THE DEVELOPMENT OF WASTEWATER ANAEROBIC DIGESTION FOR GREATER ENERGY, WATER AND NUTRIENT RECOVERY

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Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

Enhancing resource efficiency through the maximising benefit extracted from each natural resource used while minimising associated environmental burden is key for progress towards sustainable development. Anaerobic digestion (AD) is a bioprocess commonly cited to deliver towards such sustainable development owing to both its use in the upgrading of wastewater quality and the development of renewable energy. While there is potential for the integrated delivery of these targets, traditionally, AD processes have been targeted at either water treatment or renewable energy generation. This report addresses the potential for AD to deliver fit-for-purpose water while simultaneously valorising the waste components within the water stream to either energy or other products of value.

To achieve this, the project aimed to re-evaluate AD design concerning volumetric biogas productivity and effluent quality and consistency, aiming to maximise space time utilisation of the AD. Further, it set out to explore the potential of short chain volatile fatty acids (VFAs) as a product, concomitantly with, or instead of, methane owing to their potential as a carbon feedstock for the production of products of value. As resource efficiency is a key consideration in waste valorisation, the potential for the application of nitrogen and phosphorous compounds from AD effluent was considered, such as facilitating their recycling to agriculture or commodity bioprocesses. Similarly, water is an essential product from wastewater treatment and valorisation; hence the potential use of water purification techniques to harvest potable or irrigation water from the AD effluent was examined. This required assessing the persistence of pathogen contaminants through the modified AD process, addressing removal options and ensuring the availability of rapid, cheap pathogen monitoring methods. The equivalent work on micro-contaminants is carried out in an allied project. Through simple modelling of the process at its early design stage, it is desirable to model the AD process and analyse its sensitivity to feed composition and fluctuation, allowing the proposal of system operation for robustness. Using this approach, the AD-based flow sheets were then analysed for valorisation of wastewater at various scales through technoeconomic studies.

Prior research, especially WRC research on this topic, is reviewed in section 2. An overview of appropriate wastewaters is also provided, indicating the potential of AD and where this potential may be realised. To investigate optimising methane productivity through increased organic loading rate and the potential of simultaneous production of methane and VFAs, experimental Upflow anaerobic sludge blanket (UASB) methanogenic reactors were established in which bed stratification was minimised through a rapid and steady upflow velocity, facilitated through varying the internal recycle rate. It was demonstrated that increased organic loading rate led to enhanced methane productivity, up to a loading rate approaching 30 g/L/day, at which point acidification through VFA production set in, with an associated decrease in volumetric organic degradation rate. The inability to achieve stable operation of the ADs following the onset of VFA production indicated that simultaneous production of VFA and methane is not desirable, reducing space time utilisation. A decision prior to processing is required on whether to optimise a particular unit operation for methane production or VFA production, depending on desired product spectrum or nature of feedstock. Further, owing to the challenges of efficient VFA recovery, VFA production should only be considered from feedstocks sufficiently concentrated to yield high VFA concentrations.

To simultaneously maximise energy or bioproducts recovery and fit-for-purpose water recovery, pre- and post-treatment of feedstocks and digestate, gas and sludge are necessary. The potential for water recovery and recovery of potential products is considered in Chapter 4, where unit operations selected are a function of the wastewater feed and desired product spectrum. Both solid-liquid separation

through sedimentation or filtration is proposed as is a unit operation for separation of solutes from water. In the latter, micro-, ultra-, nano- filtration and reverse osmosis, precipitation (for struvite) and adsorption processes are proposed. While the nutrients N and P are readily concentrated into a single stream for re-purposing, VFAs more typically partition between the concentrate and filtrate streams in nanofiltration and reverse osmosis, requiring a subsequent adsorption step. The challenge of VFA upgrading is thus recognised, and a framework for unit operation selection is presented. In Chapter 5, further focus on 'fit-for-purpose' water is provided through the investigation of appropriate approaches to pathogen monitoring and control. In pathogen monitoring, a wide range of approaches are reviewed and the importance of selecting rapid and precise monitoring methods is highlighted. In pathogen control, the potential for the AD reactor to contribute to pathogen reduction is noted. A review of typical pathogen loads within feedstocks entering the AD reactor highlights the cases in which additional steps for pathogen removal will be required.

A simplified model for AD product prediction is provided based on the same base models as ADM1 and ADM3-P. This framework was selected to overcome the complexity of ADM1 and the restricted calibration of ADM3-P for municipal wastewater only. Such a simplified framework is essential for use with flow sheet analysis for either technoeconomic or environmental assessment. The model demonstrated the prediction of process performance to within 10% for a variety of feedstocks.

Using flow sheet analysis, a range of options were explored for the valorisation of the recalcitrant wastewater vinasse from the fermentation of molasses. This case study demonstrated the value of a decision-making approach within a flow sheeting context to optimise value recovery and burden minimisation with the desirable unit operations depending on the feedstock treated. Such analysis is also critical to identify the steps requiring improvement to either reduce cost or enhance resource efficiency.

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ACRONYMS & ABBREVIATIONS

AD	Anaerobic digestion
ADM1	Anaerobic digestion model no 1
UCT-3P	Three-phase AD model
BAM	Bacterial Analytical Manual
cfu	colony-forming unit
CHP	Combined Heat and Power
CMC	Carboxymethyl Cellulose
CMS	Concentrated Molasses Solids
COD	Chemical Oxygen Demand (kg/m$^{3)}$
CSTR	Continuous Stirred Tank Reactor
DNA	Deoxyribonucleic acid
ECD	Endocrine-Disrupting
EPS	Exogenous Polymeric Substances
ESI-MS	Electrospray Ionisation Mass Spectrometry
FCI	Fixed Capital Investment
HPWS	High Pressure Water Scrubbing
HRT	Hydraulic Retention Time (days)
HS	High Solids
IEX	Ion Exchange
IRR	Internal Rate of Return
LB EPS	Loosely-Bound Exogenous Polymeric Substances
LCFA	Long Chain Fatty Acids
LS	Low Solids
MCFA	Medium Chain Fatty Acids
MEE	Multi-Effect Evaporation
MNV	Murine Norovirus
MPN	Most Probable Number
MS	Medium Solids
MSW	Municipal Solid Waste
NADH	Cofactor Nicotinamide adenine dinucleotide – reduced form
NAD	Cofactor Nicotinamide adenine dinucleotide – oxidised form
NGS	Next-Generation Sequencing
NPV	Net Present Value
OLR	Organic Loading Rate (kgcod/m^3.day)
OOM	Order of Magnitude
PBP	Payback Period
PCE	Purchase Cost of Equipment
PCR	Polymerase Chain Reaction
pfu	plaque-forming unit (viruses)
PN EPS	Proteinaceous Exogenous Polymeric Substances
PPC	Physical Plant Cost
PS	Primary Sludge
qPCR	Quantitative Polymerase Chain Reaction

RA	Reactor A
RB	Reactor B
REFIT	Renewable Energy Feed In Tariff
RNA	Ribonucleic acid
RO	Reverse Osmosis
ROI	Return on Investment
SFP	Sludge Flotation Potential
SRT	Solids Retention Time (days)
STHS	Single Stage High Solid
STLS	Single Stage Low Solid
STMS	Single Stage Medium Solid
STP	Standard Temperature and Pressure
TCI	Total Capital Investment
TDS	Total Dissolved Solids
TIGER	Triangulation Identification for the Genetic Evaluation of Risks
TN	Total Nitrogen
TP	Total Phosphorus
UASB	Upflow Anaerobic Sludge Blanket
UCT	University of Cape Town
UCTADM1	The UCT Water Research Group's extension of ADM1
VCCR	Volumetric COD Conversion Rate
VFA	Volatile Fatty Acid
VS	Volatile Solids
WAS	Waste Activated Sludge
WRG	UCT Water Research Group

GLOSSARY

Alkalinity	The resistance of a solution's pH to addition of base or acid
Anaerobic	In the absence of oxygen
Bioproduct	Product that has been produced through a bioprocess
Chemical Oxygen Demand (COD)	The mass of oxygen required to completely oxidise all the compounds present. This quantity is usually used as the level of contamination by carbon compounds in wastewaters
Digestate	The effluent stream both liquid and solid exiting the AD system
Feedstock	The stream fed to the AD process, usually a waste stream
Genomic DNA/Genome	The total DNA originating from a cell
Hydraulic Retention Time (HRT)	The average period of time that the feedstock spends in the reactor
Metagenomic DNA/Metagenome	The total DNA extracted from the mixed community of cells present within an environment
Metagenomics	The study of the genetic potential contained within the metagenome by sequence analysis
Organic Loading Rate (OLR)	The rate at which organic carbon (measured as COD or volatile solids) is fed to the reactor per reactor volume
Rate-limiting	The step in a process consisting of a series of sequential steps that limits the rate at which the overall process proceeds
Solids Retention Time (SRT)	The average period of time that the active cells, present in the solid phase within the reactor, spend in the reactor
Substrate	The medium which the microorganisms present in the AD reactor use to grow
Volatile Fatty Acids (VFA)	Carboxylic acid molecules with five or less carbon atoms
Volatile Solids	The portion of total solids that ignites at 550 °C
Washout	Dilution of the microorganisms within the reactor at a rate faster than they can grow
Primary sludge	The solids recovered during primary treatment (settling) of raw domestic wastewater
Waste activated sludge	The solids generated during secondary aerobic treatment (conventionally the activated sludge process) of settled sewage
Whole genome sequencing	Sequencing of the complete metagenome extracted from a particular sample
Glucose	A monosaccharide with the molecular formula $C_6H_{12}O_6$
Speciation	The chemical form in which a compound is present in a system

1 INTRODUCTION

1.1 Introduction

AD is a bioprocess that has received increasing attention over the years due to its ability to treat wastewater and solid waste with concomitant energy production (Lindmark et al., 2014). Unlike its aerobic wastewater treatment alternatives, in AD systems the converted organic carbon reports to CH_4 and CO_2, ensuring a biogas product for energy recovery rather than liberating all converted carbon as CO_2 with no further value proposition. Further, it does not require the substantial energy input of the typical activated sludge process, which contributes substantially to, for example, some 4% of the USA energy supply being used for wastewater treatment (Wang et al., 2015a). A typical AD reactor produces biogas with a 60:40 ratio of methane (CH_4) and carbon dioxide (CO_2). Depending on the substrate and inoculum used, hydrogen sulphide (H_2S) can also be produced during the AD process (Kythreotou et al., 2014). AD can also produce VFAs if the CH_4-producing step of the process is inhibited or rate-limiting (Kleerebezem et al., 2015).

Studies have traditionally considered the use of AD for wastewater treatment. In recent years the combined water treatment and energy generation have been of interest. Further, AD is recognised as a potential source of VFAs for feedstock to other processes. The interaction of these competing purposes has not been fully considered, nor has the relative importance of product yield over productivity. However, optimising any of these in isolation may have its drawbacks. The treatment processes associated with high CH_4 yields require long HRTs to be effective, thus requiring large reactors. Conversely, high loading rates and associated methane productivities may result in residual VFAs. The targeted VFA production process produces a large stream of dilute VFAs and negligible or low energy output.

The high-CH_4 yield process is slow but has the advantage of improved water quality in terms of residual carbon and is beneficial as a water treatment technology. Conversely, where energy is the major aim of the AD process, it is desirable to increase CH_4 productivity. This may result in the accumulation of VFAs if not sufficiently controlled. Since methanogenesis is inhibited by low pH, accumulation of VFAs is considered a sign of process instability (Pind et al., 2003) and may lead to failure of methanogenesis. However, this could also be seen as potentially advantageous, since it may be possible to design a process with a high CH_4 productivity and simultaneous production of VFAs. While the CH_4 yield will be reduced, a portion of the COD is being converted to valuable VFAs instead. Alternatively, the process may be designed as an acidic fermentation to yield VFAs in the absence of methane. Because this viewpoint has not been a focus of studies on AD, the trade-offs between this high-CH_4 productivity process and high-CH_4 yield processes have not yet been characterised. Further, the potential benefit of combined production of VFAs and biogas for niche applications has not been compared with processes designed to yield single products. In addition to this, the point at which complete inhibition of methanogenesis results has not been well-documented for processes considering simple feedstocks with adequate cell retention.

This project is focused on the development of an intensified AD process in which water treatment is integrated through the nexus of energy-water-nutrient, producing biogas, purified water and high value bioproducts from waste resources while recycling nutrients. There is potential for value to be created by intensifying the volumetric biogas productivity at the expense of COD reduction, together with further downstream processes for recovery of valuable bioproducts and leading to clean water production. The big picture of this project is to investigate the potential to treat rural, industrial and municipal wastewaters by AD to enable some value creation from these wastewater streams and to integrate the process more successfully into actualising the energy-water-nutrient nexus. The project has potential to contribute towards the empowerment of communities, through managing waste and wastwater to

produce not only either clean water or energy, as is the case currently, but to produce fit-for-purpose water, nutrient recycle and, potentially, valuable bioproducts in addition to bioenergy.

1.2 Project aims

The following key aims will be addressed through this project:

- To re-evaluate AD design with respect to volumetric biogas productivity and effluent consistency, aiming to maximise space time utilisation of the AD.
- To investigate the application of effluent VFAs as carbon source for production of products of value.
- To explore the concentration of nitrogen and phosphorous compounds from AD effluent, facilitating their recycle to agriculture or commodity bioprocesses.
- To examine the potential use of water purification techniques to harvest potable or irrigation water from the AD effluent.
- To determine whether there is persistence of pathogens through the modified AD process and address removal options if required. Micro-contaminants are being assessed in allied projects.
- To model the AD process and analyse the sensitivity to feed composition and fluctuation, allowing proposal of system operation for robustness.
- To analyse the AD-based flow sheets for valorisation of wastewater at various scales through technoeconomic studies.

2 AD: A REVIEW

Water scarcity is one of the greatest challenges facing South Africa. Globally, water crises were positioned as the top long-term (10 year) risk by the World Economic Forum in Davos, Switzerland in January 2016 (WEF, 2016) and again in January 2017 (WEF, 2017). South Africa's National Water Resources Strategy (DWA, 2013) documents the need to elevate the importance of water as a scarce primary resource and to identify new water resources. For this reason, technologies that reduce water consumption and increase wastewater recovery to a 'fit-for-purpose' state or to potable water are of major importance.

AD has been widely deployed throughout the world, including increasingly in South Africa, to recycle wastewater, generate energy or both; as shown by Van Der Merwe-Botha et al. (2016), for example. AD is a multi-step bioprocess in which complex organic compounds are converted to methane and carbon dioxide (Kondusamy and Kalamdhad, 2014; Moraes et al., 2014). The process relies on a consortium of anaerobic bacteria and archaea to catalyse these conversions, producing biogas and water with reduced organic loading (Batstone and Virdis, 2014). Initially, the major focus of AD was in water treatment, to remove organic carbon, with a focus on water quality. More recently, the focus of AD moved to the conversion of the organic carbon present in organic wastes to a maximised yield of methane with less regard for the water stream. In this study, we focus on the combined products of both energy and compliant or fit-for-purpose water from application of AD to wastewater stream. We investigate the trade-offs between the rate and extent of conversion to methane, with the potential for production of usable carbon compounds, the recovery of nitrogen and phosphorus, and generation of fully compliant or fit-for-purpose water. The potential for this compliant or fit-for-purpose water to contribute to "new taps", or alternative water sources to surface and ground water, is key.

To address these considerations, a thorough understanding of the metabolic pathways engaged in anaerobic digestion, their response to process conditions and the overall AD process configuration is required. In this chapter, the substantive literature on AD, with particular reference to wastewater streams as feed, is reviewed.

2.1 Assessing literature trends as an indicator of the research profile of AD

2.1.1 Global publication on AD for biogas

AD has become recognised for renewable energy generation and as an environmentally responsible wastewater treatment system. A Scopus search (2018-03-29) using the key words "AD" and "biogas" identified over 7 500 research documents published since 1973. China and United States published by far the highest number of papers per country; however, Europe as a region published more than China and the US combined (Figure 2-1). Developing countries contribute mainly through India (5th) and Brazil (12th), with some participation from South East Asia. The global trend has been a steady annual increase in the number of publications since 2002, with a distinct escalation in the rate of increase from 2009 (Figure 2-2).

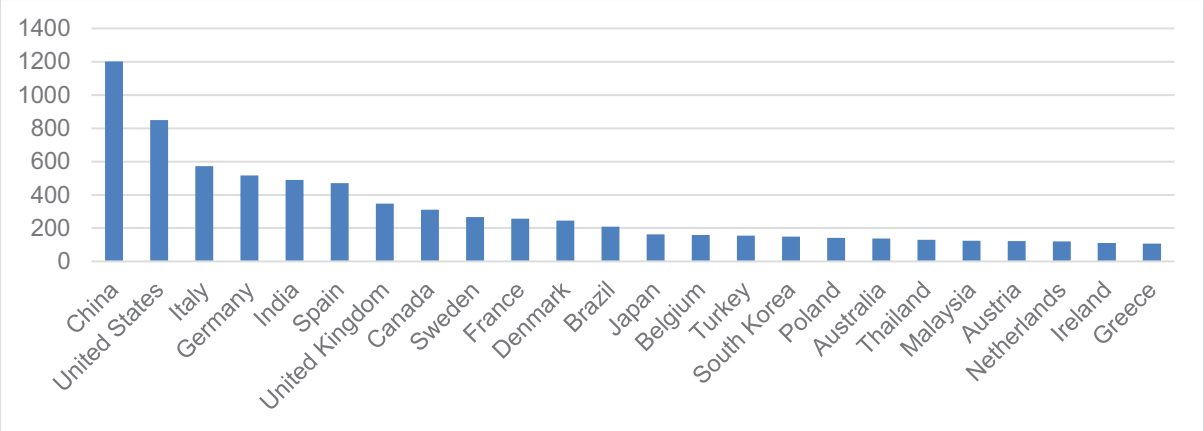


Figure 2-1 Top 25 countries according to number of publications in AD for biogas. Scopus search. (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 2018 March 29

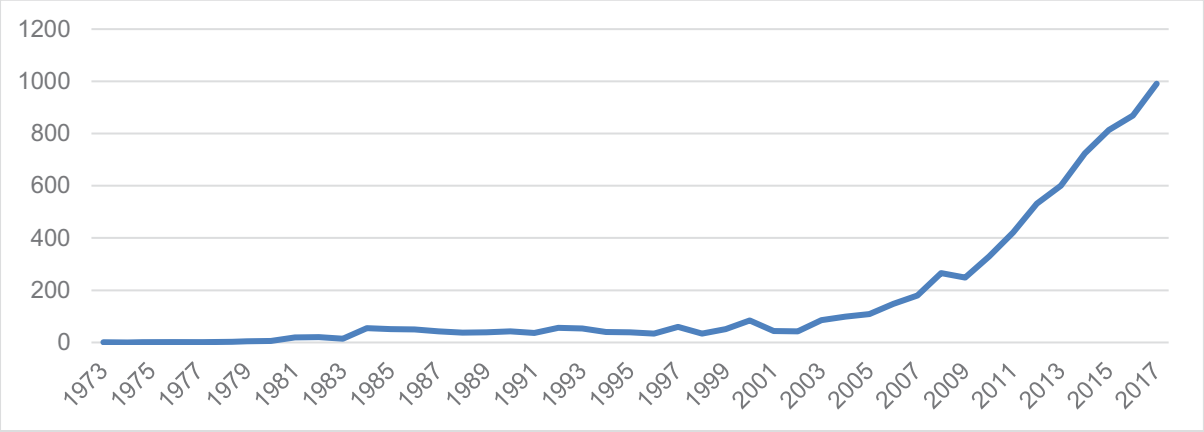


Figure 2-2 Publications in AD for biogas over the years 1973 to 2017. Scopus search. (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 2018 March 29

2.1.1.1 The South African contribution to AD for biogas publication

Globally, Nigeria and South Africa rank 30th and 31st respectively in AD for biogas publication with 68 and 67 publications respectively by the end of 2017 (Figure 2-3). The number of publications from Africa began to increase steadily from 2003 (Figure 2-4), following the global trend in increasing more sharply over the last five years. The total number of internationally available publications from Africa is classically lower than most other continents; however, pressure from growing economies leading to increases in waste streams, as well as an increase in overall environmental awareness, has led to a rise in the investigation of alternative waste management systems in Africa, including AD as a waste remediation technology.

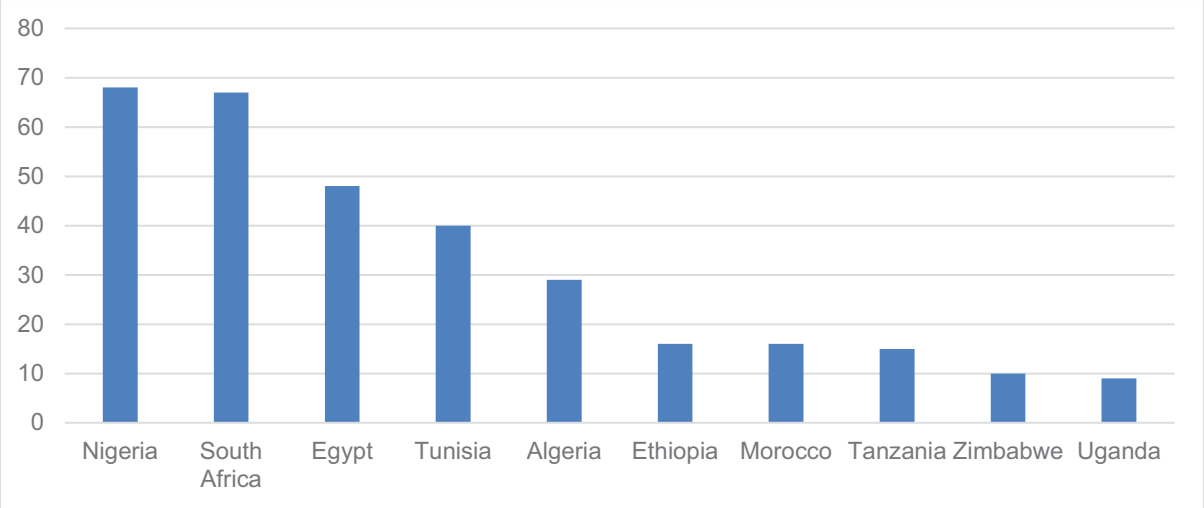


Figure 2-3 Top 10 African countries according to number of publications in AD for biogas over the years 1983 to 2017. Scopus search. (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 2018 March 29

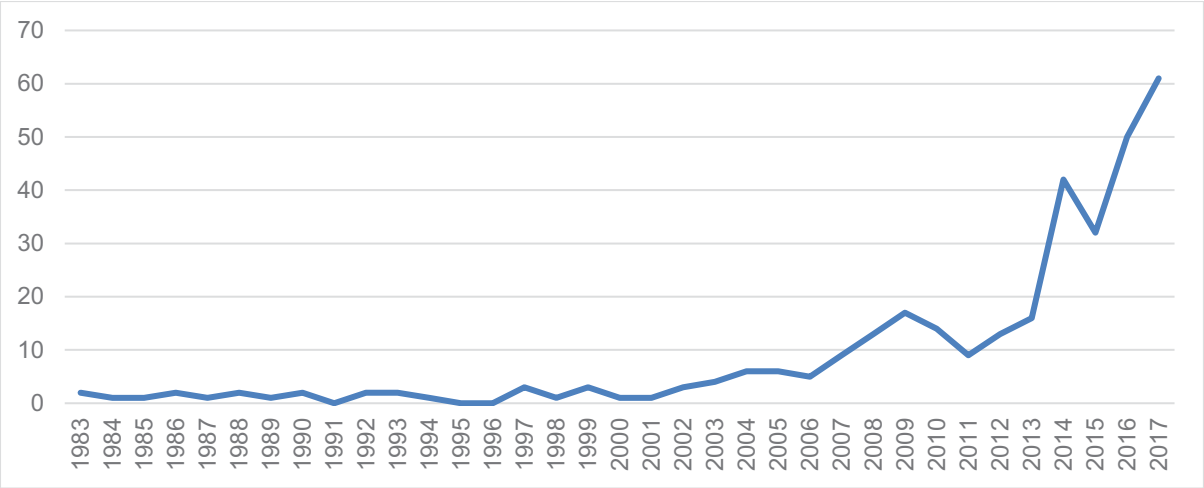


Figure 2-4 Publications in AD for biogas from Africa over the years 1983 to 2017. Scopus search. (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. African countries selected. Accessed 2018 March 29

The number of AD related publications from South Africa, although small, has been increasing, particularly over the past five years (Figure 2-5), reflecting the global and African trend. Articles on AD for biogas published with at least one South African affiliation totalled twelve up to 2007 and 51 for the 10 years 2008 to 2017 (Scopus search 2018-03-29). During the five years including 2017 eighteen South African academic research institutions were involved in at least one publication in this area; with the University of Cape Town affiliated to seven, the University of Fort Hare to five and four other institutions to four each. Of these eighteen institutions, only four were listed in AD for biogas publications in the five years up to 2012; namely, the CSIR, University of Pretoria and University of Cape Town with two publications each, and University of Kwa Zulu Natal with six publications.

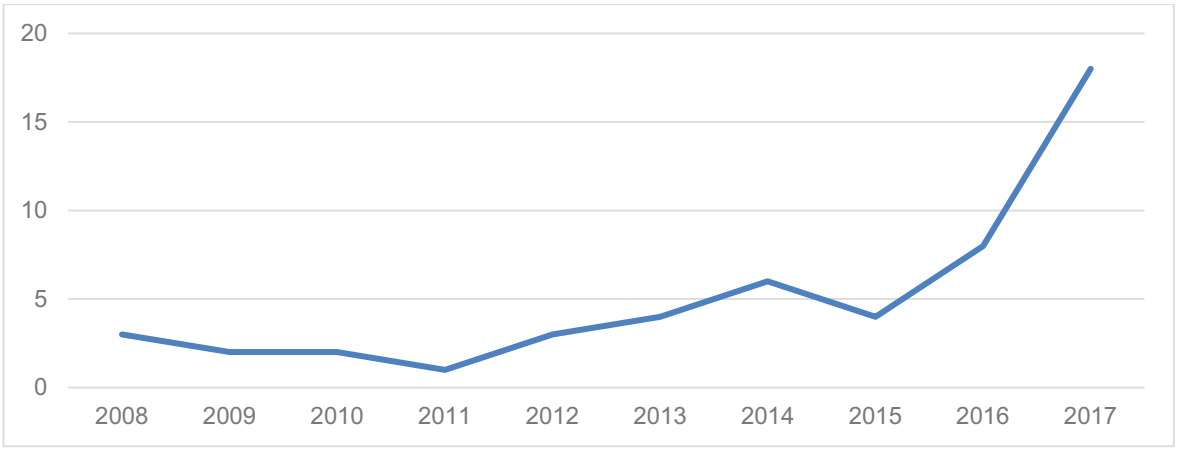


Figure 2-5 South African publications (51) in AD for biogas over the 10 years from 2008 to 2017. Scopus search Total publications listed 1983-2007: 12 (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. South Africa selected. Accessed 2018 March 29

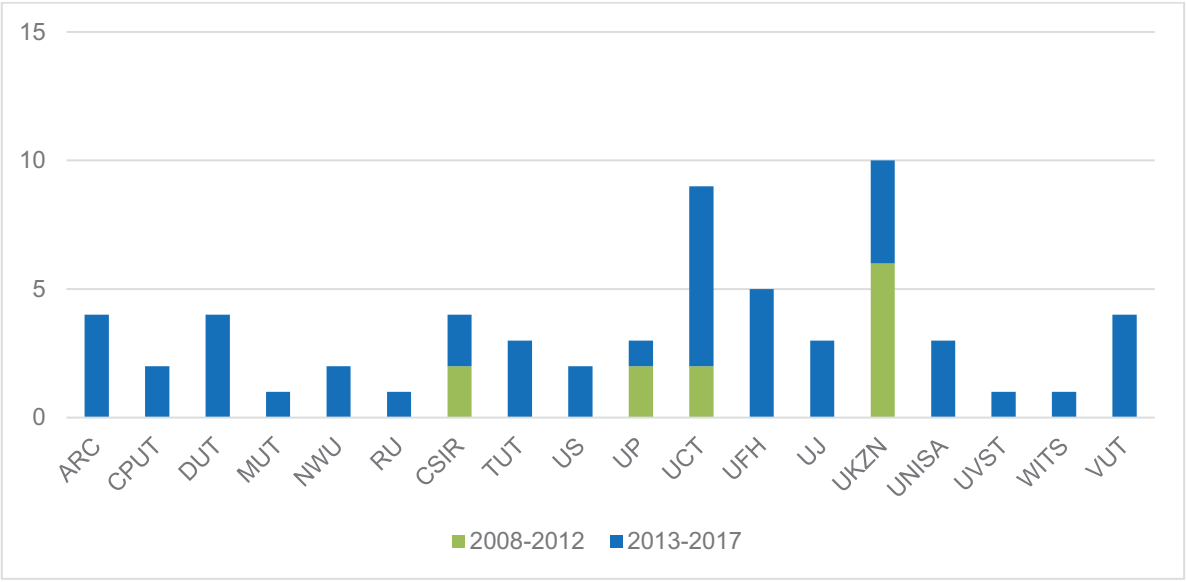


Figure 2-6 SA research initially targeted the production of institutions with publications in AD for biogas. Scopus search (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. South Africa selected. Accessed 2018 March 29

2.1.1.2 Publications targeting a multiproduct approach to AD

Global research on the AD process traditionally targeted the production of treated water with depleted organic loading. It was almost exclusively targeted on the conversion of organics to methane, sometimes with an associated exploration of methane-rich biogas use in electricity production or in other energy systems. The new awareness of the possibilities to combine value generation and enhanced resource efficiency with water treatment, presented by both energy products and non-energy products from AD, is rapidly developing. Considerable effort has been expended on the technology for increasing the yield of biogas, including pre-treatment, co-digestion and reduction of inhibitory intermediaries.

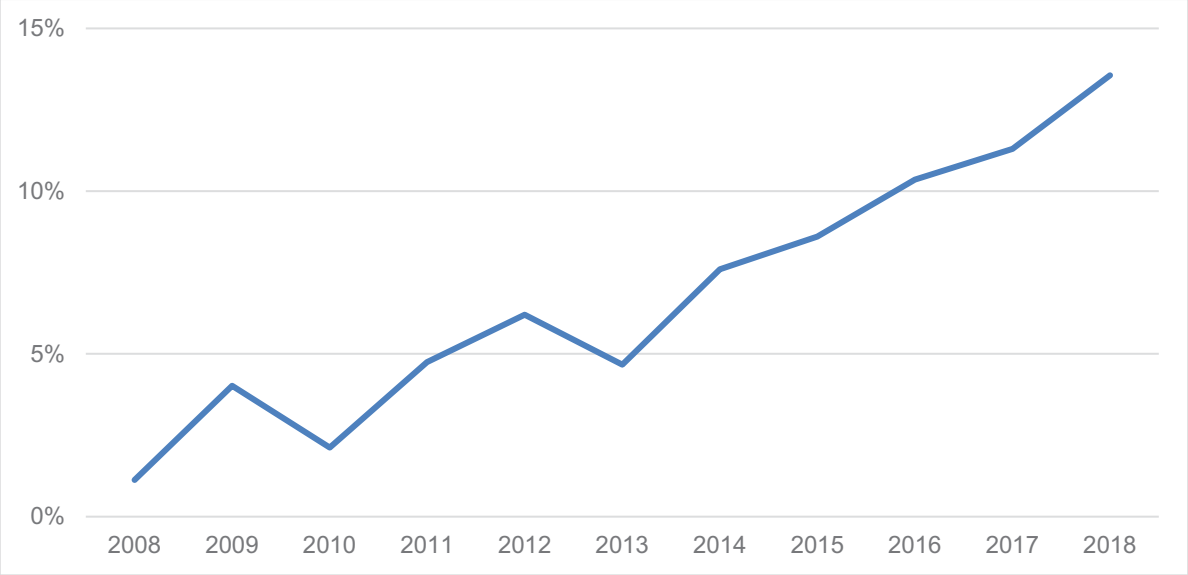


Figure 2-7 Percent of publications in AD for biogas including the biorefinery concept, 2008 -2018. Scopus search 2005 and 2007 one document each (https://www.scopus.com/) *Keywords*: "AD" + biogas + biorefinery. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 2018 March 29

As a shorthand for a multiple product approach, the keyword biorefinery was overlaid on the anaerobic digestion for biogas search. Since the term is newly coined, the references only go back to 2005, with research at UCT in the waste biorefinery field being active since 2007. From 2005, waste biorefinery-focussed publications increased as a percentage of the publications in AD for biogas (Figure 2-7), reaching 112 publications (11%) in 2017. Of these, five have South African affiliation (Ansari et al., 2017; Egieya et al., 2017; Gottumukkala et al., 2016; Inglesby et al., 2015a; Roopnarain and Adeleke, 2017).

The growing interest in non-energy products is confirmed when a secondary term indicating a non-energy product is entered into the document search. Although the number of publications found reduces by an order of magnitude (OOM), from thousands to hundreds, or even tens, the time frame generally covers the last ten years with little reported earlier. The noticeable factor in searching for publications targeting non-energy products, is that both the number of publications year on year and the percentage of the total which indicate a secondary product (Figure 2-8) is increasing. The major exception is compost, which occurs in around 10% of the publications each year for the ten-year period.

The major weakness in this analysis is that it is not possible to create a keyword search which separates publications including VFAs as products from those which address acidogenesis simply as a step in the AD process or accumulation of VFAs as an operational problem. However, VFAs are an obvious additional product, or intermediate, from AD since the only extra technological requirement for production is the recovery step. A brief reading of publication titles and abstracts yields a limited but growing (esp. since 2015) number of publications directed towards this topic. It is, however, unclear how many of these have been captured by the keywords used.

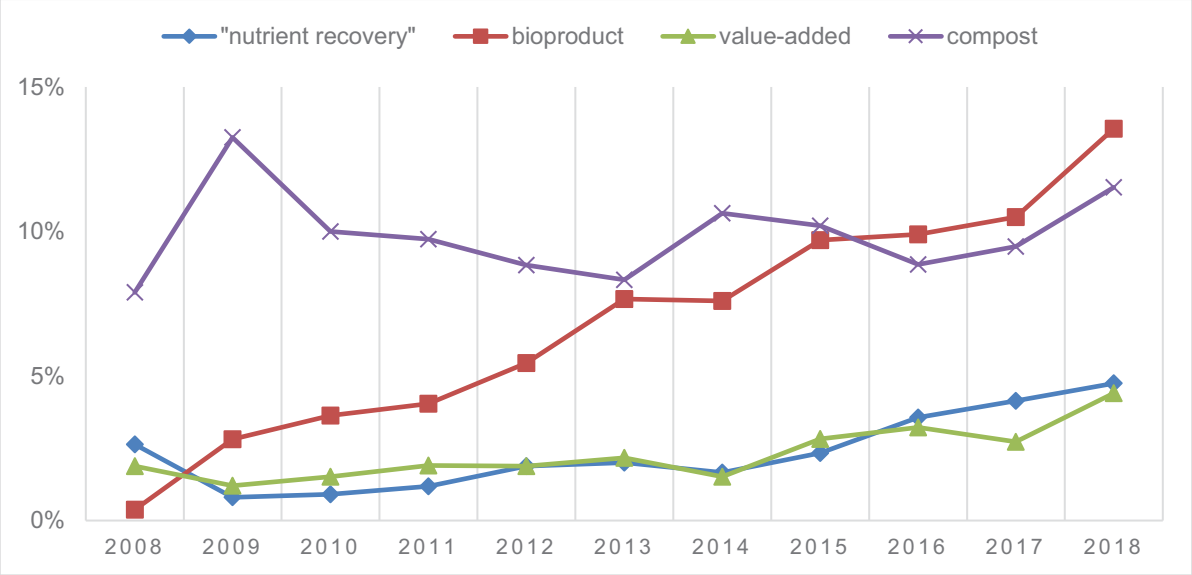


Figure 2-8 Percent of publications in AD for biogas with an extra keyword indicating non-energy products, 2008 -2018. Scopus search (https://www.scopus.com/) *Keywords*: "AD" + biogas + series name. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 2018 March 29

2.1.2 Prior WRC studies on AD

In November 2018, the final search of the WRC database for this project was conducted, using the Knowledge Hub on new WRC website. The search returned 206 research reports for *"AD"*, seven of which are dated 2017 and none 2018. Only 88 of these were returned for the search *"AD" AND biogas*, with the most relevant reports from the previous ten years (2009 – 2018) captured in Table 2-1.

Table 2-1 WRC research reports from the 10 years 2009 to 2018 most relevant to AD for biogas

Authors	WRC Research Report Title	WRC Report Number
Van Der Merwe-Botha, M; Juncker, K; Visser, A; Boyd, R	Guiding Principles in the Design and Operation of a Wastewater Sludge Digestion Plant with Biogas and Power Generation	TT681/16 2016
Sikosana, M; Randall, DG; Petrie, DJ; Oelofse, M; Russo, V; von Blottnitz, H	Nutrient and energy Recovery from Sewage: Towards an Integrated Approach	TT661/16 2016
Ntuli, N; Brouckaert, C	Micronutrient Requirements for AD of Concentrated Industrial Effluents	TT664/16 2016
Everson, TM; Smith, MT	Improving Rural Livelihoods through Biogas Generation using Livestock Manure and Rainwater Harvesting	1955/1/15 2016
Aoyi, O; Apollo, SO; Akach, J; Pete, KY	Integrated Photo-catalytic and Anaerobic Treatment of Industrial Wastewater for Biogas Production	2105/1/14 2015
Burton, S; Cohen, B; Harrison, S; Pather-Elias, S; Stafford, W; Van Hille, R; von Blottnitz, H	Energy From Wastewater – A Feasibility Study	1732/1/09 2010
Buckley, CA; Brouckaert, CJ	A Feasibility Study in eThekwini Municipality on AD for the Treatment of Toxic and High Strength Organic Wastes: A Study of the Business Case of Treating High Strength Industrial Wastes	1538/01/09 2009
Musee, N; Lorenzen, L	Market Analysis for UASB Seeding Granules: Local and International markets	KV224/09 2009

For the peer-reviewed journal Water SA, the search *"AD"* returned 106 articles (four dated 2017 and three 2018) and *"AD" AND biogas* returned 35, including none dated 2017 and all three from 2018. A Scopus search of Water SA is narrower, returning 53 articles for *"AD"* and only ten when *biogas* is added. The most relevant articles for the previous ten years (2009 – 2018) are recorded in Table 2-2.

Table 2-2 Water SA articles from the 10 years 2009 to 2018 most relevant to AD for biogas

Authors	Water SA Article Title	Water SA Reference
Erdirencelebi, D., Kucukhemek, M.	Control of hydrogen sulphide in full-scale anaerobic digesters using iron (Iii) chloride: Performance, origin and effects	(2018) Water SA, 44 (2), pp. 176-183.
Hernandez, J.E., Edyvean, R.G.J.	Toxicity and biodegradability of caffeic acid in anaerobic digesting sludge	(2018) Water SA, 44 (1), pp. 27-36.
Santos, S.L.D., Chaves, SRM, Haandel, A.V.	Influence of temperature on the performance of anaerobic treatment systems of municipal wastewater	(2018) Water SA, 44 (2), pp. 211-222.
dos Santos, S.L., Chaves, SRM, van Haandel, A.	Influence of phase separator design on the performance of UASB reactors treating municipal wastewater	(2016) Water SA, 42 (2), pp. 176-182.
Lorenzen, L., Musee, N.	Market dynamics as a driver towards the evolution of research needs: the case of up flow anaerobic sludge blanket seeding granules	(2013) Water SA, 39 (1), pp
Hernandez, J.E., Edyvean, R.G.J.	Comparison between a two-stage and single stage digesters when treating a synthetic wastewater contaminated with phenol	(2011) Water SA, 37 (1), pp. 27-32.
Machnicka, A., Grübel, K., Suschka, J.	The use of hydrodynamic disintegration as a means to improve AD of activated sludge	(2009) Water SA, 35 (1), pp. 129-132.

In early 2017 the Knowledge Hub on the WRC website (www.wrc.org.za) was searched for relevant research reports as well as papers in the peer-reviewed journal, Water SA. Using the key words “AD” as an integrated term (“Match the whole word”), only 24 entries (Table: A-1, Appendix A) were found. When “Search document content” was checked with “AD” as the search word, a total of 482 was returned, but only 201 entries appeared in the listing. These entries included all document types on the website (including research reports, Technical Briefs, Policy Briefs, Water SA and Water Wheel magazine), and were further reduced to 163 entries using only the academic entries of research reports, Technical Briefs and Water SA articles, presented in Table A-2 in Appendix A. These reports were grouped into five major categories which align with the areas of interest in this report, namely management, feedstock, products, design and modelling, and operation and control (Table 2-3). It can be seen that the major focus of prior work is on reactor design and modelling of AD (30%) and operation and control of AD (28%) with a secondary emphasis on feedstocks (18%). These major categories are comprised of different sub-categories and are further expanded in Table 2-4. Feedstocks for AD are studied in detail in section 2.5, again using WRC reporting.

