.22	12.25	12.38	1.70	10.72	11.63	11.55	11.30	12.35	12.60	12.03			11.86		11.76			11.64
PS 25oC	23.98	22.48	22.05	20.37	21.62	21.23	20.12	4.50	15.47	17.97	14.95	17.93	19.10	18.65	18.08			19.13		18.29			17.99
SS 25oC	24.38	23.80	25.00	21.23	23.00	22.55	21.88	2.15	16.65	20.95	21.35	21.27	21.60	20.93	19.83			21.19		21.34			20.47
PS nol 25oC	16.55	16.10	15.25	14.23	14.40	2.95	14.10	5.28	12.35	13.80	14.40	12.25	12.65	12.68	12.15			12.13		13.13			11.65
SS nol 25oC	18.35	17.87	16.42	15.70	16.93	4.65	12.65	3.82	14.60	15.45	15.25	13.97	16.18	15.98	15.45			15.43		15.90			13.70
Subtracting inoculum Total solids from average values to get the Total solids per assay (g/L)																							
PS 37oC	11.70	8.63	8.42	8.07	8.28	1.37	7.28	1.30	4.50	7.13	4.60	5.05	6.92	5.25	7.70			4.58		4.11			4.91
SS 37oC	12.10	10.67	11.10	10.02	9.48	7.47	9.23	1.90	5.90	5.43	7.63	5.48	8.90	8.10	7.93			7.90		7.44			8.44
PS nol 37oC	16.55	15.08	14.82	13.50	13.63	2.32	10.25	4.10	12.60	13.33	12.85	12.45	13.02	13.10	12.42			10.35		12.85			12.13
SS nol 37oC	18.35	16.82	16.70	15.55	15.87	3.45	12.90	3.70	13.93	15.12	15.30	14.57	15.25	15.40	14.55			14.17		14.60			13.88
PS 25oC	11.70	10.05	10.80	9.05	9.40	8.98	7.75	2.80	4.75	6.35	3.40	6.63	6.75	6.05	6.05			7.26		6.52			6.35
SS 25oC	12.10	11.38	13.75	9.90	10.78	10.30	9.50	0.45	5.93	9.33	9.80	9.97	9.25	8.33	7.80			9.32		9.57			8.84
PS nol 25oC	16.55	16.10	15.25	14.23	14.40	2.95	14.10	5.28	12.35	13.80	14.40	12.25	12.65	12.68	12.15			12.13		13.13			11.65
SS nol 25oC	18.35	17.87	16.42	15.70	16.93	4.65	12.65	3.82	14.60	15.45	15.25	13.97	16.18	15.98	15.45			15.43		15.90			13.70

Volatile solids (g/L)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC 1	6.70	5.70	5.40	5.05	5.05	5.55	5.95	1.60	4.90	5.20	5.50	5.45	5.20	5.70	4.65			5.10		5.60			5.40
Blank 37oC 2	5.60	5.05	5.00	4.65	4.05	5.70	5.90	0.85	4.05	4.55	5.25	5.65	5.15	6.10	4.70			4.95		5.55			5.45
Blank 37oC 3	6.30																	5.00		7.50			4.85
Blank 37oC 4	7.30																	4.60		5.55			4.95
PS 37oC 1	17.90	14.20	12.80	12.65	11.80	8.75	13.30	3.15	11.95	12.40	12.00	11.80	12.45	10.95	12.10			8.75		9.00			9.65
PS 37oC 2	17.00	13.25	13.60	12.85	13.80	8.05	12.90	2.40	7.40	11.15	9.10	10.15	10.95	10.55	10.75			9.10		9.20			9.65
PS 37oC 3	15.70																	9.40		10.65			9.45
PS 37oC 4	15.40																	9.00		9.45			9.90
SS 37oC 1	15.50	13.95	14.35	14.65	13.15	11.75	14.45	3.45	12.00	12.25	12.00	11.80	13.00	12.55	11.20			10.85		11.20			11.65
SS 37oC 2	16.90	13.85	14.90	13.45	13.35	12.55	13.60	2.85	7.35	7.90	11.00	9.15	12.40	11.55	10.85			11.05		11.30			11.50
SS 37oC 3	15.10																	11.30		11.45			11.50
SS 37oC 4	16.20																	11.30		11.55			11.45
PS nol 37oC 1	11.50	10.10	9.35	9.10	8.45	1.05	7.00	2.10	7.35	8.05	8.40	8.30	7.50	8.50	6.95			6.10		7.20			7.15
PS nol 37oC 2	10.70	8.10	8.75	8.85	7.80	2.05	7.50	0.60	7.45	7.30	8.55	8.30	7.05	7.80	7.50			6.10		7.00			7.05
SS nol 37oC 1	13.30	9.40	9.40	9.45	9.15	2.40	9.90	4.20	7.60	9.35	9.60	9.95	8.55	9.50	8.30			7.50		7.35			7.70
SS nol 37oC 2	10.80	9.45	9.55	9.85	9.90	2.35	10.30	2.15	6.65	7.25	9.00	7.90	8.15	8.15	7.80			7.70		7.15			6.90
Blank 25oC 1	6.70	5.15	4.45	5.10	5.80	5.35	6.05	0.95	4.45	5.05	5.35	5.25	5.25	5.30	4.70			5.35		4.75			5.45
Blank 25oC 2	5.60	4.35	4.75	5.80	5.25	5.55	5.70	0.40	3.90	4.40	5.25	4.80	4.90	5.95	5.00			5.05		4.95			5.55
Blank 25oC 3	6.30																	5.35		5.05			5.85
Blank 25oC 4	7.30																	5.50		4.70			5.90
PS 25oC 1	17.90	13.85	14.50	13.75	13.80	13.85	13.50	2.45	10.95	12.30	12.45	11.50	11.45	11.20	11.05			11.85		11.40			11.55
PS 25oC 2	17.00	14.50	15.30	13.95	14.35	13.50	12.20	2.20	7.65	9.90	8.20	11.25	11.65	11.25	10.40			11.85		11.70			12.10
PS 25oC 3	15.70																	11.95		10.80			
PS 25oC 4	15.40																	12.00		10.55			11.45
SS 25oC 1	15.50	14.35	15.50	14.25	14.20	13.25	14.35	1.80	12.20	13.60	13.55	13.25	12.65	12.30	12.15			22.55		12.20			13.00
SS 25oC 2	16.90	14.65	15.20	13.10	13.90	14.20	13.10	0.90	6.85	11.25	12.45	12.70	12.45	13.55	10.70			13.05		12.95			13.00
SS 25oC 3	15.10																	12.60		12.80			13.10
SS 25oC 4	16.20																	12.55		13.05			12.65
PS nol 25oC 1	11.50	9.75	10.35	9.20	8.20	2.40	9.80	3.00	7.65	8.55	9.80	8.60	7.55	7.65	6.55			7.70		7.05			7.90
PS nol 25oC 2	10.70	9.70	9.45	9.45	9.15	2.10	8.90	2.30	6.85	7.90	9.00	6.30	7.20	6.80	7.10			7.55		7.60			9.00
SS nol 25oC 1	13.30	10.35	10.15	10.15	11.55	1.75	7.70	3.00	8.20	9.20	9.75	8.85	9.45	8.95	8.50			7.85		8.70			10.15
SS nol 25oC 2	10.80	10.55	9.55	9.45	9.80	2.15	8.70	1.95	7.15	8.20	9.25	7.10	8.30	8.70	8.45			8.90		8.35			10.40
Average volatile solids per assay (g/L)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC	6.47	5.37	5.20	4.85	4.55	5.63	5.92	1.23	4.47	4.87	5.37	5.55	5.18	5.90	4.67			4.91		6.05			5.16
PS 37oC	16.50	13.73	13.20	12.75	12.80	8.40	13.10	2.78	9.68	11.78	10.55	10.97	11.70	10.75	11.42			9.06		9.58			9.66
SS 37oC	15.93	13.90	14.62	14.05	13.25	12.15	14.03	3.15	9.68	10.08	11.50	10.48	12.70	12.05	11.03			11.13		11.38			11.53
PS nol 37oC	11.10	9.10	9.05	8.97	8.13	1.55	7.25	1.35	7.40	7.68	8.47	8.30	7.27	8.15	7.23			6.10		7.10			7.10
SS nol 37oC	12.05	9.43	9.47	9.65	9.53	2.37	10.10	3.18	7.13	8.30	9.30	8.92	8.35	8.82	8.05			7.60		7.25			7.30
Blank 25oC	6.47	4.75	4.60	5.45	5.52	5.45	5.88	0.67	4.17	4.73	5.30	5.03	5.08	5.63	4.85			5.31		4.86			5.69
PS 25oC	16.50	14.18	14.90	13.85	14.07	13.67	12.85	2.32	9.30	11.10	10.32	11.38	11.55	11.22	10.73			11.91		11.11			11.70
SS 25oC	15.93	14.50	15.35	13.68	14.05	13.73	13.73	1.35	9.53	12.42	13.00	12.98	12.55	12.92	11.43			15.19		12.75			12.94
PS nol 25oC	11.10	9.73	9.90	9.33	8.68	2.25	9.35	2.65	7.25	8.23	9.40	7.45	7.37	7.23	6.83			7.63		7.33			8.45
SS nol 25oC	12.05	10.45	9.85	9.80	10.68	1.95	8.20	2.48	7.68	8.70	9.50	7.97	8.88	8.82	8.48			8.38		8.53			10.28
Subtracting inoculum volatile solids from average values to get the Volatile solids per assay (g/L)																							
PS 37oC	10.03	8.35	8.00	7.90	8.25	2.77	7.18	1.55	5.20	6.90	5.18	5.42	6.52	4.85	6.75			4.15		3.53			4.50
SS 37oC	9.45	8.52	9.42	9.20	8.70	6.52	8.10	1.92	5.20	5.20	6.13	4.93	7.53	6.15	6.35			6.21		5.33			6.36
PS nol 37oC	11.10	9.10	9.05	8.97	8.13	1.55	7.25	1.35	7.40	7.68	8.47	8.30	7.27	8.15	7.23			6.10		7.10			7.10
SS nol 37oC	12.05	9.43	9.47	9.65	9.53	2.37	10.10	3.18	7.13	8.30	9.30	8.92	8.35	8.82	8.05			7.60		7.25			7.30
PS 25oC	10.03	9.43	10.30	8.40	8.55	8.23	6.97	1.65	5.13	6.37	5.02	6.35	6.48	5.60	5.88			6.60		6.25			6.01
SS 25oC	9.45	9.75	10.75	8.23	8.53	8.28	7.85	0.67	5.35	7.70	7.70	7.95	7.47	7.30	6.58			9.87		7.89			7.25
PS nol 25oC	11.10	9.73	9.90	9.33	8.68	2.25	9.35	2.65	7.25	8.23	9.40	7.45	7.37	7.23	6.83			7.63		7.33			8.45
SS nol 25oC	12.05	10.45	9.85	9.80	10.68	1.95	8.20	2.48	7.68	8.70	9.50	7.97	8.88	8.82	8.48			8.38		8.53			10.28