Table 2-3 The number of WRC publications to 2016 relevant to AD in each of five major categories

Group		Number of publications	Percent
A:	Management	21	13
B:	Feedstocks	30	18
C:	Products	18	11
D:	Reactor design and modelling	49	30
E:	Operation and control	45	28
	Total	163	100

The sub-categories under management are policies and guidelines (76%), feasibility and economics (14%), market analysis (5%), and health, social and environmental issues (5%). The major research focuses on organic waste streams as feedstock (70%) compared to inorganic waste streams (30%). In terms of products from AD, most research reported is concentrated on bioremediation of water and pollution control (61%) rather than on valorisation through energy production (28%) or material products, such as VFAs, fertiliser, nutrients such as P and N) (11%). Reactor design and modelling is one of the most reported topics in AD research within the WRC portfolio. The emphasis is on the kinetics and modelling of the AD process (39%) followed by the different types of reactors used in AD (31%),

with pre-treatment and downstream processing (which includes water purification, product recovery, membranes, and products used in treatment) (29%) also a well-researched topic, while limited consideration has been given to the use of nanotechnology (a single document). Under the sub-categories of operation and control in AD, microbial communities together with enzymes used and pathogen removal (29%) are the most studied, followed by the operation, monitoring and maintenance of AD systems (27%), analyses used in AD such the five-point titration methods (18%), the optimisation and scale-up of AD systems (16%), and the impact of changes to the operating conditions such as temperature, pH, OLRs (11%). This categorisation is summarised in Table 2-4.

Table 2-4 The number of WRC reports relevant to AD grouped into five major categories and their subsequent sub-categories

Group		Number of publications	Percent of the major category
A:	**Management**	**21**	
	A1: Policies and guidelines	16	76
	A2: Health, Social and Environmental issues	1	5
	A3: Feasibility and Economics	3	14
	A4: Market analysis	1	5
B:	**Feedstocks**	**30**	
	B1: Inorganic waste streams	9	30
	B2: Organic waste streams	21	70
C:	**Products**	**18**	
	C1: Bioremediation (water recovery; pollution management)	11	61
	C2: Energy (biogas)	5	28
	C3: Material products (VFAs; fertiliser, nutrients)	2	11
D:	**Design and modelling**	**49**	
	D1: Reactor design (UASB, etcetera)	15	31
	D2: Reactor kinetics and modelling	19	39
	D3: Downstream processing and pre-treatment (water purification; product recovery; membranes; products used in removal)	14	29
	D4: Nanotechnology	1	2
E:	**Operation and control**	**45**	
	E1: Analyses	8	18
	E2: Operating conditions (temp, pH, loading rates, etcetera)	5	11
	E3: Microbial communities (enzymes, pathogens)	13	29
	E4: Operation, monitoring and maintenance of AD system	12	27
	E5: Optimisation and scale-up of process	7	16
	Total	**163**	

2.2 AD as a key unit operation in waste processing

AD provides the opportunity for both waste treatment and biogas formation. Biogas is a readily combustible fuel that can be used to produce heat, electricity, or further processed to a liquid fuel for automotive use (Angelidaki et al., 2003a). The Biomass Magazine (http://biomassmagazine.com/) reported in 2013 (Simet, 2013) that the World Bioenergy Association had released a Biogas Fact Sheet estimating the *potential* for annual production of biogas from waste in the EU, China and the world at

19 billion m^3 (684 PJ), 120 billion m^3 (4 319 PJ) and 370 billion m^3 (13 316 PJ) respectively. Likewise, the International Gas Union (Frick et al., 2015) reports that the Biogas Fact Sheet estimated the global electricity output from biogas installations at 14 000 MW in 2012. The report notes that China had an installed capacity of 800 MW and India 91 MW.

The World Biogas Association (http://www.worldbiogasassociation.org) has also produced a selection of country-specific fact sheets on biogas production (WBA, 2017). These contain the following statistics: the USA, over 2 200 AD units with an installed electrical capacity of 977 MW; Italy, almost 1 590 units mostly on farms; Poland, 301 AD units with a total installed capacity of 234 MW; the Netherlands, over 250 AD units with 219 MW electrical capacity and 11 905 Nm^3/hr biomethane upgrading capacity; Australia, 242 AD units mostly used for electricity and heat production.

The International Energy Agency (IEA) (https://www.iea.org) reports (IEA, 2017) that among the countries in the Organisation for Economic Cooperation and Development production of electricity from biogas in 2016 was 81.3 TWh. Germany (34.1 TWh, >4 000 units), the USA (16.3 TWh), Italy (9.0 TWh) and the UK (7.4 TWh) were the main producers.

In China in 2009 there were just over 4 000 AD plants with digester volumes over 500 m^3 and nearly 20 000 with volumes of 50-500 m^3, which collectively produced almost 1 billion m^3 of biogas, as well as almost 35 000 household-sized digesters (Jiang et al., 2011). Gu et al. (2016) show that production of biogas from household digesters decreased slightly from a peak of about 12 billion m^3 in 2011, while production from large scale industrial and agricultural installations continued to rise, reaching almost 2 billion m^3 in 2014.

From the feasibility study 'Energy from Wastewater', conducted by members of this UCT team with Burton et al (2009), the potential energy recovery from South African wastewaters was estimated to be 10 000 MWh, which represented approximately 7% of Eskom electrical power generation in 2007.

As a range is observed across the biogas production figures reported from different sources, a summary is provided in Table 2-5.

Table 2-5 Biogas capacity of selected countries (Frick et al., 2015; Gu et al., 2016; IEA, 2017; Jiang et al., 2011; Statistica, 2014; WBA, 2017)

Country (year)	Size of AD units	Number of AD units	Total annual biogas	Total electricity
Germany (2016)		4 000		34 - 80 TWh
USA (2016)		2 200		16 - 28 TWh
Italy (2016)		1 590		9 - 18 TWh
Netherlands				3 TWh
France				4 TWh
UK (2016)				7 - 18 TWh
China	>500 m^3	4 000	1 billion m^3 (2009)	90 - 170 TWh
	50 m^3 - 500 m^3	20 000	2 billion m^3 (2014)	
	household	35 000	12 billion m^3 (household)	
World				**300 – 400 TWh installed 10 000 TWh potential**

Most organic carbon-containing waste streams are appropriate as feedstocks for AD, making it an attractive option for treatment of these streams. Although energy crops are gaining interest as feedstocks (Weiland, 2003), the most popular applications of AD are for waste treatment, including wastewater treatment and favouring 'wet wastes' over woody biomass (Dowling, 2009), as follows (Angelidaki et al., 2003a):

- Treatment of primary and secondary sludge resulting from aerobic sewage and wastewater treatment, thus stabilising and lowering the quantity of sludge
- Treatment of wastewater containing organic carbon from, for example, biomass, fermentation, food processing, beverage, petrochemical, and pulp and paper industries
- Processing of livestock waste to generate methane and improve the quality of manure as a fertiliser
- Processing of the organic fraction of municipal solid waste to both decrease the need for landfilling or incineration, and to recycle nutrients to the agricultural sector

The focus of this study is on AD for integrated treatment of wastewater and wastewater sludges, with integrated potential for value recovery. The use of AD to treat organic-contaminated wastewaters is advantageous over traditional aerobic treatment for the reasons listed. Aerobic treatment consumes energy due to the power needed for aeration. It produces more sludge (biomass) than AD, which is often costly to dispose of (Angelidaki et al., 2003a; Speece, 1983) but can be used as fertiliser (Arthurson, 2008; Lens et al., 2002; Rigby and Smith, 2013). Further, it favours conversion of organic waste to CO_2 without concomitant recovery of energy (Weichgrebe et al., 2008) or other products of value. AD results in the simultaneous valorisation of the wastewater to produce energy in the form of methane-rich biogas (Angelidaki et al., 2003a) or useful chemicals in the form of VFAs (Lee et al., 2014) or other products. The use of AD processes has numerous environmental advantages, most notably that the CH_4 produced can replace conventional fuels and that the agricultural use of the nutrient rich digestate and sludge can reduce inorganic nutrient requirements (Mao et al., 2015). Because biogas is derived from geologically recently-fixed carbon in waste streams, its combustion is not considered to result in the release of additional CO_2 (Angelidaki et al., 2003a; Haberl et al., 2012). Rather, valuable energy, and potentially other products, is captured in its expected progression to CO_2.

2.3 AD process

The AD of organic materials yields a biogas, typically comprised of approximately 60% CH_4 with the remainder as CO_2 (Chynoweth et al., 2000), although concentrations of up to 89% CH_4 have been achieved (Şentürk et al., 2010). In this section, the sub-processes and biochemistry governing biogas production are provided. Further typical reactor systems used for AD are described. The impact of operating conditions on performance and the available feedstocks for AD follow in Sections 2.4 and 2.5.

2.3.1 Key stages of AD

In AD, a mixed consortium of microorganisms breakdown biodegradable matter in the absence of oxygen (Kondusamy and Kalamdhad, 2014). Generalised reactions taking place in AD, involving the commonly occurring elements found in organic waste(water), are as follows:

$$\text{Carbon (C)} \rightarrow \text{Organic acids (R·COOH)} \rightarrow CH_4 + CO_2 \qquad \text{Equation 2-1}$$

$$\text{Nitrogen (N)} \rightarrow \text{Amino acids [R·(}NH_2\text{·COOH)]} \rightarrow NH_3 + \text{amines} \qquad \text{Equation 2-2}$$

$$\text{Sulphur (S)} \rightarrow H_2S + \text{organic S compounds} \qquad \text{Equation 2-3}$$

$$\text{Phosphorous (P)} \rightarrow H_3PO_4 + \text{organic P compounds} \qquad \text{Equation 2-4}$$

The overall AD process is comprised of four main sub-processes, shown in Figure 2-9, with each involving the activity of a sub-group of the consortium of both facultative and obligate anaerobes (Lee et al., 2014). These stages are defined by the sequential biochemical conversions that occur, namely (Lee et al., 2014; Speece, 1983):

- Hydrolysis of complex organic polymers to organic monomers
- Acidogenesis of these monomers to form (predominantly) VFAs and hydrogen

- Acetogenesis of the VFAs to produce acetic acid
- Methanogenesis of acetic acid and hydrogen to produce methane

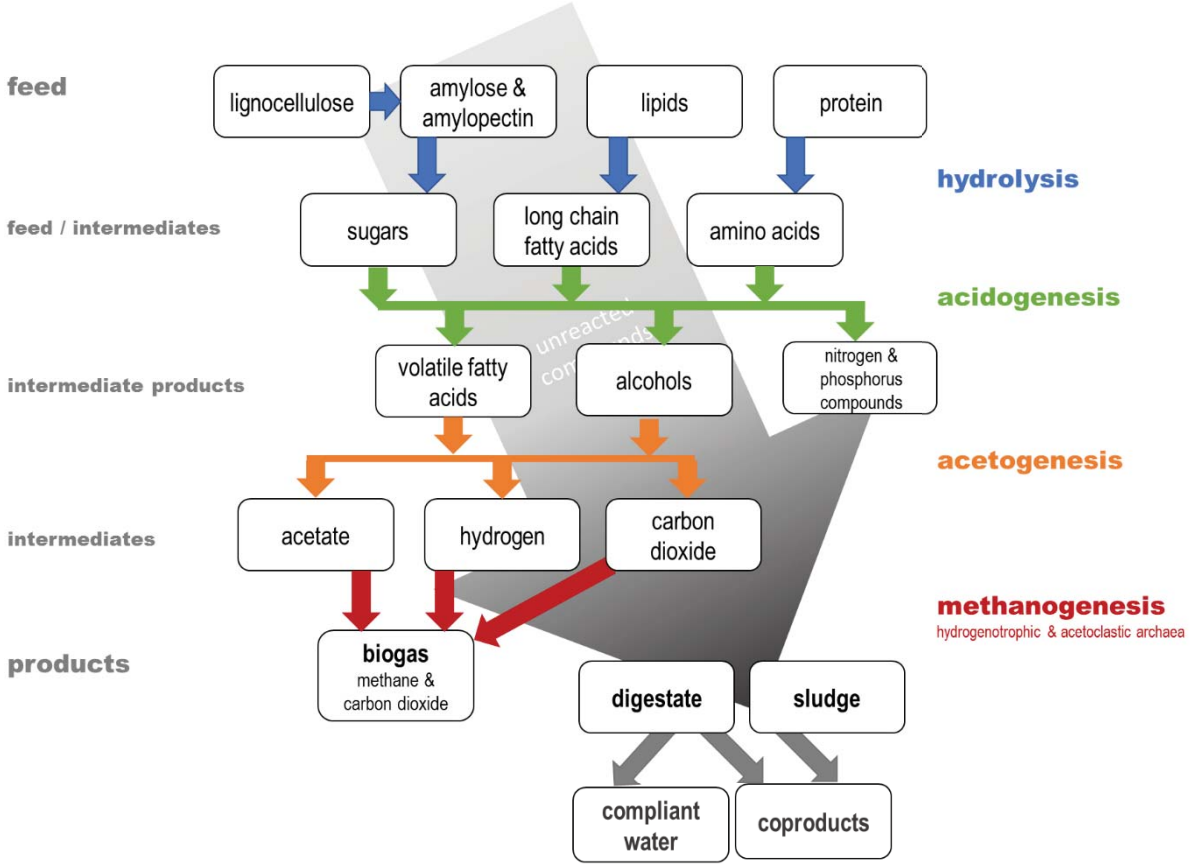


Figure 2-9 The stages of the AD process

Hydrolysis of solid particulates and inactive (dead) biomass, as well as more complex molecules such as carbohydrates, proteins and fats, occurs as a result of extracellular enzymes secreted by hydrolytic microbes (Batstone et al., 2002; Lee et al., 2014; Mao et al., 2015). The products of hydrolysis, namely simple sugars, amino acids and long chain fatty acids (LCFAs), are then converted through intracellular fermentation processes by acidogenic bacteria to VFAs (acetic, propionic, butyric and valeric acids), CO_2 and hydrogen as well as alcohol (ethanol) and ketones (Moraes et al., 2015). These reactions are thermodynamically favourable and, as a result, are primarily limited by the availability of hydrolysis products. The VFAs and alcohol are converted to acetate and more H_2 by acetogenic bacteria (Mao et al., 2015). The acetate and H_2 are then converted by acetoclastic and hydrogenotrophic methanogens respectively to form CH_4 and CO_2 (Pind et al., 2003; Speece, 1983). Acetogenesis and methanogenesis are often considered as one step since the concentrations of acetogenic products (VFAs and H_2) need to be regulated by the methanogens to keep the acetogenic reactions energetically favourable, thus the rate of acetogenesis is dependent on the rate of methanogenesis. The activity of the hydrogenotrophic methanogens is especially crucial since the hydrogen partial pressure needs to be kept lower than 0.001 atm for the conversion of higher VFAs to acetate (acetogenesis) to be thermodynamically favourable (Speece, 1983).

2.3.2 Reactor configuration for AD processes

The choice of the correct bioreactor from the many types reported for AD processes is crucial to a successful process (Speece, 1983). For wastewater treatment, a minimum HRT is preferred to minimise the reactor size required. This should be coupled with a high SRT to prevent washout of the essential microorganisms and maximise the concentration of the active microbial catalyst, thus requiring decoupling of HRT and SRT. Most reactor designs thus aim to achieve a ratio of SRT/HRT substantially greater than 1, such that a short HRT can be coupled with sludge retention (Rajeshwari et al., 2000; Speece, 1983). Common reactor types are grouped in Table 2-6 while the performance of common examples of anaerobic biomass retention reactors across different feedstocks is shown in Table 2-7.

Table 2-6 Anaerobic bioreactors operated with sludge retention (Mao et al., 2015; Rajeshwari et al., 2000)

Reactor type	Start-up period (weeks)	Description	Notes
CSTR with biomass recycle	-	Continuous stirred tank reactor followed by clarifier for solids recycling	SRT/HRT > 1 due to recycling of solids, but sludge is dilute, limiting OLR treatable
UASB	4 - 16	Substrate is fed into the reactor from the base upwards, through a blanket of suspended sludge (active biomass) granules	Long start-up period, but low investment cost and high SRT/HRT. Must allow for granulation of sludge on start-up
Fixed film or anaerobic filter	3 - 4	Packed bed on which biofilm attaches and grows	Stable to shock loads. No mechanical or hydraulic mixing leading to concentration profiles across reactor. Large reactor due to packing requiring space
Anaerobic fluidised bed (AFB)	3 - 4	Fluidised bed with packing material as biomass support	Relatively small reactors Low head loss across reactor. High SRT, well-mixed, but recycling may be needed

Table 2-7 Anaerobic bioreactors in literature (*(Bouallagui et al., 2005; Rajeshwari et al., 2000)*

Waste Type	Reactor	OLR kg-COD/m³Day	COD Reduction %	
Abattoir	UASB (granular)	11	85	
	UASB (flocculated)	5	80–89	
	Anaerobic filter	2.3	85	
	Anaerobic contact	3	92.6	
Waste Type	**Reactor**	**OLR kg-COD/m³Day**	**COD Reduction %**	
Cheese Whey and Dairy	UASB	1–28.5	95–99	
	UASB	7–9.5	90–94	
	UASB	1–6.7	90–95	
	UASB (dairy)	31	90	
	UASB (cheese whey)	–	–	
	2-stage (cheese whey)	–	36	
	UFFLR	14	95	
	DSFFR	2.6	88	
	FBR	7.7	90	
	FBR	6–40	63–87	
	AAFEB	8.2–22	61–92	
	AnRBC	10.2	76	
	SDFA	16.1	99	
	UASB	7.1	94	
	UASB	0.9–6	97–99	
	DUHR	10	97	
Waste Type	**Reactor**	**OLR kg-COD/m³Day**	**COD Reduction %**	**Specific Wastewater**
Pulp and Paper	UASB	40	40	Debarking
	Fluidised bed	0.66	50 (BOD)	Debarking
	Mesophilic UASB	12–31	60–70	Thermo-mechanical pulping
	55–70°C UASB	80 and 13	60	Chemi-thermo-mechanical pulping
		4 and 20	60	
		4.7–22	35–55	
	Contact Process Reactor	5	30–50	Sulphite condensate
Waste Type	**Reactor**	**OLR g-VS/Lday**	**VS Reduction %**	
Fruit & Veg	Batch system	1.06	65	
	Batch system	0.9	58	
	CSTR	1.6	88	One-Stage
	CSTR	3.6	83	One-Stage
	Tubular Reactor	2.8	76	Continuous
	ASBR + UASB	6.8	94	Two-stage: hydrolyser + methaniser
	ASBR + AFR	4.4	87.5	Two-stage: hydrolyser + methaniser
	CSTR + AFR	5.65	96	Two-stage: hydrolyser + methaniser

Key to reactor types:

AAFEB	Anaerobic Attached — Film Expanded — Bed Reactor
AFB	Anaerobic Fluidised Bed
AnRBC	Anaerobic Rotating Biological Contact Reactor
ASBR	Anaerobic Solid Bed Reactor
CSTR	Continuous Stirred Tank Reactor
DSFFR	Downflow Stationary Fixed Film Reactor
DUHR	Downflow Upflow Hybrid Reactor
EGSB	Expanded Granular Sludge Bed
FBR	Fluidised Bed Reactor
RBC	Rotating Biological Contactor
SDFA	Semi-continuous Digester with Flocculant Addition
UASB	Upflow Anaerobic Sludge Blanket
UFFLR	Upflow Fixed Film Loop Reactor
USSB	Upflow Staged Sludge Bed

In a single stage continuous reactor, the stages outlined in Figure 2-9 proceed simultaneously as the single system contains all microbial groups, reactants, intermediates and products (Demirel and Yenigün, 2002). This setup results in a compromise between the preferred environmental conditions of the different stages of the process and groups of microorganisms involved. Alternatively, the conditions in the reactor can be chosen to favour different microbial groups in the AD process, in particular creating preference toward the hydrolysis and acidogenesis or the acetogenesis and methanogenesis stages in

separate reactors or reactor zones through use of a two-phase (or multi-stage) digestion process (Demirel and Yenigün, 2002). Examples of reactors with separate operating zones include the packed bed with no mixing and limited axial dispersion across which a profile of reactor conditions develops, each favouring a different set of reactions and community, or the zones that develop in a plug flow anaerobic baffled reactor (Barber and Stuckey, 1999).

2.4 Factors influencing AD

2.4.1 Temperature

AD processes are usually operated at mesophilic (25-40 °C) or thermophilic (≥ 45 °C) temperatures (Angelidaki et al., 2003a); however, CH_4 production can also occur at psychrophilic (0 - 20 °C) temperatures (Angelidaki et al., 2003a; Rajeshwari et al., 2000). Increasing temperature within the limits of microbial tolerance results in an increase in microbial activity. It also affects physical properties such as viscosity, surface tension and mass transfer within the system (Angelidaki et al., 2003a). While thermophilic temperatures benefit process kinetics, limited robust systems have yet been reported under strongly thermophilic conditions. Fluctuations in temperature may hinder process performance where differing microbial consortia are required or flourish at the different temperatures, requiring time for the adaptation of communities. The microbial activity of a particular consortium is reported to decrease by roughly 50 % for every 10 °C decrease in temperature within the mesophilic range (Rajeshwari et al., 2000).

The increased microbial activity experienced by a microbial consortium at higher temperatures not exceeding its optimum temperature means that higher OLRs can be handled (Angelidaki et al., 2003a; Mao et al., 2015). Thermophilic processes have been reported to have been less stable (Mao et al., 2015), with higher VFA accumulation (Nges and Liu, 2010). Angelidaki et al. (2003) report the benefits of increased temperatures in industrial experience, through increased the rates of hydrolysis observed as well as destruction of pathogens. The accumulation of propionate during high-rate thermophilic processes suggests that the activity of propionate-degrading microorganisms does not increase in proportion to other microbial populations (Nges and Liu, 2010). While kinetics is improved with increasing temperature (up to an optimum), the microbial yield is, in general, not affected by temperature. Most notably, although the rate of methanogenesis has been seen to increase with temperatures up to 40 °C, the theoretical CH_4 yield is not affected by temperature (Angelidaki et al., 2003a). Further to rates of reaction, an optimum temperature, or ‘sweet spot’, exists at which the reactor temperature is maintained by the exothermic reactions taking place, reducing or eliminating heating and cooling costs.

Increasing temperature reduces the solubility of gases. Hence, at low temperatures an appreciable quantity of CH_4 generated can be retained in the effluent stream, especially if the feed stream is dilute in organic content, as in the case of treatment of sewage using AD (Chernicharo et al., 2015; Ferrer et al., 2012; Kim et al., 2011; Kleerebezem et al., 2015; Singh and Viraraghavan, 1998).

2.4.2 pH and alkalinity

The operating pH is one of the most important parameters in the AD process, along with temperature and substrate composition (Angelidaki et al., 2003a). Each stage of the AD process has a characteristic pH optimum. The optimal pH range for methanogens is between pH 6.8 and 7.2. Maintenance of this pH range prevents build-up of VFAs by favouring activity of the methanogens (Rajeshwari et al., 2000). It has been observed that the rate of hydrolysis is pH dependent, and that the optimal pH is dependent on the microbial community and substrate. The optimal pH range for acidogenic bacteria is pH 5.5 - 6.5. It was found that a pH of 6 was optimal in the production of VFAs using AD (Wang et al., 2014), owing to inhibition of methanogenesis under these conditions. Operation of AD in one reactor requires a compromise between the preferred pH of the different microorganisms required for the differing sub-

processes (Angelidaki et al., 2003a), whereas the dual stage process or zoned reactor system allows optimisation of the individual stages (Barber and Stuckey, 1999).

To maintain a stable pH, especially when process upsets occur and VFAs accumulate, sodium bicarbonate is usually used as a source of alkalinity (Rajeshwari et al., 2000). However, the use of calcium hydroxide as an alkalinity source is reported to increase the settleability of sludge in UASB reactors (Lettinga et al., 1980). With the desire for concomitant VFA production, the control of pH is expected to be a major control variable.

2.4.3 Substrate composition and the rate-limiting step

In a well-functioning methanogenic bioreactor, the rate controlling step is dependent on the nature of the substrate (Speece, 1983) as hydrolysis is considered the slowest process step in AD (Chernicharo et al., 2015; Fang and Chui, 1993), with the exception of methanogenesis. Grease, lipids, cellulose and lignin present in the substrate degrade very slowly, thus hydrolysis is likely to be rate-limiting in systems where these are the major components (Speece, 1983). For example, the high COD present in primary and waste activated sludges is 90-99 % insoluble, leading to a large dependence on hydrolysis which retards acidogenesis (Lee et al., 2014). Similarly, this is the case for complex food waste and the organic fraction of municipal solid waste. AD processes fed with these substrates are likely to be limited by hydrolysis (Eastman and Ferguson, 1981; Speece, 1983). Typically, fermentation, acidogenesis and acetogenesis proceed more rapidly than either hydrolysis or methanogenesis. The products of acidogenesis and acetogenesis are used in methanogenesis.

Where substrates that do not contain these recalcitrant and complex organics, methanogenesis is usually rate-limiting (Speece, 1983). For example, many food processing wastewaters (e.g., apple juice wastewater) contain high levels of simple organic compounds which are readily converted to VFAs; in these cases, methanogenesis is rate-limiting (Speece, 1983).

Potential feedstocks for the AD process is presented in detail in section 2.5, with an analysis of carbon composition given in section 2.6.

2.4.4 Nutrient requirements

Microbial consortia involved in the AD process require adequate nutrient supply in order to grow and to display optimal activity. These include both the macronutrients sulphur, nitrogen, phosphorous, magnesium and potassium and the micronutrients iron, cobalt, nickel, molybdenum, tungsten, selenium, zinc, manganese, copper and calcium (Rajeshwari et al., 2000; Speece, 1983). These are often limiting in waste and wastewater processing (Speece, 1983).

Supplementation of wastewaters with nutrients is essential where these are limiting (Speece, 1983; Von Sperling and De Lemos Chernicharo, 2005). This may be achieved cost-effectively by the co-digestion of waste streams with a complementary nutrient composition or by supplementation with a specific nutrient. Von Sperling and De Lemos Chernicharo (2005) propose that the required nutrient concentrations in the wastewater can be calculated according to Equation 2-5:

$$N_r = S_0 \cdot Y \cdot N_{bac} \cdot \frac{TSS}{VSS} \qquad \text{Equation 2-5}$$

where
N_r is the macro- or micronutrient requirement (g/L)
S_0 is the influent COD concentration (g-COD/L)
Y is the biomass yield coefficient in terms of COD (g-VSS/g-COD)
N_{bac} is the elemental concentration of the specified nutrient in the bacterial cell (g element/g-VSS)
TSS/VSS is the total solids/volatile solids ratio for the bacterial cell (usually 1.14)
(Von Sperling and De Lemos Chernicharo, 2005)

The elemental composition of methanogens is given in Table 2-8. These values can be substituted into the N_{bac} term in *Equation 2-5* to calculate the required nutrient concentrations for AD.

Table 2-8 Elemental composition of methanogens (Rajeshwari et al., 2000)

Macronutrients	Concentration (g/kg cells)	Trace nutrients	Concentration (mg/kg cells)
N	65.0		
P	15.0	Ni	100
K	10.0	Co	75
S	10.0	Mo	60
Ca	4.0	Zn	60
Mg	3.0	Mn	20
Fe	1.8	Cu	10

2.4.5 Nutrient requirements further considered: the C:N ratio for optimum biogas formation

Nitrogen is recognised as a key nutrient impacting the efficiency of the AD process. The amount of combined nitrogen required for bioprocesses such as the AD process is usually presented as a C:N ratio. Nitrogen both influences the growth of microbial communities within the AD process and can result in its inhibition, hence it is particularly important to balance the carbon to nitrogen ratio (C:N). For the complete degradation of carbohydrates, the C:N ratio required is between 20:1 and 32:1 (Adelekan and Bamgboye, 2009; Angelidaki et al., 2003b; Mao et al., 2015; Sperling and Lemos Chernicharo, 2005). However, methanogens have also been shown to tolerate even higher ratios, with a C:N of 66 tolerated for methanogenesis of VFAs (Mao et al., 2015; Sperling and Lemos Chernicharo, 2005). High C:N ratio results in rapid nitrogen depletion by methanogens and leads to lower gas production (Zhang et al., 2013). The methanogens consume nitrogen at a rapid rate to meet their protein requirements for growth. Following N limitation, biogas production rates slow. In a case of a feed stream to the AD reactor with a high C:N ratio, biogas production can be improved by co-digestion using a nitrogen-rich waste stream.

A low C:N ratio also impacts negatively on methane gas productivity in an AD system. At a low C:N ratio, ammonia accumulates, thus increasing the pH to over 8.5 which inhibits the activity of methanogens (Mao et al., 2015; Zhang et al., 2013). A pH value higher than 8.5 generates excess OH^- ions, toxic to the methanogens, resulting in a reduced methane yield (Orhorhoro et al., 2016). Furthermore, the inhibition of methanogens by high concentrations of ammonia results in the accumulation of VFAs (Shi et al., 2017) and potential acidification.

Overall, the composition of a given feedstock plays an important role in the AD process as it can influence both the methane and VFA yields. In some cases, co-digestion of various streams is necessary in order to optimise the C:N ratio, or to improve availability of other nutrients, for maximum methane or VFA yields. The optimum nitrogen requirement can be calculated in terms of these C:N ratios (on a mass basis).

2.4.6 Retention times and OLR

The HRT, defined in Equation 2-6, is the average time that the substrate-carrying liquid phase spends in the reactor.

$$HRT = \frac{V}{v} \text{ (days)} \qquad \text{Equation 2-6}$$

where V is the working volume of the reactor (m^3) and v is the volumetric flow rate of the feed (m^3/day). The HRT is closely linked to the capital investment as it defines the reactor size required per volume of feed processed (Lee et al., 2014; Speece, 1983).

The OLR is defined as the mass of biodegradable organic matter fed to the reactor per unit reactor volume per day. The mass of biodegradable organic matter is usually represented by the mass of volatile solids (VS) for solid waste treatment systems and by the COD for wastewater treatment systems. This parameter is the product of the concentration of VS or COD in the feed and the volumetric flow rate per unit reactor volume. The relationship between COD, HRT and OLR is shown in Equation 2-7.

$$OLR = \frac{COD\ (kg/m^3)}{HRT\ (days)}$$ Equation 2-7

The solid (or biomass) retention time (SRT) is used to describe systems with discrete solid and liquid phases and is defined as the average time that the solids spend in the reactor. It is most usefully defined specifically as the biomass retention time where this can be measured separately to the overall solids retention. In reactors using freely suspended biomass with no biomass recycle, the biological retention time and HRT are equivalent. As indicated in section 2.2, this is not preferred in wastewater treatment where it is ideal to minimise the HRT and maximise the SRT or BRT through biomass retention to maximise the catalytic or biologically active component in the reactor. Biomass retention may be achieved by flocculation or granulation as in the UASB, biofilm formation or use of membrane bioreactors. If there is no discrete liquid phase, then only the SRT is relevant because the substrate and active biomass do not occur in different phases (Lee et al., 2014).

The retention time of the biomass determines the relative prevalence of microbial species within the reactor (Lee et al., 2014). Cells must be replaced at a specific rate equivalent to the inverse of the biomass retention time, hence slower growing cells are more readily lost from the system. Microbial diversity can be enhanced by biomass retention or long residence times or both. For example, in CH_4 producing wastewater treating processes, it is important to ensure that the SRT is sufficiently long to prevent washout of the slow-growing methanogens and process instability (Speece, 1983). For strictly VFA production processes, the SRT is chosen to be long enough to support a large population of hydrolytic and acidogenic bacteria, but not long enough to allow methanogens to be retained in the system to prevent the conversion of the desired VFAs to CH_4 (Lee et al., 2014).

2.4.7 Inhibition of AD

Inhibition is described as a condition which adversely affects the metabolism of the microbial species of interest. In the discussion of AD, typically inhibition is focused on the production of biogas. Inhibition of methanogenesis is traditionally associated in accumulation of VFAs (Chen et al., 2008) and high nitrogen levels. There are a range of compounds with inhibitory effects on the AD process. These are discussed below.

2.4.7.1 Ammonia

Inhibition due to high concentrations of ammonia is one of the most common forms of methanogenic inhibition (Angelidaki et al., 2003a). Ammonia is present in a range of feedstocks, including manure. Elevated levels also result from the degradation of protein-rich substrates (Nielsen and Angelidaki, 2008). Inhibition is usually attributed to the non-ionised, or free, ammonia concentration (Angelidaki et al., 2003a). Concentrations of 55 mg $NH_{3(free)}$/ℓ have been found to result in inhibition. It was demonstrated that the process could be acclimatised to handle a concentration of up to 800 mg $NH_{3(free)}$/ℓ (Angelidaki et al., 2003a). The free ammonia concentration is a function of temperature and pH. Decreasing the pH has been observed to aid recovery from inhibition (Angelidaki et al., 2003a).

2.4.7.2 Sulphide

Sulphate and other S-containing compounds are commonly found in industrial process wastewater (Barrera et al. 2014) and other AD feedstocks. The anaerobic, reductive nature of the AD reactor promotes the activity of sulphate reducing bacteria (SRB), forming aqueous and gaseous H_2S,

dissolved H_2S, polysulphides and insoluble metal sulphides (Chen et al. 2008; Barrera et al. 2014). Sulphate SRB

compete with methanogens for substrate. The sulphide produced is inhibitory to methanogens (O'Flaherty et al., 1998). The COD:SO_4^{2-} ratio is important in ensuring effective methanogenesis because sulphate reduction by SRB is energetically favoured over methanogenesis. Therefore, SRBs outcompete methanogens at low COD concentrations (Speece, 1983). Concentrations of S^{2-}_{total} greater than 100-300 mg/ℓ and $H_2S_{undissociated}$ greater than 50-150 mg/ℓ were shown to result in complete inhibition of biogas production (Angelidaki et al., 2003a). O'Flaherty et al (1998) found that pH between pH 7 and 7.75 were optimal for methanogens, while pH 7.5 to 8.5 was optimal for SRBs.

2.4.7.3 LCFAs

LCFAs are the hydrolysis products of fats found in wastes (Batstone et al., 2002). Elevated levels of LCFAs as a result of insufficient β-oxidation are inhibitory to the AD process. No acclimatisation of anaerobic cultures to LCFAs has been observed, but their negative effects can be mitigated by the presence of particulates onto which the LCFAs absorb (Angelidaki et al., 2003a).

2.4.7.4 Metal toxicity

In addition to organic matter, a number of essential nutrients and micronutrients are required for cell microbial growth and survival; their presence, as well as their concentrations and bioavailability, has an effect on this microbial growth (Thanh et al., 2016). At a micronutrient level, light metal ions such as aluminium, calcium, potassium and sodium are crucial for growth and enzyme functioning; however, at high concentrations microorganisms may begin to dehydrate due to osmotic pressure, resulting in decreased growth and ultimately cell death (Chen et al., 2008). Further, specific enzymes and central functions such as transcription, translation and respiration can be inhibited by specific metals at high concentration. Although salts carry both cations and anions, the toxicity of the salts lies predominately in the cation (Chen et al., 2008). Metal resistance mechanisms are often in place to export or de-toxify these metals and adaptation to provide metal resistance is reported in some instances.

Aluminium

Aluminium binds to the cell membrane of acetogenic and methanogenic microorganisms and results in the inhibition of cell growth (Cabirol et al., 2003; Chen et al., 2008). An AD operated in the presence of 1000 mg/L of AlOH showed a 50% and 72 % decrease in the performance of methanogens and acetogenic microorganisms respectively (Cabirol et al., 2003; Chen et al., 2008). Extended studies showed microbial adaptation to the presence of the aluminium cation. However, when the cation occurred in combination with other inhibitors such as sulphate, methanogens were found to show greater inhibition with little adaptation (Cabirol et al., 2003).

Calcium

Calcium is commonly used for buffering wastewater. At a micronutrient level, i.e., less than 120 mg/L, calcium is greatly beneficial to microbial growth as it promotes biofilm growth and granulation. Its presence in the AD reactor at 120 mg/L was found to increase the rate of biogas production and the resultant quantity of biogas produced (Ahn et al., 2006). Inhibition has been observed at concentrations above 120 mg/L; increasing calcium concentrations results in an accumulation of minerals in the biofilm causing a decrease in water and ultimately cell dehydration (Ahn et al., 2006; Chen et al., 2008). Further increases of calcium concentration in the AD reactor above 400 mg/L, and in some cases as high as 8000 mg/L results in moderate to severe inhibition which is primarily characterised by inhibition of cellular activity of both acidogens, resulting in reduction in the availability of VFAs, and methanogens, resulting in a decrease in the rate of biogas production. Outside of the cellular impact of calcium accumulation, the presence of calcium salts result in scaling of reactors, scaling of biomass and a loss in buffering capacity of the AD reactor (Ahn et al., 2006; Chen et al., 2008; Kondusamy and Kalamdhad, 2014).

Potassium and sodium

At low concentrations (<400 mg/L), both potassium and sodium are essential for growth and respiration of microorganisms present in the reactors. Sodium, for instance, has been reported to be crucial for the oxidation of NADH in mesophilic anaerobes (Chen et al., 2008). However similar to calcium and aluminium increases in concentration result in cell dehydration and cell death, with negative impact on reactor stability.

Heavy metals

The presence of heavy metals in the feed to the AD process has been shown to have an overall negative impact on the AD processes, and therefore the methane production, due to the toxicity of the heavy metals towards the anaerobic microorganisms (Inyang et al., 2012). Studies have shown that accumulation of heavy metal ions leads to disruptions in enzyme structure by binding with thiol groups on proteins, or replacing the naturally occurring metal in the enzymes' active site (Chen et al., 2008). As a number of heavy metals are required by essential enzymes that facilitate the anaerobic reactions, the impacts of heavy metals are highly dependent on their concentrations, substrate, environmental conditions (pH, redox and speciation), the composition of the AD consortium and the solids loading level (Chen et al., 2008).

Heavy metals identified to be of particular concern for AD include Cd, Co, Cr, Cu, Fe, Zn and Pb, (Chen et al., 2008; Inyang et al., 2012). Generally, acidogenic bacteria have a higher resistance to heavy metal toxicity than methanogens, meaning the production of intermediate products (i.e. hydrogen, ammonia, carbon dioxide, alcohols and organic acids) is impacted less than the production of methane (Jarrell et al., 1987). In general, the sensitivity of acidogens and methanogens to heavy metals can be given as Cu > Zn > Cr > Cd > Ni > Pb and Cd > Cu > Cr > Zn >Pb > Ni respectively (Chen, Cheng, & Creamer, 2008). Altaş (2009) illustrated that metal concentrations of Zn, Cr, Ni and Cd of 7.5, 27, 35 and 36 mg/L respectively resulted in a 50% reduction of cumulative methane production over a 24-hour period.

Due to the complexity of the AD process, metals take part in numerous physico-chemical processes including sulphide precipitation, sorption onto biomass and formation of intermediates in solution (Shin et al., 1997). The concentration of these metals as soluble ions may be low if they precipitate under reactor conditions. Precipitation is common in the presence of sulphide. It has been suggested that less than 2% of the heavy metal is typically available as a toxicant (Angelidaki et al., 2003a). However, this is strongly dependent on the feedstock and culture conditions and requires further definition. Only free form, soluble metal ions have been found to be toxic to microorganisms (Chen et al., 2008; Shin et al., 1997).

2.4.7.5 VFAs

VFAs can inhibit methanogenesis in the AD process. Accumulation of VFAs is associated with lowering the reactor pH out of the optimal range for methanogens (Şentürk et al., 2010). The speciation of the VFAs is affected the pH. For this reason, VFA: alkalinity ratios between 0.1 and 0.35 $\frac{gVFAs/L}{gCaCO_3/L}$ are typically suggested to maintain a stable CH_4-producing digester (Pullammanappallil et al., 2001), although the value of this ratio required for reactor instability has been put as high as 0.8 (Şentürk et al., 2010). Undissociated VFAs can permeate the microbial cell wall, lowering cytoplasmic pH and affecting cellular functions (Pullammanappallil et al., 2001). This effect is exacerbated at low pH where a higher fraction of the VFAs are in their undissociated state (Pullammanappallil et al., 2001). Propionic acid is often considered most inhibitory to methanogens (Lee et al. 2014; Aguilar et al., 1995). Significant inhibition has been reported at a concentration of 900 mg/L by Lee et al. (2014) and above 4.5 g/L by Aguilar et al. (1995). Aguilar et al. (1995) reported that propionic acid was the most inhibitory VFA, with n-valerate being less inhibitory than propionic and n-butyric acids. It should be noted that the level of inhibition is also impacted by the composition of the anaerobic consortium.

2.5 Feedstocks for AD

Almost any form of organic matter can be used as feedstock for AD and the process has been used with a wide variety of feedstocks, both purpose-grown biomass and organic waste from diverse sources (Steffen et al., 1998). In this study, our primary focus is the organic content of wastewater streams.

2.5.1 Overview of AD feedstocks

Organic wastes can be broadly categorised as municipal waste, industrial waste and agricultural waste (Nizami et al., 2017; Steffen et al., 1998). Although these categories overlap, they form a useful basic categorisation. Table 2-9 indicates that each of these categories appear frequently in literature featuring AD for biogas. Each category is examined in the following subsections, with an emphasis on wastewater streams since a major objective of the study is water reclamation with concomitant valorisation through multiple products.

Feedstock characteristics influence the feasibility of the AD process in terms of process parameters, reactor design, possible products and downstream processing (Chandra et al., 2012; Steffen et al., 1998). This relates strongly to the type and complexity of the organic molecules and to the ratio of various components, including the carbon-nitrogen ratio (sections 2.4.3 and 2.4.4). The important organic components in any feedstock (Table 2-9) are sugars, carbohydrates, fats and oils, fatty acids and organic acids, proteins, cellulose, hemicellulose and lignin (Chandra et al., 2012; Steffen et al., 1998; Weiland, 2010). The concentration of the carbon compounds in the feedstock is also significant (Chandra et al., 2012).

Table 2-9 Publications on AD for biogas with reference to various feedstocks on Scopus (https://www.scopus.com/) *Keywords*: "AD" + biogas. *Subject Area Limits*: Environmental Science; Chemical Engineering; Agricultural and Biological Sciences; Engineering; Energy. Accessed 05 April 2018

Keyword combined with "AD" + biogas	Number publications listed in Scopus
"AD" + biogas	**7 709**
+ feedstock	1 706
+ co-digestion OR codigestion	3 048
Origin of Feedstock	
+ "municipal waste"	560
+ "municipal wastewater"	735
+ "industrial waste"	3 366
+ "industrial wastewater"	2 587
+ "agricultural waste"	3 721
+ "agricultural wastewater"	2 499
+ microalgae	516
Type of Organic Compounds	
+ sugar	1 092
+ carbohydrate	950
+ protein	1 120
+ oil	1 591
+ lignocellulosic	1 104

Important physical considerations are the consistency of the incoming stream and the presence of non-reactive solids both of which affect pumping, settling, mixing, filtration etcetera. The relative acidity and alkalinity are important variables (section 2.4.2). Cognisance must also be taken of the possible presence of substances which may inhibit one or more steps in the AD (section 2.4.6), and of compounds that are not metabolised in the AD and emerge in the effluent as environmental toxins.

To achieve the desired properties in the feedstock for AD, it has become common to operate AD with co-digestion (Mata-Alvarez et al., 2014; Steffen et al., 1998; Weiland, 2010), in which two or more waste streams are combined to form a more appropriate feedstock. It is particularly common to co-digest waste streams from different categories of waste.

2.5.2 Municipal waste as AD feedstock

AD is frequently studied as part of the treatment of municipal waste (Table 2-9), both wastewater and solid waste (MSW). Municipal wastewater tends to be more dilute and more variable in composition than industrial or agricultural wastewater streams, in addition to which, it is one of the most complex wastewaters. It may include a significant proportion of industrial effluent (Harrison et al., 2017), depending on location.

Nearly 250 WRC research reports and 150 Water SA articles focus on the treatment of municipal wastewater. Over 59 of these publications refer to both AD and biogas. The fifteen listed in Table 2-10 were selected as most relevant to the question of municipal wastewater as feedstock for AD.

Table 2-10 List of selected documents from the WRC Knowledge Hub list returned by the search "municipal wastewater biogas" including "search document contents"

Title of WRC Technical Research Document	Authors	Publication Date	Document Type
Influence of phase separator design on the performance of UASB reactors treating municipal wastewater	Dos Santos SL; Chaves S.R.M.; Van Haandel AC	2016/04/29	Water SA Manuscript
Energy from wastewater - A feasibility study technical report	Burton S; Cohen B; Harrison S (Prof); Pather-Elias S; Stafford W; Van Hille R; Von Blottnitz H	2009/07/01	Research Report No.1732/1/09
The use of hydrodynamic disintegration as a means to improve AD of activated sludge	Machnicka A; Grübel K; Suschka J	2009/01/31	Water SA Manuscript
Part 4: Process scale-up in the treatment of mine drainage wastewaters and the disposal of sewage sludge	Neba A; Whittington-Jones KJ; Rose S	2007/09/01	Research Report No.TT 198/07
The influence and mechanism of influent pH on anaerobic co-digestion of sewage sludge and printing and dyeing wastewater	Wang J; Zhang Z-j; Zhang Z-f; Zheng P; Li C-j	2007/07/01	Water SA Manuscript
The evaluation of the anaerobic baffled reactor for sanitation in dense peri-urban settlements (ABR)	Foxon KM; Buckley CA; Brouckaert CJ; Dama P; Mtembu Z; Rodda N; Smith M; Pillay S; Arjun N; Lalbahadur T; Bux F	2006/02/01	Research Report No.1248/1/06
Hydrolysis of primary sewage sludge under methanogenic, acidogenic and sulphate-reducing conditions	Loewenthal RE; Ristow NE; Soteman SW; Wentzel MC; Ekama GA	2005/01/03	Research Report No.1216/1/05
The anaerobic baffled reactor (ABR): An appropriate technology for on-site sanitation	Foxon KM; Pillay S; Lalbahadur T; Rodda N; Holder F; Buckley CA	2004/12/13	Water SA Manuscript
Pre-treatment of urban wastewaters in a hydrolytic upflow digester	Ligero P; Vega A; Soto M	2001/07/01	Water SA Manuscript
Biological sludge stabilisation Part 2: Influence of the composition of waste activated sludge on anaerobic stabilisation	de Souza Araújo L; Catunda PFC; Van Haandel AC	1998/07/01	Water SA Manuscript
Performance and biomass characterisation of an UASB reactor treating domestic wastewater at ambient temperature	Ruiz I; Soto M; Veiga MC; Ligero P; Vega A; Blázquez R	1998/07/01	Water SA Manuscript
An assessment of the effects of the dual co-disposal of phenol and activated sewage sludge with refuse on the refuse anaerobic fermentation and leachate quality	Percival LJ; Senior E	1998/01/01	Water SA Manuscript
Aspects of sewage sludge handling and disposal	Lotter LH; Pitman AR	1997/01/01	Research Report No.316/1/97
The co-disposal of wastewater sludge with refuse in sanitary landfills	Novella PH; Ross WR; Lord GE; Greenhalgh MA; Stow JG; Fawcett KS	1996/01/01	Research Report No.391/1/96
Evaluation and optimisation of dual digestion of sewage sludge Part 3: Evaluation of the technology for practical implementation	de Villiers HA; Messenger JR; Kenmuir K; Laubscher SJA; Ekama GA	1992/01/07	Research Report No.189/4/92

In a recent WRC report, Harrison et al. (2017) used previous WRC reports (Burton et al., 2009; Cloete et al., 2010; Verster et al., 2013) together with information from the Department of Water and Sanitation "Green Drop Report 2014" (DWS SA, 2016) to estimate the total nutrients (carbon, nitrogen and phosphorus) available in municipal wastewater across South Africa. The total volume of municipal wastewater is estimated at 5 000 ML/day. While it is treated at over 800 WWTW across the country, 4 000 ML/day (80%) is treated in the 62 largest plants which each treat over 25 ML/day. The estimated totals for nutrients available from municipal wastewater are shown in Table 2-11.

Table 2-11 Estimated daily average amount of nutrients available in municipal wastewater across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/L)	Estimated Amount Available - All WWTW (t/day)	Estimated Amount Available - 62 Largest WWTW (t/day)
Total Carbon	2 550	12 750	10 200
Total Nitrogen	65	325	260
Total Phosphorus	15	77	62

The biogas production potential of municipal WWTW in South Africa has been extensively researched in a collaboration between Gesellschaft für Internationale Zusammenarbeit (GIZ, German International Cooperation) and South African Local Government Association (SALGA). This was reported as an initial study of nine selected municipalities (Ferry and Giljova, 2015) and, more recently, as a study assessing 130 plants (Gifford and Visser, 2016). The latter identified 87 WWTW with biogas potential, of which 39 were deemed feasible for Combined Heat and Power (CHP) projects (Gifford and Visser, 2016). All except one of those with CHP potential had a plant capacity of > 25 Mℓ/day. By completion of the study, Johannesburg Water had upgraded the anaerobic digesters at their Northern Wastewater Treatment Works and installed a CHP plant to utilise the biogas produced (Franks et al., 2013; Van Der Merwe-Botha et al., 2016).

In the initial GIZ-SALGA study, it was noted that a source of supplementary (solid) organic waste can be used in co-digestion to enhance the feasibility of AD using municipal wastewater sludge as primary feedstock, allowing additional energy generation (Ferry and Giljova, 2015). Since the study was focused on municipal wastewater, supplementary information was collected on municipal solid waste, in particular ascertaining whether there was current separation of the organic fraction and whether separate collection of suitable co-digestate fractions was possible. The Biogas Project List (SABIA, 2014) gives five AD plants in the Western Cape which were co-digesting municipal solid waste organics and sewage.

The municipal solid waste organics fraction can also be used as feedstock for production of biogas (Bolzonella et al., 2006). In the Biogas Project List (SABIA, 2014), two planned projects using AD with municipal solid waste as well as eleven planned and two built landfill gas projects are reported.

2.5.3 Industrial waste as AD feedstock

2.5.3.1 Overview of industrial wastewaters in South Africa

Between 1984 and 1990, the WRC ran the first phase of a project entitled "The National Industrial Water and Wastewater Survey", commonly referred to as NATSURV (http://search.wrc.org.za/srch/#!/). A series of twelve reports on focused industries across South Africa were published: malt brewing, metal finishing, soft drink, dairy, sorghum malt and beer, edible oil, red meat, laundry, poultry, tanning and leather finishing, sugar, paper and pulp. In addition, a similar report on the fruit and vegetable processing industry was published. Reports on the textile and wine industries were published in 1993 and on oil refining and re-refining and power generating in 2005. Revisions of the National Surveys have been compiled over the last four years with sixteen second edition surveys published to date, as well as a survey of the iron and steel industry, not covered in the first project

(http://www.wrc.org.za/?s=NATSURV). These reports contain data on water usage, effluent quantities and quality in the various industries. The new series of reports makes more applicable information available. The issue of stakeholder reluctance to participate was found in both rounds of research. Albeit reduced in the second round, the reluctance still restricts the level of data available.

Three more general inventories in the WRC collection are helpful in assessing the potential of industrial wastewaters for AD. In 2009, a report was published on the feasibility of energy production from wastewater in South Africa (Burton et al., 2009). Also in 2009, an assessment was published evaluating industries for non-point sources of pollution (Heath et al., 2009). In 2010, an inventory was published of water use and effluent production in South African industries (Cloete et al., 2010). In this report, it was noted specifically that data was outdated, being based largely on the original NATSURV reports from over 30 years before. It was also noted that stakeholder participation had been poor.

Between 2015 and 2016, a team at the Centre for Bioprocess Engineering Research at UCT compiled relevant data as part of WRC project WRC2380 to assess the potential across South Africa for the implementation of wastewater biorefineries (Harrison et al., 2017). As far as possible the data was updated and extrapolated using industry-published figures and values such as specific effluent volume and average concentrations reported in research literature.

Considering the industrial sector with the exclusion of mining and power generation, the highest industrial effluent producers by volume are listed in Table 2-12. The pulp and paper industry accounts for 51% and the petroleum industry 31%, by volume. These warrant particular attention as providers of wastewater feedstock for conversion to value through AD and are addressed further, including COD load, in the following sections.

Table 2-12 Proportion of industrial wastewater by industry sector excluding Power Generation and Mining (Cloete et al., 2010)

Industry	Effluent Volume %	Comments
Pulp and Paper	50.5	
Petroleum	31.0	
Food and Beverage	9.5	Animal-based & Plant-based
Other	9.0	Organics-based & Non-organics-based

2.5.3.2 Pulp and Paper Industry

The pulp and paper industry as the producer of the highest volume of industrial wastewater in South Africa is not only large, but also centralised in terms of both ownership (five major owners, and six independent) and plant locations (22 mills in four regions) (Van Der Merwe-Botha et al., 2017). The industry is also demonstrated to be proactive in water saving and in effluent treatment, with reductions in specific water intake, specific effluent volume and average effluent COD load between 1990 and 2017 (Steffen Robertson & Kirsten, 1990; Van Der Merwe-Botha et al., 2017). This makes it an ideal industry for further targeted interventions centred on resource efficiency and water use reduction.

In the NatSurv project focused on pulp and paper (Van Der Merwe-Botha et al., 2017), it was reported that sixteen of twenty-two mills gave treated effluent volumes, totalling 340 000 m^3/d. Of these, ten mills reported average COD for treated effluent which totalled 167 t-COD/d, accounting for approximately half the total volume for the sixteen mills. Treated effluent was variously re-used in the process, used in irrigation, discharged to municipal treatment system or discharged to marine outfall. The sludge generated during treatment was not generally mentioned but uses mentioned included consumption in a multi-fuel boiler, composting after combination with cattle dung, and recycling of dried fibres. At about half of the mills, reference was made to ongoing projects for upgrading of water treatment. The fate of SO_4^{2-}, Cl^- and Na^+ were not reported

The total available nutrients as feedstock is higher than this because the valorisation takes place using untreated effluent. Harrison et al. (2017) estimated the total carbon, nitrogen and phosphorus across the pulp and paper industry as listed in Table 2-13.

Table 2-13 Estimated daily average nutrients available in pulp and paper industry wastewater across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/L)	Estimated Total SA Wastewater Volume ML/d	Estimated Amount Available (t/day)	Comments
Total Carbon	2 850	930	2 650	Lignocellulosic carbon Phenols; chlorinated hydrocarbons; colour compounds adsorbable organic halogen (AOX)
Total Nitrogen	9.04		8.4	
Total Phosphorus	1.30		1.2	

2.5.3.3 Petroleum industry

Like the pulp and paper industry, the petroleum industry is centralised with only six refinery sites in South Africa and owned or co-owned by seven companies. The NATSURV 15 study (Pearce et al., 2005) lists four re-refinery sites. This, together with the large amounts of effluent water and significant organic nutrient inventory, makes this industry important in terms of research for AD. Burton et al (2009) included petrochemical wastewater as a case study for the feasibility of energy from wastewater in South Africa. The petrochemical group Sasol has already developed and piloted an AD process for production of biogas from gas-to-liquid petrochemical effluent (Harrison et al., 2017). Similarly, PetroSA runs an anaerobic digester at its Mossel Bay gas-to-liquids refinery (Burton et al., 2009). Indeed, the feasibility study presented by Burton et al. (2009) noted that the potential for energy from wastewater lies in the synfuel refineries rather than the crude oil refineries. This was further considered in a subsequent WRC project (Ntuli and Brouckaert, 2016). However, consideration of recovery of carbon loading from specific streams within crude oil refineries is mooted as a research possibility. Use of these carbon sources for bioproduct production has been studied through the 1990s.

Harrison et al (2017) estimated the total carbon, nitrogen and phosphorus across the pulp and paper industry as listed in Table 2-14.

Table 2-14 Estimated daily average amount of nutrients available in petroleum refinery wastewater across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/ℓ)	Estimated Total SA Wastewater Volume ML/d	Estimated Amount Available (t/day)	Comments
Total Carbon	23 688	210	5 000	Oils & grease; solvents phenols; sulphides; heavy metals
Total Nitrogen	48		10.1	
Total Phosphorus	1.3		0.3	

2.5.3.4 Food and beverage industry

The food and beverage industry is extremely disparate; however, these multiple specific industries can be grouped. Thus, the primary division is made between animal-based and plant-based industries because the type of organic content in the effluent is very different (Table 2-15 and Table 2-16). Although this has not been done here, one could include the food services commercial sector (restaurants, hotels, hospitals and other food outlets) as the waste from these enterprises is similar to the industries listed here.

Off-the-shelf AD process equipment for simultaneous water treatment and biogas production is readily available from a number of companies, including Talbot and Talbot, Veolia Water Technologies, iBert and others. These are very suitable for small scale use with most waste-based feedstocks available in this industry. There are estimated to be in the order of 400 small to medium scale units in operation across South Africa, but accurate data are not available (Harrison et al., 2017).

One of the features of food and beverages industry is a cycle of wastewater rich in primary organics (untreated food: dissolved, in suspension, as sludge or as pieces) followed by wastewater containing mainly cleaning agents ((Issa-Zacharia et al., 2010; Manzocco et al., 2015; Pavón-Silva et al., 2009). An important aspect of some of the food and beverage industries is seasonality (Carucci et al., 2005; Heaven et al., 2011; Litchfield, 2009); shared with the agricultural sector, this can be challenging in terms of running a system. Other noteworthy factors, depending on the exact industry in question, are the possibility of a high proportion of particulate matter (Bacenetti et al., 2015; Frenkel et al., 2013), the potential presence of fat, oil and grease (FOG) (Klaucans and Sams, 2018; Long et al., 2012) and the possible absence of complex organics (Comelli et al., 2016).

Animal-based food and beverages industries

Animal-based food and beverages industry include red meat abattoirs, poultry abattoirs, fisheries and primary and secondary dairies. All, except fisheries, appear in the NATSURV series. All these industries have wastewaters with high organic carbon content, as well as specific contaminants and valorisation options (Table 2-15). Research on AD for effluent treatment in the dairy industry (Strydom et al., 2001) and the red meat industry (Goosen, 2013; Swanepoel, 2014) in South Africa has been undertaken. AD processes have been installed for treating abattoir effluent (iBert, 2016).

The industries manufacturing processed meat, fish and poultry processed products also require consideration. The South African Meat Processors Association (SAMPA, n.d.) lists 28 members and fourteen associates; however, this is not a comprehensive list with a number of major producers not represented. Information on this industry is not readily available and primary research is most likely necessary.

Table 2-15 Estimated daily average amount of nutrients available in wastewaters of some animal-based food and beverages industries across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/ℓ)	Estimated Total SA Wastewater Volume ML/d	Estimated Amount Available (t/day)	Comments
Red Meat Abattoirs				
Total Carbon	17 000	22	374	FOG, protein fat, viscera, blood, skin, hair, flesh, manure, grit and undigested feed
Total Nitrogen	12.4		0.3	
Total Phosphorus	NA			
Poultry Abattoirs				
Total Carbon	13 200	15	198	FOG, protein fat, viscera, blood, skin, feathers, flesh, manure, grit and undigested feed
Total Nitrogen	175		2.6	
Total Phosphorus	57		0.8	
Fisheries				
Total Carbon	17 400	5	87	FOG, protein scales, flesh, blood, bones brine (sea water)
Total Nitrogen	35		0.2	
Total Phosphorus	NA			
Primary Dairies				
Total Carbon	45 000	237	11 000	fats, protein, sugars manure, grit
Total Nitrogen	350		83	
Total Phosphorus	40		9.5	
Secondary Dairy Processing				
Total Carbon	12 000	12	144	fats, protein, sugars
Total Nitrogen	NA			
Total Phosphorus	NA			

Plant-based food and beverages industries

The plant-based food and beverage industries are even more varied than the animal-based ones, hence some secondary grouping is possible. The raw-food processing industry includes milling of grains and the processing of fruit and vegetables for packaging, freezing and drying. More complex processing is required in the sugar and edible oils industries as well as for the fruit juice and canning industries. Industries which further process plant-based foods include manufacturers of confectionery, snacks and baked goods. Soft drinks manufacturing includes (in addition to fruit juice) bottled water, carbonated drinks, energy drinks and concentrates. Manufacturers of alcoholic beverages are breweries, wineries and spirits distilleries.

Specific research on AD for the treatment of effluent from the brewing industry (Cohen, 2006; Nkadimeng, 2015) and from fruit processing (Strohwald, 1993a) in South Africa has been undertaken. The major breweries in South Africa are already operating AD units, using the energy in the brewing process for steam generation (Nkadimeng, 2015).

Table 2-16 Estimated daily average amount of nutrients available in wastewaters of a few plant-based food and beverages industries across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/ℓ)	Estimated Total SA Wastewater Volume ML/d	Estimated Amount Available (t/day)	Comments
Edible oil				
Total Carbon	400 000	3.5	1 400	Oils
Total Nitrogen	31		0.1	
Total Phosphorus	2 500		8.8	
Canning				
Total Carbon	11 000	3.0	33	Sugars, Carbohydrate
Total Nitrogen				
Total Phosphorus				
Wineries				
Total Carbon	20 400	6.5	133	
Total Nitrogen	110		0.7	
Total Phosphorus	52		0.3	
Breweries				
Total Carbon	12 000	23	276	
Total Nitrogen	52		1.2	
Total Phosphorus	30		0.7	

2.5.3.5 Other organics-based industries

Other organics-based industries include biofuel, cleaning agents, cosmetics, dying and colouring, laundry, paint, pharmaceuticals, plastic, tanning and leather, and textiles. This division of industries is varied and cannot be generalised. These wastewaters may contain highly specific components which can include heavy metals, solvents, enzymes and catalysts, high concentrations of salts, extreme pH, and toxic materials or pathogens (Harrison et al., 2017). Comprehensive data on the number and size of manufacturers in these industries were not available in the previous studies consulted nor through extensive internet searches.

There are WRC documents specifically engaging the use of AD in treating effluent from the printing and dyeing industry (Aoyi et al., 2015; Wang et al., 2007), the textile industry (Aoyi et al., 2015; Barclay and Buckley, 2004) and the manufacture of acetic acid (Strohwald, 1993b). An investigation into the co-digestion of high strength or toxic industrial organic wastewaters with municipal wastewater is of particular interest and included a protocol for evaluation of these effluents (Remigi and Buckley, 2006); it includes assessment of textile and dye manufacturing effluents. Aoyi et al. (2015) also considered heavy metals and pharmaceutical wastes for AD biogas production.

A relatively new industry is the use of microalgae for the generation of biofuels such as biodiesel which produces a 60 – 70% residual biomass waste (Richardson 2009, Ward et al. 2014). The AD treatment with the generation of biogas results in a N- and P-rich digestate which can further be used as fertiliser (Ward et al. 2014, Inglesby et al. 2015). The combination of the liquid biofuel and biogas generation could be cost effective in the current climate.

Table 2-17 Estimated daily average amount of nutrients available in wastewaters of some other organics-based industries across South Africa (Harrison et al., 2017)

	Estimated Average Concentration (mg/L)	Estimated Total SA Wastewater Volume ML/d	Estimated Amount Available (T/day)	Comment
Ethanol production from sugar cane (vinasse)				
Total Carbon	42 000 [1,2]	13 [3]	546	
Total Nitrogen	1 940 [1]		25	
Total Phosphorus	355 [1]		5	
Textiles				
Total Carbon	150 000	82	12 300	dyes
Total Nitrogen	0.1		< 0.1	
Total Phosphorus	6.5		0.5	
Cleaning products				
Total Carbon	160 000	1	160	
Total Nitrogen	8		< 0.1	
Total Phosphorus	18		< 0.1	

1. (SMRI and DST, n.d.)
2. Total Carbon calculated at COD/3 see Harrison et al (2017)
3. (Davis, 2014)

2.5.4 Agricultural waste as AD feedstock

The agricultural sector is an important source of potential feedstock (Rupf et al., 2016) for the purposes of this project because the waste generated is all organic. Although much of the waste is not wastewater *per se*, slurries and even dry biomass can be important as co-digestates. This may be particularly important for distributed rural or small town applications. This sector is less well represented in the WRC literature, possibly because the waste often is not waterborne; however, it features strongly in the general literature (Table 2-9).

Agricultural waste is considered in three categories, which each feature different characteristics: animal husbandry wastes, field crop and orchard residues and forestry residues (Chandra et al., 2012; Steffen et al., 1998).

2.5.4.1 Animal husbandry wastes

Animal husbandry wastes consist largely of manures and the slurries formed when manure is combined with liquid wastes or the wastewater from hose-down cleaning. The AD of manure and manure-slurry is a very well-developed technology at multiple levels, from single household to major industrial units (Rupf et al., 2016).

Not many WRC reports deal with animal slurries as a feedstock for AD and biogas production. Remigi and Buckley (2006) review the literature around use of farm animal manure as a co-digestate in their study of high strength organic effluents. Poulsen et al. (2017) demonstrate the effect of various co-digestion combinations of brewery spent grains, sewage sludge, cow dung and pig manure. A 2014 review of co-digestion in AD for biogas has a whole section on manure-based systems (Mata-Alvarez et al., 2014). One of the appendices in Burton et al. (2009) surveys animal wastes, including feedlots, piggeries and poultry farms, for inherent potential for energy recovery as biogas.

AD units on farms are not uncommon. Recently a biogas-for-CHP unit was installed near Cape Town using piggery waste for AD and supplying power to the cheese factory on the same site (iBert and GIZ, 2017). Another AD facility using feedlot waste supplies power to a nearby car production plant (Cokayne, 2015). Both units feed a fertiliser product back into the agriculture sector. The water component of the digestate is not recovered, but applied with the fertiliser (iBert and GIZ, 2017).

2.5.4.2 Crop residue wastes

A multitude of parent crops producing residues exist; however, globally maize, wheat, rice and sugarcane produce the majority of the biomass in this category (Chandra et al., 2012). In South Africa rice is not cultivated and may be discounted and fruit must be added as a major crop. The United Nations Food and Agriculture Organisation statistics for 2014 for South African crops (FAO UN, 2014) place production of sugarcane and maize as the 18 and 14 million tonnes respectively, with grapes, oranges, potatoes and wheat between 1.75 and 2.25 million tonnes each and soybeans and sunflower seeds at just under 1 million tonnes each.

Two issues must be addressed when assessing the use of crop residues. One is the current value obtained through use of the residue, usually as feed. The second requires assessment of the amount of residue which can be harvested sustainably without causing deterioration in soil properties (Gobin et al., 2011; Gregg and Izaurralde, 2010; Smil, 1999). Further to these, it must be recognised that dry crop residues typically require pre-treatment to liberate the organics (Kumar et al., 2018). It has been shown that such feedstocks are better suited to energy generation by other approaches such as combustion and gasification rather than co-digestion (Dowling 2009). Fruit and vegetable waste are suited to AD.

These agricultural wastes are almost always solid wastes from harvesting, handling and packaging, with the wastewater occurring mainly in the agro-industrial sector during processing. Solid wastes can be important for co-digestion with wastewaters for improved feedstock quality (Anjum et al., 2016; Matuszewska et al., 2016). AD for biogas and production of compost and fertiliser is used, especially in farming-intensive areas, to improve the overall handling of waste for simultaneous environmental enhancement and valorisation (Bouallagui et al., 2005; Chandra et al., 2012).

2.5.4.3 Forestry residue wastes

Forestry residues offer particular problems in AD because the biomass is largely lignocellulosic and thus requires (usually expensive) pre-treatment for effective biogas production. Combustion and gasification are typically better options, if appropriately located (Dowling, 2009). Forestry residues can be grouped together with elements of other waste sources which are largely wood-based. This would include pruning from orchards (crop residues), offcuts and sawdust from sawmills and manufacturing industries using wood (other industrial wastes), and the wood in municipal solid wastes (especially from the building industry, as well as garden and park tree care) (Chandra et al., 2012). These will not be considered further as AD feedstocks in this work.

2.6 The influence of the carbon composition of feedstocks

2.6.1 Carbon complexity in feedstocks

The complexity of the organic carbon in the feed stream strongly affects the first step in the AD process (sections 2.3.1 and 2.4.3). The hydrolysis of complex carbon compounds must take place to form substrate suitable for the acidogenic bacteria which provide the second step in the AD process. Using a single stage reactor, the more complex organic compounds are hydrolysed largely through extracellular enzymes produced by the microbial consortium. However, when these compounds dominate, the hydrolysis step of the AD process can become rate-limiting to the extent of non-feasibility for the entire AD process. Dowling (2009) demonstrated that the impact of reduced biodegradability on energy generation by AD is significant. Ohemeng-Ntiamoah and Datta (2018) have studied the relative effect of the feed concentration of lipids, proteins and carbohydrates on biogas production. Jankowska et al (2017) found that substrate complexity affected the distribution of VFAs produced during acidogenesis.

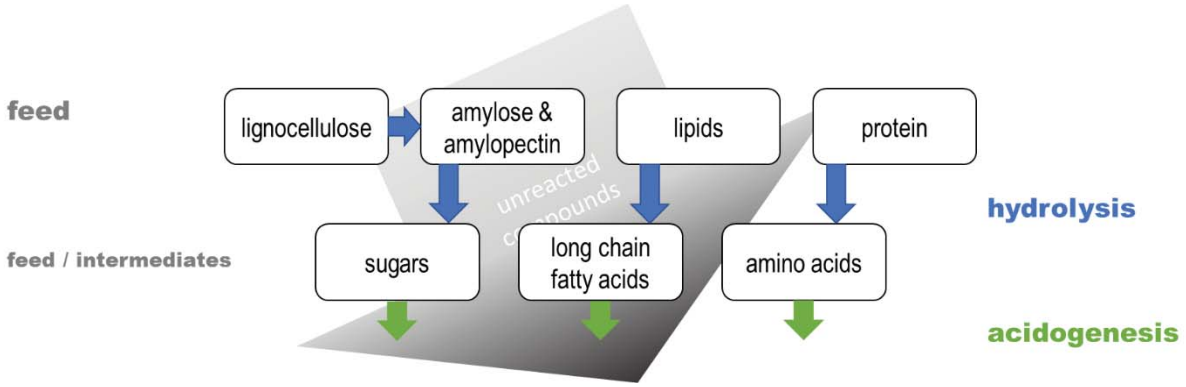


Figure 2-10 The relationship of the complexity of organic compounds to hydrolysis and acidogenesis in AD

The type of carbon compound in the feed interacts with the microbial community in the anaerobic digester, with lignocellulose-, lipid- and protein-rich substrates causing changes in the species prevalence in the consortium of microbes (De Francisci et al., 2015; Wagner et al., 2013).

2.6.2 Lignocellulosic feedstocks

Lignocellulosic biomass is composed of a combination of 35-50% cellulose, 20-35% hemicellulose and 10-20% lignin and these feedstocks are, globally, a large and sustainable carbon source (Feng and Lin, 2017). Lignocellulosic waste streams include those from the pulp and paper industry (Meyer and Edwards, 2014), some biofuel production residues such as vinasse (Feng and Lin, 2017), and most crop and forestry residues (Chandra et al., 2012; Schroyen et al., 2018). A lignocellulosic component of municipal sludge is toilet tissue paper and other sanitary products (Crutchik et al., 2018; Roman et al., 2002).

Research on lignocellulosic biomass has shown the presence of lignin to impact the biomethane potential (BMP) negatively (Carrere et al., 2015). This is because lignin polymers, which are strongly cross-linked and therefore recalcitrant, usually surround the carbohydrate components (Schroyen et al., 2018) However, waste feedstocks where the organic carbon has a high lignocellulosic component are abundant and, as the circular economy becomes increasingly non-negotiable, the need to improve the efficiency of remediation of lignocellulosic wastes has led to the investigation of various methods of degradation.

For AD, the most common route is investigation of pre-treatment for lignocellulosic feedstocks, focused on breaking cell walls and breaking down lignin compounds and polymeric substances (Carrere et al., 2015). Methods proposed include chemical, mechanical, thermal and biological pre-treatment, occasionally with two types of treatment combined (Achinas et al., 2017; Choi et al., 2018; Feng and Lin, 2017; Gagliano et al., 2018; Martín Juárez et al., 2018). Biological pre-treatment methods include enzymatic (Schroyen et al., 2018) and microbial (Barua and Kalamdhad, 2018) approaches. There has also been research into adding supplementary hydrolytic enzymes or microbial cultures in the AD process reactor rather than in a separate pre-treatment stage (Čater et al., 2015; Nzila, 2017; Roman et al., 2002; Yang et al., 2010), as well as the stimulation of enzyme secretion (Fu et al., 2018). One further difficulty posed by lignin-rich feedstocks is that the hydrolysis of lignin via some methods may produce phenols, which are inhibitory to the AD process (Schroyen et al., 2018).

2.6.3 Lipid-rich feedstocks

Lipid-rich waste feedstocks are produced largely in the food processing industry, with the main contributors being abattoirs, edible oil producers and dairies (both primary and secondary) (Cirne et al., 2007). In addition, grease trap waste from the commercial hospitality industry can be a significant source of FOG (Long et al., 2012).

FOG can cause mechanical issues in wastewater systems since it forms hard deposits through chemical reactions and floating aggregations through physical processes (Long et al., 2012; Williams et al., 2018). In some systems it is possible to mechanically recover the FOG in a pure enough form for direct use in other processes (Catarino et al., 2007). This direct valorisation may be advantageous, since FOG may cause clogging in an AD system (Cirne et al., 2007). However, where valorisation through removal is not feasible, use in AD is an attractive option since the BMP of lipids is higher than other organic carbons.

The difficulty is the hydrolysation step in the AD of lipids. Lipids are hydrolysed by lipases excreted by acidogenic bacteria producing LCFAs (Cirne et al., 2007). LCFAs are inhibitory to methanogenesis; however, the exact mechanism is debated, and may include toxic effects on methanogenic bacteria, sludge flotation and washout, and diffusion limitation resulting from adsorption onto other substrates (Cirne et al., 2007; Long et al., 2012).

The high potential conversion to methane coupled with the potential inhibition of the AD process has led to considerable research into co-digestion of lipid-rich wastes with other AD feedstocks, in particular municipal sludge (Cirne et al., 2007; Grosser, 2018; Long et al., 2012; Maragkaki et al., 2018; Ohemeng-Ntiamoah and Datta, 2018). Grosser (2018) demonstrated that the proportion of methane to carbon dioxide in the biogas product is also affected by the presence of lipids in the feed. A further consideration is the interaction around acclimation-inhibition-recovery of the microbial community (Cirne et al., 2007; Long et al., 2012; Williams et al., 2018) which affects how the streams are combined.

2.6.4 Protein-rich feedstocks

Protein-rich feedstocks are produced in all animal-based industries, including all meat, fish and dairy production. Moreover, microalgae cultivated for biogas production is also protein-rich (Mahdy et al., 2017).

It has been found that protein-rich substrates can be difficult in AD because of a lower C/N ratio than other feedstocks (Ács et al., 2013; Mahdy et al., 2017). The high nitrogen content results in the formation of ammonia and ammonium ions, high concentrations of which inhibit the methanogenesis step in the formation of biogas (Ács et al., 2013; Bojti et al., 2017). However, protein-rich feedstocks allow a high methane yield if the challenges can be overcome (Wagner et al., 2013), in addition to being sourced from treatment-imperative waste streams. Further, the high potential of algal cultivation does not compete with food sources.

Therefore, considerable literature can be found around methods of dealing with high-protein substrates in AD for biogas (Tian et al., 2017). Almost all include some level of co-digestion with carbohydrate-rich feed streams. In addition, pre-treatment to remove nitrogen rich urea, continual stripping of the liquid phase or adsorption onto biochar, zeolite or clay to reduce ammonia or ammonium ions and precipitate struvite are all examined (Bojti et al., 2017; Cuetos et al., 2017; Tian et al., 2017). Recently the adaptation of the microbial community to high-ammonium conditions has come to the fore (Ács et al., 2013; Solli et al., 2014; Tian et al., 2017) and some research suggests the benefit of bioaugmentation (Fotidis et al., 2017; Mahdy et al., 2017; Nzila, 2017).

2.6.5 Phenolic feedstocks

Phenol-rich wastewaters are found mostly in the petrochemical industry, or industries which use petrochemicals (Haak et al., 2016; Razo-Flores et al., 2003). However, they do occur in other chemical industries (Poirier et al., 2018) as well as in olive processing wastewaters (Ntougias et al., 2013). Pre-treated lignocellulosic streams frequently contain phenolic compounds (Schroyen et al., 2018).

Phenols are environmentally problematic (Choromański et al., 2016; Maletić et al., 2018; Razo-Flores et al., 2003) and must thus be removed from wastewaters. Phenols (and many petrochemical mixtures) exhibit toxicity to the AD microbial processes (Choromański et al., 2016; Haak et al., 2016; Poirier et al., 2018; Razo-Flores et al., 2003). The usual solution offered for the mitigation of these waste streams

through AD is via co-digestion (Mehryar et al., 2017; Siddique et al., 2015, 2014), although the addition of support media such as zeolites and activated carbon have also been researched (Poirier et al., 2018) as has pre-treatment (Haak et al., 2016). However it has been demonstrated that AD of phenol-containing streams can be effective in a continuous process after an initial adaptation period (Razo-Flores et al., 2003).

2.6.6 Sugar-rich feedstocks

On the AD of simple sugar containing wastewaters, methanogenesis is the limiting reaction. Hence acetogenesis can occur faster than methanogenesis with accumulation of VFAs and accompanying acidification, leading to the inhibition of methanogenesis and AD failure. A potential route to overcome this is two-stage AD.

2.6.7 Co-digestion and the optimisation of feedstocks

The AD process operates across a wide spectrum of substrates; however, several factors may reduce the efficacy of AD for specific feedstocks. As outlined in sections 2.6.1 to 2.6.6, the dominant form of carbon compounds in a feedstock can result in low conversion of the organic component, inhibition of one or more parts of the AD process, or instability of the system (Mata-Alvarez et al., 2014).

2.7 Additional products from AD

The final result of the AD for biogas is two streams: the biogas and the digestate (Figure 2-11), as well as a sludge stream comprised of excess biomass and potentially undigested material. The biogas consists of methane and carbon dioxide, with the proportions dependent on various process factors. If the feed stream is sulphur-bearing, the biogas may also contain hydrogen sulphide (section 2.4.6.2). Depending on the operating conditions, some H_2 may be present, typically in lesser amounts. The digestate in a system using wastewater as feed is water-rich and contains other constituents of potential value. The composition of the digestate is dependent on many factors and it may include recalcitrant compounds from the feed, un-utilised products of hydrolysis, intermediate products of acidogenesis and products of acetogenesis that were not fully converted into methane. Most importantly, the digestate carries un-metabolised N and P components, including ammonia.

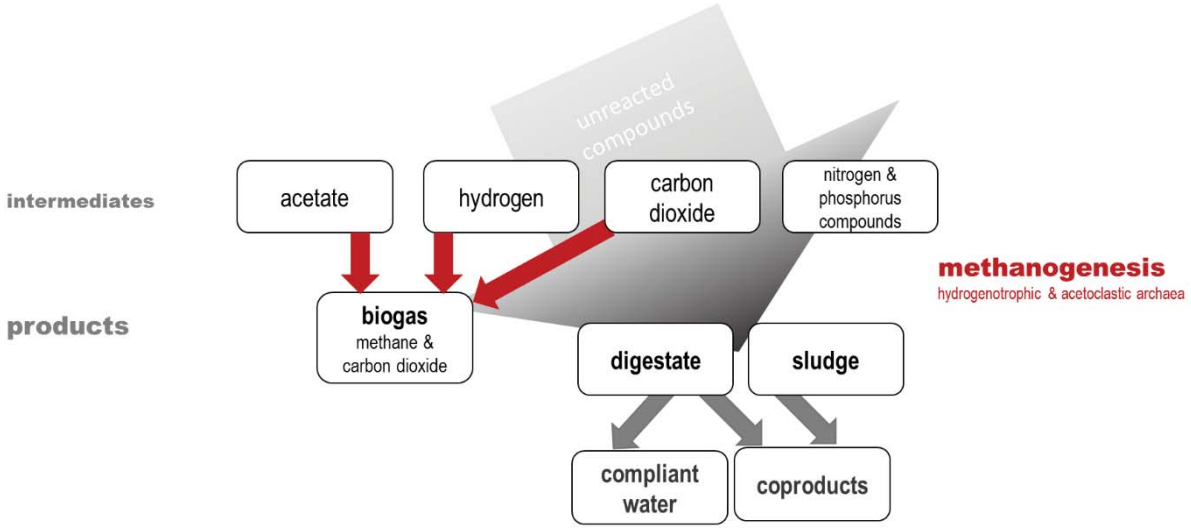


Figure 2-11 Relationship between methanogenesis, biogas and co-products in AD

Increasingly studies are focusing on AD as part of the development of the waste biorefinery, either as the central process (Crutchik et al., 2018; Hagman et al., 2018; Martinez et al., 2016; Sawatdeenarunat et al., 2016; Strong et al., 2016; Richardson, 2011) or as a pre-treatment to liberate VFAs as platform

chemicals or as a downstream process to convert residual carbon based compounds to valuable biogas (Nizami et al., 2017; Raheem et al., 2018; Reis et al., 2017; Schwede et al., 2017; Uggetti et al., 2014; Wang et al., 2015b).

Hagman et al. (2018) conclude that "(their) research suggests that the most interesting, and more impactful, contribution of these solutions lies in their potential for product valorisation and material upcycling". A review of these multiple-product studies suggests a lengthy list of potential co-products, in addition to biogas, from AD. Ultimately, therefore, some form of decision making regarding the various options is needed (Eikelboom et al., 2018).

2.7.1 Biogas valorisation

Biogas is a product with direct applications for heating, that can also be valorised further (Kleerebezem et al., 2015). The options for valorisation reported in the literature are CHP, electricity generation, upgrading to biomethane, use as transportation fuel (Ardolino et al., 2018; Khoshnevisan et al., 2018), and conversion to methanol as a platform chemical (Sawatdeenarunat et al., 2016).

2.7.2 Biofertiliser as product

As can be seen from Figure 2-8, compost is the traditional "co-product" with biogas in AD. Some 10% of AD biogas publications include compost, typically derived from the AD sludge component, in their analysis. In addition, many AD processes produce a digestate which is rich in N and P and hence is suitable for immediate application to land as a liquid biofertiliser. Further, composting as a process is also considered as a pre- or post treatment for AD with lignocellulosic feedstocks (Wagner et al., 2013; Zou and Kang, 2018).