Average VFA concentrations per assay (mg/L)																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC	0.00	0.00	40.00	157.50	0.00	56.50	57.50	56.00	65.00	41.00	77.00	41.00	74.00	36.00	106.00		28.50	31.00		9.00			20.75
PS 37oC	356.00	993.00	1061.50	676.00	1090.50	1543.00	1691.50	1728.50	1660.00	1612.00	1511.00	0.00	1273.00	1091.00	744.00		626.50	380.00		107.00			194.25
SS 37oC	238.33	519.50	508.50	355.00	377.00	637.50	662.00	605.00	443.50	610.00	532.00	133.00	255.00	142.00	185.00		51.75	101.00		4.00			64.00
PS nol 37oC	285.00	830.50	1025.50	1030.00	1108.50	1343.50	1668.00	1629.50	1717.00	1641.00	1829.00	887.00	1559.00	1371.00	1069.00		766.50	660.50		769.50			1042.00
SS nol 37oC	256.00	540.50	649.00	634.50	615.50	944.50	1079.00	1012.00	953.00	876.00	780.00	521.50	636.00	482.00	398.00		398.00	322.00		172.50			334.50
Blank 25oC	0.00	0.00	58.50	89.50	7.50	86.50	61.00	37.00	35.00	28.00	47.00	26.00	68.00	46.00	99.00		32.50	5.50		5.50			41.50
PS 25oC	349.75	872.00	894.00	922.00	1004.00	1269.00	1528.00	1492.50	1511.00	1468.00	1557.00	818.00	1264.00	996.00	713.00		361.50	319.00		205.00			183.50
SS 25oC	240.00	489.50	489.00	459.00	486.00	575.00	563.50	435.00	337.00	233.00	222.00	136.00	240.00	109.00	159.00		48.75	27.00		28.00			75.50
PS nol 25oC	276.00	788.00	869.50	886.50	1132.00	1361.00	1481.50	1552.50	1525.00	1665.00	1846.00	1736.00	1807.00	1670.00	1056.00		1180.50	1585.00		1048.50			730.50
SS nol 25oC	223.00	405.00	580.00	360.00	612.00	722.00	846.00	868.00	828.00	826.00	943.00	712.00	799.00	634.00	453.00		231.50	334.50		313.50			141.00
Substracting VFA from blanks to get VFA concentrations per assay (mg/L)																							
PS 37oC	356.00		1021.50			1486.50	1634.00	1672.50		1571.00	1434.00		1199.00	1055.00	638.00		598.00	349.00		98.00			173.50
SS 37oC	238.33		468.50			581.00	604.50	549.00		569.00	455.00		181.00	106.00	79.00		23.25	70.00					43.25
PS nol 37oC	285.00		1025.50			1343.50	1668.00	1629.50		1641.00	1829.00		1559.00	1371.00	1069.00		766.50			769.50			1042.00
SS nol 37oC	256.00		649.00			944.50	1079.00	1012.00		876.00	780.00		636.00	482.00	398.00		398.00	322.00		172.50			334.50
PS 25oC	349.75		835.50			1182.50	1467.00	1455.50		1440.00	1510.00		1196.00	950.00	614.00		329.00	313.50		199.50			142.00
SS 25oC	240.00		430.50			488.50	502.50	398.00		205.00	175.00		172.00	63.00	60.00		16.25	21.50		22.50			34.00
PS nol 25oC	276.00		869.50			1361.00	1481.50	1552.50		1665.00	1846.00		1807.00	1670.00	1056.00		1180.50			1048.50			730.50
SS nol 25oC	223.00		580.00			722.00	846.00	868.00		826.00	943.00		799.00	634.00	453.00		231.50	334.50		313.50			141.00

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Total solids per assay (g/L) as fractions of initial Total solids																							
PS 37oC	1.00	0.74	0.72	0.69	0.71		0.62		0.38	0.61	0.39	0.43	0.59	0.45	0.66			0.39		0.35			0.42
SS 37oC	1.00	0.88	0.92	0.83	0.78		0.76		0.49	0.45	0.63	0.45	0.74	0.67	0.65			0.65		0.61			0.70
PS nol 37oC	1.00	0.91	0.90	0.82	0.82		0.62		0.76	0.81	0.78	0.75	0.79	0.79	0.75			0.63		0.78			0.73
SS nol 37oC	1.00	0.92	0.91	0.85	0.87		0.70		0.76	0.82	0.83	0.79	0.83	0.84	0.79			0.77		0.80			0.76
PS 25oC	1.00	0.86	0.92	0.77	0.80		0.66		0.41	0.54	0.29	0.57	0.58	0.52	0.52			0.62		0.56			0.54
SS 25oC	1.00	0.94	1.14	0.82	0.89		0.79		0.49	0.77	0.81	0.82	0.76	0.69	0.64			0.77		0.79			0.73
PS nol 25oC	1.00	0.97	0.92	0.86	0.87		0.85		0.75	0.83	0.87	0.74	0.76	0.77	0.73			0.73		0.79			0.70
SS nol 25oC	1.00	0.97	0.90	0.86	0.92		0.69		0.80	0.84	0.83	0.76	0.88	0.87	0.84			0.84		0.87			0.75
Line of best fit for Total solids per assay (g/L) as fractions of initial Total solids																							
PS 37oC	1.00	0.90	0.81	0.74	0.69	0.64	0.60	0.57	0.54	0.52	0.50	0.49	0.48	0.47	0.46	0.44	0.43	0.42	0.42	0.42	0.42	0.42	0.42
SS 37oC	0.99	0.92	0.87	0.83	0.79	0.77	0.74	0.73	0.71	0.70	0.70	0.69	0.68	0.68	0.68	0.67	0.67	0.67	0.66	0.66	0.66	0.66	0.66
PS nol 37oC	1.01	0.92	0.86	0.82	0.79	0.78	0.76	0.76	0.75	0.75	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74
SS nol 37oC	1.00	0.93	0.88	0.85	0.83	0.81	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
PS 25oC	1.01	0.89	0.81	0.74	0.70	0.66	0.63	0.61	0.59	0.58	0.57	0.56	0.55	0.55	0.55	0.54	0.54	0.54	0.54	0.53	0.53	0.53	0.53
SS 25oC	1.01	0.93	0.87	0.83	0.80	0.78	0.77	0.76	0.75	0.74	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
PS nol 25oC	1.00	0.96	0.93	0.91	0.88	0.86	0.84	0.83	0.81	0.80	0.79	0.78	0.77	0.76	0.75	0.74	0.73	0.72	0.71	0.71	0.71	0.70	0.70
SS nol 25oC	0.99	0.95	0.92	0.90	0.88	0.86	0.85	0.84	0.83	0.82	0.82	0.81	0.81	0.81	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.79	0.79