Most studies assume direct application of the digestate, either as a slurry digestate or the separated solids or liquid fraction (Hagman et al., 2018; Tesfamariam et al., 2015; Uggetti et al., 2014). The composition of the digestate has been shown to depend in part on the feed, with co-digestion an important option for optimising digestate as biofertiliser (Kheira et al., 2017). All necessary nutrients have been demonstrated to be present in the effluent from the municipal food waste AD process, with this use of the whole digestate being an improvement on more usual disposal methods (Paul et al., 2018). The overall feasibility of an AD for biogas and compost system has been demonstrated (Ranieri et al., 2018); however, an LCA (Neri et al., 2018) has suggested that sustainability depends on the geographical proximity of the various components of the life cycle.

A number of studies go further than quantifying the composition of the digestate as potential biofertiliser, to investigating the actual effect on the soil, especially in comparison with the use of undigested manure. Some studies suggest the importance of co-digestion for increasing the effectiveness of digestate as biofertiliser (Muscolo et al., 2017; Myburgh and Howell, 2014; Venanzi et al., 2018). It is also apparent that the comparative effectiveness of AD digestate and undigested manure used as biofertiliser varies with the soil type (Rigby and Smith, 2013; Risberg et al., 2017). Other comparisons have been made, with leaching of selected metals shown to be less (Dragicevic et al., 2017) and nitrogen-compound emission higher (Nicholson et al., 2018; Thomas and Hao, 2017) with digestate than with undigested composts or manures.

Tilvikienė (2018) demonstrated an increase biomass yield and quality over a five year period, fertilising with AD digestate relative to using inorganic fertilisers. While Lin (2018) showed that, for large scale centralised treatment, AD for biogas and biofertiliser offers an improvement in sustainability over straight composting. The social impact of widespread small scale AD for biogas and compost was assessed to be positive in India (Sfez et al., 2017). However, Arthurson (2008) sounds a warning with regard to using digestate from AD of sewage sludge because of possible pathogen recalcitrance and possible presence of heavy metals.

In addition to the more general consideration of AD digestate for direct use as biofertiliser, several researchers consider the option of post treatment of the digestate slurry using a composting process (Cucina et al., 2017; Czekała et al., 2018; Sawatdeenarunat et al., 2016; Wang et al., 2017).

2.7.3 VFAs as product

Within the complexity of the AD process, VFAs form a crucial intermediate which can also be exploited as a co-product with biogas (Kleerebezem et al., 2015; Zacharof and Lovitt, 2013; Zhou et al., 2018) or may be selected as the more major product over biogas. The technoeconomic potential of balancing production of biogas and VFAs during remediation of wastewater is considered as one of the focal points of this project. VFAs can be viewed as a product stream or intermediate product stream from this process or an intermediate within the methane production process. These VFAs may be used either as final product or as an intermediate forming feedstock to a second process e.g., for platform chemicals, feedstock for polymer production (de Kreuk et al., 2007; Lin et al., 2010; van Loosdrecht and Brdjanovi, 2014) or feedstock for biological sulphate reduction as part of Acid mine drainage (AMD) remediation (Van Hille et al., 2015), Where VFAs are used as product of the AD process, acetogenesis is allowed to be incomplete, with VFA-COD in the digestate recovered in downstream processing (Chapter 4). Khan et al (2016) compare using the full AD process for production for methane with using AD to produce only the “intermediates” VFAs and hydrogen gas concluding that the latter is an improvement over the former in terms of economics, society and environment.

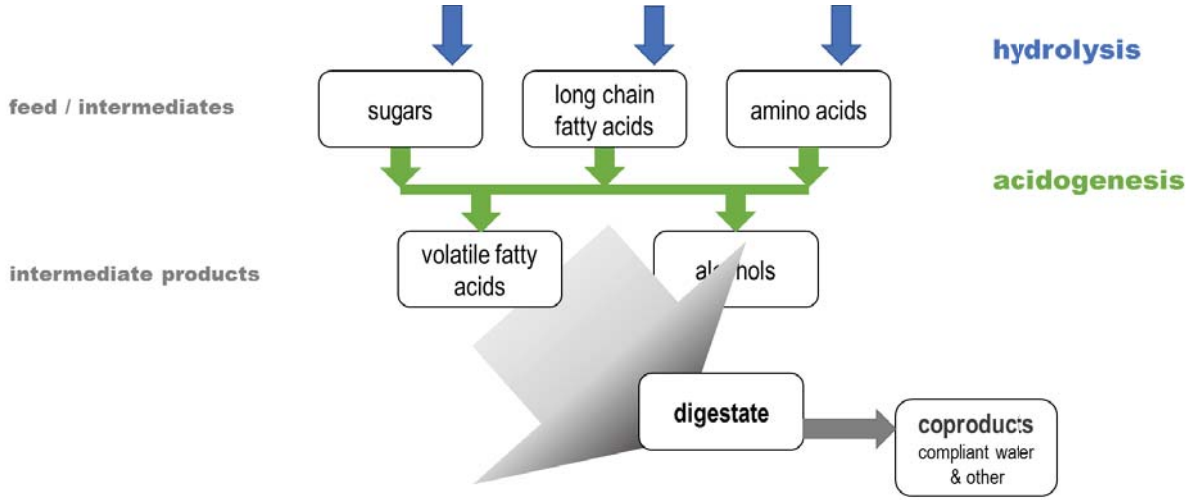


Figure 2-12 VFAs as intermediate product: from acidogenesis

Kleerebezem et al. (2015) reviewed the concept of a two-stage AD, with the first stage focused on VFA production and the second on biogas production. They note that research into selective production of organic acids from various feedstocks is growing and identify key issues which need to be resolved:

- Product spectrum – for separation of the two process stages and control of which VFA dominates the first stage
- Reactor development for adequate use of wastewater as feed
- Bioprocess optimisation – pre-treatment for some feedstocks and optimal conditions for acidogenesis
- Valorisation – downstream recovery of VFAs and product utilisation route

Zhou et al (2018) specifically review the metabolic pathways which have been identified in acidogenic fermentation, summarising seven pathways and some of the associated microbial acidogens. In order to improve VFA production, they specify the need for improvement of the hydrolysis step or careful choice of substrate, promotion of acidogenesis through optimisation of process conditions and removal inhibitors, notably the product itself.

Choice of substrate for VFA production

Jankowska et al. (2017) investigated the impact of substrate complexity on VFA production and distribution with varied pH and retention times, using maize silage, cheese whey, microalgal biomass and glucose solution. Their results suggest that process conditions are more important for generation of VFAs than the substrate complexity.

Pre-treatment for improved VFA production

There are a number of studies of the efficacy of various pre-treatment options for enhanced VFA production in AD. Yin et al. (2014) report successful hydrothermal pre-treatment of food waste with an optimal temperature of 160 °C; however, Shen et al. (2017) found hydrothermal pre-treatment ineffective for protein-rich substrates. Alkaline (Janke et al., 2016), thermal-alkaline (Liu et al., 2016), and thermal-acidic (Kuruti et al., 2017) pre-treatment were all effective in raising VFA yield with different substrates. Liu et al. (2016) demonstrate successful use of protease/EDTA-2Na hydrolysis with waste activated sludge.

Optimisation of process conditions for VFA production

Multiple studies reseach process conditions for VFA production in AD. Sawatdeenarat et al. (2017) achieved an increase of VFA production during AD of cattle manure by choice of inoculum and combined adjustment of micro-oxygenation and incubation time. In temperature studies, Gruhn et al. (2016) found that mesophilic conditions were more favourable than thermophilic conditions for VFA production on AD of microalgal biomass. However, Hao et al. (2015) found improved VFA production under thermophilic conditions on AD of municipal sludge, while Jiang et al. (2013) reported a negligible improvement at 45°C, over 35°C.

Process conditions also affect the distribution of VFAs, with these influenced by both pH control (Begum et al., 2018; Hussain et al., 2017; Jankowska et al., 2017) and OLR (Cavinato et al., 2017; Jiang et al., 2013). OLR affects both the stability of the AD process and the rate of production of VFAs with VFA production increasing with increasing OLR up to a point beyond which a higher volumetric rate, impacted by the microbial activity available for AD, cannot be sustained (Begum et al., 2018; Cavinato et al., 2017; Jiang et al., 2013) without *in situ* recovery. Jankowska et al. (2017) showed that VFA production can be optimised without costly adjustment of pH by careful consideration of substrates used.

There is increasing interest in two-stage AD with reactors configured for different operating conditions for acidogenesis and methanogenesis (Bharathiraja et al., 2018; Stoyanova et al., 2017; Yuan and Zhu, 2016) in order to reduce the inhibition of the methanogenesis phase of the AD process by VFA accumulation. Although Lindner et al. (2016) conclude that the two-stage process is only economic for methane production with simple carbohydrate substrates, their conclusion may be reversed with the possibility of partial recovery of VFA product from the first stage and the methane product from the second stage (Cavinato et al., 2017; Crutchik et al., 2018; Martinez et al., 2016; Peces et al., 2016).

DSP for VFA recovery

Zacharof and Lovitt (2013), in considering complex waste streams as a source of VFAs, review the literature on VFA extraction. Singhania et al. (2013) remark that separation of VFA from the AD liquid is a major barrier to use of this technology and they review a similar list of recovery technologies. López-Garzón and Straathof (2014) in their review of recovery methods include recovery of specific organic acids, noting that for commercial application compounds may need purification after extraction.

The various options for recovery of the VFAs which these reviews suggest receive specific attention from other researchers: liquid-liquid extraction (Andersen et al., 2016; Reyhanitash et al., 2016; Rocha et al., 2017; Yin et al., 2014), reactive extraction (Prochaska et al., 2014), distillation and evaporation, esterification (Andersen et al., 2016), adsorption (Da Silva and Miranda, 2013; Li et al., 2015; Rebecchi et al., 2016), precipitation and crystallisation, membrane processes (Aydin et al., 2018; Trad et al., 2015;

Tugtas, 2014), electrodialysis (Jones et al., 2015; Prochaska et al., 2014; Scoma et al., 2016; Zhang et al., 2015), reverse osmosis, gas stripping (Garrett et al., 2014; Li et al., 2015), and microfiltration, ultrafiltration or nanofiltration (Prochaska et al., 2014; Zacharof and Lovitt, 2014). The last of these receives the most attention from Zacharof and Lovitt (2013, 2014) and they demonstrate that the cost of AD and filtration is considerably lower than the cost of producing medium suitable for VFA production (Zacharof and Lovitt, 2014). Pervaporation for recovery of VFAs is reported by van Baelen et al (2005) using a membrane with a combination of liquid sorption, diffusion and desorption into the gas phase.

DSP for VFA recovery is fully explored in Chapter 4.

2.7.4 Nitrogen and phosphorus as products

Acetogenesis and methanogenesis utilise the intermediate organic products in the AD process, hence the residual liquid phase of the digestate is often rich in ammonium ions and phosphates (Sawatdeenarunat et al., 2016; Uggetti et al., 2014). This limits both the release potential and the resource recovery from the digestate unless due care is taken and appropriate uses considered. Depending on the nutrient concentrations, the digestate may be used directly as a nutrient source, provided through irrigation (Dube et al., 2016; García-González et al., 2016; Sawatdeenarunat et al., 2016); however, at high concentrations this may negatively impact soil quality through over-supply of nutrients or salinisation. One suggestion is to use the nitrogen and phosphorus-rich digestate (Monfet et al., 2017; Sawatdeenarunat et al., 2016; Uggetti et al., 2014) as a substrate in microalgae production, choosing a suitable algal species for utilisation of the high nutrient concentrations (section 2.7.5). Zhang and Ogden (2017) and Richardson (2011) suggest nutrient recovery from an algal-based AD for recycle to algal growth. These nutrients can be recovered directly as N and P compounds, thus balancing the nutrients in the remaining digestate (Cantrell et al., 2012), although the economic scale-up of the technologies may be challenging (Raheem et al., 2018) and the relative valorisation presented by different options must be considered.

Monfet et al. (2017) review the technologies available for phosphorus and nitrogen removal and recovery in AD effluent, while Krakat et al. (2017) concentrate on ammonia removal and Sikosana et al. (2016) tabulate phosphorus removal and recovery technologies. Cantrell et al. (2012) look at nitrogen and phosphorus recovery from a “green farming” perspective using an industrial ecology basis. These reviews (Krakat et al., 2017; Monfet et al., 2017; Raheem et al., 2018; Sikosana et al., 2016) consider the following recovery techniques for nitrogen, phosphorus or both: nanofiltration, reverse osmosis, forward osmosis, ion exchange, electrodialysis, membranes, precipitation and crystallisation, ozonation, chemical and electrical oxidation, adsorption and biosorption, thermal extraction from sludge ash, sonication and microwave, air stripping and steam stripping, hollow fibre membranes and microbial fuel cells.

Phosphorus recovery is partly driven by the desire to close the phosphorus cycle since phosphorus is a non-renewable resource (Raheem et al., 2018; Wang et al., 2015b). Most studies consider the recovery of calcium phosphate or struvite (Crutchik et al., 2018; Raheem et al., 2018; Sikosana et al., 2016), with the latter combining P and N recovery.

Nitrogen recovery is mostly as ammonia or an ammonium salt since the AD process produces ammonium ions (Figure 2-9) which are inhibitory to methanogenesis (Krakat et al., 2017; Yuan and Zhu, 2016). Air stripping of ammonia (Jiang et al., 2014; Zhao et al., 2015) is a commonly investigated recovery method, demonstrated to be straightforward and cost effective. Several researchers (García-González et al., 2016; Gerardo et al., 2013) also present membrane-based ammonia recovery, while Cerrillo et al. (2018) investigate a novel integrated system using microbial electrolysis.

The options for value-added products through nitrogen and phosphorus recovery are central to the focus of Chapter 4.

2.7.5 Fit-for-use water as the non-negotiable product

This project is centred around remediation of wastewater streams and regenerating the water in the process as a product. This is particularly important in water scarce environments and in closing the water cycle in processes; however, attaining compliant water quality is essential in all environments prior to return to the natural environment. Despite this, from an assessment of the literature, it is clear that consideration of water as a product or outgoing stream of importance is seldom included and is an area needing attention. Khan et al (2011) review the use of UASB for treatment of municipal wastewater sludge favourably for discharge of compliant water. However, in evaluating the options for the necessary post treatment, they comment that "most of these upcoming post treatment systems lack in scale-up studies and implementation on full-scale" (Khan et al., 2011) and conclude that adaptation for developing countries which favour low cost treatment options is necessary. In considering typical of studies of UASB technology for waste(water) valorisation, Kleerebezem et al. (2015) look at biogas and VFAs as products, but ignore the potential value of reusable water. Wang et al. (2015b), in a study evaluating resource recovery in wastewater treatment, do not consider the water as a resource for potential recovery.

In studies which specifically focus on DSP for water quality from AD effluent, focus tends to be on water as compliant for environmental discharge, rather than as a product for re-use. Liu et al (2015) report the development of a system using electro-coagulation for simultaneous reclamation of water and upgrading of biogas. In Ethiopia, a pilot scale system including an aerobic reactor and constructed wetlands (Alemu et al., 2016) after the anaerobic digestor producing biogas from tannery wastewater, again, was evaluated for quality of water for release, not re-use. In India, Tyagi and colleagues (Prakash et al., 2007; Tyagi et al., 2010) investigated flocculation of AD effluent, but only for water-for-discharge. The WRC publication by Swartz et al. (2014) reports eighteen South African water authorities reclaiming water for re-use rather than discharge, with five of these specifying potable water. This report does not consider the biorefinery approach of multiple products and it is not known whether any of these reclaim water after AD.

In Chapter 4 the technologies for valorising the water contained in the digestate after biogas production are evaluated.

2.7.6 Other potential products

In addition to the products of interest in this project, a number of other potential co-products for the waste-based AD for biogas biorefinery are presented in the literature (Hagman et al., 2018; Raheem et al., 2018; Sawatdeenarunat et al., 2016; Strong et al., 2016).

Co-products may involve recovery of otherwise inhibitory or pollutant compounds present because of the nature of the particular waste feedstock. These include sulphur compounds (Khan et al., 2011; Yuan and Zhu, 2016) and metals (Gerardo et al., 2013), as well as pre-treatment for recovery of phenols (Martinez et al., 2016; Serrano et al., 2017). Strong et al. (2016) and Harrison et al. (2017) suggest that careful selection of the microbial culture could produce a number of products directly (single-cell protein, biopolymers, ectoine and lipids among others) which would otherwise only be produced in a downstream process. Other products mentioned essentially require downstream valorisation of one of the primary products or by-products. Thus the digestate, in part or whole, can be used as a substrate for microalgae, or other microbial biomass, which are then upcycled for any of a number of products (Richardson, 2009; Ledda et al., 2016, 2015; Uggetti et al., 2014; Yan and Zheng, 2013). The liquid fraction of the digestate may be used in a microbial fuel cell (Abourached et al., 2014). Enzymes could be produced as pre-treatment for AD or as valorisation of digestate (Pletschke et al., 2016; Roman et al., 2002). The solid fraction or sludge, instead of direct use as compost, can be used for valorisation by recovering or producing animal feed, cellulose products, bio-oil, biochar for example.. (Awedem Wobiwo et al., 2017; Monlau et al., 2015; Nizami et al., 2017; Pelaez-Samaniego et al., 2017; Sawatdeenarunat et al., 2016).

2.8 Value addition with AD of wastewater

AD is a mature technology with a considerable body of academic research and numerous industrial technologies and operators. The existing operations producing biogas cover the full range of solid waste, waste slurries and wastewater as feedstock coming from agricultural, industrial and municipal sources. AD for biogas is operational at all scales, from major city wastewater down to household level. A number of major units are operating in South Africa with different financial models and from several technology providers.

The factors influencing the outcomes of AD are well-researched and readily applied to predicting the factors which may need optimisation in specific situations. However, the application of AD as the core process in a waste biorefinery for multiple value-added products is relatively new and under-researched. The anticipated trade-off in optimising productivity for biogas, VFAs, nutrients, fit for purpose water, including the DSP needed for recovery of each, is key to this project.

3 IMPACT OF OLR ON YIELD, PRODUCTIVITY AND DISTRIBUTION OF CARBON TO METHANE AND VFAS

AD is traditionally used as a waste treatment process with key focus on water quality. In fact, many industrial plants utilising AD for treatment of their waste flare is the biogas produced (Kleerebezem et al., 2015). This is because the advantages gained through treatment of the waste are enough to justify the installation of a digester, whereas the value addition of the biogas has not been sufficiently economically attractive or sufficiently assessed to justify the inclusion of systems to capture and use it. Further, resource efficiency has not been considered when water treatment is a central focus. The acidogenic part of the AD process may present an alternative or additional means of valorisation for carbon-contaminated waste streams to form products of higher value than biogas. Further, on including the maximising of resource productivity as a key focal area, in addition to the focus on water quality, the trade-off between rate and yield of biogas production becomes an important consideration.

Carbon, measured as COD is typically only used in AD processes for biomass growth, sulphate reduction and methane production. The consortium of microbes in AD processes grow very slowly, with only 5-10 % of COD degraded being used for biomass growth (Angelidaki et al., 2003a; Angelidaki and Sanders, 2004; Speece, 1983). Therefore, the removal of influent COD is achieved almost exclusively by its conversion to biogas, provided the sulphate concentration of the feed is not significant. This results in high CH_4 yields for successful treatment processes.

There is also potential for AD processes to be operated at higher OLRs such that complete COD removal and high CH_4 yields are not achieved, but CH_4 productivity is increased resulting in decreased capital costs due to smaller reactors (Speece, 1983). Where energy production is the focus of the AD process, maximising CH_4 productivity is key and this approach deserves further exploration to assess approaches to maximise resource efficiency. As discussed in this chapter, operation at high OLR may also result in the accumulation of VFA in the effluent stream. Alternatively, operational conditions can be selected to favour VFA production over CH_4. These may present a valuable carbon source for valorisation or bioremediation processes. This scheme may present an AD process in which space time productivity of CH_4 is maximised and the VFAs produced make an economic contribution.

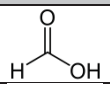
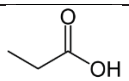
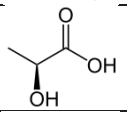
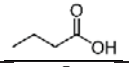
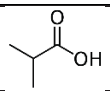
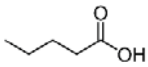
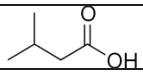
The information presented in this chapter firstly elaborates on acidogenesis in its own right (section 3.1). Thereafter, the impact of OLR on biogas yield and productivity is reviewed with associated consideration of the partitioning of the feed carbon to methane, CO_2 and VFAs (section 3.2). Finally, an experimental study is presented in which the impact of OLR on the operation of AD is investigated in terms of methane yield, productivity and potential to provide methane, VFA or both as products, with potential to manipulate their relative abundance and space time utilisation in the reactor (sections 3.3 and 3.4).

3.1 AD for production of VFAs

3.1.1 VFA as metabolic intermediates

VFAs are carboxylic acids with an aliphatic tail of five or less carbon atoms, as shown in Table 3-1. They form the main soluble metabolic compounds generated through the AD process (Zacharof and Lovitt, 2013). Lactic acid is not usually included under the definition of VFA due to its extra hydroxyl group, but it has been included here due to its close relationship to VFAs and its role as an AD process intermediate.

Table 3-1 List of VFAs and their chemical and structural formulae

No. of C atoms	Common name	Systematic name	Common name of conjugate base	Structural formula	Diagram
1	Formic acid	Methanoic acid	Formate	HCOOH	
2	Acetic acid	Ethanoic acid	Acetate	CH3COOH	
3	Propionic acid	Propanoic acid	Propionate	CH3CH2COOH	
3	Lactic acid	2-Hydroxypropanoic acid	Lactate	CH_3CHCO_2H	
4	Butyric acid	Butanoic acid	Butyrate	CH3(CH2)2COOH	
4	IsobutyricIso-butyric acid	2-Methylpropanoic acid	Isobutyrate	(CH3)2CHCOOH	
5	Valeric acid	Pentanoic acid	Valerate	CH3(CH2)3COOH	
5	Iso-valeric acid	3-Methylbutanoic acid	Isovalerate	(CH3)2CHCH2COOH	

VFAs can be used as a carbon source and hydrogen donor for other biological processes, including for the removal of pollutants through bioremediation, microbial fuel cells and production of biodegradable plastics such as PHA, PHB and PLA (Lee et al., 2014; Zacharof and Lovitt, 2013) as well as platform chemicals. VFAs represent an attractive alternative to CH_4 owing to their economic value and their derivatives. The relative values of some common energy-carrying organic compounds and platform chemicals are listed in Table 3-2 for context. The global market sizes and prices of VFAs are shown in Table 3-3. As can be seen, higher order VFAs and their derivatives hexanoic acid and PHB are worth up to an OOM more than CH_4 by mass. These VFA markets are currently supplied by the petroleum industry (Lee et al., 2014).

Table 3-2 The http://www.wrc.org.za/?s=NATSURV of organic energy carriers and platform chemicals (Kleerebezem et al., 2015)

Organic compound	Chemical formula	Price (€/tonne)	Price (US$/tonne*)	Price (US$/tonne-C*)
Coal	C	0.05	50	50
Methane (US June 2013)	CH_4	0.20	210	160
Methane (Europe June 2013)	CH_4	0.40	420	320
Oil (June 2013)	CH_2	0.64	68	60
Hydrogen	H_2	2.0	2 100	-
Sugar (June 2013)	$C_6H_{12}O_6$	0.28	300	63
Ethanol (2013)	C_2H_6O	0.52	550	290
Hexanoic acid	$C_6H_{12}O_2$	1.00	1 060	660
PHB	CH_3O	2.00	2 120	820
*Prices were converted to US$/ton using an exchange rate of 1.06 US$/€ (X-RatesTM, 2015)				

Table 3-3 Global production rates and prices of VFA (Zacharof and Lovitt, 2013)

VFA	Chemical formula	Market size (ton/year)	Price (US$/ton)	Price (US$/tonne-C)
Formic	HCOOH	30 000	800 - 1200	210 - 310
Acetic	CH_3COOH	6 500 000	400 - 800	160 - 320
Propionic	CH_3CH_2COOH	180 000	1 500 - 1 650	730 - 800
Butyric	$CH_3(CH_2)_2COOH$	30 000	2 000 - 2 500	1090 - 1360
Lactic	$CH_3CHOHCOOH$	120 000	1 000 - 1 800	400 - 720

However, the anaerobic production of VFA has some disadvantages compared to conventional AD for CH_4 production. Firstly, CH_4 is a sparingly soluble gas separating naturally from the liquid and solid reactor contents (Kleerebezem et al., 2015) making downstream processing costs negligible. Secondly, the presence of VFAs in the reactor effluent implies that the downstream process is required to remove or utilise the VFAs to ensure depletion of COD in the final outgoing water stream. Lastly, the use of waste streams for the production of VFAs or VFAs-derivatives limits the independent scalability of the process and the implementation of a generalised process flowsheet. In application of the waste stream as a feedstock for local niche products and markets, the demand for products must be matched to the supply (production) potential of the waste stream to make the process practical (Kleerebezem et al., 2015).

3.1.2 Production and recovery of VFA

VFAs are produced through AD by selecting environmental conditions that exclude the acetogenesis and methanogenesis stages or by adding methanogenic-specific inhibitors or a combination (Wang et al., 2014). This ensures that the VFAs produced through hydrolysis and subsequent acidogenesis are not (completely) consumed (Lee et al., 2014; Zacharof and Lovitt, 2013). Because methanogenesis is rate-limiting when simple substrates are used, increasing the OLR results in accumulation of VFAs due to methanogens not being able to keep up with the rate of acidogenesis (Rajeshwari et al., 2000). VFA concentrations as high as 29 g-COD/L have been reported using a semicontinuous process (Lee et al., 2014). Concentrations of VFAs found in conventional biogas AD processes usually vary between 0.1 g-COD/L and 5 g-COD/L(for an 'overloaded' process) (Nges and Liu, 2010).

Few studies have looked at recovering the VFAs accumulating in biogas AD processes (Zacharof and Lovitt, 2013). The liquid effluent from anaerobic VFA production processes has been used as a feed for biological nutrient removal with success (Zheng et al., 2010). According to Zacharof and Lovitt (2013), the use of nanofilters offers the greatest potential for separating relatively concentrated VFA from the AD effluent since neither ultrafiltration nor reverse osmosis can separate salts from small organic molecules (Zacharof and Lovitt, 2013), although anion exchange and liquid-liquid extraction are other options (Kleerebezem et al., 2015). The processing of the AD liquid-solid stream, with associated potential for VFA recovery, is considered in Chapter 4. There also exists the potential to use the VFA-rich effluent stream directly for the production of PHA or medium chain fatty acids, which are more easily recovered than the VFA themselves (Kleerebezem et al., 2015).

3.1.3 Control of the acidogenesis product spectrum

Downstream processing options for the VFAs produced from acidogenesis are sensitive to the spectrum of AD products formed (Kleerebezem et al., 2015). Acidogenesis products from carbohydrates include VFAs, medium chain fatty acids (MCFAs), lactate, alcohols, H_2 and CO_2 (Kleerebezem et al., 2015). Environmental and operational parameters such as pH, temperature, retention time, substrate composition, VFA concentration and mode of operation all affect the spectrum of acidogenesis products (Kleerebezem et al., 2015).

It has been observed that at moderately acidic pH values of pH 4.5 – 7 and mesophilic temperatures (25-40°C), acidogenesis of glucose produces mixtures of acetate and butyrate typical of *Clostridium*

fermentations (Kleerebezem et al., 2015). Acidogenesis of potato starch at a pH of 3.9 was found to select for *Lactobacillus* strains which resulted in lactate dominating the VFA spectrum (Kleerebezem et al., 2015). Although Lee et al. (2014) claim that temperature has no effect on the relative proportions of the various VFAs, higher pH values (7 – 8.5) and thermophilic temperatures have been noticed to result in ethanol being the dominant product (Kleerebezem et al., 2015). Investigations into the pH-dependency of the VFA spectrum so far suggest that, even at a single pH, different VFAs form based on the feedstock used (Lee et al., 2014).

First attempts to understand the distribution of VFA from acidogenesis considered the hydrogen partial pressure as defining the oxidation state of the electron carrier NADH/NAD, which determined which VFAs were produced (Mosey, 1983). However, experimental evidence suggested the presence of other electron carriers (Kleerebezem et al., 2015). The present state of modelling of the VFA product spectrum has been expanded to include the transfer of electrons through electron bifurcation, as well as product-forming metabolic pathways and transport considerations (Kleerebezem et al., 2015). The results of experimental and modelling efforts show that not all the mechanisms by which these products are formed have been discovered.

3.2 Influence of OLR on production of CH_4 and VFA in AD

3.2.1 Productivity and yield of CH_4 and VFA

The productivity of CH_4 is defined as the volume of CH_4 produced per volume of reactor per day as shown by Equation 3-1:

$$Productivity = \frac{V_{CH_4}\ (Nm^3_{CH4})}{V\ (m^3_{rctr}) \cdot day} \qquad \text{Equation 3-1}$$

where V_{CH4} is the volume of CH_4 produced (measured in Nm^3_{CH4}), adjusted to STP conditions and V is the working volume of the reactor (m^3_{rctr}).

The yield of CH_4 is defined as the volume of biogas produced per mass of COD (or VS) fed to the reactor as shown by Equation 3-2:

$$Yield = \frac{Nm^3_{CH4}}{kgCOD_{feed}} \qquad \text{Equation 3-2}$$

These can readily be converted to a mass basis. Similarly, the productivity and yield of VFA are defined as the mass of VFA produced per volume of reactor per day and the mass of VFA produced per mass of COD (or VS), respectively.

Because the masses of VFA and CH_4 produced can also be converted to their equivalent CODs (COD_{VFA} and COD_{CH4} respectively), one can also determine the recovery of influent COD to VFA and CH_4. To convert concentrations of VFA and CH_4 to their COD equivalents, the values in Table 3-4 are used. These conversion factors can be generated by balancing a combustion reaction between each compound and determining the mass of oxygen required per mass of compound.

Table 3-4 Conversion factors used to determine COD_{VFA} and COD_{CH4} from VFA and CH_4 concentration and volume respectively

Compound	Conversion factor for [product] to COD (g-COD/g)
Acetic acid	1.067
Propionic acid	1.514
Butyric acid	1.818
Valeric acid	2.039
Methane	2.857 kgcod/Nm^3_{CH4}

To determine the recovery of influent COD to CH_4, the productivity of CH_4 is converted to productivity of COD_{CH4} and divided by the OLR according to Equation 3-3 (units are included in brackets).

$$Recovery_{COD_{CH4}} = \frac{V_{CH_4}\ (Nm^3_{CH4})}{V\ (m^3_{rctr} \cdot day)} \cdot \frac{2.857 kg\text{-}COD}{Nm^3_{CH4}} \cdot \frac{1\ (m^3_{rctr} \cdot day)}{OLR\ (kg\text{-}COD)} = \left(\frac{kg\text{-}COD_{CH4}}{kg\text{-}COD_{fed}}\right) \quad \text{Equation 3-3}$$

To determine the recovery of influent COD to VFAs, the effluent VFAs are converted to their concentration in terms of COD using the conversion factors in Table 3-4. These COD_{VFA} values are then summed, with the summed value finally being divided by the influent COD concentration as shown in Equation 3-4.

$$Recovery_{COD_{VFA}} = \frac{\sum COD_{VFA}(kg\text{-}COD_{VFA}/L)}{kgCOD_{influent}(g\text{-}COD/L)} = \left(\frac{COD_{VFA}}{COD_{influent}}\right) \quad \text{Equation 3-4}$$

There is a clear negative correlation between the HRT and the productivity of CH_4 (Şentürk et al., 2010). This is because the HRT is the parameter with the largest influence on the availability of reactants and intermediate products of the process (Zacharof and Lovitt, 2013). Decreasing the HRT at a constant feed substrate concentration result in an increased OLR, which represents an increased quantity of biodegradable substrate fed to the reactor. If the feedstock comprises mainly easily biodegradable components, this leads to increased concentrations of process intermediates, especially VFAs, since methanogenesis is rate-limiting where hydrolysis is not necessary (Mao et al., 2015; Nges and Liu, 2009; Zacharof and Lovitt, 2013). In turn, this increases the rate of methanogenic activity or the cell concentration in the reactor or both, resulting in increased CH_4 productivity in accordance with Monod kinetics used in AD modelling (Batstone et al., 2002). However, in well-mixed reactors, increased concentrations of intermediates result in increased concentrations of these compounds in the liquid effluent stream and a decrease in the CH_4 yield attainable from the influent substrate.

At low HRTs, the yield of VFA is typically increased, provided the substrate carbon contains a significant fraction of easily biodegradable compounds, owing to the limitation in rate of methanogenesis leading to not all these acids are being converted to biogas (Zacharof and Lovitt, 2013). In addition to this, the VFA productivity is higher due to the combination of increased VFA concentration and volumetric flow rate. As mentioned in Section 2.4.6.5, the presence of VFA also inhibits methanogenesis, hence this trend of increased CH_4 productivity is only observed up until the point at which the accumulated VFA concentration becomes inhibiting (Nges and Liu, 2010). These effects are substantiated by studies performed on the effect of HRT or OLR on the AD process, some of the results of which are documented in Appendix B (Jiménez et al., 2003; Lettinga et al., 1980; Nges and Liu, 2010; Salminen and Rintala, 2002).

However, the coupling of increased productivity of VFA with decreased HRT holds provided the rate at which organic matter is fed is also increased i.e., the OLR increases and washout does not occur.

3.2.2 Previous studies on the influence of OLR in AD

The results of previous studies are summarised in Appendix B and provide a clear starting point for understanding the interaction between methane yield, VFA yield, methane productivity and VFA concentration and productivity.

Two of the laboratory case studies presented in Appendix B, using different feeds and different reactor types are presented in Sections 3.2.2.1 and 3.2.2.2 with further discussion tying the overall trends together in Section 3.2.3.

3.2.2.1 A case study of AD of poultry waste in a CSTR

Salminen and Rintala (2002), from the University of Jyväskylä in Finland, conducted a study on the digestion of diluted poultry slaughterhouse solids waste. They used a CSTR and investigated the changes in digester performance that occurred when changing HRT while keeping OLR constant (by manipulating the feed concentration) and with changing OLR. The results of their study are shown in Table 3-5.

Table 3-5 Results of the mesophilic digestion of poultry slaughterhouse solid waste diluted with water (Salminen and Rintala, 2002)

HRT	SRT	Influent Conc	OLR	VS Reduction	CH_4		VFA	
days	days	kg-VS/m^3	kg-VS/m^3.day	%	Yield Nm^3/kg-VS_{added}	Productivity Nm^3/m^3.day	Yield kg/kg-VS_{added}	Productivity kg/m^3.day
100	100	80	0.8	76	0.52	0.42	0.08	0.066
50	50	40	0.8	74	0.55	0.44	0.08	0.061
25	25	52	2.1	63	0.31	0.65	0.34	0.704
13	13	27	2.1	31	0.09	0.19	0.41	0.869

At a constant OLR of 0.8 kg-VS/m^3.day, changing the HRT from 100 to 50 days results in effectively no change in treatment efficiency (VS reduction), CH_4 yield or productivity. This supports the argument in Section 3.2.1 that these parameters are only affected when the OLR changes, provided washout is avoided.

In contrast to this, at a constant OLR of 2.1 kg-VS/m^3.day, decreasing the HRT from 25 to 13 days resulted in CH_4 yields and productivities of less than 30% of their previous values, as well as a 50 % decrease in the percentage VS removed. However, two other parameters changed aside from HRT, being influent VS concentration and SRT (because in a CSTR, SRT = HRT). The authors attribute this lowered reduction in VS to an inhibition of hydrolysis caused by elevated propionate levels. It does appear that hydrolysis was limited by the decrease in HRT since the total VFA concentration dropped from 17.6 g-COD/ℓ to 11.3 g-COD/ℓ and the percentage VS reduction dropped from 63 to 31%. However, there were also other factors that could influence the inhibition. An alternative reason could be that hydrolysis was retarded by the shorter time in which the complex organic particles in the feed had to react. Furthermore, increased propionate levels may still be experienced under these conditions in spite of a decrease in the total VFA concentration due to the syntrophic propionate-converting bacteria being known to grow very slowly (O'Flaherty et al., 1998), thus their populations likely suffered at the lower HRT, owing to their washout.

Further, at the constant OLR of 2.1 kg-VS/m^3.day, the yield and productivity of VFAs increased slightly from an HRT of 25 to 13 days, while the yield and productivity of methane decreased substantially. This suggested that some washout or inhibition of the methanogens occurred, but that the activity of the acidogens was not affected much. This could be expected since methanogens are the slowest growing and most sensitive of the AD microbes (Speece, 1983).

Although CH_4 productivity increased as the HRT was decreased from 50 to 25 days, increasing the OLR resulted in a decrease in CH_4 yield. This may be due to partial washout of the AD consortia, as

well as insufficient contact time between these microbes or hydrolytic enzymes and the substrate, resulting in residual substrate in the effluent. In the context of this project, the most significant result shown in this study was achieved at a HRT of 25 days and an OLR of 2.1 kg-VS/m^3.day. At this OLR, the productivity of CH_4 peaked, and an extremely high concentration of VFAs of 17.6 g-COD/ℓ occurred. This shows that high concentrations of VFAs can occur while the CH_4 productivity is maximised.

3.2.2.2 A case study of AD of a simple synthetic waste using a UASB

Fang and Chui (1993), from the University of Hong Kong, conducted a study where they increased the OLR of three 8.5 ℓ UASB reactors (reactors A, B and C) at 37°C until they eventually 'failed'. They used a simple synthetic feedstock, with 50% of the COD coming from each of sucrose and dried milk powder (of which, 40% of the COD was soluble) respectively. The results of this study at the higher OLRs investigated are shown in Table 3-6.

Table 3-6 The results for the degradation of sucrose and milk powder in a UASB (Fang and Chui, 1993)

OLR	HRT	COD_{Feed}	$COD_{Effluent}$	COD Removal Efficiency	$COD_{totalVFA}$	COD_{VFA}/ $COD_{Influent}$	CH4 Productivity	COD-CH4/ Codfeed	Reactor
g/ℓ. day	h	g/ℓ	g/ℓ	-	g/ℓ	-	Nm^3/m^3.day	-	-
54	2.8	6.3	1.39	0.78	0.07	1%	13.5	71%	A
84	1.8	6.3	1.95	0.69	0.20	3%	16.0	54%	B
83	1.8	6.3	1.89	0.70	0.22	3%	16.5	57%	C
100	2.8	12	3.12	0.74	0.48	4%	16.5	47%	A
130	2.2	12	2.76	0.77	0.40	3%	25.5	56%	B
160	1.8	12	3.00	0.75	0.67	6%	24.0	43%	C
160	3	20	12.6	0.37	5.44	27%	13.5	24%	A
210	2.3	20	13.4	0.33	4.74	24%	17.5	24%	B
260	1.8	20	14.8	0.26	5.49	27%	18.0	20%	C

This study achieved the highest OLR with reasonable removal efficiency (160 g-COD/ℓ.day OLR with 75 % removal efficiency) of any of the studies reviewed, and was performed with the purpose of testing the maximum OLR attainable in a mesophilic UASB. The results of the three reactors after 'failing' are shown as the last three rows in Table 3-6, where the influent COD was 20 000 mg/ℓ and COD removal efficiencies dropped from ~0.75 to ~0.3 for all three reactors. The reason for this failure was attributed to extremely turbulent conditions in the reactors caused by vigorous biogas production, resulting in washout of the granules in the upper region of the reactors. While the soluble COD removal efficiencies were well over 90% prior to reactor failure, the total COD removal efficiencies were typically under 80% after reactor start-up (data not shown in Table 3-6). This can be attributed to the low capacity of the system to hydrolyse the insoluble COD (representing ~30 % of the total COD) at the low HRT under which the experiment was conducted.

An interesting result in the context of this project is that, even at the highest OLR before failure, VFAs failed to build-up to an appreciable extent. This can be seen explicitly in the column representing the recovery of influent COD to VFA (in the column headed COD_{VFA}/COD_{Feed} in Table 3-6). Likewise, the efficiency of methanogenesis can be appreciated most explicitly by determining the recovery of influent COD to CH_4 (shown in the second-last column on the right in Table 3-6). At the highest OLR before failure (160 g-COD/ℓ. day), only 6 % of the influent COD is recovered as VFA in the effluent stream, while 43 % is recovered as CH_4. After failure and washout of ~half the accumulated biomass, the VFA production increased dramatically, and the CH_4 productivity fell to 56% of its previous value, clearly showing that methanogenesis has become rate-limiting. At this point, recovery of influent COD as VFA increases to ~25 %, while recovery of CH_4 decreases to just under 25 %. This performance after failure is therefore comparable to that before failure in terms of recovery of the influent COD to VFA or CH_4. The overall recovery of influent COD as VFA or CH_4 is <50 % at both the OLR corresponding to

maximum CH_4 productivity and after failure where significant VFA production occurs. In comparison, a recovery of 71 % of the influent COD as CH_4 is achieved at an OLR of 54 g/ℓ. day, which is more typical of results achieved at lower OLR (data not shown). The methane productivity of the former condition, however, approaches two-fold that of the latter condition.