Volatile solids per assay (g/L) as fractions of initial Volatile solids																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
PS 37oC	1.00	0.83	0.80	0.79	0.82		0.72		0.52	0.69	0.52	0.54	0.65	0.48	0.67			0.41		0.35			0.45
SS 37oC	1.00	0.90	1.00	0.97	0.92		0.86		0.55	0.55	0.65	0.52	0.80	0.65	0.67			0.66		0.56			0.67
PS nol 37oC	1.00	0.82	0.82	0.81	0.73		0.65		0.67	0.69	0.76	0.75	0.66	0.73	0.65			0.55		0.64			0.64
SS nol 37oC	1.00	0.78	0.79	0.80	0.79		0.84		0.59	0.69	0.77	0.74	0.69	0.73	0.67			0.63		0.60			0.61
PS 25oC	1.00	0.94	1.03	0.84	0.85		0.70		0.51	0.64	0.50	0.63	0.65	0.56	0.59			0.66		0.62			0.60
SS 25oC	1.00	1.03	1.14	0.87	0.90		0.83		0.57	0.81	0.81	0.84	0.79	0.77	0.70			1.04		0.83			0.77
PS nol 25oC	1.00	0.88	0.89	0.84	0.78		0.84		0.65	0.74	0.85	0.67	0.66	0.65	0.61			0.69		0.66			0.76
SS nol 25oC	1.00	0.87	0.82	0.81	0.89		0.68		0.64	0.72	0.79	0.66	0.74	0.73	0.70			0.70		0.71			0.85
Line of best fit for Volatile solids per assay (g/L) as fractions of initial Volatile solids																							
PS 37oC	0.99	0.93	0.87	0.82	0.77	0.73	0.70	0.67	0.64	0.62	0.60	0.58	0.56	0.55	0.53	0.50	0.48	0.47	0.46	0.45	0.45	0.45	0.44
SS 37oC	0.99	0.95	0.92	0.89	0.86	0.83	0.81	0.79	0.77	0.75	0.74	0.73	0.71	0.70	0.69	0.67	0.65	0.64	0.63	0.62	0.62	0.62	0.62
PS nol 37oC	0.99	0.90	0.84	0.79	0.76	0.73	0.71	0.69	0.68	0.67	0.67	0.66	0.66	0.66	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
SS nol 37oC	0.98	0.94	0.91	0.87	0.84	0.82	0.79	0.77	0.75	0.74	0.72	0.71	0.70	0.69	0.68	0.66	0.64	0.63	0.62	0.61	0.61	0.61	0.61
PS 25oC	1.02	0.94	0.88	0.82	0.78	0.75	0.72	0.70	0.68	0.66	0.65	0.64	0.63	0.63	0.62	0.61	0.60	0.60	0.60	0.60	0.60	0.60	0.60
SS 25oC	1.00	0.96	0.92	0.89	0.87	0.85	0.84	0.83	0.82	0.81	0.80	0.80	0.79	0.79	0.79	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
PS nol 25oC	1.00	0.94	0.89	0.84	0.81	0.78	0.76	0.75	0.73	0.72	0.71	0.71	0.70	0.70	0.69	0.69	0.68	0.68	0.68	0.68	0.68	0.68	0.68
SS nol 25oC	1.00	0.91	0.85	0.81	0.78	0.76	0.74	0.73	0.72	0.72	0.71	0.71	0.71	0.71	0.71	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70

pH values																							
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	24	27	31	34	38	40
Blank 37oC	7.77	7.72	7.67	7.72	7.69	7.69	7.68	7.70	7.68	7.66	7.74	7.71	7.67	7.65	7.63			7.63		7.58			7.50
PS 37oC	7.58	7.08	7.05	6.98	7.04	6.97	6.98	7.03	7.04	7.00	7.23	6.98	7.18	7.13	7.24			7.44		7.47			7.37
SS 37oC	7.81	7.26	7.36	7.26	7.18	7.16	7.15	7.22	7.24	7.32	7.39	7.37	7.31	7.35	7.37			7.37		7.36			7.23
PS nol 37oC	7.83	6.66	6.73	6.70	6.70	6.63	6.65	6.70	6.69	6.67	6.75	6.76	6.77	6.84	6.85			7.09		6.99			6.98
SS nol 37oC	7.78	7.05	7.01	6.95	6.92	6.78	6.76	6.80	6.85	6.90	7.02	7.04	7.01	7.05	7.07			7.04		7.08			7.07
Blank 25oC	7.55	7.79	7.82	7.80	7.81	7.74	7.76	7.80	7.78	7.76	7.83	7.82	7.78	7.77	7.78			7.73		7.70			7.66
PS 25oC	7.57	6.95	6.91	6.89	6.91	6.88	6.72	6.74	6.72	6.77	6.79	7.04	6.89	6.98	7.03			7.15		7.21			7.15
SS 25oC	7.85	7.25	7.25	7.16	7.16	7.04	7.10	7.17	7.17	7.22	7.21	7.24	7.15	7.24	7.21			7.18		7.16			7.04
PS nol 25oC	7.73	6.59	6.58	6.55	6.51	6.50	6.45	6.52	6.49	6.43	6.29	6.21	6.15	6.13	6.13			6.28		6.56			6.60
SS nol 25oC	7.63	6.91	6.86	6.80	6.75	6.65	6.62	6.63	6.63	6.62	6.64	6.68	6.69	6.72	6.78			6.86		6.84			6.79

	Non linear model Equation 2.22 - fh*Po*exp(-kh*t) + Po*(1-fh)													Remaining Fraction		Reduced Fraction		Temperature	
	Data points	kh	kh bounds (95%)		fh	fh bounds (95%)		1 - fh		1 - fh bounds (95%)		RMSE	SSE	R2	Adjusted R2	Actual	Model	Actual	Model	Ln kh	T^(-1)
PS 37oC	13	0.1921	0.0302	0.3539	0.5783	0.3319	0.8246	0.4189	0.4217	0.2916	0.5463	0.1042	0.1086	0.7323	0.6788	0.42	0.42	0.58	0.58	-1.6497	0.0270
SS 37oC	12	0.2365	0.0960	0.3770	0.3299	0.2527	0.4072	0.6641	0.6701	0.6165	0.7117	0.0384	0.0133	0.9138	0.8947	0.70	0.66	0.30	0.34	-1.4418	0.0270
PS nol 37oC	15	0.3924	0.0000	0.7994	0.2662	0.1412	0.3913	0.7389	0.7338	0.6878	0.7900	0.0616	0.0456	0.6632	0.6071	0.73	0.74	0.27	0.26	-0.9355	0.0270
SS nol 37oC	14	0.4087	0.0404	0.7770	0.2165	0.1279	0.3050	0.7860	0.7835	0.7487	0.8232	0.0426	0.0200	0.7405	0.6933	0.76	0.79	0.24	0.21	-0.8948	0.0270
PS 25oC	14	0.2688	0.0726	0.4651	0.4704	0.3248	0.6160	0.5347	0.5296	0.4617	0.6076	0.0739	0.0601	0.8249	0.7931	0.54	0.53	0.46	0.47	-1.3138	0.0400
SS 25oC	15	0.3372	0.0000	0.8643	0.2817	0.0955	0.4679	0.7308	0.7183	0.6516	0.8101	0.0935	0.1050	0.4869	0.4014	0.73	0.73	0.27	0.27	-1.0871	0.0400
PS nol 25oC	13	0.1249	0.0358	0.2139	0.2954	0.2036	0.3872	0.7021	0.7046	0.6177	0.7866	0.0382	0.0146	0.8671	0.8406	0.70	0.70	0.30	0.30	-2.0802	0.0400
SS nol 25oC	12	0.2099	0.0000	0.4772	0.1983	0.1090	0.2877	0.7934	0.8017	0.7225	0.8644	0.0437	0.0172	0.7394	0.6815	0.75	0.79	0.25	0.21	-1.5611	0.0400
PS 37oC	13	0.1278	0.0315	0.2242	0.5541	0.3621	0.7461	0.4407	0.4459	0.2917	0.5898	0.8100	0.0656	0.8103	0.7724	0.45	0.44	0.55	0.56	-2.0573	0.0270
SS 37oC	11	0.1098	0.0062	0.2134	0.3827	0.2370	0.5285	0.6111	0.6173	0.4925	0.7297	0.6570	0.0345	0.8301	0.7877	0.67	0.62	0.33	0.38	-2.2091	0.0270
PS nol 37oC	14	0.2828	0.0807	0.4849	0.3388	0.2232	0.4544	0.6465	0.6612	0.5941	0.6989	0.0537	0.0317	0.7981	0.7613	0.64	0.65	0.36	0.35	-1.2630	0.0270
SS nol 37oC	11	0.1157	0.0569	0.1744	0.3808	0.2904	0.4712	0.6040	0.6192	0.5412	0.6669	0.0364	0.0106	0.9224	0.9030	0.61	0.61	0.39	0.39	-2.1568	0.0270
PS 25oC	13	0.2089	0.1118	0.3059	0.4229	0.3424	0.5034	0.5982	0.5771	0.5510	0.6453	0.0411	0.0169	0.9325	0.9190	0.60	0.60	0.40	0.40	-1.5659	0.0400
SS 25oC	12	0.2194	0.0100	0.4288	0.2233	0.1258	0.3209	0.7773	0.7767	0.7241	0.8306	0.0420	0.0158	0.7490	0.6932	0.77	0.78	0.23	0.22	-1.5169	0.0400
PS nol 25oC	15	0.2257	0.0268	0.4247	0.3247	0.1931	0.4562	0.6789	0.6753	0.6107	0.7470	0.0643	0.0497	0.7126	0.6647	0.76	0.68	0.24	0.32	-1.4885	0.0400
SS nol 25oC	14	0.3320	0.0450	0.6190	0.2941	0.1733	0.4149	0.7030	0.7059	0.6521	0.7540	0.0551	0.0334	0.7315	0.6827	0.85	0.70	0.15	0.30	-1.1026	0.0400

Appendix C – RTD experiment results

Tracer calibration (mg/L)					
Standard	**1st**	**2nd**	**3rd**	**4th**	**5th**
1000.000	1014.400	1017.400	1027.290	1015.267	
750.000	783.700	774.730	765.267		
500.000	511.700	516.270	509.320	559.542	
250.000	261.400	264.230	258.790	263.400	278.817
200.000			233.779		
150.000			194.275		
125.000	139.400	138.850			
0.000	7.900	7.980	8.000	8.300	

60 min HRT - 60 min pumping time - Continuous flow - 1000 mg tracer							Normalized		
i	**δt_i**	**t_i**	**C_i**	**$C_i * \delta t_i$**	**$t_i * C_i * \delta t_i$**	**$(t_i)^2 * C_i * \delta t_i$**	**δt_i**	**t_i**	**C_i**
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	5.000	5.000	0.000	0.000	0.000	0.000	0.083	0.083	0.000
3.000	5.000	10.000	0.000	0.000	0.000	0.000	0.083	0.167	0.000
4.000	5.000	15.000	0.000	0.000	0.000	0.000	0.083	0.250	0.000
5.000	5.000	20.000	0.000	0.000	0.000	0.000	0.083	0.333	0.000
6.000	5.000	25.000	45.611	228.053	5701.336	142533.397	0.083	0.417	0.046
7.000	5.000	30.000	76.145	380.725	11421.756	342652.672	0.083	0.500	0.076
8.000	5.000	35.000	84.542	422.710	14794.847	517819.656	0.083	0.583	0.085
9.000	5.000	40.000	103.244	516.221	20648.855	825954.198	0.083	0.667	0.103
10.000	5.000	45.000	103.435	517.176	23272.901	1047280.534	0.083	0.750	0.103
11.000	5.000	50.000	102.672	513.359	25667.939	1283396.947	0.083	0.833	0.103
12.000	5.000	55.000	84.351	421.756	23196.565	1275811.069	0.083	0.917	0.084
13.000	5.000	60.000	73.282	366.412	21984.733	1319083.969	0.083	1.000	0.073
14.000	5.000	65.000	58.397	291.985	18979.008	1233635.496	0.083	1.083	0.058
15.000	5.000	70.000	41.412	207.061	14494.275	1014599.237	0.083	1.167	0.041
16.000	5.000	75.000	35.115	175.573	13167.939	987595.420	0.083	1.250	0.035
17.000	5.000	80.000	33.397	166.985	13358.779	1068702.290	0.083	1.333	0.033
18.000	5.000	85.000	27.863	139.313	11841.603	1006536.260	0.083	1.417	0.028
19.000	5.000	90.000	22.901	114.504	10305.344	927480.916	0.083	1.500	0.023
20.000	5.000	95.000	0.000	0.000	0.000	0.000	0.083	1.583	0.000
21.000	5.000	100.000	0.000	0.000	0.000	0.000	0.083	1.667	0.000
22.000	5.000	105.000	0.000	0.000	0.000	0.000	0.083	1.750	0.000
23.000	5.000	110.000	0.000	0.000	0.000	0.000	0.083	1.833	0.000
24.000	5.000	115.000	0.000	0.000	0.000	0.000	0.083	1.917	0.000
25.000	5.000	120.000	0.000	0.000	0.000	0.000	0.083	2.000	0.000
			892.366	4461.832	228835.878	12993082.061			0.892

180 min HRT - 45 min pumping time - Intermittent flow - 400 mg tracer							Normalized		
i	δt_i	t_i	C_i	$C_i * \delta t_i$	$t_i * C_i * \delta t_i$	$(t_i)^2 * C_i * \delta t_i$	δt_i	t_i	C_i
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	30.000	30.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000
3.000	30.000	60.000	0.000	0.000	0.000	0.000	0.167	0.333	0.000
4.000	30.000	90.000	44.771	1343.130	120881.679	10879351.145	0.167	0.500	0.112
5.000	30.000	120.000	70.992	2129.771	255572.519	30668702.290	0.167	0.667	0.177
6.000	30.000	150.000	66.050	1981.489	297223.282	44583492.366	0.167	0.833	0.165
7.000	30.000	180.000	57.473	1724.198	310355.725	55864030.534	0.167	1.000	0.144
8.000	30.000	210.000	51.660	1549.809	325459.924	68346583.969	0.167	1.167	0.129
9.000	30.000	240.000	38.674	1160.210	278450.382	66828091.603	0.167	1.333	0.097
10.000	30.000	270.000	31.221	936.641	252893.130	68281145.038	0.167	1.500	0.078
11.000	30.000	300.000	23.989	719.656	215896.947	64769083.969	0.167	1.667	0.060
12.000	30.000	330.000	0.000	0.000	0.000	0.000	0.167	1.833	0.000
13.000	30.000	360.000	0.000	0.000	0.000	0.000	0.167	2.000	0.000
			384.830	11544.905	2056733.588	410220480.916			0.962