3.2.3 Discussion of data from previous studies

The results of the two studies discussed above, as well as nine other studies, are detailed in Appendix B. These data clearly follow the trends discussed in Section 3.2.1, namely that productivity of CH_4 increases with increasing OLR, up until some point at which either washout or inhibition occurs, or hydrolysis limits the conversion of complex organics. It must be noted that the variations in the extent to which the above trends are present result from these studies being performed using a variety of reactor types, for a variety of substrates with different rates of biodegradability and concentrations of inhibitors.

As seen in the two case studies discussed above, the reactor and substrate types greatly affect the result of the study. “High-rate” sludge bed reactors, such as the UASB, can typically treat waste at a far higher OLR (van Lier et al., 2015). The reason for this is that sludge bed reactors decouple the HRT from the SRT through either solid supports or the generation of settleable sludge, which allows the sludge (mainly active biomass) to be retained within the reactor (Fang and Chui, 1993; Lettinga et al., 1980; Speece, 1983). However, these reactor schemes are limited to wastewater treatment, since feedstocks containing a large fraction of solids (e.g., manure, sewage sludge) do not exhibit discrete liquid and solid phases.

In the study by Fang and Chui (1993), the UASB reactor type exhibited the capacity to deal with extreme OLR, producing proportional quantities of biogas (see Appendix B). There are, however, challenges to the simultaneous production of VFA and CH_4 efficiently within the same reactor. Similar challenges have been reported for CSTRs treating feedstocks containing a large fraction of solids. These challenges result from inter-linked rate-limiting processes and the need for effective control strategies. The results of some laboratory scale studies look promising for this dual purpose; however, the production of VFAs in combination with methane has mostly been reported when the reactor is operating with a compromised methanogen community due to unfavourable conditions, hence risking wash out of the methanogens and process instability. As the methanogens grow slowly, recovery from instabilities may be slow in the AD plant. From studies such as those by Jiménez et al. (2003), where the SRT is decoupled from HRT and simultaneous production of VFA and CH_4 occurs, it is observed that feedstocks that include methanogen inhibitors (such as the alcohol fermentation wastewater called vinasse) result in production of both products.

Lettinga et al. (1980), and indeed many studies documented in the review of Lee et al. (2014), have shown the potential for VFA to be generated using high-rate reactors by using environmental conditions that optimise hydrolysis and acidogenesis and suppress methanogenesis. The use of two anaerobic reactors (hybrid AD reactor) for production of both VFA and CH_4 may be desirable, such that the environmental conditions in the two reactors can be tailored to optimise hydrolysis/acidogenesis and acetogenesis/methanogenesis separately. Such an arrangement also allows the scheme of VFA and CH_4 production to be extended to substrates where hydrolysis is rate-limiting. Through understanding the conditions required for these two conditions and by understanding the trade-off between achieving high biogas productivity or high biogas yield as well as between achieving high VFA productivity or high VFA yield, it is possible to explore the best conditions to optimise simultaneously water quality and resource efficiency as well as productivity and resource efficiency. Further control of the reactor or reactor train can be better achieved.

3.3 Methane productivity and the partitioning of feedstock carbon into methane and VFAs production

3.3.1 Experimental approach

3.3.1.1 Reactor design and operation

Experiments that investigate the distribution of influent COD of a synthetic wastewater to CH_4 and VFA production were designed to explore the use of the AD in maximising CH_4 productivity, the focus of this section, and the maximised valorisation of the wastewater resource. A short review of the AD literature revealed the UASB reactor to be the most popular type of AD wastewater treatment reactor (Chernicharo et al., 2015; van Lier et al., 2015). This reactor's popularity stems from its ability to treat wastewater at high OLR and short HRT (van Lier et al., 2015), thus it was selected for use in this project. A study conducted by Fang and Chui (1993) was successful in testing the limits of OLR in a UASB reactor, hence was used as a basis for the experimental design in this project. For comparison purposes, two identical UASB reactors were operated in parallel i.e. Reactor A (RA) and Reactor B (RB). Figure 3-1 illustrates the reactor design, with dimensions are given on Table 3-7, with specific details given in Appendix C. The development of the reactor design is detailed in Appendix C and by Stott (2019). Each reactor had a working volume of 2.5 L.

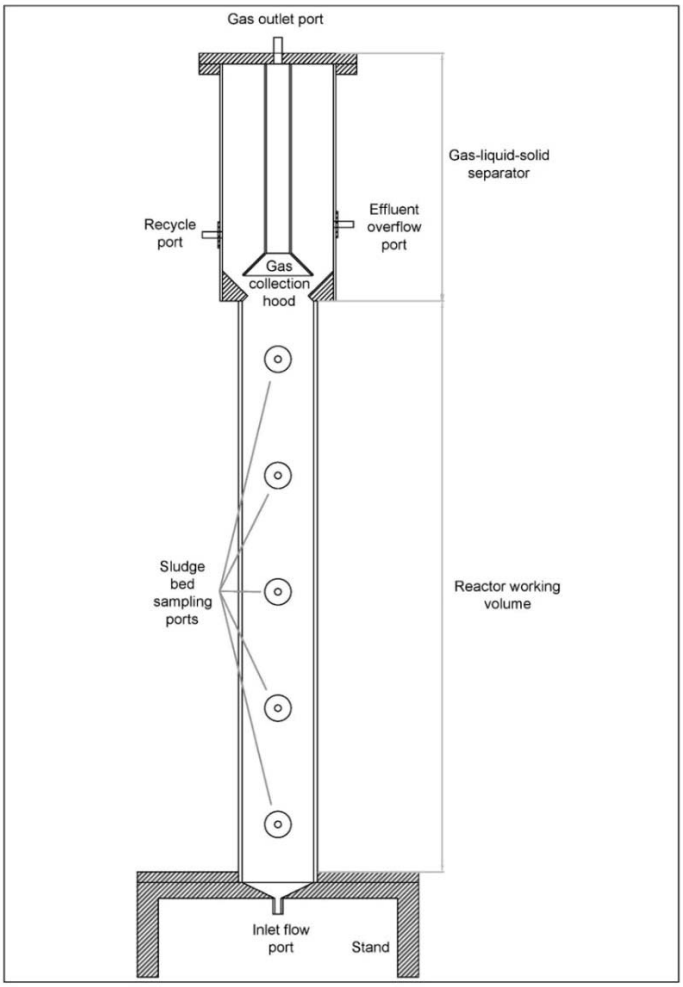


Figure 3-1 Schematic diagram of a UASB reactor

The reactor body dimensions are as follows: inner diameter of 67.8mm, height of 554 mm and diameter of constriction of 57.8mm. The gas-liquid-solid separating hood had the following dimensions: inner diameter of 106 mm, height of 226 mm, gas collection hood diameter of 65 mm.

Table 3-7 Dimensions of UASB reactor used in this study

Reactor body	Inner diameter	67.8 mm
	Height	554 mm
	Diameter of constriction	57.8 mm
Gas-liquid-solid separation	Inner diameter	106 mm
	Height	226 mm
	Gas collection hood diameter	65 mm

The reactors were inoculated with a granular sludge obtained from Prospecton Brewery (SA Breweries, KZN) and from Tabolt and Tabolt (Durban), for the first and second sets of experiments, respectively (Figure 3-2). The ratio of inoculum to feed was 1:1 and 1:1.5 for the first and second sets of experiments, respectively. Following inoculation, the reactors were sparged with nitrogen for 10 minutes to ensure that all oxygen in the sludge and headspace was displaced; thus creating an anaerobic environment.

Figure 3-2 Granular sludge used as inoculum

Temperature in the reactors was maintained at 37°C using thermostat controlled heating coils. Some of the effluent was recycled from the recycle port (as indicated on Figure 3-3), in order to maintain a constant up flow velocity and mixing in the reactors. Liquid samples were taken from the effluent port and the biogas volume produced was measured by a wet tip gas meter. Feed reservoirs were prepared in 10 L Schott bottles (Duran®, Schott). Masterflex peristaltic pumps (Cole Parmer, Item EW-77521-47) together with the Masterflex precision tubing were used for the feed, effluent as well as the to recycle some of effluent.

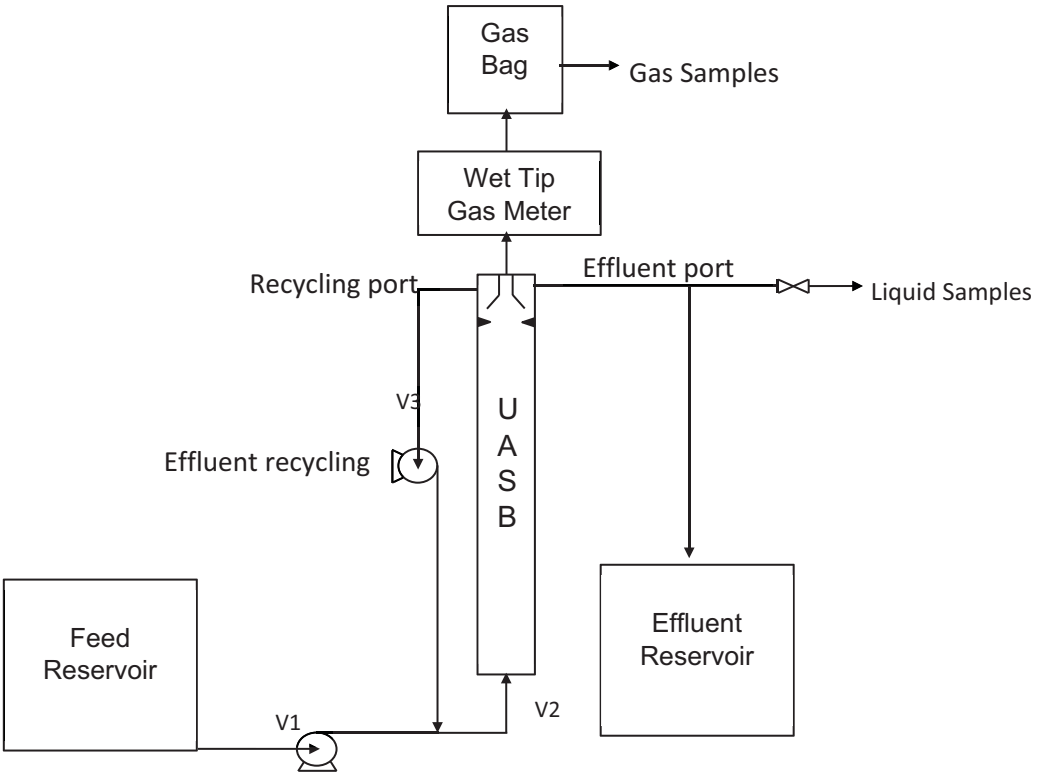


Figure 3-3 Schematic diagram of the reactor set up

Although UASB reactors are relatively well-mixed through the production of biogas at higher OLRs (Fang and Chui, 1993), mixing was not considered adequate to achieve complete mixing. This poses problems when trying to model the system or use data generated using a UASB system for model development, since accounting for spatial variations in concentration adds to the model complexity. To overcome this drawback, some of the effluent was recycled. A recycle ratio (defined by Equation 3-5) allowed for a constant up flow velocity to be maintained at any given feed rate. The added benefit of a recycle stream is that the alkalinity generated in the reactor through the production of carbonate species is recycled, thus reducing likelihood of regions of acidification, and hence associated regions of inhibition of methanogenesis (Chui et al., 1994).

$$\boldsymbol{Recycle\ ratio} = \frac{\dot{v}_{recycle}+\dot{v}_{feed}}{\dot{v}_{feed}} \qquad \text{Equation 3-5}$$

where
$\dot{v}_{recycle}$ is the volumetric flow rate of the recycle stream
$\dot{v}_{feed}$ is the volumetric flow rate of the feed stream

During the start-up stages of the experiment, it is preferable that a high upflow velocity is maintained so as to allow for proper mixing. The OLR is varied by varying the feed rate and the feed concentration. Initially feed rate was increased to increase OLR as a constant feed concentration. Once the maximum desired feed rate has been achieved, further increases in the OLR can be controlled by increasing the influent COD concentration. Accordingly, during the start-up stages of the first and second sets of experiments, a high recycle ratio was maintained (Table 3-8 and 3-9). Two experimental plans are shown in Table 3-8 and Table 3-9, for the first and second sets of experiments respectively. In the first set of experiments, the OLR was increased through the ramping up of a feed rate, whereas in the second set of experiments the OLR was ramped up by increasing the COD concentration in the feed and keeping the feed rate constant.

Table 3-8 Experimental design for the first stage of the project: operational specifications. At this stage the OLR was ramped up by increasing the feed rate while the COD concentration in the feed was kept constant

Phase	Start-up/acclimatisation				1	2	3	4	5	6	7	8	9	10	11
OLR (g/ℓ/day) =	1.0	1.5	2.5	4.0	6.0	9.0	14.0	22.0	30.0	45.0	65.0	95.0	130.0	160.0	200.0
HRT (h) =	36.00	24.00	14.40	9.00	6.00	4.00	2.57	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64
Up flow rate (ℓ/h) =	2.10	2.80	3.50	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.77	6.77
COD (g/ℓ) =	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	2.05	3.07	4.43	6.48	8.86	10.91	13.64
v1* (ℓ/h) =	0.06	0.08	0.14	0.22	0.33	0.50	0.78	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22
v3* (ℓ/h) =	0.70	0.93	1.12	2.22	2.11	1.94	1.67	1.22	1.22	1.22	1.22	1.22	1.22	1.22	1.22
Feed rate (ℓ/day) =	1.30	2.00	3.30	5.30	8.00	12.00	19.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00

Table 3-9 Modified experimental design during the second stage of the project: operational specifications. At this stage the OLR was ramped up by increasing COD concentration while the feed rate was kept constant

Phase	Start-up/acclimatisation				1	2	3	4	5
OLR (g/ℓ/day) =	7	15	21	16	20	25	27	29	31
HRT (h) =	44.2	22.1	15.7	12.6	10.2	10.2	10.2	10.2	10.2
Up flow rate (ℓ/h) =	2.36	2.42	2.42	2.41	2.39	2.39	2.39	2.39	2.39
COD (g/ℓ) =	13.5	13.5	13.5	8.5	8.5	10.5	11.5	12.4	13.1
v1* (ℓ/h) =	0.06	0.11	0.16	0.20	0.25	0.25	0.25	0.25	0.25
v3* (ℓ/h) =	2.31	2.31	2.26	2.21	2.14	2.14	2.14	2.14	2.14
Feed rate (ℓ/day) =	1.36	2.72	3.83	4.75	5.90	5.90	5.90	5.90	5.90

3.3.1.2 Feed preparation

The feed composition used in this project was based on the composition used in a study by Fang and Chui (1993), with slight modifications. For instance, the concentration of ammonium chloride was decreased while the calcium chloride concentration was increased in order to compensate for the calcium contained in the milk powder, which was used as a calcium source by Fang and Chui (1993). The concentration of calcium chloride was fixed at 413 mg/L, since this yields a calcium concentration of 149 mg/L, corresponding to the concentration found to be optimal for granule formation in a study by Yu et al. (2001). In the first set of experiments, two carbon sources were initially used i.e. sucrose in RA and a mixture of sucrose and carboxymethyl cellulose (CMC) in RB (Table 3-10). However, CMC was eventually replaced by sucrose in RB owing to its poor metabolism and associated effective under-feeding of the reactor. In the second set of experiments, sucrose and glucose were used as carbon sources (Table 3-11). The carbon sources in both sets of experiments were supplemented with protein (in the form of yeast extract), macro and micronutrients as indicated in Tables 3-10 and 3-11. Sodium bicarbonate and potassium dihydrogen phosphate were included in the feed to buffer the pH in the event of VFA accumulation. The feed was prepared by autoclaving deionised water, in 10 L Schott bottles, at 120ºC for 20 minutes. This was followed by the addition of sterile solutions of organics, macro and micronutrients under a laminar flow. In the second set of experiments, the pH in the feed was adjusted between 8 and 8.5, using 10 M NaOH.

Table 3-10 Composition of synthetic wastewater used in the first set of experiments

Medium Components	mg/L	Stock Solution
Sucrose	1389	Sucrose
Sodium bicarbonate ($NaHCO_3$)	1500	Macronutrients & Buffers
Ammonium chloride (NH_4Cl)	390	
Potassium monohydrogen phosphate (K_2HPO_4)	170	
Sodium citrate ($Na_3C_6H_5O_7$))	116	
Ferric chloride ($FeCl_3.6H_2O$)	10	Fe
EDTA ($C_{10}H_{16}N_2O_8$)	10	
Magnesium sulphate ($MgSO_4.7H_2O$)	25.0	Mg
Calcium chloride ($CaCl_2.2H_2O$)	191	Ca
Nickel sulphate ($NiSO_4.7H_2O$)	0.50	Micronutrients
Manganese (II) chloride ($MnCl_2.4H_2O$)	2.0	
Zinc chloride ($ZnCl_2$)	0.50	
Cobalt chloride ($CoCl_2.2H_2O$)	0.50	
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	0.50	
Copper (II) chloride ($CuCl_2.2H_2O$)	0.50	
Sodium borate ($Na_2B_4O_7.10H_2O$)	0.50	
Sodium selenite ($Na_2SeO_3.5H_2O$)	0.50	
EDTA	5.0	

Table 3-11 Composition of synthetic wastewater used in the second set of experiments

Medium Components	mg/L	Stock Solution
Sucrose	1000	Organics
Glucose	1000	
Yeast extract	2000	
Propionic acid	100	
Acetic acid	400	
Starch (soluble)	1000	
Sodium bicarbonate ($NaHCO_3$)	100	Macronutrients
Ammonium chloride (NH_4Cl)	200	
Potassium monohydrogen phosphate (K_2HPO_4)	250	
Magnesium sulphate ($MgSO_4.7H_2O$)	150	
Calcium chloride ($CaCl_2.2H_2O$)	191	
Nickel chloride $NiCl_2.6H_2O$	35	Micronutrients
Manganese (II) chloride ($MnCl_2.4H_2O$)	250	
Zinc chloride ($ZnCl_2$)	25	
Cobalt chloride ($CoCl_2.6H_2O$)	1000	
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	45	
Copper (II) chloride ($CuCl_2.2H_2O$)	15	
Boric acid powder (H_3BO_3)	25	
Sodium selenite ($Na_2SeO_4.10H_2O$)	93	
Ferric chloride ($FeCl_3.6H_2O$)	1154	
EDTA ($C_{10}H_{16}N_2O_8$)	500	
HCl 32%	1.13 [mL/L]	

3.3.1.3 Analytical techniques

COD measurements

In the first experiment, soluble COD was measured at least once a week for the first three months, after which it was recorded intermittently. The COD analysis was performed according to the closed reflux, colorimetric method (5220 D.) (APHA, 1999). The Merck COD reagent set (1.14555 HR) was used in conjunction with a digestion block and the Nova Spectroquant photometer. Samples were centrifuged and the supernatant used for determination of the soluble COD. Potassium hydrogen phthalate (PHP) (1,175 g-COD/g-PHP) in deionised water was used as a standard, at concentrations of 85, 212.5, 425, 637.5 and 850 mg-PHP/L, corresponding to COD values of 100, 250, 500, 750 and 1000 mg-COD/L respectively. Deionised water was used as a reagent blank, and all COD measurements were made in duplicate. The volumes of samples and reagents were halved for economy and waste reduction.

In the second set of experiments, total and soluble COD from the feed and effluent were measured daily by using COD reagents A (Merck, 114679) and B (Merck, 114680). Briefly, reagents A and B were mixed in glass vials at a volume of 1.10 mL and 0.90 mL, respectively, according to the manufacturer's instructions. Thereafter, 0.50 mL of sample were added to the vial. De-ionised water was used a blank. The volumes of samples and reagents were halved for economical and waste production purposes. The vials were then incubated in a heating block (HI 839800 COD reactor 2008 series) at 150ºC for 2 hours. Following the incubation period, the samples were left at room temperature to cool down and the absorbances were measured in a spectrophotometer at 605 nm. The COD results are presented as the percentage of soluble COD removed from the system, which is calculated as the difference between the influent and effluent soluble CODs, divided by the influent soluble COD.

pH measurements

The influent and effluent pH were measured on a daily basis using the Jenway 3510 pH metre (Lasec).

VFA analysis

The concentrations of lactic, acetic, propionic and butyric acids were measured using high performance liquid chromatography. A Waters Breeze 2 system equipped with a Bio-Rad Organics Acids ROA column and a UV (210 nm wavelength) detector. The system was run isocratically using a mobile phase of 0.01 M H_2SO_4 at a flow rate of 0.6 mL/min. Standards containing the abovementioned VFAs at concentrations of 100-600 mg/L in 100 mg/L intervals were used to generate standard curves to allow quantification of the VFA measured.

Biogas production and composition

The volume of biogas produced was measured using Wet Tip Gas Meters (http://wettipgasmeter.com/), which utilise the principle of liquid displacement. These metres were calibrated to count every 35 mL and 45 mL of biogas produced, for reactor A and B, respectively. Gas counts were recorded on a daily basis, along with the time of reading to allow for the calculation of biogas productivity. Gas samples were collected in syringes, for the measurement of methane. A Perkin-Elmer Auto-system gas chromatograph equipped with a Supelco wax column (1.2 mm x 37 m) and flame ionisation detector (FID) was used for the determination of the methane fraction in the biogas. The FID and oven temperatures were set at 280°C and 50°C respectively. Nitrogen was used as a carrier gas at a flow rate of 1.5 mL/min. Standards containing 25% and 50% methane were used to generate a standard curve with each analysis. Measurements were performed by injecting 50 µL of gas and each sample was injected three times.

3.3.2 Experimental results

3.3.2.1 First set of experiments

Reactor performance

In the first part of the project, the OLR was gradually increased from 1 to 9 g/L/day (Table 3-12) by increasing the feed rate while keeping the COD concentration in the feed constant. The impact of gradually increasing the OLR on COD removal efficiency and methane production was assessed by measuring the effluent COD and methane content in the biogas produced, respectively. At the start-up OLR of 1 g/L/day more than 90% and 50% of the COD was removed from Reactor A (RA: sucrose only) and Reactor B (RB: sucrose and CMC), respectively. Although, more than 90% of COD was removed from RA, the COD removal efficiency in RB (which had a mixture of sucrose and CMC as carbon sources) never increased above 50%. This was attributed to incomplete degrading of CMC.

There were various operational issues encountered in RB, for instance the sludge bed tended to float due to entrapped biogas within the sludge bed. A low recycle rate and a viscous feed (due to the inclusion of CMC), are possible causes for this entrapment of biogas. In an attempt to increase the turbulence within the sludge bed, it was decided that the recycle rate be increased to ~40 mL/min and the OLR increased to ~2 g/L/day. Due to the complexity of CMC as a substrate, it was removed from the feed composition of RB and sucrose was left as a sole carbon source as in RA. Hereafter, the sucrose concentration in RB was gradually increased until it was equivalent to the sucrose concentration in RA. The COD removal efficiency at an OLR of ~2 g/L/day was above 90% in both reactors. The OLR was further increased (to ~3.4 g/L/day) for both reactors on day 80 and 87 respectively. At this point, the HRT could not be further decreased due to the restricted capacity of the feed preparation and storage vessel. Another operational issue encountered was the frequent contamination of the feed. Hence, both reactors were often fed a slightly acidic substrate, which lowered the reactor pH, detrimental to reactor performance, in terms of the activity of the methanogens. While establishing the appropriate operational protocols for the correct feed maintenance procedures, reactor performance was dissatisfactory in that floating sludge incidents were observed. In addition, the presence of non-granulated biomass on the surface of the reactor liquor caused blockage in either the recycle or effluent ports, interrupting the designed recycle rate or, in a few cases, causing the washout of some sludge. Despite these issues, more than 90% of soluble COD entering the reactors was concerted and removed from solution. The OLR was further increased to ~9 g/L/day by increasing the flow rate from 8 L/day to 12 L/day. However, the problem with floating sludge persisted and, by this stage, clear degradation of the structure of the granules was evident. Many granules were damaged and a fluffy white layer coated them. Several attempts were made to flush out the fluffy white substance (initially thought to be a contaminant); however, it would reappear by the day after flushing.

Throughout the start-up phase, the effluent pH of both reactors steadily decreased until RA and RB had an effluent pH of 6.31 and 6.43 respectively. The feed's pH and buffer capacity was increased by adding only K_2HPO_4 instead of part K_2HPO_4 and part KH_2PO_4, as well as the addition of $Na_3C_6H_5O_7$ and by increasing the $NaHCO_3$ concentration from 0.66 g/g-COD to 1.0 g/g-COD. This caused the effluent pH to increase above pH 6.6. Although reactor performance increased slightly, there was little observable improvement in the sludge condition over the next month.

Table 3-12 Performance of UASB reactors in Experiment 1: a) performance during reactor acclimatisation; b) performance as of August 2017

a)

Date	OLR (g/L/day)	HRT (h)	pH	Soluble COD* removal (%)	Biogas composition (% CH4)
2016-11-08	1	36	6.8-7	>90%	
2017-01-17	1.5	24	6.6-6.8	>90%	
2017-02-03	3.6	10	~6.5	>90%	
2017-04-27	5.4	6.7	~6.5	80-90%	50% (RA) and 56% (RB)
2017-09-01	9	4	~6.5	70-80%	55%

b)

Reactor	OLR (g/L/day)	pH	COD removal (%)	Biogas composition (% CH4)	Biogas productivity (L/day)
A	5.6	6.61	87	56	4.57
B	5.6	6.67	94	56	4.86

The COD balance

To test the accuracy of the gas collection metre and COD tests and goodness of data, a COD balance was performed on the August 2017 data (Table 3-12). For this system, the COD balance is described in Equation 3-6.

$$COD_{Feed} = COD_{Effluent} + COD_{Cell\ growth} + COD_{[CH4]aq} + COD_{CH4,biogas} COD_{CH4,biogas} \quad \text{Equation 3-6}$$

where
COD_{Feed} is the rate at which COD is fed to the reactors (g-COD/day)
$COD_{Effluent}$ is the rate at which COD leaves the reactors in the liquid effluent
$COD_{Cell\ growth}$ is the rate at which COD is incorporated into the biomass synthesised through the degradation of organic COD-containing compounds
$COD_{[CH4]aq}$ is the rate at which COD leaves the reactor as methane dissolved in the effluent
$COD_{CH4,biogas}$ is the rate at which COD is removed from the reactor as methane in the biogas

Because the amount of COD used for cell growth is extremely difficult to measure accurately, the COD of the other terms is measured, and a value is calculated for the portion of COD used for cell growth that allows Equation 3-6 to balance. The value of the COD used for cell growth can then be compared to the expected cell yield to ensure that all other terms are being measured correctly. The results from performing the COD balance at an OLR of 5 g/L/day are shown in Table 3-12 for RA.

Table 3-12 Results from a preliminary COD balance for RA

In (g-COD/day)	Out (g-COD/day)				
Feed	Effluent	Cell Growth	$[CH_4]_{aq}$	CH_4, Biogas	Total
10.07	1.27	0.66	0.84	7.31	10.07

3.3.2.2 Second set of experiments

In the second set of experiments modifications were made to the experimental design and reactor operation to address the issues encountered during the first set of experiments. For instance, the constant contamination of the feed was minimised by changing the feed daily and regularly cleaning and autoclaving the feed lines. Furthermore, the feed pH was adjusted to between 8 and 8.5 to avoid the feed's acidification. The OLR was ramped up by increasing the COD concentration in the feed. The system's performance was assessed over140 days, in terms of COD removal efficiency and methane performance. The run, in both reactors, was started at a feed COD concentration of 13.5 g/L and a feed rate of 1.36 L/day, which corresponds to an OLR of

7 g/L/day. The COD removal efficiencies in this start-up phase were at an average of 89.9 and 89.3% for RA and RB respectively (Figure 3-4 and Figure 3-6). The COD removal efficiency in RA remained stable (COD removal efficiencies of between 80 and 98% were observed) over a gradual ramp up of the OLR; at least until the OLR of 25 g/L/day. Over this period, the volumetric COD conversion rate (VCCR) increased with increasing OLR, as required to maintain the consistent conversion. At an OLR of 30 g/L/day, there was a sudden drop in COD removal efficiency and VCCR (Figure 3-4); this coincided with a drastic decrease in pH, between day 100 and 120 (Figure 3-5).

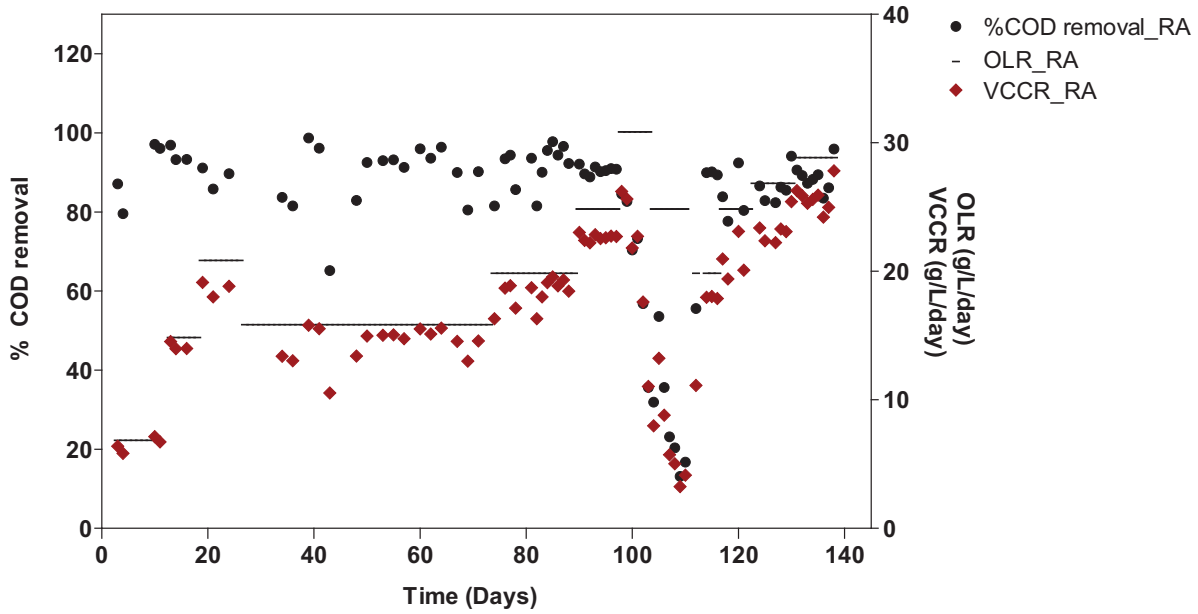


Figure 3-4 COD removal efficiencies from RA and associated VCCR under various OLRs over 140 days

The pH dropped to a low of 6.03 during the period with an OLR of 30 g/L/day (Figure 3-5). This suggested that the VFAs were accumulating. Despite reducing the OLR back to 25 g/L/day, the decrease in conversion and VCCR continued; hence, the system had to be re-inoculated with the activated sludge from the same batch used during start-up. With this re-inoculation, the OLR was lowered back to 20 g/L/day for the system to recover. As expected, with these adjustments, the system was recovered such that the COD removal efficiency was stable between 80 and 95% at OLRs of 27 and 29 g/L/day (Figure 3-4 and Figure 3-5).

The system's performance in RB differed from that of RA during the early stages of operation (Figure 3-6). The removal efficiency was stable at the start-up OLR of 7 g/L/day. After that, there was a major drop in COD removal efficiency as the OLR was increased. At day 25, the system was re-inoculated with fresh sludge. This resulted in a slight, but temporary, improvement in COD removal efficiency, whereafter the system deteriorated again. Between day 20 and day 80, the COD conversion rate remained low and the system's performance was compromised with substantial time in the conversion range of 10 to 40% interspersed by two short periods of 80 – 90% conversion and of 50 – 65% conversion. Performance improved only after day 80. Similarly to RA, the COD removal efficiency decreased with low pH values (Figure 3-5). Eventually, on achieving a stable pH in RB in excess of pH 7.0, the system performance improved and a stable COD removal efficiency of 90.5% was observed at 25 g/L/day (Figure 3-6). Further increase of OLR to 27 and 29 g/L/day COD resulted in decreased removal efficiencies of 80% and 73.3%, respectively (Figure 3-6). On increasing the OLR from 25 g/L/day through 27 to 29 g/L/day, the VCCR varied over the range of averages of 20.1 to 22.2 g/L/day, suggesting a consistent performance based on volumetric rate but decreasing conversion owing to increasing OLR.

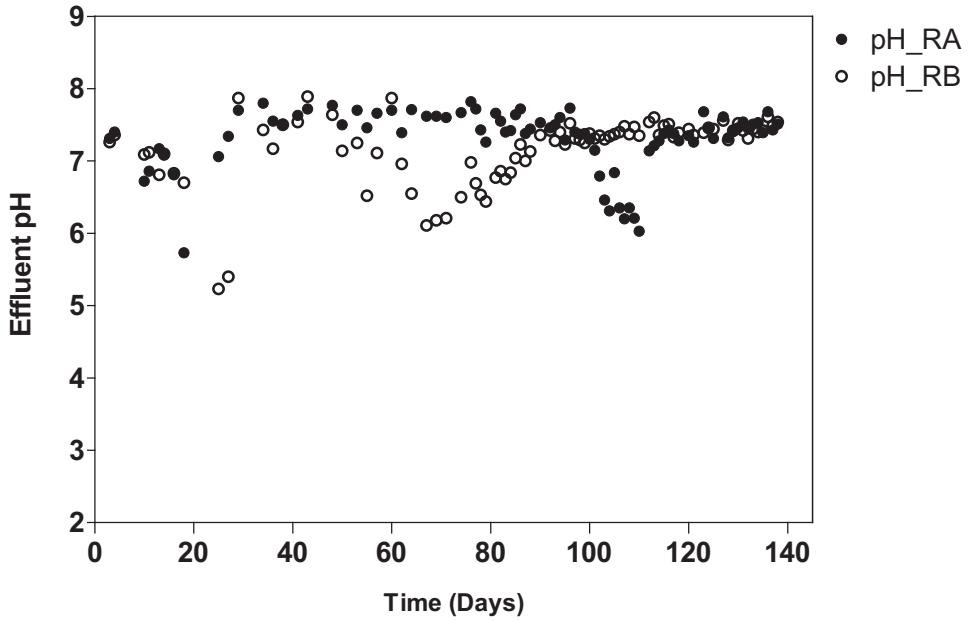


Figure 3-5 Effluent pH for RA and RB over the time period of 0 to 140 days

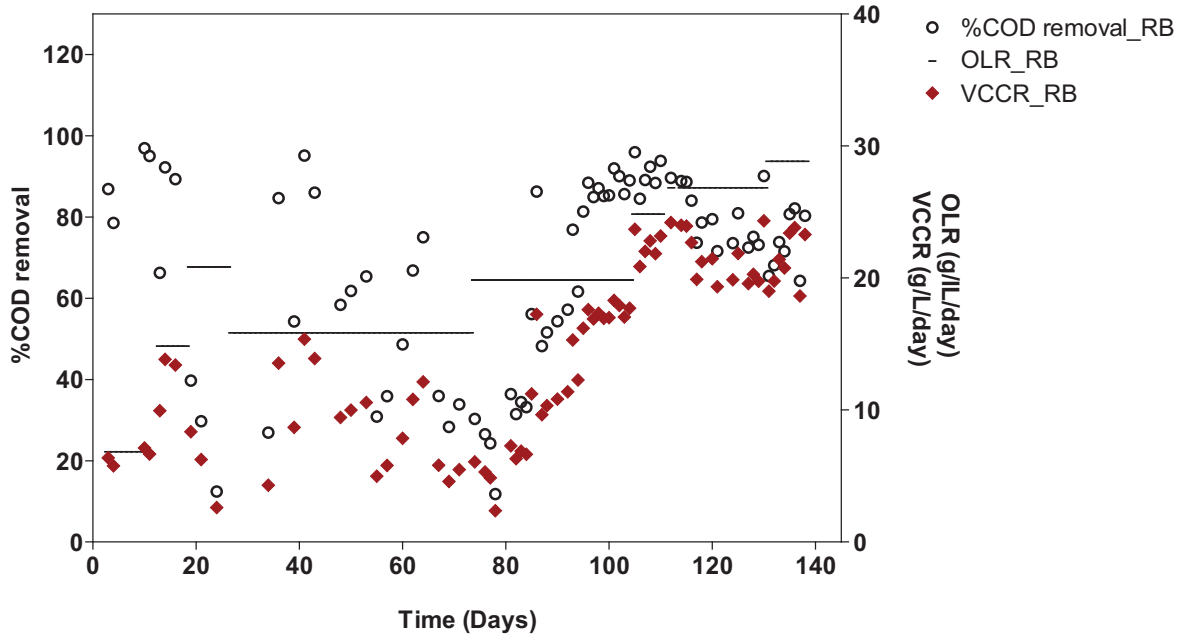


Figure 3-6 COD removal efficiencies and VCCR in RB under various OLRs over 140 days

As shown in Figure 3-7, increasing the OLR resulted in a linear increase in VCCR in both reactors, except where reactor pH fell below pH 7.0. This increase in VCCR coincided with an increase in the rate of methane production per unit reactor (Figure 3-7). As mentioned above, at an OLR of 30 g/L/day, the performance in RA was compromised and as a result the OLR was brought back down to 25 g/L/day and 20 g/L/day. This OLR is circled in red in Figure 3-7 to indicate that the VCCR and methane productivity were low because the system was still recovering from perturbation and the associated low pH. Maximum methane productivities of 6.59 L/L/day and 5.65 L/L/day were observed at an OLR of 29 g/L/day in RA and RB, respectively (Figure 3-7). Data suggests that further increase in OLR beyond 29 g/L/day are not associated with further increase in VCCR, owing to limitation in metabolic activity; hence conversion efficiency decreases.

In general, increased CH_4 productivity was observed with increased OLR. Low methane productivities at OLRs between 15 and 21 g/L/day coincided with low methane yields per gram of COD under these OLRs (Figure 3-8). Maximum methane yields of 0.32 and 0.26 L/g-COD were obtained at OLRs of 20 and 29 g/L/day, for RA and RB respectively (Figure 3-8). These actual methane yield values are not far off from the methane yield theoretical value of 0.35 L/g-COD.