180 min HRT - 60 min pumping time - Intermittent flow - 800 mg tracer							Normalized		
i	δt_i	t_i	C_i	$C_i * \delta t_i$	$t_i * C_i * \delta t_i$	$(t_i)^2 * C_i * \delta t_i$	δt_i	t_i	C_i
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	30.000	30.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000
3.000	30.000	60.000	44.916	1347.481	80848.855	4850931.298	0.167	0.333	0.056
4.000	30.000	90.000	95.174	2855.210	256968.893	23127200.382	0.167	0.500	0.119
5.000	30.000	120.000	107.571	3227.118	387254.198	46470503.817	0.167	0.667	0.134
6.000	30.000	150.000	111.693	3350.782	502617.366	75392604.962	0.167	0.833	0.140
7.000	30.000	180.000	107.819	3234.561	582220.992	104799778.626	0.167	1.000	0.135
8.000	30.000	210.000	77.830	2334.905	490329.962	102969291.985	0.167	1.167	0.097
9.000	30.000	240.000	54.574	1637.233	392935.878	94304610.687	0.167	1.333	0.068
10.000	30.000	270.000	49.656	1489.695	402217.557	108598740.458	0.167	1.500	0.062
11.000	30.000	300.000	42.920	1287.595	386278.626	115883587.786	0.167	1.667	0.054
12.000	30.000	330.000	39.389	1181.679	389954.198	128684885.496	0.167	1.833	0.049
13.000	30.000	360.000	0.000	0.000	0.000	0.000	0.167	2.000	0.000
			731.542	21946.260	3871626.527	805082135.496			0.914

180 min HRT - 180 min pumping time - Continuous flow - 1200 mg tracer							Normalized		
i	**δt_i**	**t_i**	**C_i**	**$C_i * \delta t_i$**	**$t_i * C_i * \delta t_i$**	**$(t_i)^2 * C_i * \delta t_i$**	**δt_i**	**t_i**	**C_i**
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	12.000	12.000	0.000	0.000	0.000	0.000	0.067	0.067	0.000
3.000	12.000	24.000	0.000	0.000	0.000	0.000	0.067	0.133	0.000
4.000	12.000	36.000	0.000	0.000	0.000	0.000	0.067	0.200	0.000
5.000	12.000	48.000	0.000	0.000	0.000	0.000	0.067	0.267	0.000
6.000	12.000	60.000	0.000	0.000	0.000	0.000	0.067	0.333	0.000
7.000	12.000	72.000	64.313	771.756	55566.412	4000781.679	0.067	0.400	0.054
8.000	12.000	84.000	72.137	865.649	72714.504	6108018.321	0.067	0.467	0.060
9.000	12.000	96.000	77.481	929.771	89258.015	8568769.466	0.067	0.533	0.065
10.000	12.000	108.000	92.748	1112.977	120201.527	12981764.885	0.067	0.600	0.077
11.000	12.000	120.000	100.954	1211.450	145374.046	17444885.496	0.067	0.667	0.084
12.000	12.000	132.000	97.137	1165.649	153865.649	20310265.649	0.067	0.733	0.081
13.000	12.000	144.000	88.168	1058.015	152354.198	21939004.580	0.067	0.800	0.073
14.000	12.000	156.000	73.473	881.679	137541.985	21456549.618	0.067	0.867	0.061
15.000	12.000	168.000	59.924	719.084	120806.107	20295425.954	0.067	0.933	0.050
16.000	12.000	180.000	52.290	627.481	112946.565	20330381.679	0.067	1.000	0.044
17.000	12.000	192.000	44.466	533.588	102448.855	19670180.153	0.067	1.067	0.037
18.000	12.000	204.000	38.168	458.015	93435.115	19060763.359	0.067	1.133	0.032
19.000	12.000	216.000	35.878	430.534	92995.420	20087010.687	0.067	1.200	0.030
20.000	12.000	228.000	30.344	364.122	83019.847	18928525.191	0.067	1.267	0.025
21.000	12.000	240.000	26.527	318.321	76396.947	18335267.176	0.067	1.333	0.022
22.000	12.000	252.000	25.191	302.290	76177.099	19196629.008	0.067	1.400	0.021
23.000	12.000	264.000	22.901	274.809	72549.618	19153099.237	0.067	1.467	0.019
24.000	12.000	276.000	22.137	265.649	73319.084	20236067.176	0.067	1.533	0.018
25.000	12.000	288.000	0.000	0.000	0.000	0.000	0.067	1.600	0.000
26.000	12.000	300.000	0.000	0.000	0.000	0.000	0.067	1.667	0.000
27.000	12.000	312.000	0.000	0.000	0.000	0.000	0.067	1.733	0.000
28.000	12.000	324.000	0.000	0.000	0.000	0.000	0.067	1.800	0.000
29.000	12.000	336.000	0.000	0.000	0.000	0.000	0.067	1.867	0.000
30.000	12.000	348.000	0.000	0.000	0.000	0.000	0.067	1.933	0.000
31.000	12.000	360.000	0.000	0.000	0.000	0.000	0.067	2.000	0.000
			1024.237	12290.840	1830970.992	308103389.313			0.854

180 min HRT - 90 min pumping time - Intermittent flow - 800 mg tracer							Normalized		
i	δt_i	t_i	C_i	$C_i * \delta t_i$	$t_i * C_i * \delta t_i$	$(t_i)^2 * C_i * \delta t_i$	δt_i	t_i	C_i
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	30.000	30.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000
3.000	30.000	60.000	72.691	2180.725	130843.511	7850610.687	0.167	0.333	0.091
4.000	30.000	90.000	190.668	5720.038	514803.435	46332309.160	0.167	0.500	0.238
5.000	30.000	120.000	171.794	5153.817	618458.015	74214961.832	0.167	0.667	0.215
6.000	30.000	150.000	103.550	3106.489	465973.282	69895992.366	0.167	0.833	0.129
7.000	30.000	180.000	73.073	2192.176	394591.603	71026488.550	0.167	1.000	0.091
8.000	30.000	210.000	51.336	1540.076	323416.031	67917366.412	0.167	1.167	0.064
9.000	30.000	240.000	31.660	949.809	227954.198	54709007.634	0.167	1.333	0.040
10.000	30.000	270.000	22.901	687.023	185496.183	50083969.466	0.167	1.500	0.029
11.000	30.000	300.000	20.420	612.595	183778.626	55133587.786	0.167	1.667	0.026
12.000	30.000	330.000	0.000	0.000	0.000	0.000	0.167	1.833	0.000
13.000	30.000	360.000	0.000	0.000	0.000	0.000	0.167	2.000	0.000
			738.092	22142.748	3045314.885	497164293.893			0.923

180 min HRT - 60 min pumping time - Intermittent flow - 800 mg tracer							Normalized		
i	δt_i	t_i	C_i	$C_i * \delta t_i$	$t_i * C_i * \delta t_i$	$(t_i)^2 * C_i * \delta t_i$	δt_i	t_i	C_i
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	30.000	30.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000
3.000	30.000	60.000	0.000	0.000	0.000	0.000	0.167	0.333	0.000
4.000	30.000	90.000	137.996	4139.885	372589.695	33533072.519	0.167	0.500	0.172
5.000	30.000	120.000	165.078	4952.347	594281.679	71313801.527	0.167	0.667	0.206
6.000	30.000	150.000	136.349	4090.477	613571.565	92035734.733	0.167	0.833	0.170
7.000	30.000	180.000	97.828	2934.847	528272.519	95089053.435	0.167	1.000	0.122
8.000	30.000	210.000	64.357	1930.706	405448.282	85144139.313	0.167	1.167	0.080
9.000	30.000	240.000	53.113	1593.378	382410.687	91778564.885	0.167	1.333	0.066
10.000	30.000	270.000	36.691	1100.725	297195.802	80242866.412	0.167	1.500	0.046
11.000	30.000	300.000	30.584	917.519	275255.725	82576717.557	0.167	1.667	0.038
12.000	30.000	330.000	22.311	669.332	220879.580	72890261.450	0.167	1.833	0.028
13.000	30.000	360.000	0.000	0.000	0.000	0.000	0.167	2.000	0.000
			744.307	22329.218	3689905.534	704604211.832			0.930

240 min HRT - 240 min pumping time - Continuous flow - 1200 mg tracer							Normalized		
i	δt_i	t_i	C_i	$C_i * \delta t_i$	$t_i * C_i * \delta t_i$	$(t_i)^2 * C_i * \delta t_i$	δt_i	t_i	C_i
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	17.000	17.000	0.000	0.000	0.000	0.000	0.071	0.071	0.000
3.000	17.000	34.000	0.000	0.000	0.000	0.000	0.071	0.142	0.000
4.000	17.000	51.000	0.000	0.000	0.000	0.000	0.071	0.213	0.000
5.000	17.000	68.000	0.000	0.000	0.000	0.000	0.071	0.283	0.000
6.000	17.000	85.000	31.489	535.305	45500.954	3867581.107	0.071	0.354	0.026
7.000	17.000	102.000	64.313	1093.321	111518.702	11374907.634	0.071	0.425	0.054
8.000	17.000	119.000	72.137	1226.336	145933.969	17366142.366	0.071	0.496	0.060
9.000	17.000	136.000	77.481	1317.176	179135.878	24362479.389	0.071	0.567	0.065
10.000	17.000	153.000	92.748	1576.718	241237.786	36909381.298	0.071	0.638	0.077
11.000	17.000	170.000	100.954	1716.221	291757.634	49598797.710	0.071	0.708	0.084
12.000	17.000	187.000	97.137	1651.336	308799.809	57745564.313	0.071	0.779	0.081
13.000	17.000	204.000	88.168	1498.855	305766.412	62376348.092	0.071	0.850	0.073
14.000	17.000	221.000	73.473	1249.046	276039.122	61004645.992	0.071	0.921	0.061
15.000	17.000	238.000	59.924	1018.702	242451.145	57703372.519	0.071	0.992	0.050
16.000	17.000	255.000	52.290	888.931	226677.481	57802757.634	0.071	1.063	0.044
17.000	17.000	272.000	44.466	755.916	205609.160	55925691.603	0.071	1.133	0.037
18.000	17.000	289.000	38.168	648.855	187519.084	54193015.267	0.071	1.204	0.032
19.000	17.000	306.000	35.878	609.924	186636.641	57110812.214	0.071	1.275	0.030
20.000	17.000	323.000	30.344	515.840	166616.221	53817039.504	0.071	1.346	0.025
21.000	17.000	340.000	26.527	450.954	153324.427	52130305.344	0.071	1.417	0.022
22.000	17.000	357.000	25.191	428.244	152883.206	54579304.580	0.071	1.488	0.021
23.000	17.000	374.000	22.901	389.313	145603.053	54455541.985	0.071	1.558	0.019
24.000	17.000	391.000	22.137	376.336	147147.328	57534605.344	0.071	1.629	0.018
25.000	17.000	408.000	0.000	0.000	0.000	0.000	0.071	1.700	0.000
26.000	17.000	425.000	0.000	0.000	0.000	0.000	0.071	1.771	0.000
27.000	17.000	442.000	0.000	0.000	0.000	0.000	0.071	1.842	0.000
28.000	17.000	459.000	0.000	0.000	0.000	0.000	0.071	1.913	0.000
29.000	17.000	476.000	0.000	0.000	0.000	0.000	0.071	1.983	0.000
30.000	17.000	493.000	0.000	0.000	0.000	0.000	0.071	2.054	0.000
			1055.725	17947.328	3720158.015	879858293.893			0.880

360 min HRT - 45 min pumping time - Intermittent flow - 200 mg tracer							Normalized		
i	**δt_i**	**t_i**	**C_i**	**$C_i * \delta t_i$**	**$t_i * C_i * \delta t_i$**	**$(t_i)^2 * C_i * \delta t_i$**	**δt_i**	**t_i**	**C_i**
1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.000	60.000	60.000	0.000	0.000	0.000	0.000	0.167	0.167	0.000
3.000	60.000	120.000	0.000	0.000	0.000	0.000	0.167	0.333	0.000
4.000	60.000	180.000	19.660	1179.618	212331.298	38219633.588	0.167	0.500	0.098
5.000	60.000	240.000	27.143	1628.588	390861.069	93806656.489	0.167	0.667	0.136
6.000	60.000	300.000	26.844	1610.611	483183.206	144954961.832	0.167	0.833	0.134
7.000	60.000	360.000	23.803	1428.206	514154.198	185095511.450	0.167	1.000	0.119
8.000	60.000	420.000	23.706	1422.366	597393.893	250905435.115	0.167	1.167	0.119
9.000	60.000	480.000	23.002	1380.115	662454.962	317978381.679	0.167	1.333	0.115
10.000	60.000	540.000	20.380	1222.786	660304.580	356564473.282	0.167	1.500	0.102
11.000	60.000	600.000	0.000	0.000	0.000	0.000	0.167	1.667	0.000
12.000	60.000	660.000	0.000	0.000	0.000	0.000	0.167	1.833	0.000
13.000	60.000	720.000	0.000	0.000	0.000	0.000	0.167	2.000	0.000
			164.538	9872.290	3520683.206	1387525053.435			0.823

Appendix D – ABR bench experiments results

pH							
Day	R1	1st	2nd	3rd	4th	5th	6th
0	48 hrs	6.91	8.45	8.45	8.45	8.45	8.45
1		5.82	6.70	6.90	7.19	7.28	7.33
2		5.69	6.50	6.76	7.06	7.17	7.42
34		6.09	6.18	6.29	6.44	6.51	6.55
35		5.83	6.02	6.08	6.20	6.22	6.25
40		6.07	6.25	6.32	6.49	6.62	6.68
41		6.30	6.33	6.37	6.52	6.49	6.56
44		6.21	6.32	6.53	6.57	6.63	6.68
45		6.23	6.46	6.55	6.58	6.71	6.78
48		6.13	6.44	6.62	6.76	6.90	6.94
49		6.00	6.27	6.45	6.60	6.69	6.77
52		6.09	6.52	6.65	6.76	6.88	6.75
53		6.35	6.70	6.85	6.93	6.98	6.99
56		6.21	6.64	6.77	6.77	6.83	6.80
57		6.20	6.62	6.77	6.86	6.99	6.93
60		6.59	6.83	6.88	6.95	6.95	6.95
61		6.55	6.65	6.69	6.74	6.85	6.84
62		6.63	6.63	6.77	6.96	6.78	6.88
63		6.61	6.80	6.88	6.91	6.93	6.96
64		6.71	6.92	7.05	7.09	7.07	7.06
65		6.64	6.94	7.05	7.09	7.10	7.15
66		6.97	7.01	6.97	7.00	6.96	6.96
67	48hrs	6.68	6.93	7.05	7.09	7.09	7.11
68	36hrs						
81		6.70	6.86	6.89	6.94	6.92	6.87
82		6.64	6.75	6.84	6.89	6.84	6.86
83		6.72	6.74	6.86	6.89	6.88	6.85
84		6.59	6.66	6.79	6.85	6.88	6.86
85		6.83	6.84	6.95	6.92	6.98	7.04
86		6.63	6.78	6.88	6.93	6.90	6.89
87		6.63	6.87	6.97	7.03	7.05	7.03
88		6.96	7.01	7.06	7.11	7.15	6.99
89		6.91	6.99	7.13	7.17	7.14	7.04
90	36hrs	6.80	6.90	6.97	7.02	7.03	6.94
91	24 hrs						
98		6.23	6.27	6.26	6.41	6.58	6.74
99		6.32	6.32	6.41	6.46	6.53	6.78
100		6.27	6.43	6.54	6.59	6.75	6.84
101		6.35	6.56	6.60	6.65	6.74	6.96
102		6.42	6.59	6.69	6.74	6.79	6.90
103		6.60	6.74	6.84	6.89	6.90	6.85
104		6.55	6.70	6.80	6.89	6.92	6.91
105	24hrs	6.58	6.72	6.82	6.89	6.91	6.88
106	12 hrs						
113		6.45	6.46	6.51	6.58	6.64	6.72
114		6.41	6.50	6.56	6.65	6.74	6.76
115		6.35	6.52	6.61	6.66	6.72	6.77
116		6.40	6.42	6.50	6.59	6.67	6.73
117		6.41	6.45	6.54	6.66	6.74	6.78
126	12hrs	6.42	6.51	6.59	6.67	6.75	6.82
127	6 hrs						
134		6.39	6.46	6.53	6.56	6.55	6.56
135		6.13	6.23	6.26	6.31	6.36	6.37
136		6.30	6.33	6.37	6.52	6.49	6.56
137		6.07	6.09	6.17	6.28	6.34	6.40
138		6.19	6.27	6.32	6.41	6.43	6.49
139	6hrs	6.22	6.28	6.33	6.42	6.43	6.48