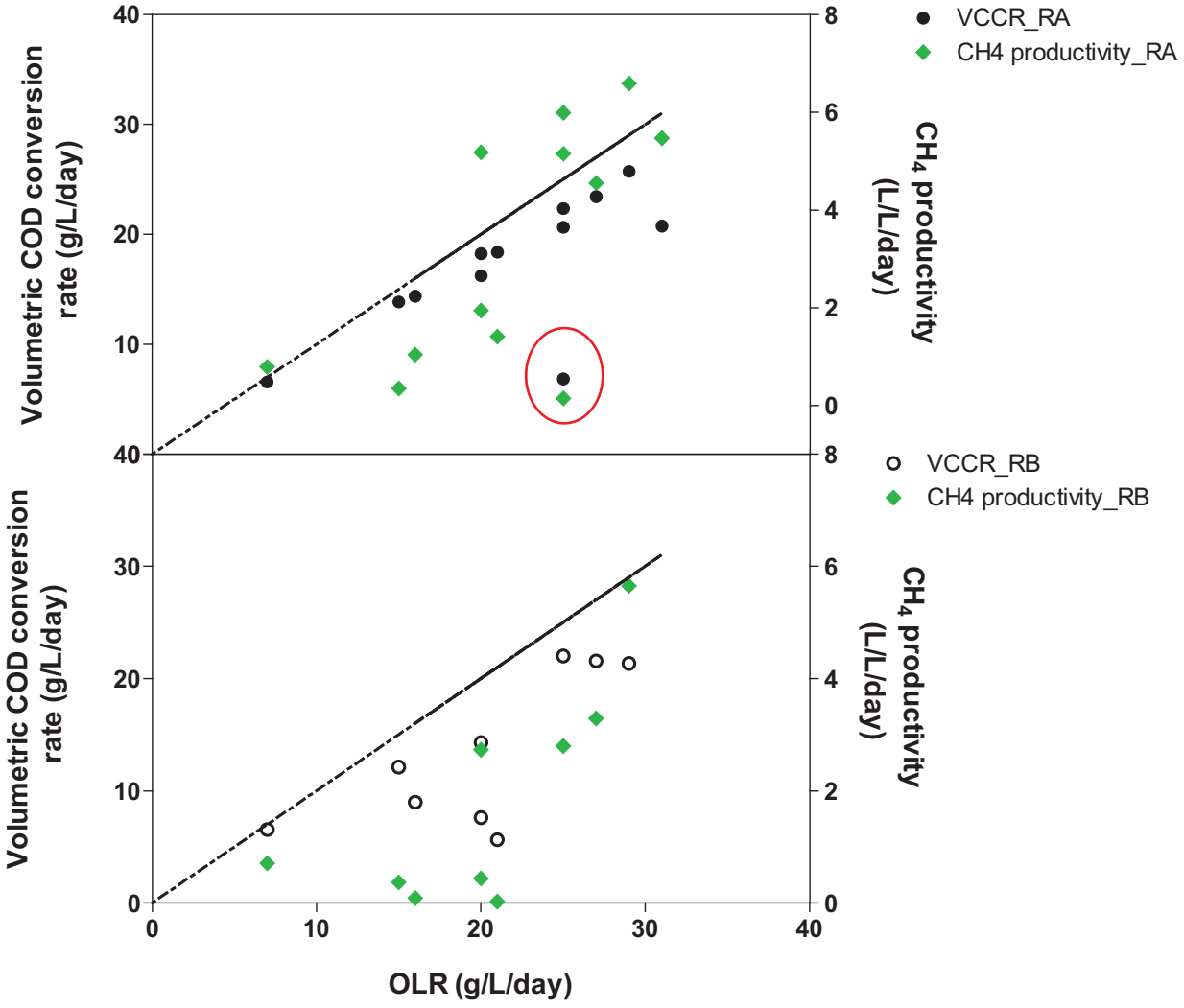


Figure 3-7 VCCR (g/L/day) and methane productivity per reactor volume (L/L/day) for RA and RB at various OLRs (g/L/day). The circled data points indicate a point (OLR 25 g/L/day) when RA was still recovering after a failure at an OLR of 30g/L/day

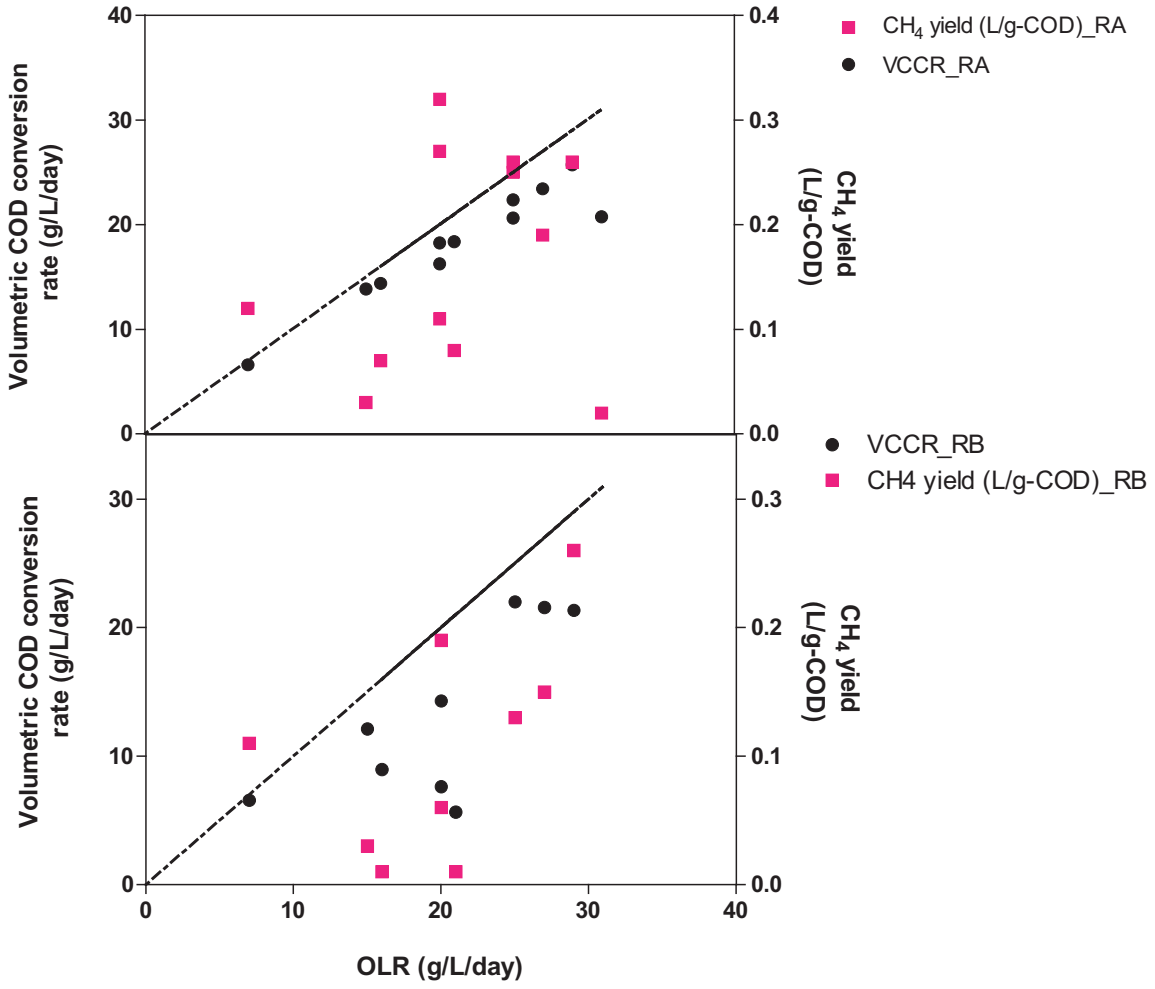


Figure 3-8 COD VCCR (g/L/day) and methane yield per reactor volume (L/L/day) for RA and RB at various OLRs (g/L/day) over the 140 day period

The relationship between OLR, COD conversion and VFA concentrations is specified in Figure 3-9. In reactors that were performing well in terms of COD conversion rate, VFA concentrations were low, suggesting that methanogenesis was not limited and the methanogens were active. Under periods where the system performance was compromised in terms of COD conversion and methane production, VFAs accumulated in the systems. For RA this was observed when the system was pushed from an OLR of 25 g/l/day to 30 g/L/day. In this period, the rate of methanogenesis was probably limited within the existing microbial community while VFA formation rate continued to increase, hence a VFA concentration of 1 g/L was observed (Figure 3-9). In RB the VFAs accumulated to a high of 7 g/L at an OLR of 20g/L/day when the system was underperforming (Figure 3-9).

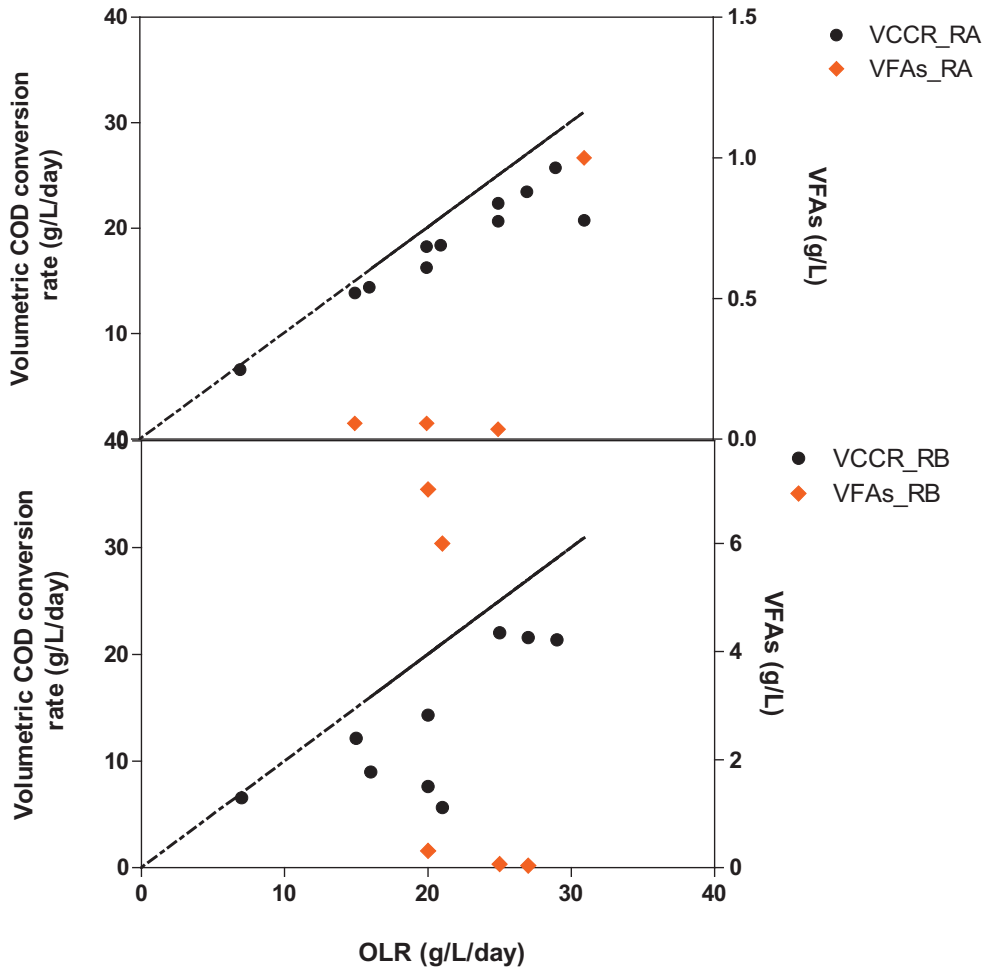


Figure 3-9 VCCR (g/L/day) and VFA productivity per reactor volume (L/L/day) for RA and RB at various OLRs (g/L/day)

3.3.3 Discussion of results

This part of the project was conducted to evaluate the impact of increasing the OLR on COD removal efficiency, VCCR, and methane productivity in a UASB reactor. Further, factors influencing the partitioning of carbon between methane and VFAs are considered. The study was conducted in two UASB reactors, where a synthetic domestic wastewater feedstock was used. The study consists of two sections, with the second section incorporating modifications to address the challenges encountered from the first section.

In the first section, the conditions thought to have caused the deterioration of the granular inoculum included under-feeding the sludge and under-supplying calcium. The reactors were initially started at an OLR of 1 g/L/day, which according to Alphenaar (1994) is quite a low OLR. For a successful start-up of the UASB system, it has been recommended that the OLR should be between 4 and 8 g/L/day (Alphenaar, 1994). Moreover, under-feeding the sludge results in a less turbulent sludge bed environment due to a lower specific biogas production per mass of sludge. In this low-shear environment, the filamentous growth of the hydrophobic methanogenic *Methanosaeta spp.* is not limited to shorter filaments, and the growth of loosely-bound (LB) exogenous polymeric substances (EPS) with a higher proteinaceous (PN) EPS content is favoured (Wang et al., 2017). RB was more adversely affected by this phenomenon due to being fed only half its COD content as readily-biodegradable COD initially, resulting in roughly half the biogas production compared to RA. This explains RB's higher frequency of sludge bed flotation incidents over RA.

Contamination of the feed also compromised performance of the reactors in the first experiment. The feed was often contaminated by opportunistic fermentative bacteria, resulting in organic acid production and decreased feed pH, which interfered with the pH within the reactor system. Although a partially fermented or “sour”, substrate has been treated effectively in UASB reactors (Lettinga et al., 1980), feeding reactors with substrate containing the acidogenic biomass has been documented as being detrimental to the granule formation (Vanderhaegen et al., 1992). In the fourth month of the first set of experiments, this situation was remedied by installing feed drums in a walk-in fridge. However, by this stage the sludge granules had developed a fluffy white outer layer, which was viscous and prone entrapping gas bubbles, giving the sludge bed a tendency to float. Even at a low pH of 6.5 and under conditions not conducive to granulation, the majority (70-90%) of the soluble COD was being converted into either sludge (biomass and EPS) or CH_4. This indicates the robustness of the microbial consortia present. The type of sludge produced under these substrate-limited conditions makes for poor use in a UASB reactor, where settleability and stability are required. Towards the end of the first set of experiments, most of these solids were present as sludge agglomerates. This sludge meets the visual criteria of LB EPS, described by Fukuzaki et al. (1995) as a “thick polymeric coat, resulting in fluffy granule formation” and by Wang et al. (2017) as “loose and dispersible slime layer found in the outer layer of sludge flocs without an obvious edge”.

Also worth noting is that within two weeks of inoculating RB in the first set of experiments, the sludge bed began to float. This shows that the characteristics of the sludge agglomerates respond relatively quickly to environmental changes. This phenomenon was also observed in the study by Wang et al. (2017), where sludge granules with positive characteristics (summarised by a low sludge flotation potential (SFP)) were developed from a flotation-prone sludge in 30 days.

In the second section, the reactors were started at an OLR of 7 g/L/day, which is in accordance with the recommended starting OLR by Alphenaar (1994). Once stable removal efficiencies of 89.9% and 89.4% were obtained for RA and RB, the OLR was gradually ramped up. OLR increases up to 25 g/L/day showed no disturbances in the system's performance in terms of COD removal efficiency, at least for RA. In RA, an average COD removal efficiency of 90.4% was obtained between the OLRs of 7 g/L/day and 25 g/L/day. This removal efficiency was slightly higher that the efficiency observed by Torkian et al. (2001), where an average COD removal efficiency of 85% was observed at OLRs of between 14 g/L/day and 25 g/L/day. Due to high COD conversion efficiency, VCCR increased linearly with OLR from 7 to 22.5 g/L/day across the OLR range 7 to 25 g/Lday, showing the improved space time utilisation of the reactor.

On increasing the OLR from 25 to 29 g/L/day, a further increase in VCCR was initially obtained, followed by a plateauing or decrease in VCCR at 29 g/L/day. This was accompanied by a decrease in conversion efficiency and methane production and an increase in VFA production. This, as well as the system failure to increase the OLR from 25 to 30 g/L/day, suggests that a maximum microbial activity for COD conversion and methane productivity had been reached. To further enhance the productivity of the reactor system, it would be necessary to enhance the volumetric microbial activity by either achieving a higher specific activity through optimising operating conditions simultaneously for both acidogenesis and methanogenesis or by increasing the loading of the active granulated microbial consortium.

While the UASB has been successfully shown to be an effective method in treating various effluents (Amin et al., 2016; Mirsepasi et al., 2006; Montes et al., 2019; Musa et al., 2018), there are some challenges that are encountered during its operation and these impede the overall system performance. For instance, the UASB is quite sensitive to major perturbations i.e. in some cases when the OLR was increased substantially, this negatively impacted COD removal rate and the methane production rate (Govahi et al., 2012). The sensitivity of the UASB system was observed in this project, specifically in RA when the OLR was increased from 25 g/L/day to 30 g/L/day. With this change there was a considerable drop in COD removal efficiency and in methane productivity, while the VFAs accumulated in the system. With the accumulation of VFAs in the system, the pH dropped drastically, resulting in the complete failure of the system in terms of methane production. According to Alphenaar (1994), if the OLR is abruptly increased prior to steady state being reached, this can result in the decrease in granule

integrity and, as a consequence, result in the decrease in reactor performance. This agrees with what was observed in our study, when the system was recovered by lowering the OLR back to 25 g/L/day and then 20 g/L/day, the system maintained stable COD removal efficiencies of above 80%. After having recovered, the OLR in the system was again increased further to 27 and 29 g/L/day and with this change, the system continued to run effectively in terms of COD removal efficiency and methane production. This suggested that the system was merely affected by too big a change in OLR. Similar findings have been recently reported in a study by Montes et al. (2019), where they observed stable high efficiencies to be reached at 28 g/L/day with a maximum methane productivity of 8.4 L/L/day. However, in the same study, when the OLR was further increased to 32 g/L/day, the system became unstable and the reactor performance decreased dramatically; demonstrated by the inhibition of methanogenesis (Montes et al., 2019). Both the system failures observed in this study and in a study by Montes et al. (2019) at an OLR of ~30 g/L/day were probably a result of the drastic increase in the OLR that shocked the system. With smaller increments in the OLR the system remained stable i.e. at 29 g/L/day, the system did not show any signs of failure. If time permitted, the system could perhaps still be pushed even further by a more gradual increment in OLR. Fang and Chui (1993) successfully showed that the OLR in a UASB system could be pushed up to an OLR of 160 g/L/day under a short HRT, before it became unstable.

3.4 Potential of VFAs as an AD product from acidogenic fermentation

To assess the feasibility of VFA production from the acidogenic fermentation of biomass or organic-bearing wastewaters, a short term study on the production of VFAs from grass was performed. The VFA yields obtained from this study, were compared to the yields achieved from the AD of Spirulina (a cyanobacterium often termed “algae”) by Inglesby (2011).

The results presented in section 3.3 suggest that if VFA production is desired, it is unlikely that sufficient VFA yields would be achieved when performing AD as a complete process. Also, the presence of excessive VFAs in the methanogenesis process can lead to process instability. Thus for efficient recovery of either methane or VFAs, the process conditions should be tailored for recovery of the desired product under optimal operating conditions for whichever is selected. The conditions favouring methane production have been highlighted and discussed in detail in the preceding sections. In this section, the production of VFAs from the acidogenic fermentation of organic material is considered and discussed.

Acidogenic fermentation relies on the hydrolysis of complex organic material to more soluble substrates followed by the acidification of the soluble organics by acid-forming bacteria which utilise these soluble substrates for growth. This results in the production of VFAs (Yuan et al., 2011). The rate of VFA production is influenced by operating parameters such as feedstock characteristics, loading and dilution rate, inoculum activity and microbial community structure, hydraulic and SRT, pH and temperature (Jiang et al., 2013; Yuan et al., 2011; Zhang et al., 2005). The optimisation of the operating conditions for high VFA production yields and rates are required to ensure the feasibility of the process.

Inhibiting methanogenesis is key to the accumulation of VFAs in the process, should these be the desired product. Methanogens operate strictly between pH 6.5 and 8 (Cheng, 2009; Chynoweth and Isaacson, 1987). Outside this pH range, the products of hydrolysis and acidogenesis are less easily converted to methane. Care should be taken when considering the correct operating pH. At very low pH values, a build-up of VFAs, in the undissociated form, may inhibit acidogenic fermentation (Xiao et al., 2016). VFAs in the dissociated form, prevalent at higher pH values, are less inhibitory to the acidogenic process. Operation at an increased pH also facilitates the hydrolysis of recalcitrant feedstocks, often believed to be the rate-limiting step of acidogenic fermentation (Chen et al., 2007; Liu et al., 2012; Wang et al., 2014; Wu et al., 2009; Zhang et al., 2005).

Together with operation at specific pH ranges, the inclusion of a methanogen inhibitor such as 2-bromoethanesulfonic acid (BESA) (Bouwer and McCarty, 1983) or the addition of small amounts, up to

100 mg/L, of heavy metals can result in the inhibition of methanogens (Lin and Shei, 2008; Lin and Chao, 1996). However, caution needs to be taken to avoid the inhibition of hydrolysis and acidogenesis by metal toxicity.

3.4.1 Experimental approach

The feasibility of using grass as a biomass feedstock for the production of VFAs using acidogenic fermentation was investigated by performing a batch fermentation test in a 1 L reactor consisting of a Schott bottle. Grass blades, cut into approx. 1 cm lengths, were added as biomass for VFA production at a 30 g/L COD loading. The working volume in the reactor was 900 mℓ and it was fitted with an airtight cap containing a metal insert with two ports. The first port was connected to an 8 mm internal diameter tube submerged two thirds of the way into the reactor fluid to allow for the sampling of the reactor slurry. The outside of the port was connected to a 10 mL syringe allowing for the reactor fluid to be withdrawn. This tube was clamped shut except during sampling periods. The second port was connected to a 50 mℓ syringe to allow for the collection and volume measurement of any gas produced. The reactors were maintained in a 30°C constant temperature room at pH 6. Preceding gas volume measurements and sampling of the reactors, the reactors were mixing thoroughly to allow a homogenous representation of the solid and liquid phases of the reactor and release any gas trapped within the sludge. The reactor was sampled on 0, 1, 4, 5 and 11 days. Sampling involved the removal of 2 mL of the slurry from the reactor. Samples were centrifuged and supernatants were combined and filtered through a 0.22 µm syringe filter into 2 mL Eppendorf tubes for .VFA samples were stored at -20°C until analysis. VFA analyses were performed as detailed in section 3.3.1.3.

The inoculum applied in these tests were obtained from a combination of environmental enrichments containing microorganisms capable of efficient conversion of complex organics to readily metabolisable substrates. Manure samples from domestic and game ruminant animals, a sample of sludge from a working commercial-scale AD treating kitchen waste, cow rumen fluid, sludge and liquid effluent from a Spirulina-fed AD and a sample of sludge from the commercial-scale AD reactor at SAB Newlands was used. Additionally, a sample of compost was included to further enrich for cellulolytic microorganisms. To inhibit methanogens, BESA to a final concentration of 1 mM was added to the culture and the pH was dropped to 6.

3.4.2 Results: Feasibility of grass as biomass for VFA production using acidogenic fermentation

Figure 3-10 shows the VFA production and profile over an 11 day period. The initial acetate present on day 0 was most probably a result of the build-up of acetic acid in the inoculum following the inhibition of the methanogens present with BESA. Further production over the next 11 days was assumed to be as a result of the hydrolysis of the grass feedstock. A total concentration of 6.6 g/L VFAs were achieve from the fermentation over the 11 days. 0.61 g/L VFAs were produced per day over the 11 day period. During the first four days acetic acid was the predominant VFA present within the acidogenic reactor with lesser proportions of butyric and isobutyric acid. Valeric and isovaleric acid was produced from day 4 onwards, with valeric acid becoming the predominant VFA in the reactor by day 11.

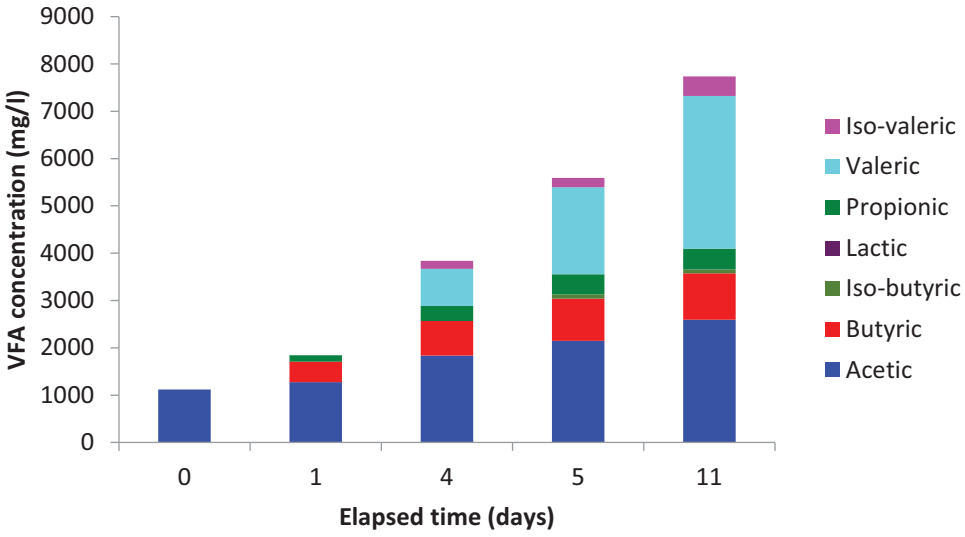


Figure 3-10 VFAs profile of acidogenic fermentation of grass over a 11 day period

A study by Inglesby (2011) reported the VFA profile released from the AD of cyanobacterial, Spirulina, biomass. Over the first four days, the acetic acid increases similarly to that reported for the grass study here. However, acetic acid remained the predominant VFA present within the reactor. This is possibly due to the increased activity of acetogens in this reactor compared to the acidogenic reactor. Although butyric acid becomes a significant fraction of the VFAs present, unlike in the case of the acidogenic fermentation of grass, valeric acid is not accumulated in the reactor. The VFA concentration increased at a rate of 1.4 g/L day^{-1} over the first four days of the AD. Although this reactor was operated as a complete AD system, minimal gas production was observed for the first 18 days of the experiment. The VFA production rate is higher than that achieved for the acidogenic grass fermentation possibly because grass contains a large proportion of recalcitrant lignocellulosic material in the plant cell walls. These lignocellulosics are much harder to solubilise to accessible organic compounds than the easily degradable cyanobacterial biomass.

Operating a separate acidogenic fermentation process for the production of VFAs may be feasible for especially feedstocks that are considered waste streams or are inexpensively acquired. In the case of recalcitrant feedstocks such as the grass used here, a number of pre-treatment steps may be considered to increase the rate of hydrolysis and accessibility to the carbon contained in the biomass (Bohutskyi and Bouwer, 2013). Similarly low rates of VFA production 0.52 g/ℓ day^{-1} was observed for a recalcitrant substrate, carboxy-methyl cellulose (CMC), used as a control test during this study. An increase in temperature and pH would facilitate liberation of organics from recalcitrant feedstock used as biomass (Chen et al., 2007; Liu et al., 2012; Zhang et al., 2005) and should be considered when using recalcitrant biomass. Care should be taken to avoid any inhibition which may result from a build-up of VFAs. By operating a continuous, or semicontinuous, system the VFA concentrations may be maintained below inhibitory concentrations to ensure maximum VFA production. But these conditions should be experimentally determined for each feedstock.

For the recovery of VFAs from acidogenic fermentation, a few options may be considered. Should VFA concentrations be sufficiently high, adsorption to activated charcoal, filtration or electrodialysis may be used to further concentrate the VFA stream and produce a separate nutrient rich stream. This nutrient rich stream can used as a feedstock for other processes or as a liquid agricultural fertiliser. The VFA concentrating methods are mentioned in more detail in sections 4.2 and 4.3. Alternatively, more dilute VFA streams may be considered as electron donors and carbon source for bioprocesses such as biological sulphate reduction (BSR). Literature suggests that SRB favour acetic, propionic and butyric acid as electron donors (Barnes et al., 1994, 1991; O'Flaherty and Colleran, 1999; Widdel, 1988), thus the acidogenic fermentation of grass or other feedstocks such as Spirulina will produce VFA streams

sufficient to sustain the BSR community. The nutrient content of these streams, containing essential macro- and micronutrients, may further enhance SRB growth for efficient BSR. Suggestions of the use of the VFA stream for sustaining BSR communities are further discussed in section 4.6.

3.5 AD productivity and the partitioning of feedstock carbon to methane and VFAs production: chapter conclusions

Overall, the results obtained from this study showed that with an increasing OLR, the COD conversion rate and methane productivity also increase. Increasing OLR resulted in high COD removal efficiencies and biogas production. Maximum COD conversion rates of 26 and 21 g/L/day and methane productivities of 6.59 and 5.65 L/L/day in RA and RB, respectively, were measured at an OLR of 29 g/L/day. It can be concluded from the system failures observed in both reactors, that a robust one-stage UASB system is not feasible to develop to produce VFAs and methane gas in a one-stage UASB reactor simultaneously, as the production of VFAs limits or inhibits the production of the CH_4 production. The operation of an acidogenic reactor for the production of VFAs is suggested and an experimental approach was followed to test the feasibility of VFA production from grass biomass.

The results presented show the benefit of operating the UASB at elevated OLR to maximise methane productivity and space time utilisation of the reactor. However, at high OLRs, it is important that the system is run steadily, without shock loadings, to maintain the robust process. Further, the plot of VCCR against OLR suggests that a maximum VCCR is attained for a specific reactor configuration of sludge loading. In our data, it appears that this is being approached at 29 g/L/day. Increase of OLR beyond this (system-specific) maximum will result in no further increase in VCCR and hence a decrease in conversion efficiency. Because methanogenesis is the rate-limiting step in the AD reactor metabolising simple organics, acidification is also likely to occur under these high OLR conditions leading to reactor failure.

While VFAs have been identified as an additional or alternative product to methane, this study supports literature studies in demonstrating the inability to achieve a stable, two product system owing to the differing operating pHs of the two processes. It is recommended that either a single product is selected and targeted or a two-stage process is operated with differing conditions used in each reactor to optimise VFA production in the 1st reactor and methane production in the 2nd.

4 DOWNSTREAM PROCESSING OF DIGESTATE – FOCUS ON RECOVERY OF ORGANIC PRODUCTS, NUTRIENTS AND WATER

To fully valorise a wastewater stream and to ensure meeting necessary environmental standards to prevent ecosystem burden, the downstream processing of the AD digestate is critical. The recovery of valuable components present in the effluent is desirable in making these wastewater processing options viable, both in terms of the circular economy and in economic terms. In addition, the recovery of the water fraction as 'fit-for-purpose' is non-negotiable in a water-stressed country such as South Africa. As outlined in Section 2.7, the compounds typically of most interest for recovery from AD effluent streams are VFAs, phosphates and nitrates (Milan et al., 1997; Mostafa, 1999; Yoshino et al., 2003; Zacharof and Lovitt, 2014), in addition to the non-negotiable fit-for-purpose water product. Potential for upgrading of the digestate to extended products of value also exists.

4.1 Post AD processing

AD effluent water, also termed 'digestate', contains organic, inorganic and particulate compounds in significant levels; the quantities and concentrations present are highly dependent on the feedstock to the AD and the nature of AD reactor used. Therefore, the effluent streams may have residual levels of COD, total nitrogen (TN) and total phosphorous (TP) in addition to sulphides or metals, dependent on feedstock. Most anaerobic digesters operate under mesophilic conditions while some are thermophilic. Some produce an intermittent sludge product and others a slurry comprised of microbial cells and undigested substrate or a combination, which may be combined or separate depending on reactor design. Due to this, AD effluents are often unsuitable for direct discharge into the environment and, in some cases, are unfit for irrigation purposes (Cheng et al., 2015; Liu et al., 2015). AD digestors treating wastewater, particularly using granulated systems, are less prone to a high solids content in the digestate. In this project, our focus is on potential for recovery of VFAs as an intermediate for conversion to products of value or use as a feedstock for remediation processes, recovery of the nutrients phosphorus and nitrogen for re-purposing and recovery of 'fit-for-purpose' water for re-purposing from the digestate, as well as recovery of methane as biogas, for electricity generation or steam production and recovery of sludge with potential as a soil conditioner. Production of methane and VFAs is the focus of Chapter 3. Recovery of methane is straightforward owing to its partitioning to the gaseous phase; its further valorisation is discussed in Chapter 7. For recovery of nutrients, valorisation of a portion of the organics beyond methane and recovery 'fit-for-purpose' water, further treatment of the resultant digestate stream is required. These treatments are discussed in this chapter. In Chapter 5 the further processing of water for safe use is discussed.

Treatments have been reported from the perspective of meeting environmental legislation (Webb et al., 2003; Frischmann, 2012). Here we focus on the recovery of nutrients and organic products from the digestate for safe re-use to enhance resource productivity with simultaneous recovery of water. The attainment of water of acceptable quality for its purpose is further expanded in Chapter 5. AD sludge may either be dried or used wet as fertiliser, incinerated with energy capture or disposed of to land fill, the latter being least desirable. Chemical, biological, thermal and physical methods such as flocculation, coagulation, sedimentation, ozone treatment, adsorption and filtration have been used for the purpose of reclaiming water from digestate. The juxtaposition of these downstream unit operations and their purpose in the conditioning of the water or recovery of nutrients is shown schematically in Figure 4-1 and the associated unit operations are listed in Table 4-1.

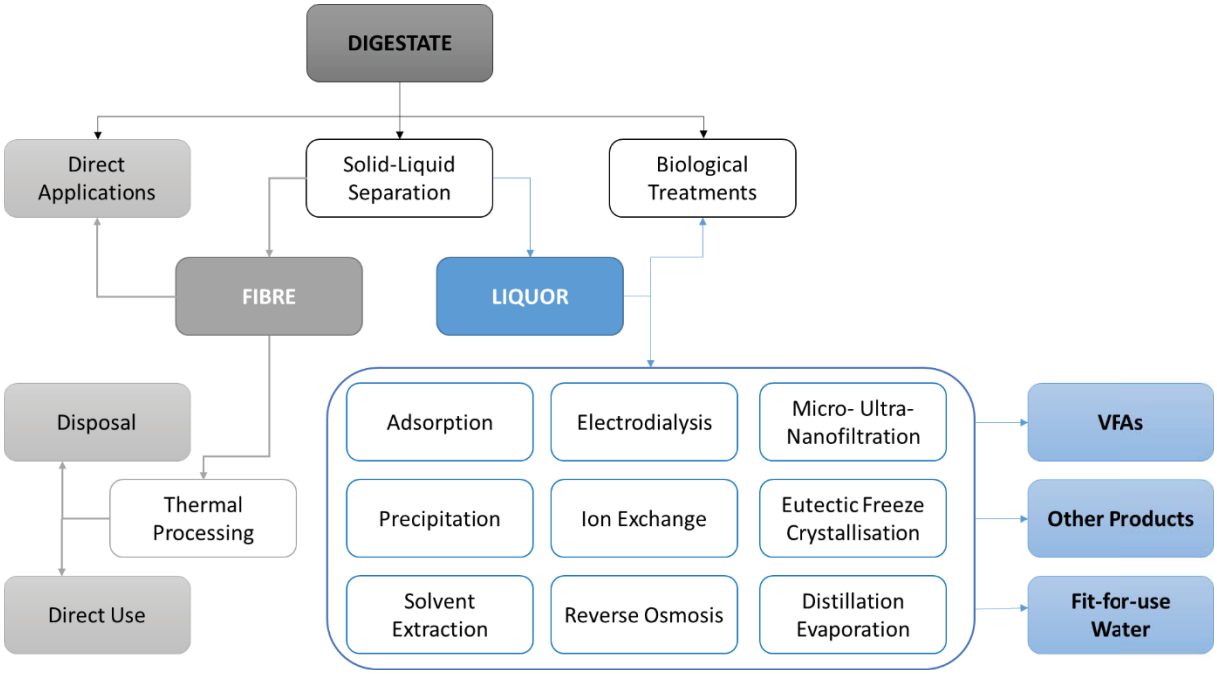


Figure 4-1 AD digestate treatment techniques

In general, the treatment options used to date are focused on one of four applications: solid-liquid separation; solid sludge treatment and potential beneficial usage; biological removal of biological oxygen demand (BOD) and nutrients, such as nitrates and phosphates; and physico-chemical removal of trace chemicals (for recovery) and contaminants (for removal) (Colorado School of Mines, 2009; Frischmann, 2012; Gupta et al., 2012). In this study,we extend their consideration to focus on the recovery of water from wastewater and its re-purposing to 'fit-for-purpose' water (Chapter 5), organic products of value, nutrients N and P for re-use and sludge with potential as fertiliser.

Table 4-1 Summary of techniques used for treatment of AD effluent
Red- Solid-liquid separation; **Green-** Solid sludge treatment options;
Blue- Treatment of BOD, nitrates and phosphates; **Black-** Non-biological liquid treatment options
Compiled from Colorado School of Mines(2009), Frischmann (2012) and Gupta et al. (2012)

Physical	**Thermal**
Thickening (belt press, centrifuge) Dewatering (belt press, centrifuge, Bucher press) Purification (filtration, reverse osmosis) Ion exchange Electrodialysis Adsorption	Drying (rotary drying, belt drier, solar, j-vap) Conversion (incineration, pyrolysis) Evaporation Distillation Eutectic freeze crystallisation
Biological	**Chemical**
Composting Reed beds Biological oxidation Biofuel production (algae) Microbial fuel cells	Coagulation Flocculation Precipitation (struvite, numerous others) Solvent extraction

A number of WRC reports and Water SA papers deal with downstream processing or pre-treatment for recovery of valuable components from wastewater processed by AD (Table 4-2).

Table 4-2 WRC reports and Water SA articles most relevant to recovery of valuable components of wastewater

Title of WRC document	Reference
Integrated photocatalytic and anaerobic treatment of industrial wastewater for biogas production	Aoyi et al. (2015)
Beneficiation of agri-industry effluents: extraction of anti-oxidant phenolics from apple and citrus wastewaters coupled with fermentation of residual sugars to ethanol or other value-added products	Burton et al. (2012)
Fate and behaviour of engineered nanoparticles in simulated wastewater and their effect on microorganisms	Chaúque et al. (2016)
The performance and kinetics of biological nitrogen and phosphorus removal with ultrafiltration membranes for solid-liquid separation	du Toit et al. (2010)
Nanotechnology for the treatment of industrial scale effluents – particularly the removal of organic contaminants from textile effluents using nano-TiO_2	Greyling et al. (2017)
Membrane bioreactors for metal recovery from wastewater: A review	Mack et al. (2004)
Performance of tubular reverse osmosis for the desalination / concentration of a municipal solid waste leachate	Schoeman and Strachan (2009)
A technological and economic exploration of phosphate recovery from centralised sewage treatment in a transitioning economy context	Sikosana et al. (2017)
Nutrient and energy recovery from sewage: towards an integrated approach	Sikosana et al. (2016)
Technical and social acceptance evaluation of microfiltration and ultrafiltration membrane systems for potable water supply to rural communities	Swartz Water (2009)
The removal of N and P in aerobic and anoxic-aerobic digestion of waste activated sludge from biological nutrient removal systems	Vogts et al. (2014)
The treatment of wastewaters with high nutrients (N and P) but low organic (COD) contents	Musvoto et al. (2003)

4.2 Solid-liquid separation and solids dewatering

AD digestate streams typically contain particulate material, therefore a separation step is required before the liquid phase can be recovered for further downstream processing. The solids are composed of both undigested substrate and microbial biomass formed in the AD process (Bonmati and Flotats, 2002; Dosta et al., 2008; Frischmann, 2012). Consequently, the first step in all treatment options is physical separation of the liquid and solid fractions as shown in Figure 4-1 (Colorado School of Mines, 2009; Gupta et al., 2012). The separated liquids and solids, now termed liquor and fibre or sludge, can be treated separately, allowing more options for purification and energy recovery. Physical techniques, such as thickening and dewatering, capture the solids and allow for manageable transport and storage of the digester sludge and residual fibre in a significantly reduced volume (Frischmann, 2012).

Coagulation and flocculation are chemical treatment methods that may be applied prior to filtration or sedimentation of wastewater to improve the solid-liquid separation step. Without this prior treatment, very small particles require micro- or ultrafiltration for removal. An alternative is to make use of their high surface areas and the fact that their behaviour in solution is governed by their surface properties (Gray, 2010) to use surface processes such as dissolved air flotation.

Coagulation works by destabilising the charges on the particle surface, disrupting the repulsion forces between suspended solids (Mazille and Spuhler, 2012). In its absence, particles form micro-flocs. Coagulation allows for aggregation of particles into large visible flocs that can settle out of solution (Dosta et al., 2008; Mazille and Spuhler, 2012). Some of the more commonly used coagulants include lime, alum, ferric chloride and ferrous sulphate (Dosta et al., 2008). However, these chemicals must be consistent with the further use of the water and cost effective for treatment of large volumes.

Flocculation occurs by collisions of micro-flocs to form macro-flocs. High molecular mass polymers are, in some cases, added at this point to strengthen the floc, add mass and increase settling rate (Dosta et al., 2008; Mazille and Spuhler, 2012). Advantages of this system include its simplicity, low cost and wide applicability. However, disadvantages include the potential transfer of toxic chemicals into the sludge requiring additional treatment. Also, chemicals used could inhibit downstream biological treatment.

Thermal drying is used to reduce the remaining water in dewatered digested fibre (extracted solid sludge) to produce a drier product, as much as 98% dried solid. The dried fibre has several uses, many of which revolve around land application. In lieu of land application, dried digestate fibre or sludge can be incinerated; a process for which the efficiency is determined by the efficiency of the drying process. This incineration of digestate fibre or sludge can become an auto-thermal process, dependent on moisture content (Dowling, 2009). Phosphorous can also be obtained from the ash through acid leaching (Frischmann, 2012).

The liquid fraction may then be treated to obtain 'fit-for-purpose' water and to retrieve other products.

4.3 Liquid separation for resource streams and fit-for-purpose water

Following solid-liquid separation, the liquor stream may undergo an intermediate step of solvent extraction or precipitation to recover high value products or a pre-treatment step in preparation for more exacting purification. Whether or not this median step is used, water treatment options must be implemented to produce water of a high quality, 'fit-for-purpose'. In the light of this, chemical additions to the water need to be carefully considered and, typically, minimised.

The recovery of wastewater to potable water is the ideal goal for any wastewater treatment facility, yet it is challenging and needs tight process control. An example is the Windhoek municipality which has utilised reverse osmosis to treat municipal wastewater for potable consumption since 1968 (Asano, 2002; Wintgens et al., 2005). A second example includes the reverse osmosis plants in the Mpumalanga area treating acid mine drainage to potable water through reverse osmosis, the best known being the Emalangeni plant. Economically, the cost of processing required to produce potable water may become excessive; in these cases it can be preferable to process the water to be 'fit-for-purpose' for agricultural irrigation, industrial re-use or similar operations (Asano, 2002; Gupta et al., 2012; Levine and Asano, 2004).

Biological options are typically required for liquid streams with high residual BOD, although this should not be required for most AD processes (Frischmann, 2012; Gupta et al., 2012). A 2010 WRC report has investigated the coupling of biological reactors with membranes to achieve BOD removal and water treatment simultaneously (Edwards et al., 2010). More recently the concept of wastewater biorefineries has modelled the placement of multiple biological processes, including AD, in series for simultaneous production of 'fit-for-purpose' water and valuable products (Verster et al., 2016).

The following discussion only considers downstream processing of the AD effluent liquor assuming that further biological treatments are not required. All the technologies produce a purified water stream and a stream containing concentrated nutrients and other components which can be purified for recovery or valorisation (see Chapter 7) or discarded.