Day	R2	1st	2nd	3rd	4th	5th	6th
0	48 hrs	6.91	8.45	8.45	8.45	8.45	8.45
1		5.89	6.82	7.01	7.10	7.34	7.60
2		5.88	6.10	6.35	6.52	6.82	7.01
34		5.09	5.19	5.62	5.74	5.90	6.15
35		4.94	5.38	5.72	5.94	6.15	6.26
44		4.63	5.52	5.85	5.82	6.12	6.28
45		4.74	5.02	5.24	5.45	5.70	6.13
52		5.19	6.19	6.27	6.30	6.40	6.36
53		5.32	5.98	6.05	6.09	6.13	6.25
56		5.72	5.98	6.05	6.09	6.13	6.25
57		5.97	6.02	6.04	6.06	6.07	6.09
60		5.90	5.94	6.00	6.05	6.07	6.17
61		6.03	6.11	6.02	6.07	6.06	6.07
65		5.64	5.75	5.78	5.82	5.85	5.90
66		5.71	5.79	5.80	5.86	5.88	5.89
67		5.93	6.00	6.05	6.05	6.05	6.04
74		5.92	6.07	6.15	6.19	6.24	6.27
75		6.03	6.19	6.27	6.30	6.40	6.36
83		6.04	6.18	6.27	6.33	6.37	6.41
84		6.25	6.31	6.37	6.45	6.51	6.67
87		6.13	6.26	6.36	6.40	6.46	6.65
88		6.03	6.17	6.25	6.27	6.34	6.46
89		6.03	6.18	6.23	6.31	6.37	6.51
92		6.18	6.32	6.34	6.37	6.35	6.56
93		6.09	6.20	6.35	6.42	6.44	6.61
94		5.97	6.14	6.24	6.29	6.37	6.48
100		6.07	6.19	6.29	6.35	6.40	6.49
101		5.97	6.15	6.26	6.31	6.39	6.53
102		6.04	6.20	6.33	6.39	6.46	6.61
103		6.07	6.26	6.41	6.44	6.53	6.70
104		6.04	6.23	6.30	6.36	6.47	6.57
105	48hrs	6.06	6.25	6.36	6.40	6.50	6.64
106	36hrs						
117		5.97	6.10	6.20	6.29	6.39	6.53
118		6.03	6.14	6.23	6.28	6.36	6.51
119		6.02	6.11	6.20	6.27	6.34	6.51
121		5.99	6.10	6.20	6.25	6.36	6.54
122		5.94	6.05	6.15	6.21	6.31	6.45
123		5.97	6.13	6.21	6.31	6.40	6.55
124	36hrs	6.01	6.18	6.32	6.43	6.55	6.71
125	24 hrs						
132		6.01	6.16	6.27	6.37	6.48	6.60
133		6.08	6.22	6.31	6.44	6.51	6.65
136		6.23	6.56	6.52	6.69	6.69	6.96
137		5.49	5.75	6.03	6.28	6.42	6.55
138		5.97	6.11	6.25	6.37	6.47	6.61
139	24hrs	5.97	6.11	6.25	6.37	6.47	6.61

R3	1st	2nd	3rd	4th	5th	6th
48.00	6.06	5.90	6.13	6.33	6.40	6.46
48.00	6.40	6.23	6.33	6.44	6.53	6.63
36.00	5.97	6.32	6.55	6.65	6.69	6.74
36.00	6.01	6.30	6.54	6.71	6.80	6.91
24.00	6.30	6.50	6.73	6.77	6.96	6.99
24.00	5.87	6.25	6.44	6.54	6.70	6.82
12.00	6.08	6.34	6.39	6.45	6.50	6.56
6.00	4.82	5.16	5.39	5.50	5.54	5.57

VFA							
Day	R1	1st	2nd	3rd	4th	5th	6th
0	48 hrs	64.00	157.00	157.00	157.00	157.00	157.00
34	48 hrs	460.00					57.00
35	48 hrs	583.00					141.00
40	48 hrs	596.00					173.00
41	48 hrs	539.00					70.00
52	48 hrs	467.00					65.00
53	48 hrs	435.00	192.00	96.00	74.00	37.00	15.00
66	48 hrs	455.00	261.00	118.00	58.00	47.00	9.00
67	48 hrs	300.00	142.00	52.00	24.00	23.00	34.00
89	36hrs	298.00	182.00	110.00	59.00	44.00	41.00
90	36hrs	307.00	165.00	78.00	37.00	49.00	50.00
104	24 hrs	295.00	219.00	137.00	87.00	62.00	80.00
105	24 hrs	274.00	170.00	103.00	118.00	107.00	59.00
125	12 hrs	461.00	315.00	270.00	234.00	163.00	137.00
126	12 hrs	490.00	427.00	354.00	246.00	187.00	152.00
138	6 hrs	528.00	498.00	489.00	474.00	481.00	472.00
139	6 hrs	329.00	364.00	371.00	318.00	373.00	329.00

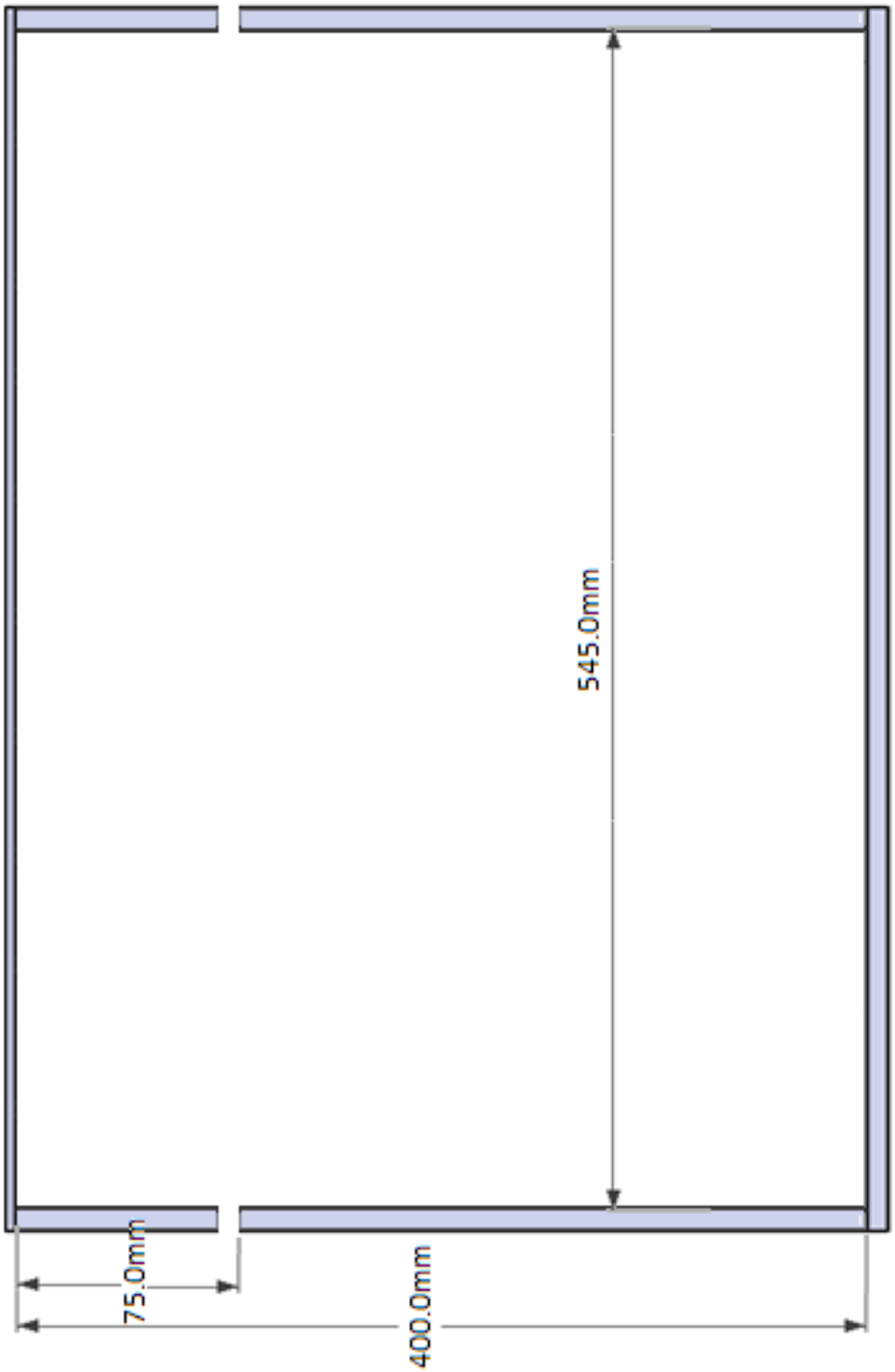
Day	R2	1st	2nd	3rd	4th	5th	6th
0	48hrs	64.00	157.00	157.00	157.00	157.00	157.00
34	48hrs	387.00					457.00
35	48hrs	421.00					488.00
44	48hrs	565.00					497.00
45	48hrs	485.00					452.00
52	48hrs	454.00					459.00
53	48hrs						425.00
66	48hrs	474.00					406.00
67	48hrs	481.00	453.00	373.00	334.00	275.00	230.00
89	48hrs	589.00	480.00	422.00	380.00	312.00	224.00
104	48hrs	540.00	464.00	381.00	317.00	253.00	180.00
105	48hrs	558.00	494.00	423.00	383.00	282.00	209.00
123	36hrs	560.00	544.00	503.00	457.00	4020.00	326.00
124	36hrs	604.00	593.00	461.00	364.00	271.00	185.00
138	24 hrs	550.00	504.00	424.00	336.00	241.00	179.00
139	24 hrs	546.00	430.00	419.00	272.00	235.00	139.00

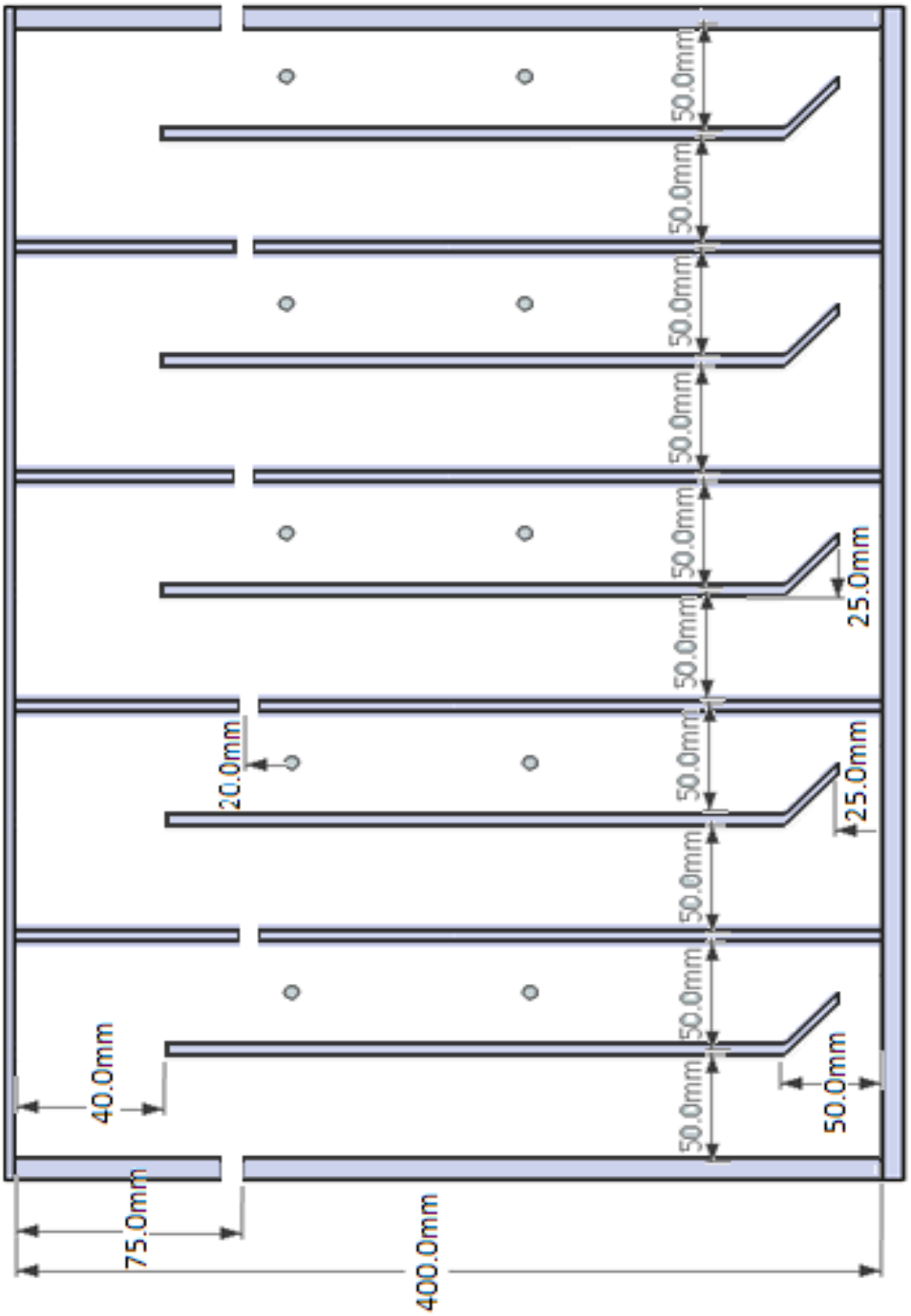
R3	1st	2nd	3rd	4th	5th	6th
48.00	461.00	444.00	310.00	197.00	139.00	93.00
48.00	333.00	314.00	221.00	106.00	63.00	28.00
36.00	480.00	340.00	184.00	110.00	69.00	42.00
36.00	458.00	349.00	241.00	157.00	77.00	26.00
24.00	511.00	418.00	299.00	238.00	190.00	121.00
24.00	477.00	368.00	277.00	189.00	135.00	71.00
12.00	241.00	245.00	207.00	220.00	211.00	132.00
12.00	433.80	441.00	372.60	396.00	379.80	237.60
6.00	449.00	420.00	404.00	389.00	385.00	402.00

COD							
Day	R1	1st	2nd	3rd	4th	5th	6th
67	48 hrs	1109.00	725.00	462.00	351.00	313.00	287.00
90	36hrs	914.00	640.00				248.00
105	24 hrs						396.00
125	12 hrs						534.00
126	12 hrs						570.00
138	6 hrs						1240.00
139	6 hrs	1452.00	1292.00	1372.00	1080.00	1128.00	1008.00
Day	R2	1st	2nd	3rd	4th	5th	6th
67	48hrs	1826.00	1557.00	1448.00	1296.00	1170.00	1104.00
89	48hrs	1591.00	1331.00	1219.00			740.00
105	48hrs						668.00
123	36hrs						1077.00
124	36hrs						735.00
138	24 hrs						409.00
139	24 hrs	1888.00	1424.00	1508.00	988.00	864.00	620.00
	R3	1st	2nd	3rd	4th	5th	6th
	48.00	1868.00	1716.00	1404.00	912.00	690.00	534.00
	48.00	1437.00	1422.00	1007.00	654.00	481.00	368.00
	36.00	1736.00	1332.00	847.00	595.00	438.00	356.00
	36.00	1832.00	1563.00	1206.00	880.00	637.00	446.00
	24.00	1966.00	1516.00	1191.00	1020.00	837.00	648.00
	24.00	1887.00	1435.00	1122.00	864.00	682.00	485.00
	12.00	1026.00	864.00	795.00	736.00	775.00	660.00
	12.00	1846.80	1555.20	1431.00	1324.80	1395.00	1188.00
	6.00	2180.00	1936.00	1700.00	1670.00	1640.00	1580.00

Methane							
Day	R1	1st	2nd	3rd	4th	5th	6th
60	48hrs	60.0	64.0	53.0	12.0	35.0	3.2
61	48hrs	70.0	74.0	70.0	30.0	43.0	24.6
62	48hrs	63.3	58.2	71.4	29.1	40.0	41.0
63	48hrs	60.0	48.3	67.1	23.8	49.6	13.3
64	48hrs	76.5	42.3	47.2	20.7	7.4	27.4
65	48hrs	36.8	4.0	18.1	11.6	12.3	5.7
66	48hrs	27.0	4.5	29.9	35.8	0.0	18.0
67	48hrs	13.2	17.4	32.4	9.9	0.0	9.1
Day	R2	1st	2nd	3rd	4th	5th	6th
60	48hrs	0.0	10.6	13.2	25.4	26.0	4.0
61	48hrs	0.0	15.0	16.0	42.0	16.8	2.4
62	48hrs	2.4	25.5	27.5	30.8	26.9	7.1
63	48hrs	0.0	31.2	29.6	37.7	20.3	3.0
64	48hrs	7.2	46.4	46.1	42.1	21.0	27.0
65	48hrs	3.0	3.2	33.0	36.3	28.2	7.6
66	48hrs	5.8	33.9	43.7	23.5	15.3	7.2
67	48hrs	14.6	50.0	51.6	54.9	42.6	30.8

Appendix E – ABR bench model design details

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