4.3.1 Distillation and evaporation

Distillation and evaporation both work on the principle of vapourisation of the liquid stream which is then condensed to form a near pure water stream (Cheremisinoff, 2002; Colorado School of Mines, 2009; Gupta et al., 2012). In single stage vapourisation and condensations, components more volatile than water, such as formic acid, are recovered to the water stream as impurities. Evaporative systems for water recovery are less common than distillation and differ in that vapourisation takes place below the boiling point at the ambient pressure of the system. Hence, it is characteristically slower.

Numerous options exist, including evaporation, membrane distillation, multiple effect distillation, mechanical-vapour-compression distillation and multi-stage flash (Colorado School of Mines, 2009). Membrane distillation (pervaporation) uses hydrophobic membranes in combination with the thermal process and, as such, can handle streams with higher total dissolved solids than other processes. It can also selectively separate a higher proportion of water than traditional techniques like distillation. Consequently, the need for extensive pre-treatment and post treatment is reduced and the system is able to handle a higher degree of process fluctuation (Colorado School of Mines, 2009). However, this technique is still relatively new and un-proven on large scales.

Multiple effect distillation and multi-stage flashing use a series of stages to minimise the energy consumed within the process. The energy used for vapourisation at a stage with a lower pressure is recovered from the heat released by condensation from a stage with a higher pressure. Multi-stage flashing differs from multiple effect distillation in that the liquid entering a new stage almost instantaneously vaporises. In both cases, steam is the primary source of heat. Mechanical-vapour-compression distillation is similar to multiple effect distillation but uses mechanical compression of the vapour from the last stage and recovers the heat from condensation in the same stage. Vapour compression distillation can also handle most water sources and is not particularly sensitive to total dissolved solids (Colorado School of Mines, 2009).

Both evaporation and distillation are well established technologies. The theoretical minimum amount of energy required to purify water by desalination is 0.86 kWh m^{-3} but values of 5 to 26 fold are observed in practice. Thermal processes require more overall energy input than mechanical processes such as reverse osmosis or mechanical-vapour-compression distillation; hence, some claim these not to be feasible for water recovery processes (Cheremisinoff, 2002; Colorado School of Mines, 2009). In the AD system, the methane generated can be used provide the thermal energy needed for heating and evaporation in evaporative and distillation processes; however, evaluation of the energy cycle remains important.

4.3.2 Ion exchange

Ion exchange takes advantage of the reversible adsorption of ions of the same charge from a solution onto a solid ion exchange medium (Gray, 2010). This method has been tested on high strength (high salts) water and on complex wastewaters such as vinasse and has been found to show a salts removal efficiency ranging from 87 to 99 % (Kleerebezem et al., 2015; Zhang et al., 2012).

Ion exchange is commonly carried out in downflow fixed bed reactors, where the resin is housed in a tank similar to that of a sand filter in design. Some of the most common exchange media are in the form of polymer resins; however, naturally occurring and synthetic zeolites (aluminosilicates) are also used (Gray, 2010; Kesraoui-ouki et al., 1994; Kotsopoulos et al., 2008). Exchange resins are capable of selectively removing cations, anions, or both from a solution (Gray, 2010; Zhang et al., 2012). Cations are most commonly replaced with Na^+ or H^+ while anions are exchanged for OH^- ions. These are refreshed once the solute has been eluted from the resin (Gray, 2010; Wang et al., 2007).

Ion exchange is a well-established technology and the resins can be regenerated effectively since the adsorption is reversible (Colorado School of Mines, 2009; Gupta et al., 2012). However, the regeneration solutions require extensive treatment, and the resins can be fouled by suspended solids, metals, organics and sulphates. Furthermore, if the impurity levels in the wastewater are high, the resins are exhausted quickly. Consequently, the need for pre-treatment is extensive (Colorado School of Mines, 2009; Gupta et al., 2012; Kapoor and Viraraghavan, 1997). Ion exchange systems also cannot remove non-charged particles, but they are effective at removing dissociated organic acids (Colorado School of Mines, 2009; Gupta et al., 2012; Teella, 2011). Ion exchange resins can therefore be used to selectively remove organic acids which allows for their recovery (Alkaya et al., 2009).

Adsorption in the form of ion exchange has been used in the removal of ammonium nitrogen from AD effluent using natural zeolite (Milan et al., 1997; Sánchez et al., 1995). Sánchez et al. (1995)

investigated the efficiency of AD of piggery waste and the feasibility and efficiency of subsequent ion exchange using zeolite columns to selectively remove NH_4^+ and PO_4^{3-} for later recovery. The results of this study showed a 90% removal in ammonium nitrogen after 20 hours of operation. In addition, a 70 to 80% reduction was observed for phosphate. Further increasing operating time resulted in an increase in the concentration of phosphate and ammonia in the effluent, owing to the saturation of active sites (Milan et al., 1997; Sánchez et al., 1995). This technique can be employed to recover valuable phosphates and nitrogen for sale. It is currently being explored in CeBER for the recovery of K^+ from high salt vinasse, prior or post-AD.

4.3.3 Filtration

Physical purification in the form of filtration involves the separation of water and suspended contaminants or large solutes using a physical barrier such as a membrane which acts as a molecular sieve retaining the suspended material or large solute molecules (concentrate) and allowing the water (permeate) through

Types of filtration membranes are usually categorised according to pore size as they operate on size exclusion (Cheremisinoff, 2002; Koch, 2013; Williams, 2003). Conventional filter membranes remove particles that are visible to the eye such as sand (particles larger than 10^{-2} mm). Microfiltration membranes are capable of separating particles with sizes between 0.1 and 10 µm including some microorganisms. Ultrafiltration removes particles and solutes down to a size of 0.01 µm, including macromolecules and most microorganisms. While nanofiltration pores are small enough to separate salts and ions (Gray, 2010; Koch, 2013; Lameloise et al., 2015).

All filtration methods are well established with fouling of the membranes being the chief obstacle to overcome. Technological advances have meant that fouling is reduced with newer membranes (Cheremisinoff, 2002; Colorado School of Mines, 2009; Jaiyeola and Bwapwa, 2016; Zacharof and Lovitt, 2014). To reduce fouling and ensure effective operation, the filtration methods are used in series with the largest pore sizes first and each filter removing smaller and smaller particles and solutes. As such microfiltration and ultrafiltration are used as pre-treatments for nanofiltration systems (Colorado School of Mines, 2009; Gupta et al., 2012; Zacharof and Lovitt, 2014).

4.3.3.1 Microfiltration

Microfiltration (MF) is marketed in a ready to use cartridge or a plate and frame configuration and can separate or retain particles between 0.05 and 5 µm in size. MF is widely used for the removal of chlorine resistant pathogens, large colloids and bacteria.

The membrane can either be installed as tubular, capillary, hollow fibre or spiral wound sheets in the cartridge or flat sheet in the plate and frame configuration. The untreated water is pumped into the filter system at high pressure, between 100 and 400 kPa, forcing clarified liquid (permeate) through the membrane while retaining particles that are too big to pass through the membrane (Gray, 2010).

MF membranes are designed with uniform pores at a high pore density of approximately 80 %. This high density results in a low hydrodynamic resistance during operation, allowing for high flow rates. The concentration of sludge build-up increases through operation and the volume of permeate recovered is inversely proportional to the build-up of the sludge on the membrane. As a result, periodic backwashing is required to clean the system. Backwashing is achieved with either pressurised water or gas and involves using reverse pressure to clean or remove particles trapped in the membrane (Gray, 2010). After several backwashes the membrane integrity is lost and it has to be replaced.

4.3.3.2 Ultrafiltration

Ultrafiltration (UF) works on the same principle as MF, with the major difference lying in the pressure used and the size of the micropores (0.002 – 0.1 µm). UF membranes are made of thin (0.1 – 1 µm) polymer membranes which can withstand pressures as high as 700 kPa (Colorado School of Mines, 2009; GEA Process Engineering, 2012; Van Der Bruggen et al., 2003). UF has different applications to

MF, with mainly solvents and salts being able to cross the membrane. As such, in industry, it is most commonly used for the concentration of proteins and hormones or their removal, removal of colour or humic substances and the removal of bacteria, viruses and cellular fragments (Frischmann, 2012).

4.3.3.3 Nanofiltration

Nanofiltration (NF) works on the same principle as ultrafiltration and due to the pore size (0.5-10 nm) can achieve liquid streams almost as pure as reverse osmosis (Colorado School of Mines, 2009; Williams, 2003; Yangali-quintanilla et al., 2011). As with all filtrations systems, membrane fouling is the design issue and hence micro and ultrafiltration is required for pre-treatment (Zacharof and Lovitt, 2014). NF also requires very high pressures (300-2000 kPa) for operation and hence the operating expenses are relatively high when compared with other filtration methods (Colorado School of Mines, 2009; Gupta et al., 2012; Van Der Bruggen et al., 2003; Williams, 2003). NF effectively retains multivalent ions, such as nitrates and phosphates, (Colorado School of Mines, 2009; Gupta et al., 2012; Van Der Bruggen et al., 2003) but is less efficient with low molecular weight VFAs, such as acetic acid, which partition into both the retained and filtered liquid (Teella, 2011; Williams, 2003; Yangali-quintanilla et al., 2011). This is because acetic acid has a molecular size of less than 0.4 nm which is below the size scale of NF membranes (Green and Perry, 2008; Welty et al., 2008).

4.3.4 Reverse osmosis

Reverse osmosis (RO) works on separating compounds based on their diffusivity and solubility and can be used effectively to separate both inorganic and organic molecules from water (Colorado School of Mines, 2009; Gupta et al., 2012). RO is the standard technique in desalination technology and hence has developed fast with improved membranes and technologies (Ghaffour et al., 2013; James and Boriah, 2010). During the process of osmosis, the solvent flows from a dilute solution through the membrane to a more concentrated solution. This will occur until an equal concentration is reached on both sides of the membrane. During RO, however, water particles are forced to move in the opposite direction when osmotic pressure is applied (Gray, 2010). When pressure equal to the osmotic pressure is applied to the liquid, the process of osmosis can be stopped. By increasing pressure beyond this point, osmosis can be reversed and water will move from a concentrated salt solution to a low concentration solution.

RO removes particles, solutes and ions between 0.1 and 0.5 nm; this includes total solids, metal ions (removal efficiency above 95%), organics such as trihalomethanes, pesticides, benzene and nitrates (Gray, 2010; Morin Couallier et al., 2006). However, very small inorganic and organic particles remain in the purified water stream. This means that RO cannot effectively retain all low molecular weight organic acids, such as formic and acetic acid, although relatively high retentions have been demonstrated (Cheremisinoff, 2002; Colorado School of Mines, 2009; Diltz et al., 2007; Ragaini et al., 2005). The retentions of low molecular weight VFAs is dependent on the membrane and pore size used with better retentions obtained for small pore size systems that operate at higher pressures (Colorado School of Mines, 2009; Diltz et al., 2007; Yangali-quintanilla et al., 2011).

Practically, application of RO on wastewater has shown that it can be used for the elimination of fermentation product molecules such as alcohols, acetic, propionic and butyric acids at a retention rate higher that 85%. This process will allow for the elution of clarified water without the use of toxic chemicals while meeting the concentrations required by industry. In addition to its high performance RO can also operate at a wide pH range (Morin Couallier et al., 2006). Morin Couallier et al. (2006) looked at the application of RO for the treatment of the complex wastewater vinasse at four different pHs. Their study showed that as pH increases there is an increase in the rejection of difficult organic molecules.

As with other membrane systems, fouling is the primary issue for RO operation, with organic acids, phenols, metal oxides and low solubility salts being the chief fouling agents (Colorado School of Mines, 2009). Total dissolved solids also need to be kept low to ensure the membrane does not clog too quickly (Kapoor and Viraraghavan, 1997). Therefore, extensive pre-treatment, typically in the form of

ultrafiltration, and the addition of anti-scaling compounds is required (Colorado School of Mines, 2009; Gupta et al., 2012) as the performance of the RO system is dependent on the quality of the influent wastewater (Lameloise et al., 2015). Due to its wide application, the technology is robust and can produce high quality water at a stable rate when operated correctly. It is also typically one of the cheapest available options for the production of potable water (Colorado School of Mines, 2009; Williams, 2003; Zhang et al., 2012). Scale-up is easy due to the modular design (Williams, 2003).

In a 2010 study, UF, RO and MF were coupled with a membrane bioreactor (MBR). The main finding of this report was that RO and UF were capable of operating at 91 % removal of salts and a 92% rejection rate of TDS and approximately 94% reduction in turbidity (Edwards et al., 2010). This indicated that RO was the best choice for downstream processing of the microbial bioreactor effluent. It was also suggested by Edwards et al. that water purification using membrane systems would require coupling the systems in series. Thus MF, UF, then RO could be ideal for the remediation of water for re-use.

4.3.5 Eutectic freeze crystallisation

In eutectic freeze crystallisation, the liquid stream is chilled to produce pure ice crystals and a concentrated liquid containing the other components. The technique can also recover pure salt crystals if the solution is brought to the specific compound's eutectic point (Fernández-Torres et al., 2012; Padhiyar and Thakore, 2013; Randall et al., 2011). The technique has typically only been implemented for brine solutions and applicability for multi-component streams is largely unknown (Fernández-Torres et al., 2012; Randall et al., 2011). Furthermore the technology is still novel (Padhiyar and Thakore, 2013; Randall et al., 2011). Following piloting by Eskom in 2017, the first large scale plant was commissioned Prentec at the Glencore Tweefontein Water Treatment Plant in 2017-8 to treat the brine of an RO plant reclaiming potable water from acid mine drainage (Nicolson, 2017; Nkuna, 2016). This plant treats 750 000 litres per day. Although eutectic freeze crystallisation has almost exclusively been used for inorganic salts, it is possible to separate organics, such as organic acids, from solution (Padhiyar and Thakore, 2013).

The complexity of the stream determines the equipment and cooling required and the economics depends entirely on the waste stream itself. Due to the process being more complex than other separation methods, the capital investment is, however, known to be high (Padhiyar and Thakore, 2013; Randall et al., 2011; van der Ham et al., 1998) but expected to reduce on increased implementation.

4.3.6 Adsorption

Adsorption involves the removal or transfer of molecules from a liquid phase to a solid surface. This process has been found to be a viable solution for the treatment of various solutes. The solid surface, or adsorbent, provides a surface where species can be adsorbed. These species termed the adsorbate can selectively be removed by changing the properties of the liquid phase in a process known as desorption (Worch, 2012). When the adsorbent is correctly chosen, the adsorbed molecules can be recovered in this desorption process.

Since the performance of this technique is focused on surface retention, surface properties of the adsorbent are highly important. Surfaces typically need to provide high surface area through high porosity with numerous active sites (Worch, 2012). Adsorption has previously been used for the removal of organics such as phenols, chlorinated hydrocarbons, and pesticides from drinking water, swimming pool water, ground water and landfill leachates. This has mainly been done using activated carbon, aluminium oxide and polymeric adsorbents. In AD waste, the absorption of difficult to remove organic acids can be completed effectively using activated charcoal (Cheremisinoff, 2002).

Materials that exhibit high surface area to mass ratio plus stable physical and chemical properties such as nanomaterials, metal oxide nanoparticles and zeolites have been used in the removal of heavy metals, organics and biological impurities from wastewater (Liang et al., 2011; Worch, 2012). Although efficient, these adsorbents suffer from two primary limitations: low adsorption capacity and slow adsorption rates (Thakur and Voicu, 2016). The nanostructure adsorbents are now being immobilised

into membranes to improve performance and develop cost effective and fast water purification. Immobilisation into membranes increases contact between the adsorbent surface and the liquid phase, thus increasing the contact time between the surface and the surface active sites (Liang et al., 2011).

4.3.7 Electrodialysis

This process is, by definition, a membrane process that uses an electrical current to separate ions. Ions in solution are attracted and attach to fixed charge groups that are secured to the polymer matrix of the ion selective membranes. Each electrodialysis unit is made up of numerous flat membrane sheets (approximately 400) arranged in a layout of alternating cation and anion sheets, 0.5 to 2.0 mm thick and sandwiched between an anode and a cathode (Gray, 2010). The anion membranes in the system are only permeable to anions and impermeable to cations, while the cation membrane is permeable to cations and impermeable to anions. As a result, when an electrical current is applied, the membranes restrict movement of specific ions and result in an ion rich solution and an ion depleted solution. Studies have shown that this process is not applicable for high salt water e.g., sea water, but it has been used in the metal plating and pharmaceutical industries as well as for the remediation of wastewater such as vinasse (Decloux et al., 2002; Gray, 2010), the latter carrying a high salt content.

This technique requires the liquid to be extensively pre-treated with prior ultrafiltration being the typical method (Kapoor and Viraraghavan, 1997). The technology has already been extensively developed and further improvements are unlikely (Kapoor and Viraraghavan, 1997). As with ion exchange, only charged molecules are separated by this technique. Electrodialysis does allow for the removal of organic acids in solution (Cheremisinoff, 2002; Huang et al., 2007; Tao et al., 2016; Yu et al., 2000).

4.3.8 Comparison of technologies available for recovery of fit-for-purpose water and associated compounds

The costs and applicability of the different treatment options are presented in Table 4-3. The exact treatment train required is highly dependent on the effluent stream produced from the anaerobic digester. Most systems require initial separation of the solid and liquid fractions using filtration or screening. The production of potable water from wastewater requires evaporation, distillation, ion exchange, nanofiltration or RO as the final purification step (Asano, 2002; Colorado School of Mines, 2009; Gupta et al., 2012; Levine and Asano, 2004). Some general concluding remarks follow about treatment selection; however, final decisions require analysis of the AD effluent being treated.

Due to the complex nature of the AD effluent with its mixture of inorganic, organic and biological components, any system producing potable water requires step-wise treatment culminating in the treatment, yielding potable water. The number of treatment steps required is maximum for ion exchange, nanofiltration and RO, with these systems probably requiring microfiltration and ultrafiltration before the final processing step. Evaporation and distillation also require some form of filtration, with microfiltration typically sufficing. Electrodialysis, adsorption and eutectic freeze crystallisation all require pre-treatment using MF and, potentially, UF.

Table 4-3 Cost and applicability of various wastewater treatment options. Abbreviations: Sol Soluble Sus- Suspended I- Inorganics O- Organics V- Volatiles B-Biologicals *-Rarely used technology (Gupta et al., 2012)

Technology	Applicability	Costs US$ /ML treated	Principle of separation
Evaporation*	Sol & Sus IOB	15-200	Boiling point
Distillation	Sol IOB	15-2000	Boiling point
Ion Exchange	Sol IO	50-200	Charge of particles
Micro-, ultra- and nanofiltration	Sol IOB	15-400	Particle size
Reverse osmosis	Sol IOB	20-450	Diffusivity and solubility
Crystallisation*	Sol IO	50-150	Melting point, solubility
Electrodialysis	Sol IO	15-400	Charge of particles
Precipitation*	Sol IO	20-500	Chemical reaction, solubility
Adsorption	Sol & Sus IOB	50-150	Attraction to surface
Solvent extraction	Sol OV	250-2500	Solubility difference; partition coefficient

Ion exchange is only capable of removing charged particles. It does, however, require less intense pre-treatment than RO (Cheremisinoff, 2002; Williams, 2003). As can be seen from Table 4-3, its entry level cost is typically greater than RO. Nanofiltration and RO are similar processes, with similar process issues and produce similar quality water. The water product from RO is usually of a slightly higher standard (Colorado School of Mines, 2009; Williams and Follows, 2011; Yangali-quintanilla et al., 2011). RO has less down time than ion exchange systems (Cheremisinoff, 2002) and does not require the use of hazardous regeneration chemicals (Colorado School of Mines, 2009).

Evaporation and distillation both require large amounts of energy for the heating and vapourisation of water, hence are typically considered unfavourable. The biogas produced in AD processes can be used for energy integration to produce the heat or steam required for these systems (Colorado School of Mines, 2009). In terms of energy usage and ability to handle process variations, vapour compression distillation appears to be the best option for AD effluent. It is significantly more energy efficient than evaporation processes (Colorado School of Mines, 2009). The cost of distillation is highly dependent on the contaminants found within the incoming water but can compete with RO as shown in Table 4-3.

The carryover of small organic molecules, such as formic and acetic acids, is probable if VFAs are not removed earlier for recovery. If at high concentrations, needing removal, the use of absorbents, such as activated charcoal, or ion exchange resins are feasible (Alkaya et al., 2009; Cheremisinoff, 2002). Efficient recovery of VFAs requires their extraction into a solvent such as ethyl acetate and subsequent energy intensive distillation, owing to the high volumes resulting from the partition coefficients found (Petersen et al., 2018). Refinement of solvents for this process which have improved partition coefficients and low water solubility are being addressed. The VFA removal by extraction or adsorption can be done before the final water purity treatment, or as the final treatment step.

The production of high quality potable water from AD effluent is most likely to be achieved economically using RO or vapour compression distillation. The potential for eutectic freeze crystallisation requires investigation. RO is well established and relatively cheap for treating all types of organic and inorganic contamination. Vapour compression distillation allows for energy integration with biogas production and is the most energy efficient type of evaporation or distillation process.

The complete treatment option will require: solid-liquid separation with drying and potentially processing of the solid waste; microfiltration and potentially ultrafiltration to ensure TDS and contaminant levels are low enough for the final processing; activated charcoal absorption or ion exchange to remove organic acids; and, potentially, final purification using vapour compression distillation or RO. The recovery of phosphates and nitrates would be most likely be achieved through precipitation of the resulting concentrated stream from either of these two processes.

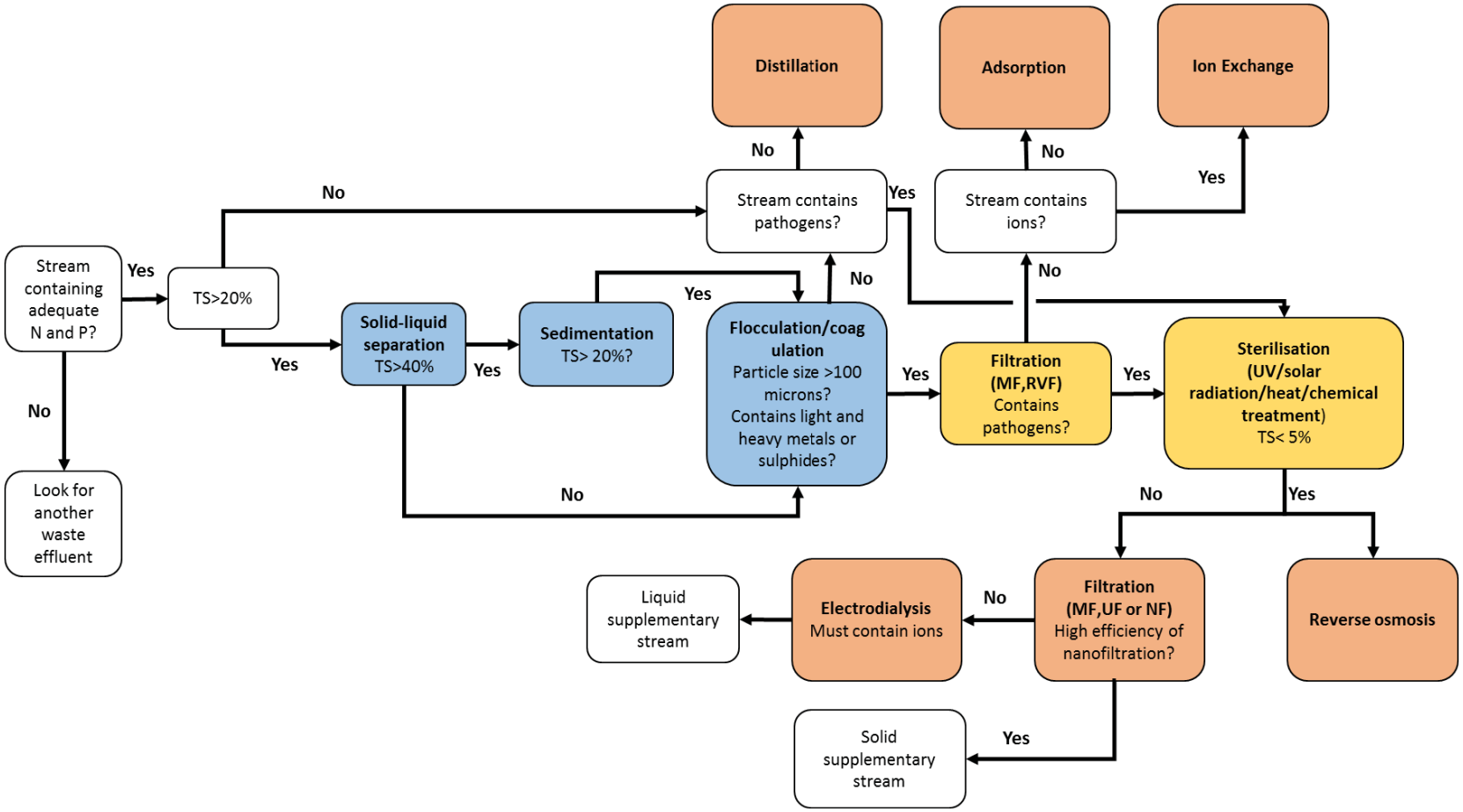


Figure 4-2 Decision making tree for selection of appropriate liquid separation technology for recovery of nitrogen and phosphorus (TS – Total solids, MF- Membrane filtration, NF – nanofiltration, UF – ultrafiltration)

4.3.9 Selection of liquid separation technology for further assessment

The selection of the most appropriate separation technology is dependent on a number of factors which vary with context. These factors include the composition of the stream and the products to be recovered in addition to the fit-for-purpose water, the volume of the stream, its BOD and the final proposed use of the water. A key factor in setting up the DSP train is the total solids in the effluent which determines the initial separation steps (blue in Figure 4-2). Thereafter the size of remaining particulates and solutes present, the presence of heavy metal contaminants and the presence of pathogens determine whether intermediate filtration or sterilisation are needed (yellow in Figure 4-2). The final separation step(s) (orange in Figure 4-2) is determined by whether product or contaminant is ionic, whether there are residual solids, their particle size, solubility and reactivity of the compounds present.

From the review of separation operations and knowledge of typical components within the digestate, it has been concluded that the key steps for DSP, subsequent to solids removal, are the filtration processes: micro-, ultra-, nano- filtration and RO, precipitation (for struvite) and adsorption. The ionic species, including P and N compounds report to the concentrate of nanofiltration and RO while the short chain VFAs e.g., formic and acetic acid may be found in both streams; hence post-retentate adsorption is proposed for final polishing of the water and recovery of VFAs, if not recovered earlier in the process train.

4.4 Recovery of valuable products through separation operations

Ion exchange and eutectic freeze crystallisation can be used to remove and recover the high value charged compounds from solution selectively and simultaneously with water purification (Colorado School of Mines, 2009; Frischmann, 2012; Gupta et al., 2012; Milan et al., 1997). In particular, recovery of N as ammonium ions, P as phosphate ions and metal cations is reported. The adsorption of VFAs for recovery is also a viable option although the purity of the VFAs produced is dependent on the selectivity of the adsorbent used (Cheremisinoff, 2002; Worch, 2012). Any of these three processes can be used at an appropriate point in a step-wise purification process.

During distillation and evaporation processes, the phosphorus and nitrogen compounds are partitioned to the concentrate from which they may be recovered. VFAs can report to both the purified water and the concentrate streams due to their low boiling points. This complicates VFA extraction, implying that their extraction should either be complete prior to one of these processes, or that a final water purification for extraction of VFAs should follow, typically using adsorption.

With nanofiltration, RO and electrodialysis, the concentrate produced contains most of the phosphates and nitrogen compounds found in the incoming effluent, hence these compounds can be recovered from this stream. VFAs, particularly formic and acetic acid, have small size particles and can therefore be found in both the purified water and concentrate stream. However, the VFAs will be mostly retained in the concentrate stream, particularly in the case of RO. Larger organic molecules will also report to the concentrate stream. The exact retention obtained depends on the membrane used, its pore size and pressures used.

4.5 Recovery of products of value from the concentrate formed on water purification

In cases where the water purification methods do not allow adequate recovery of the selected products, it is possible to use precipitation or solvent extraction (Figure 4-2). However, these methods introduce new compounds into the waste stream which make further processing of the liquid stream harder to achieve. To ensure the chemicals added to mediate the separation are not present in the purified water stream, precipitation and solvent extraction would be carried out on the concentrated component stream produced during the DSP step which is used to recover fit-for-purpose water.

The precipitation of phosphate and ammonia is possible using struvite precipitation or through other salts. The precipitated compounds can be sold as raw ingredients for the fertiliser industry (De-Bashan and Bashan, 2004; Tao et al., 2016; Yoshino et al., 2003) or further processed for other uses. Organic acids can be recovered through solvent extraction and sold (Alkaya et al., 2009, Petersen et al. 2018). Solvent extraction and precipitation are typically concentration-based, motivating their application to the concentrated waste stream produced through prior water purification processes (Mostafa, 1999; Tao et al., 2016; Yoshino et al., 2003; Zacharof and Lovitt, 2014). Economically, precipitation is the more favourable option for recovery of phosphates and nitrogen compounds (Gupta et al., 2012). Alternatively, the use of concentrated liquid streams rich in N and P as fertilisers is gaining popularity, as demonstrated by the 'Liquid Gold' fertiliser produced from urine (Galloway et al., 2008; Randall and Naidoo, 2018).

4.6 Valorisation of organic compounds and nutrients recovered from the AD digestate

The potential for further value creation from residual components in the digestate is recognised as is the potential necessity for their removal, based on the planned use of the water product generated. This valorisation can be carried out by recovery of key compounds or by their conversion into new compounds of interest and value. To investigate this, in Table 4-4 examples of the concentrations of N, P and VFAs entering and leaving the AD digestor are shown, demonstrating the potential for additional value recovery. Further, operation of the AD, or pre-treatment prior to AD, can impact the potential for value recovery of organic products, in addition to methane. This potential is shown in Figure 4-3. It is proposed that to deliver potential for value recovery from VFAs in the digestate effluent, the cut-off of minimum feasible VFA concentration lies in the range 30 to 400 g VFA per litre (Chang, 2009) (Chang 2009).

From Table 4-4, it is seen that additional recovery and processing of VFAs from algal and piggery digestate is unlikely to be feasible unless designed into the circuit to displace some of the methane generation, for example through acidogenic digestion (Section 3.4). A number of industrial effluents are possible sources for direct valorisation of VFAs from digestate. Concentrating processes are typically too costly for the value generated; however, these may be required for polishing to potable water.

Table 4-4 Example compositions of the feed and effluent streams to and from AD

FEEDSTOCK	FEED NTOTAL [mg/L]	FEED PTOTAL [mg/L]	FEED VFA [mg/L]	EFFLUENT NTOTAL [mg/L]	EFFLUENT PTOTAL [mg/L]	EFFLUENT VFA [mg/L]
Algal	664	376	4 175	330	298	5 084
Pig slurry	6	-	11	5	-	<1
Agricultural (mixed)	-	-	-	5 027	216	-
Fruit/Veg waste	-	-	1 170	-	-	6 620
Industrial & Agri waste	1 000	-	3 564	7 800	-	25 114
Slaughterhouse	74	28	-	-	-	-
Cattle manure	4 574	-	1 608	3 105	-	2 030
Brewery	11 900	2 200	-	19 500	14 000	-
Opaque brewery	0.02	59	-	100	130	-

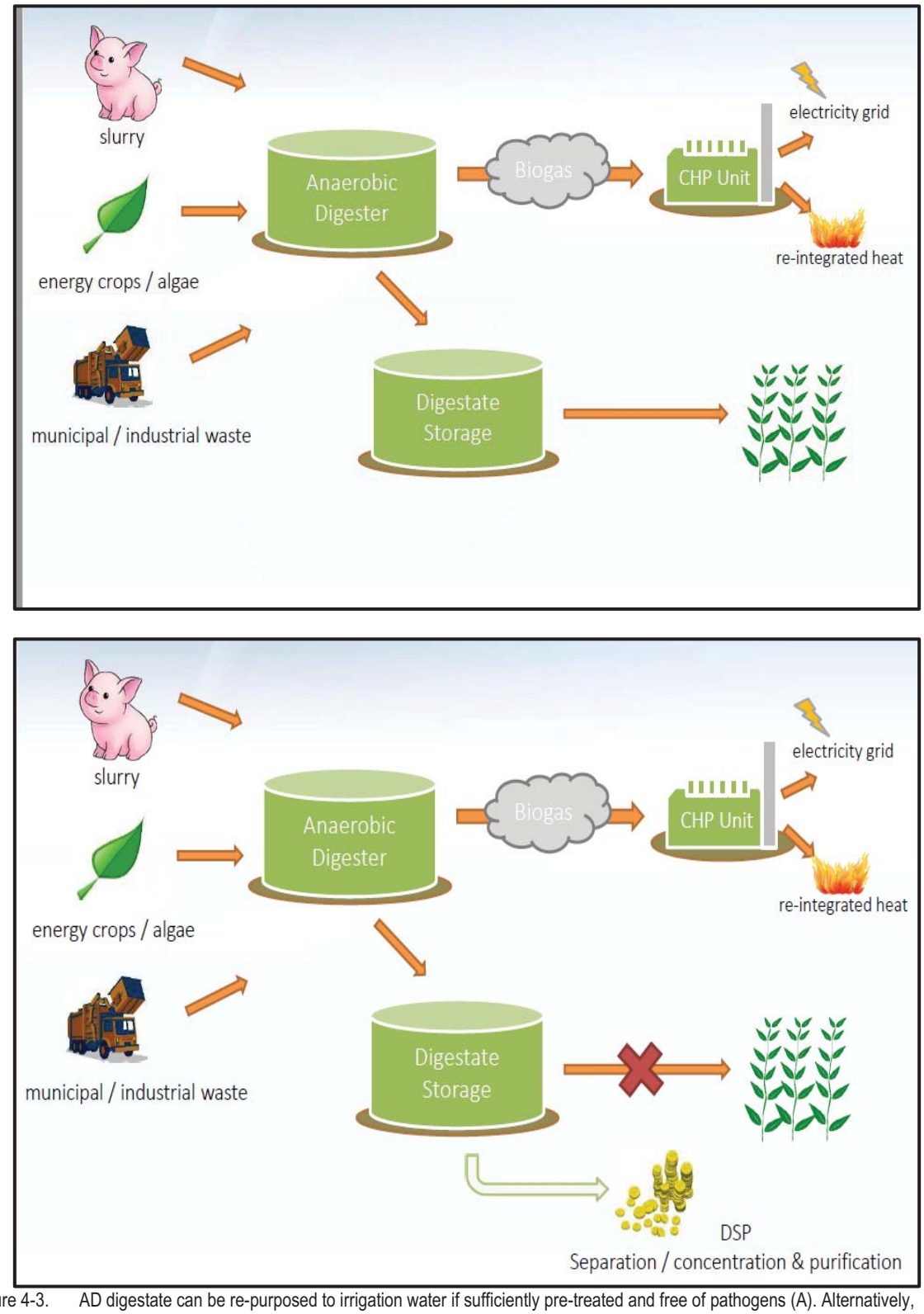


Figure 4-3. AD digestate can be re-purposed to irrigation water if sufficiently pre-treated and free of pathogens (A). Alternatively, further post AD DSP may allow recovery of organics and their conversion to products of interest

The VFA stream generated by AD or by acidogenic AD can be used as the carbon source for remediation processes, such as the sulphate reduction-mediated bioremediation of acid mine drainage (Van Hille et al., 2015). These digestate streams contain a mix of VFAs. For example, digestate of the cyanobacterium Spirulina leads to the presence of the following VFAs: isovaleric acid, valeric acid, propionic acid, isobutyric acid, butyric acid and acetic acid (Inglesby et al., 2015b), allowing potential for an integrated process for sulphur recovery from AMD concurrent with its remediation, as illustrated in Figure 4-4 and proposed by Van Hille and Harrison in joint publications (Harrison et al., 2014; Van Hille et al., 2015).

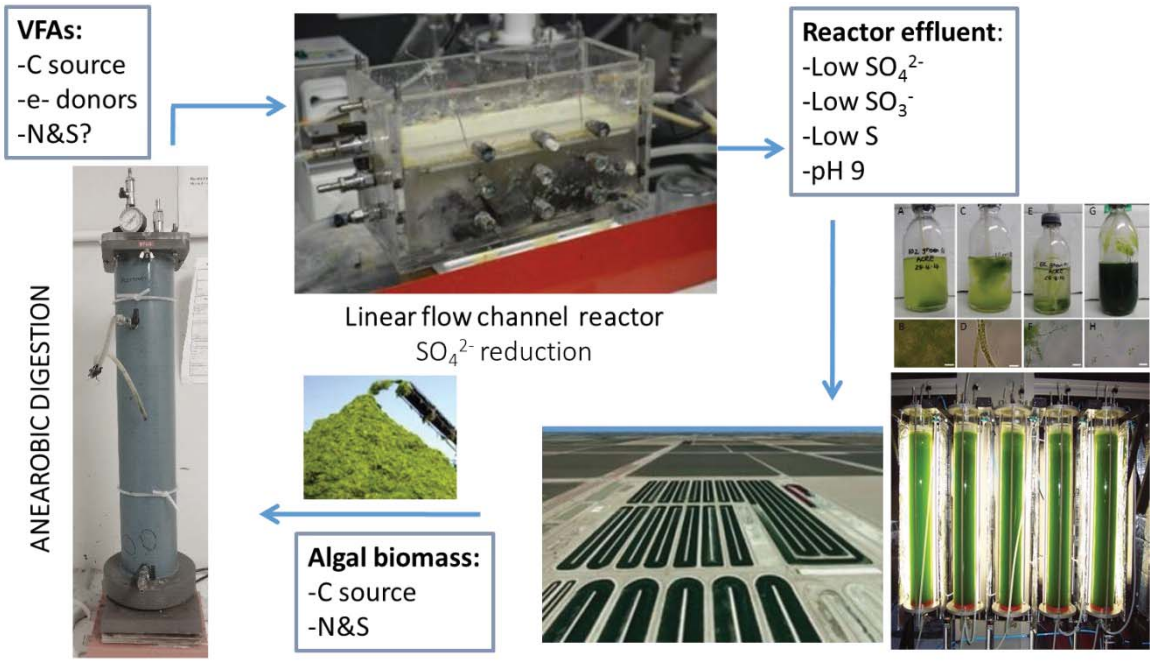


Figure 4-4 Integrated process for remediation of acid mine drainage by BSR and partial oxidation to recover elemental sulphur and treated water, using the VFAs generated on AD of algal biomass as carbon source and electron donor.

An alternative integrated process is presented in Figure 4-5 in which the N and P in the AD digestate and the CO_2 in the biogas are used as feedstocks for autotrophic algal growth to provide algal biomass. This algal biomass can be used for the recovery of a range of high value algal products, including algal oils, pigments and vitamins, medium value products including protein feeds and fertilisers or to provide further feedstock for AD to enhance methane production (Griffiths et al., 2016; Uysal et al., 2015).

There is also potential for conversion of residual organic components such as the VFAs in the AD digestate to products through heterotrophic metabolism (Lee et al., 2014). This is only a valid option where the residual organic (or VFA) concentration is sufficiently concentrated for development of an efficient bioprocess.

Considering platform chemicals, reduction of the VFAs to alcohols can be achieved using the co-formed H_2 from the AD process as electron donor. Such alcohols can form intermediates for a number of more complex bio-based products, including bioPET, provided production is at a large enough scale. An alternative route is the production of biodegradable polymers such as polyhydroxyalkanoates, with a range of uses as ‘one-use’ plastics, including diapers, disposable food utensils and personal hygiene products. The polymer alginate also has a range of uses in the construction environment. VFAs can also for the building blocks for additional energy compounds to biogas. VFAs can be used in both microbial fuel cells for electricity production and microelectrochemical systems. The VFA-rich stream from dark fermentation can be converted to H_2 via light fermentation (Han and Shin, 2004) (Han & Sim, 2003), They can also be converted to lipids by oleaginous microorganisms. Following analysis of product demand, the most promising of these can be investigated through flow sheet analysis.

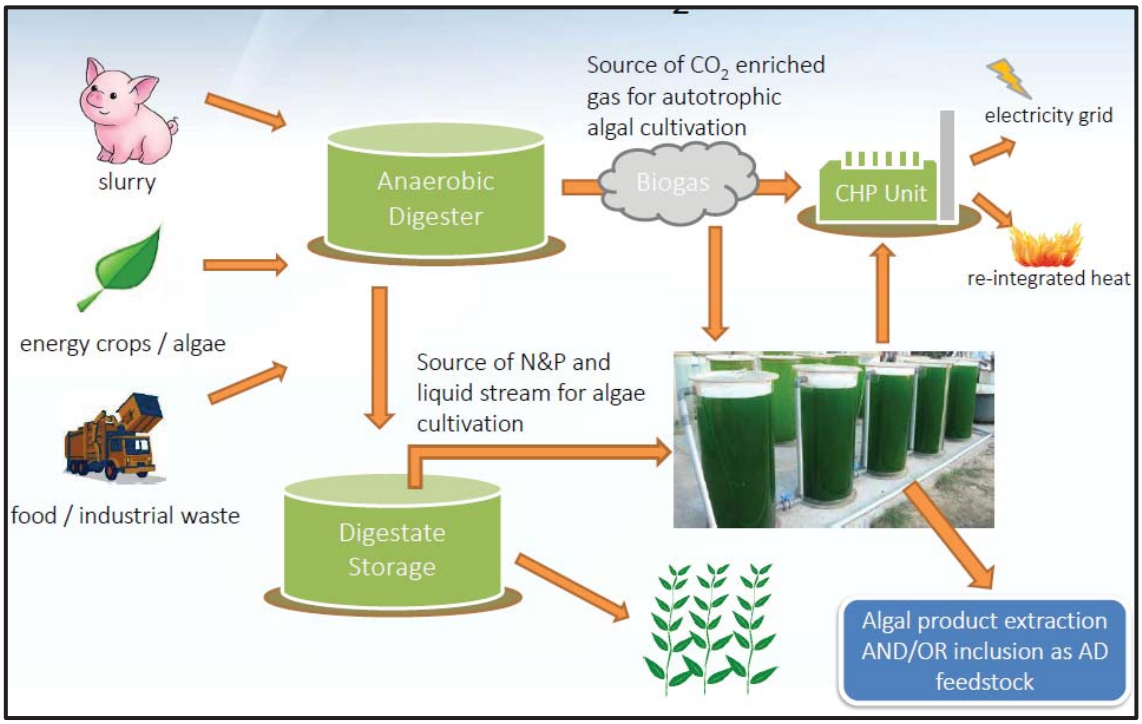


Figure 4-5 Integrated process for the re-purposing of N and P in the AD digestate and CO_2 in the biogas stream into algal biomass with the potential for recovery of algal products or further provision of AD feedstock or both

5 DOWNSTREAM PROCESSING OF DIGESTATE – A FOCUS ON RECOVERY OF PATHOGEN-FREE WATER AND OTHER PRODUCTS

5.1 Difficulties associated with re-use of wastewater

Due to the growth of the human population and the decrease and pollution of natural resources the world is facing formidable challenges in meeting the growing demand for clean water. This, along with an increase in environmental awareness and the need for improved resource efficiency, has prompted the need for new approaches to wastewater streams. In this project, we have focused on the combined removal of pollutants, recovery of energy, re-purposing of organic carbon, recovery of nutrients and recovery of clean water, as discussed in Chapter 4. This 'industrial ecology'-based approach requires that the prior demands of wastewater treatment are still met i.e., to convert waste materials present in wastewater into stable compounds that can easily be disposed (or preferably re-purposed) and to ensure a safe, reliable water stream for release to the environment or, preferably, re-purposing as a resource. Through this, we plan to recycle and recover valuable components of wastewater, to protect public health and to comply with legal standards and consent conditions placed on dischargers (Asano, 2002; Gray, 2010; Gupta et al., 2012). As we increasingly seek to derive value from wastewater, the key product of this treatment process remains compliant quality water. Re-purposing of available nutrients is an important secondary goal.

AD systems operated on domestic waste streams, mixed municipal wastewaters, abattoirs, some food industry waste streams and some agricultural waste streams may contain human pathogens originating from the presence of human and animal faecal matter and other pathogen-bearing materials. The quality of the digestate generated by AD therefore needs to be assessed for the presence of pathogenic microorganisms before the downstream processing needs can be determined. In addition to microorganisms such waste streams can also contain endocrine-disrupting (ECD) substances, such as oestrogen,17β-oestradiol and ethinyl oestradiol from female contraceptive pills. Changes in the reproductive processes and feminisation of male fish are some of the observed changes resulting from contamination of rivers by endocrine-disrupting hormones (Gray, 2010). These compounds also pose risks to human health if consumed in large quantities, due to their pharmaceutical properties (Webb et al., 2003). Methods to test for the presence of human pathogens and the proposed removal these from wastewater are presented in Sections 5.3 and 5.4. Removal of micro-contaminants such as ECDs is not covered in this report, owing to its coverage in related WRC projects.

In order to meet environmental and health standards (*National Water Act [No. 36 of 1998]*, 1998) of the resultant water stream for re-purposing, as detailed in Section 5.2, and to ensure effective recovery of nutrients for re-use, secondary treatment processes are required to treat the AD digestate.

5.2 Standards for re-use of product water

There is a considerable body of literature on the assessment of water quality in terms of fit-for-purpose requirements. The standards required for different purposes and the assay methods required are laid out in both WHO and DWS literature. The South African limits for drinking water are up to date and in line with the WHO recommendations. These "Blue Drop" limits for drinking water are presented in Table 5-1., including the risk posed by exceeding the limit. These limits include those for microbial and organic indicators.

Table 5-1 2015 Blue Drop Limits – Drinking Water (SANS, 2015)			
	Unit	**Risk**	**Standard limits**
MICROBIOLOGICAL DETERMINANDS			
Bacteriological			
Escherichia coli (E. coli)	count/100mL	Acute Health	Not Detected (ND)
Faecal coliforms	count/100mL	Acute Health	ND
Protozoan			
Cryptosporidium species	count/10mL	Acute Health	ND
Giardia species	count/10mL	Acute Health	ND
Total coliforms	count/100mL	Operational	< 10
Heterotrophic plate count	count/1mL	Operational	< 1 000
PHYSICAL & AESTHETIC DETERMINANDS			
Free Chlorine	mg/L	Chronic Health	≤ 5
Monochloramine	mg/L	Chronic Health	≤ 3
Colour	Pt-Co	Aesthetic	< 15
Conductivity at 25°C	mS/m	Aesthetic	≤ 170
Total Dissolved Solids	mg/L	Aesthetic	≤ 1 200
Turbidity	NTU	Operational Aesthetic	≤ 1 ≤ 5
pH at 25°C	pH units	Operational	5.0 to 9.7
CHEMICAL DETERMINANDS – MACRO-DETERMINANDS			
Ammonia as N	mg/L	Aesthetic	≤ 1.5
Calcium			—
Chloride as Cl^-	mg/L	Aesthetic	≤300
Fluoride as F^-	mg/L	Chronic Health	≤ 1.5
Magnesium as Mg			—
Nitrate as N	mg/L	Acute Health	≤ 11
Nitrite as N	mg/L	Acute Health	≤ 0.9
Nitrite-nitrate ratio		Acute Health	≤ 1
Nitrate and Nitrite as N	mg/L	–	–
Sodium as Na	mg/L	Aesthetic	≤ 200
Sulphate as SO_4^{2-}	mg/L	Acute Health Aesthetic	≤ 500 ≤ 250
Zinc as Zn	mg/L	Aesthetic	≤ 5
CHEMICAL DETERMINANDS – MICRO-DETERMINANDS			
Aluminium as Al	µg/L	Operational	≤ 300
Antimony as Sb	µg/L	Chronic Health	≤ 20
Arsenic as As	µg/L	Chronic Health	≤ 10
Barium as Ba	µg/L	Chronic Health	≤ 700
Boron as B	µg/L	Chronic Health	≤ 2 400
Cadmium as Cd	µg/L	Chronic Health	≤ 3
Chromium (total) as Cr	µg/L	Chronic Health	≤ 50
Cobalt as Co	µg/L	–	–
Copper as Cu	µg/L	Chronic Health	≤ 2 000
Cyanide (recoverable) as CN^-	µg/L	Acute Health	≤ 200
Iron as Fe	µg/L	Chronic Health Aesthetic	≤ 2 000 ≤ 300
Lead as Pb	µg/L	Chronic Health	≤ 10
Manganese as Mn	µg/L	Chronic Health Aesthetic	≤ 400 ≤ 100
Mercury as Hg	µg/L	Chronic Health	≤ 6
Nickel as Ni	µg/L	Chronic Health	≤ 70
Selenium as Se	µg/L	Chronic Health	≤ 40
Uranium as U	µg/L	Chronic Health	≤ 30
Vanadium as V	µg/L	–	–
CHEMICAL DETERMINANDS – ORGANIC DETERMINANDS			
Dissolved organic carbon as C	mg/L	–	–
Total organic carbon as C	mg/L	Chronic Health	≤ 10
Trihalomethanes (total)	mg/L	–	–
Trihalomethanes:			

Table 5-1 2015 Blue Drop Limits – Drinking Water (SANS, 2015)			
	Unit	**Risk**	**Standard limits**
Chloroform	µg/L	Chronic Health	≤ 300
Bromoform	µg/L	Chronic Health	≤ 100
Dibromochloromethane	µg/L	Chronic Health	≤ 100
Bromodichloromethane	µg/L	Chronic Health	≤ 60
Trihalomethane ratio		Chronic Health	≤ 1
Total Microcystin as LR	µg/L	Chronic Health	≤ 1
Phenols	µg/L	Aesthetic	≤ 10
DISINFECTANT RESIDUALS			
Free Chlorine:			
Treatment works	mg/L	Operational	> 0 to ≤ 0.5
Points of consumption	mg/L	Operational	> 0 to ≤ 0.2
Monochloramine:			
Treatment works	mg/L	Operational	> 0 to ≤ 0.5
Points of consumption	mg/L	Operational	> 0 to ≤ 0.2

The older South African Water Quality Guidelines (DWAF, 1996a), summarised in Table 5-2 are more comprehensive, giving standards for drinking water (DWAF, 1996b), four qualities of industrial water (DWAF, 1996c), two aspects of irrigation water (DWAF, 1996d) and livestock drinking water (DWAF, 1996e) as well as recreational water (DWAF, 1996f), aquaculture (DWAF, 2007, 1996g), and aquatic ecosystems (DWAF, 1996h). These come in seven volumes with the 8th volume being a field guide. In addition, there are four volumes referring to marine environments. Similar categories are used in the Initiative for Responsible Mining Assurance (IRMA, 2018) water guidelines, which also includes aquatic and aquaculture guidelines.

Table 5-2 South Africa Water Quality Guidelines Summary (from SAWQG Volumes 1-6, 1996)

Legend Biological Metals Non-metals Pesticides	Drinking Water	Industry				Irrigation		Livestock
		1 high quality	*2 intermediate quality*	*3 = domestic water*	*4 poor quality*	*crop yield and quality*	*equipment*	note: some species can tolerate more than the target
Algae								
Chlorophyll a *µg/L*	0 - 1							
Blue-green *cells/mL*	0 - 50							no visible blue-green scum < 6 *colonies /0.5 mL*
Microcystin *µg/L*	0 - 0.8							< 2000 *cells/mL*
Alkalinity *mg-CaCO3/L*		0 - 50	0 - 120	0 - 300	0 - 1200			
Aluminium *mg/L*	0 - 0.15					< 5.0		0 - 5
renal dialysis or preparation of intravenous fluids, TWQR: 0 - 0.003 mg/L								
Ammonia *mg-N/L*	0 - 1.0							
Arsenic *µg/L*	0 - 0.010					< 100		0 - 1000
Asbestos Fibre count *(fibres/L)*	0 - 1 x 10^6							
Atrazine *µg/L*	0 - 2							
Beryllium *mg/L*						< 0.10		
Boron mg/L						< 0.5		
Cadmium *µg/L*	0 - 5					< 10		0 - 10
Calcium *mg/L*	0 - 32							0 - 1 000
COD *mg-O_2/L*		0 - 10	0 - 15	0 - 30	0 - 75			
Chloride *mg/L*	0 - 100	0 - 20	0 - 40	0 - 100	0 - 500	< 100		0 - 1500
Chromium(VI) *mg/L*	0 - 0.050							0 - 1
Cobalt *mg/L*								0 - 1
Colour *Pt-Co units*	0 - 0.050							
Copper *mg/L*	0 - 1					< 5.0		0 - 0.5
Corrosion & Scaling								
Langelier index = pHa - pHs	~ 0						-0.4	
Ryznar index = 2pHs - pHa	~ 6.5						~ 6.5	
Corrosion ratio R = {me/L (Cl- + SO42-)}/{me/L alkalinity as CaCO3}	< 0.1							
Aggressiveness index AI = pH + log (AH) where: A = total alkalinity in mg/L CaCO3 H = calcium hardness as mg/L CaCO3	>12 Non-aggressive 10.0 - 11.9 Moderately aggressive < 10 Highly aggressive						> 12	
Dissolved Organic Carbon *mg-C/L*	0 - 5							
Fluoride *mg/L*	0 - 1.0					< 0.2		0 - 2
Indicator Organisms								
Heterotrophic Bacteria Plate Counts *counts/mL*	0 - 100						< 10 000	

Legend Biological Metals Non-metals Pesticides	Drinking Water	Industry				Irrigation		Livestock
		1 high quality	*2 intermediate quality*	*3 = domestic water*	*4 poor quality*	*crop yield and quality*	*equipment*	note: some species can tolerate more than the target
Total Coliforms *counts/100 mL*	0 - 5							
Faecal Coliforms *counts/100 mL*	0					< 1		0 - 200 & 200 - 1000 for < 20 % of samples
Coliphages *counts/100 mL*	0 - 1							
Enteric Viruses *TCID50/10L*	0							
Virological analysis is recommended only for situations in which there is reason to suspect the presence of viruses, such as outbreaks of enteric viral disease New methods may have changed this								
Protozoan Parasites *cysts or oocysts/10L*	0							
Iron *mg/L*	0 - 0.1	0.0 - 0.1	0.0 - 0.2	0.0 - 0.3	0.0 - 10.0	< 5.0	< 0.2	0 - 10
Lead **µg/L**	0 - 10					< 0.2		0 - 100
potentially acute and/or irreversible effects on human health								
Lithium *mg/L*						< 2.5		
Magnesium *mg/L*	0 - 30							0 - 500
Manganese *mg/L*	0 - 0.05	0.0 - 0.05	0.0 - 0.1	0.0 - 0.2	0.0 - 10.0	< 0.02	< 0.1	0 - 10
Mercury **µg/L**	0 – 1							0 - 1
potentially acute and/or irreversible effects on human health								
Molybdenum *mg/L*						< 0.01		0 - 0.01
Nickel *mg/L*						< 0.2		0 - 1
Nitrate *mg/L*	0 – 6							0 - 100
Nitrogen *mg/L*						< 5	< 0.5	
Odour *threshold odour number (TON)*	1							
there is a move away from odour measurement by TON in favour of sensory profile analysis								
pH	6.0 - 9.0					6.5 - 8.4		
Phenols *mg/L*	0 – 1	7.0 - 8.0	6.5 - 8.0	6.5 - 8.0	5.0 - 10.0			
Potassium *mg/L*	0 – 50							
Radioactivity alpha								
Total α Bq/L	0 - 0.5							
Thorium -232	0 - 0.228							
Radium-226	0 - 0.42							
Radioactivity beta								
Total β Bq/L	0 - 1,38							
Radium-228	0 - 0.42							
Selenium **µg/L**	0 – 20					< 0.02		0 - 50
Settleable Matter								
The distribution system should not show any visible sediments upon inspection; The quality of the water reaching the end-user should not be lower than that of the water leaving the treatment plant.								

Legend Biological Metals Non-metals Pesticides	Drinking Water	Industry				Irrigation		Livestock
		1 high quality	*2 intermediate quality*	*3 = domestic water*	*4 poor quality*	*crop yield and quality*	*equipment*	note: some species can tolerate more than the target
Silica *mg/L*		0 - 5	0 - 10	0 - 20	0 - 150			
Sodium *mg/L* potentially acute and/or irreversible effects on human health	0 – 100					< 70		0 - 2000
Sodium Adsorption Rate SAR = [sodium]/([calcium] + [magnesium])0.5 (concentrations in mmol/L in solution)						< 2.0		
Sulphate *mg-SO42-/L*	0 – 200	0 - 30	0 - 80	0 - 200	0 - 500			0 - 1000
Suspended Solids *mg/L*		0 - 3	0 - 5	0 - 5	0 - 25		< 50	
Trihalomethanes *µg/L*	0 - 100							
Total Dissolved Solids								
TDS Range *mg/L*	0 - 450	0 - 100	0 - 200	0 - 450	0 - 1 600			0 - 1000
EC Range *mS/m*	0 - 70	0 - 15	0 - 30	0 - 70	0 - 250	< 40		
Total hardness (mg CaCO /L) = 2.497 x [mg Ca/L] + 4.118 x [mg Mg/L]	0 - 50	0 - 50	0 - 100	0 - 250	0 - 1000			
Turbidity nephelometric turbidity units (NTU)	0 - 1							
Uranium *mg/L*						< 0.01		
Vanadium *mg/L* potentially acute and/or irreversible effects on human health	0 - 0.1					< 0.10		0 – 1
Zinc *mg/L*	0 - 5					< 1.0		0 - 20
Pesticides **µg/L**								
Chlorinated Hydrocarbons:								
Aldrin								1
Chlordane								3
DDT								50
Dieldrin								1
Endrin								0.5
Heptachlor								0.1
Lindane (BHC)								5
Methoxychlor								1 000
Toxaphene								5
Organophosphates:								
Parathion								100
Malathion								100
Herbicides:								
2.4 -D								20
2.4.5-T								2
2,4,5-TP								30

5.3 Assessing microbial quality of feed and product water

5.3.1 Presence of microorganisms in AD feedstocks

Some commonly used AD feedstocks originating from domestic wastewaters, abattoir wastewaters, food waste or animal manure may contain a variety of bacteria, fungi, parasites and viruses. Some of these organisms may pose a health risk in the form of pathogenic organisms often reported as the presence of faecal coliforms (Mitchell et al., 2015). Zoonotic pathogens, which cause infection in both humans and animals, are the main concern when animal-derived wastes are used as AD feedstock. The potential for presence of pathogens in some common AD feedstocks is given in Table 5-3.

5.3.1.1 Wastewater AD feedstocks

Domestic wastewater, also termed municipal wastewater, is the effluent from domestic and industrial operations that enters the sewer and is subsequently treated in a wastewater plant. Domestic wastewater may also refer to black and grey water from domestic dwellings that is treated 'on-site' i.e., not connected to reticulated waterborne sewers. This wastewater carries the greatest risk of human pathogen infection. The variation in the concentration of organisms reported in literature for this waste stream may be dependent on the contributory streams. One of the main contributors to pathogens in these feedstocks is the presence of faecal matter, human and animal, which also increases the risk of parasitic nematodes and their eggs being present. Not many studies refer to the viral and helminth loads present within waste streams, and thus this information is only included for some feedstocks in Table 5-3.

5.3.1.2 Solid AD feedstocks

The risk of human pathogens within agricultural biomass is low; however, pathogens may be present due to the application of livestock wastes or sewage sludge to the soil as fertiliser. An additional consideration for the use of the AD digestates originating from the digestion of agricultural wastes may be the persistence of plant pathogenic fungal spores and weed seeds, especially if the AD residues are to be applied as fertiliser or soil enhancer for agriculture.

Incorrect storage of feedstock may create conditions under which the pathogenic load becomes higher than that reported in Table 5-3. No clear regulation exists for residue standards. Recycling residue requires an understanding of the potential organisms it may contain. Where the stream may contain a dilute pathogenic load, incorrect storage of the material or other conditions may increase the pathogenic load post initial analyses; these need to be considered. Most industrial effluent streams are not separated from other waste streams such as domestic and faecal waters disposed of through the same effluent stream (Vrhovšek et al., 1996). This potentially introduces pathogens or increases the pathogen load. It raises the question of the value or sense of mixing effluents, particularly where the quality of effluent varies substantially.

No single indicator bacterium has been identified which gives a good overall picture of the bacteria present (Sahlström, 2003). The identification of one single organism to fulfil this function is also highly unlikely. Here we consider the risks associated with not only bacterial load, but virus and phage as well as intestinal parasites which may be present or become enriched within the digestate as a result of incorrect handling before application as a fertiliser or recovery of additional value adding products. The pathogen presence in both digestate and residues is directly related to pathogen load in the AD feedstocks. Where co-digestion is used to achieve desirable C:N ratios and overcome seasonal variability of these with agricultural feedstocks, the pathogen load of each feedstock and its impact on subsequent valorisation of digestate and sludge must be considered in its selection. For example, *Salmonella* tolerates a broad temperature range and can infect both animals and humans. It is present at high levels in sewage and animal manure slurries and can persist to AD digestate and sludge in the absence of additional treatment.

Table 5-3 The load of pathogenic contaminants in commonly utilised AD feedstocks

AD feedstock wastewaters	Microbial cfu/100 mL*							Viral (pfu/L)	Helminth (eggs/ ℓ)	References
	Total coliforms	*Faecal coliforms*	*E. coli*	*Faecal enterococci*	*Salmonella*	*Listeria*	*Campylobacter*	*Coliphages*		
Domestic wastewater	1.8 x10^6 - 5.3 x10^{10}	3.4 x10^5 - 2.5 x10^{10}	2.0 x10^5 - 3.4 x10^{10}	2.6 x10^4	Present			6.4 x10^3	1.9 x10^2 - 7.4 x10^3	(Latrach et al., 2016, 2015; Quiñónez-Díaz et al., 2001; Yaya-Beas et al., 2016)
Livestock wastewaters	7.1 x10^6	15 - 148	1 x10^4 - 4.4 x10^4		4 x 10^2					(Gorra et al., 2014; Howard et al., 2017; Vanotti et al., 2005)
Abattoir wastewaters	2 x10^6 - 3.2 x10^7	7 x10^5 – 2.1 x10^7	4.0 x10^5		Present					(De Nardi et al., 2011; Mittal, 2004; Saddoud and Sayadi, 2007)
Food processing wastewaters†	8 x10^5 – 1.6 x10^9	2 x 10^3 – 8 x 10^7								(Vrhovšek et al., 1996)
Brewery, winery and beverage waste streams	12	0	<5							(Ediget Wendimagegn Advisor and Leta, 2016; Ikhajiagbe et al., 2014; Nyilimbabazi et al., 2011)
Pulp and paper wastewater	2.3 x10^4 - 1.6 x10^7	<2.0 x10^3								(Gauthier and Archibald, 2001)

AD feedstocks solids streams	Microbial cfu/g*							Viral (pfu/g)	Helminth (eggs/ g)	References
	Total coliforms	*Faecal coliforms*	*E. coli*	*Faecal enterococci*	*Salmonella*	*Listeria*	*Campylobacter*	*Coliphages*		
Manure		10^5 - 10^8	5.0 x10^2 – 1.6 x10^5		1.6 x10^3 – 2.9 x10^7	1.6 x10^2 – 3.3 x10^7	1.3 x10^2 – 1.6 x10^4			(Erickson et al., 2014; Nicholson et al., 2005; Resende et al., 2014)
Agricultural biomass waste*	10 – 3.2x10^9		4 – 1x10^4	10 – 1.6x10^6	low	low	0			(Abadias et al., 2008; Johnston et al., 2006, 2005; Thunberg et al., 2002)
Municipal waste	1.4x10^3 – 3.7x10^3		1x10^6 – 2x10^7		2.6x10^5 – 3.2x10^6				0	(Hassen et al., 2001; Soobhany, 2018)

†Data presented for "Food processing wastewaters" includes faecal and meteor waters.
*Data represented for raw produce. Where 'low' is recorded as a measure the cases of positive detection by qualitative methods were less than 1% of the total sample number tested.
Where 'present' is recorded as a measure, qualitative methods were used for detection.
Where data are missing, specific values were not available in the literature reviewed.

The persistence of human pathogens in the form of bacteria (Busta et al., 2003; Harris et al., 2003), viruses (Brassard et al., 2012) and the eggs of human parasites (Arora et al., 2011) is a risk to fresh agricultural produce and thus their exclusion from digestate used as irrigation water is required (see irrigation water requirements for SA in Table 5-2).. A persistence of these pathogens into agricultural wastes is therefore also a risk, especially in the case where it may be enriched by feedstocks which can act as a growth medium for these organisms. An increase in the bacterial numbers of *E. coli, L. monocytogenes*, *Salmonella* spp has been recorded following the incubation at various temperatures of raw fruit and vegetables on which these organisms were present (tubulised by Harris et al. (2003)), highlighting the importance of their exclusion or minimisation in agricultural practice.

5.3.2 Proposed methods for assessing feedstocks and digestates for the presence of pathogens

Water sourced from potentially contaminated AD digestate streams, where these originate from feedstocks with heavy pathogen loads, require rigorous screening to ensure the absence of potential disease causing organisms. Standard microbiological techniques can be used to test for the presence of specific microorganisms using selective media. In general, indicator organisms are used to assess the safety of drinking water or water used for the irrigation of agricultural food crops. Total coliform bacteria include a range of aerobic and facultative anaerobic, heterotrophic, Gram-negative, non-spore forming bacilli and is used to assess the level of cleanliness of water streams. The coliform count is frequently used to advise on the “potability” of water. Most typically, the coliform method relies on the filtration of microorganisms from the water to be tested, incubation of the membranes on selective media and the counting of emerging colonies following a 24 h incubation period at 37°C. Alternatively the production of acid from lactose or the production of the β-galactosidase enzyme is monitored which allow the production of field test kits such as those distributed by many companies including LaMotte (USA) and Simpltek (USA). However, relying only on the total coliform count as an indication of water safety is not ideal as these coliforms are more sensitive to disinfectants than many gram positive spore forming anaerobic organisms typically found as faecal pathogens (Gorchev and Ozolins, 2011).

Due to the complexity of the microbial community associated with the AD digestate, it may not be possible to choose a single pathogen as a reference of the pathogenicity of the liquid. The factors which may affect the nature of pathogens present, if any, and their persistence in the liquid include the source of feedstock and the nature of the AD process. It is therefore advisable to perform a complete analysis of the possible pathogens, including bacteria, viruses and helminths, present within the AD digestate for each feedstock used for AD. It is proposed that the testing focus be on feedstocks in the first instance, and on digestate at the level of polishing once the risk is clearly defined.

Once suitable reference organisms have been selected for the microorganisms and eukaryotes which may be present within the specific feedstock, specific assays may be designed for these. Assays for bacterial pathogens, represented by faecal coliforms, are well defined and categorised as traditional microbiological techniques (Section 5.3.2.1) and DNA based methodologies (Section 5.3.2.4). However, reference organisms for the other pathogenic organism groups are less well defined and often not included when routine testing is performed. Here, of particular concern are viruses and helminths. Some of the viruses which may be suitable reference organisms for AD digestate are rotaviruses, enteroviruses and noroviruses. Helminths, or enteric parasites, are macroscopic organisms prevalent in water sources contaminated with human or animal faeces. The following helminths are most often screened for and should form part of the array of organisms included in the initial pathogen assessment of the AD feedstock: *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum* and *Ascaris* spp.

5.3.2.1 Conventional detection methods

As mentioned above, standard practice for the assessment of the bacterial pathogenic load within a water or solid sample relies on the preparation of selective media types for specific organisms and spread plating of the sample at various dilutions to achieve ‘countable’ cell colonies. Either the plating

method or the Most Probable Number (MPN) method is most often used for measuring the reduction of bacterial numbers between the influent feed and effluent digestate of AD systems. Specific media types and methodologies can be obtained from the *Bacterial Analytical Manual* (BAM) published by the US Food & Drug Administration (https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm; accessed 28/12/2018). The plating method is highly selective for live cells, thus only cells with the ability to grow and divide form visible bacterial colonies following incubation of the plates at the appropriate temperature. This is one of the benefits of using this assay for assessing the bacterial load. The data is presented as colony-forming units (cfu) per mL or L of liquid sample. However, some assumptions are made when only relying on the plating method for the assessment of pathogenic, or coliform, load. The greatest assumption is that all pathogenic organisms will be able to proliferate on these plates at the temperature chosen for incubation.

When plating of samples is not desirable, or when samples contain particles that will interfere with plate counting, the MPN method can be employed. This method is especially attractive when low bacterial numbers are expected. In short, it relies on the serial dilution of the sample to be tested, inoculation of selective broth medium with the prepared dilutions in triplicate and incubation of the inoculated broth. The turbidity of the inoculated tubes are monitored and the results presented as a series of numbers indicating the number of tubes that turned turbid. Although the method appears qualitative, based on the multiple dilutions used, a quantitative number is derived for the MPN score based on the MPN table published by the US Food & Drug Administration (https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm109656.htm; accessed 26/12/2018). The MPN has been shown to be more rapid and reliable than plate counting (Sutton, 2010); however, it suffers from the same limitations as all culture based techniques. Firstly, a knowledge of the pathogens present is required. Secondly there is a need for the preparation of numerous media types suitable for the growth of each organism to be tested. Thirdly “hitting” the correct dilution range is required to allow the quantitative assessment of the microbes present.

The presence of helminth eggs in samples can be determined by their hatching and counting emerging larvae using microscopy. Methodologies for detection of parasites in food products have been detailed in the BAM (https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071468.htm) and can be amended for solid feedstocks. A standard method for the detection and enumeration of helminth eggs is also well described and detailed by Moodley et al. (2008) in a WRC report. Helminth eggs and ova are easily distinguishable and counted by trained microscope users, making them easier to identify microscopically than bacterial species.

A recent paper detailed the testing of various viruses for persistence through anaerobic digestion by performing small scale simulation experiments that mimicked the AD environment (Sassi et al., 2018). The survival of viruses following AD treatment was determined by standard mammalian viral assays using the permissive mammalian cell culture host for each virus, while bacteriophage assays were performed using the double-overlay agar method and the permissive bacterial host.

Although well defined and detailed, the standard methods, except for the helminth enumeration method, are often tedious, time-consuming (2-3 days) and require a knowledge of the specific pathogens present within the feedstock and digestate.

5.3.2.2 Immunology based methods

Immunology based methods can be applied to a wide range of targets; however, antibodies to the specific organisms are required and these may be costly or unavailable. A commonly used method is the enzyme-linked immunosorbent assay (ELISA), which uses a secondary antibody which binds the specific pathogen antibody coupled to an easily assayed enzyme (Lazcka et al., 2007). The principle of immunology based methods has become the inspiration for many biosensor assays.

5.3.2.3 Biosensors

Many of the traditional pathogen detection techniques are tedious, time-consuming and require skilled personnel and possibly specialised equipment. A more desirable technology is one which incorporates the identification of specific cell characteristics leading to the identification of the possible pathogen associated with an output such as a colour change or signal transmission that identifies and quantifies the pathogen. The characteristics of such a 'biosensor' are well defined and reviewed by Habimana et al. (2018). A number of biosensors for specific microorganisms have been developed, including some for *E. coli* (Ali et al., 2018; Hashemi and Forouzandeh, 2018), *Pseudomonas aeruginosa* (Hanke et al., 2018), *Staphylococcus aureus* (Suaifan et al., 2017) and *Salmonella typhimurium* (Ali et al., 2018); however, none of these are yet available for commercial use.

Biosensors can make use of nucleic acids, such as DNA and RNA, for detection and quantification of specific organisms. Towards the development of a universal biosensor for the detection of a broad range of pathogens with a high degree of specificity, Isis Pharmaceuticals initiated TIGER (Triangulation Identification for the Genetic Evaluation of Risks) developed for the Department of Defence in the USA (Blyn, 2005). The technology relies on the extraction of the total nucleic acid contained within a sample, followed by the specific amplification of desired targets in an high-throughput format. The mass of the amplicons are thereafter determined by mass spectrophotometry allowing detection of pathogens down to strain level. The method is referred to as PCR/ESI-MS (PCR followed by electrospray ionisation mass spectrometry) and is applicable for the detection and strain (or serotype) identification of a number of bacteria, viruses, fungi and protozoa (Sampath et al., 2007). The TIGER biosensor technology has successfully been applied to the typing of adenovirus in a military setting (Russell et al., 2006). The system was developed firstly under the commercial name Ibis T5000 (Ecker et al., 2006), then further developed into the Abbott/Ibis PLEX-ID (https://www.wmddetectorselector.army.mil/PDFs/219.pdf). A similar technology, making use of nucleic acid probes is available through the company Early Warning Inc. as an inline analysis unit (https://www.earlywarninginc.com/products.php; accessed 28/12/2018). The biochip carries probes for up to 25 specific bacteria, protozoa and virus probe types and the automated system differentiates between total and viable cells.

The two biosensor technologies reviewed here are not suitable for field use and although entirely automated, thus requiring less trained on-site personnel, the Abbott/Ibis PLEX-ID was priced at USD 450,000 per system unit and USD 50 to 90 per analysis in 2018 (https://www.wmddetectorselector.army.mil/detectorPages/219.aspx; accessed 28/12/2018). Although the analysis cost is possibly cheaper than the cost of pathogen detection performed by an outside laboratory, the initial cost of acquiring the instrumentation is severe. This makes this type of technology currently inaccessible for routine pathogen monitoring within AD feedstocks and digestates.

5.3.2.4 DNA and RNA methods

A number of AD studies have investigated the use of quantitative DNA based techniques such as qPCR (quantitative Polymerase Chain Reaction) (Chen et al., 2012; Van Lint et al., 2013; Verweij et al., 2004; Wang et al., 2018) or multiplex PCR followed by amplicon size and peak height determination (similar to the Abbott/Ibis PLEX-ID) (Wang et al., 2018) for pathogen enumeration. Following the harvesting of cells from the sample to be analysed, DNA may be extracted using either standard extraction methods or commercially available extraction kits. This metagenomic DNA, representing all the genetic information from the organisms within the sample, is then used as template with the application of organism specific primer pairs. By preparing a serial dilution of a standard with a known copy number, the copy number of the target organism within the sample can be determined. Comparative MPN determination and qPCR analysis has shown similar trends for *E. coli*, *Salmonella* spp. and *Shigella* spp. (Chen et al., 2012) in AD influent and effluent samples. qPCR has also been used to detect *Collinsella aerofaciens* and *Streptococcus salivarius* (Wan et al., 2018) and has been applied to the screening of three common human intestinal parasites, *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* (Van Lint et al., 2013; Verweij et al., 2004). Recently, Wan et al. (2018) developed a highly specific, sensitive and validated high-throughput multiplex genetic detection system

for the detection and enumeration of seventeen major diarrhoeal pathogens. This assay was shown to be specific for twelve bacteria (including six different pathogenic *E. coli* strains) and four viral types including norovirus, rotavirus, adenovirus and astrovirus.

Most of the techniques used for conventional pathogen enumeration, immunology assays, biosensor analyses and the qPCR based DNA method require a good understanding of the pathogens which may be present within the feedstock to further inform which methods can routinely be applied to test for the persistence of the these through the AD system. A holistic view of the organisms present in the feedstock or the AD digestate or both can be obtained from applying metagenomic DNA or RNA techniques. The use of these metagenomic methods for pathogen detection has recently been reviewed by Gu et al. (2018). The un-biased approach to pathogen detection using Next-Generation Sequencing allows for a broad identification of the organisms present within AD feedstock or digestate. The use of NGS for the broad spectrum identification of pathogens in AD feedstock and digestate carries a similar limitation highlighted by Gu et al. for the application of NGS to the detection of pathogens in clinical samples from humans. The nucleic acids extracted from the AD feedstock are dominated by DNA originating from the feedstock type such as plant residues, animal manure or human origin if faecal sludge is considered. Similarly, the nucleic acids extracted from digestate would contain a high number of organisms associated with the AD process. High background host DNA levels reduce sensitivity of NGS for pathogen detection. However, NGS is still one of the only ways to obtain a complete profile of the pathogens available. A number of methods are available to reduce the background host DNA. NGS was used to identify and enumerate pathogen numbers successfully in a thermophilic alkaline fermentation followed by mesophilic AD of waste activated sludge from a wastewater treatment facility (Wan et al., 2018). The application of NGS resulted in a more detailed pathogen profile than that obtained from the qPCR assays applied to the samples, hence it plays an important role in targeting future qPCR assays..

The drawback of using DNA based methods for pathogen detection is that they do not differentiate between live and dead cells, as the traditional plating and MPN methods do. This is currently being addressed through the application of similar techniques targeting RNA, thus only measuring the presence of cells with metabolic activity (Avery et al., 2014). Furthermore, new NGS approaches using the origin of replication allow actively dividing cells to be determined. However, depending on the HRT under which the AD is operated, dead or non-proliferating cells are “washed” from the system. Although the DNA is initially detected by using DNA based techniques, time course data soon shows a reduction in the DNA obtained from these organisms. This was demonstrated by the parallel MNP and qPCR study performed by Chen et al. (2012). Additionally, as culture based methods often only consider cells in their viable form, and not spores or dormant cells, resilient and persistent pathogens may remain undetected. The presence of DNA from these pathogenic organisms indicate that they may be present within the feedstock and should warrant investigation of their numbers in the digestate.

5.4 Pathogen removal strategy

5.4.1 AD as a pathogen removal strategy for waste feedstocks

AD is an efficient treatment for the removal of many microorganisms; however, especially resilient organisms such as the spore forming organisms *Clostridium* and *Bacillus* spp. and heat resistant fungi may persist subsequent to AD (Johansson et al., 2005). For some feedstocks containing a large pathogenic load, thermophilic AD at 55 to 60°C may be considered as an alternative to mesophilic AD between 30 and 40°C to reduce the number of microbes that persist through digestion and remain viable in the digestate (Smith et al., 2005). Numerous mesophilic AD studies focusing on the stabilisation of sewage sludge have demonstrated a two OM reduction in the number of *E. coli* (Horan et al., 2004; Smith et al., 2005)*, Salmonella* spp (Horan et al., 2004; Smith et al., 2005), *Listeria monocytogenes* (Horan et al., 2004), *Clostridium* spp (Romanazzi et al., 2016) and *Methanobrevibacter smithii* (Romanazzi et al., 2016) following the mesophilic AD process. However, species such as

Campylobacter jejuni (Horan et al., 2004) and *Shigella* spp (Chen et al., 2012) showed no reduction following mesophilic AD.

Consideration needs to be given to the initial pathogenic load within the feedstock. For high bacterial loads contained within some feedstocks (Table 5-1), a two OM reduction in bacterial numbers, as reported for the mesophilic AD processes above, results in organisms still being present at between 10^2 and 10^4 organisms per 100 mL in the liquid phase following AD. These numbers are higher than the 10^3 colony-forming units (cfu) permissible per 100 mL for the irrigation of crops to be eaten raw detailed by the WHO guidelines (WHO, 2006). To reduce these to within limits, alterations within the operating temperature and residence time would be required. Thus, although a reduction in certain organisms can be expected following AD, the number of pathogenic organisms remaining in the liquor depends on the feedstock and specific AD process used and requires testing before deciding on the use of the liquid effluent and sludge formed..

A similar study on a thermophilic operated digester, however, showed no survival of any of these organisms after a 24 hr exposure to the reactor conditions (Wagner et al., 2008). Thermophilic digestion at 70°C was shown to result in the inactivation of *E. coli* and *Salmonella* spp, both laboratory and isolates from sewage sludge, within 10 s of exposure, and digestion at 55°C could achieve pathogen inactivation after 20 to 60 mins depending on the strain tested (Smith et al., 2005). The decrease in pathogenic organisms was achieved partially by more efficient mixing, sludge stabilisation resulting from the thermophilic process and minimising dead zones and by-pass flow, in addition to being linked to the increase in temperature increasing the death rate of microorganisms. It is suggested that for the less heat resistant pathogens, mesophilic AD is sufficient when digesters are well-managed and efficiently mixed; however, for feedstocks containing a large proportion of more heat resistant, Gram positive spore forming organisms, thermophilic digestion should be considered.

Helminth egg removal has been shown to be successful in a number of AD studies. A low temperature UASB reactor (12 to 14°C) operated on domestic wastewater containing initial helminth (*Ascaris* sp) loads of 100 to 260 eggs/L could achieve between 80 and 95% reduction at HRT of between 14.2 and 4 hours (Yaya-Beas et al., 2016). A study investigating the fate of *Ascaris suum* ova during mesophilic AD at 35°C of swine manure reported the near complete inactivation of ova after 16 days if the ova were embryonated through exposure to aerobic conditions preceding AD (Manser et al., 2015). This study is in contradiction with many other mesophilic studies where unsatisfactory reduction in helminth eggs were reported (Méndez-Contreras et al., 2009; Rojas Oropeza et al., 2001). Thermophilic AD, or a thermophilic treatment preceding AD, has been shown to be effective at helminth inactivation in a number of studies (Aitken et al., 2005; Rojas Oropeza et al., 2001; Rubio-Loza and Noyola, 2010). In all these studies the efficacy of helminth ova inactivation was directly related to temperature and the duration of sludge contact at the specified temperature, in accordance with thermal death kinetics. This is in agreement with laboratory studies on the temperature dependent inactivation of ova from *Ascaris* spp. (Naidoo and Foutch, 2018) Although helminths appear to be significantly reduced during thermophilic AD, if mesophilic AD is used and the digestate is to be used on food crops as either fertiliser or irrigation water, the persistence of eggs through to consumer level should be investigated and decontamination of either the digestate preceding application or final produce should be considered.

5.4.2 Proposed methods for removal of pathogenic contaminants

The liquid fraction resulting from AD has been identified as a high value fertiliser due to the presence of high levels of nutrients. Similarly, the sludge also still contains organic value, including complex organics, in the form of undigested feedstock and microbial biomass generated during AD. Literature suggests that dependent on AD feedstock and the efficiency of the AD process, some pathogens may remain in the AD digestate. If this is so, its direct use as a nutrient-containing irrigant for agricultural crops may result in the contamination of food crops or animal feeds with the pathogens (Murphy et al., 2016). The presence and persistence of microorganisms in AD digestate streams should therefore be

determined before the downstream usage of the AD liquid fraction or the sludge can be decided. Where necessary, remedial action should be taken to prevent persistence into the follow-on use.

Changing the AD operating conditions in terms of temperature, OLR (and associated pathogen load should the feedstock contain these) and HRT may facilitate more efficient pathogen removal during AD. A number of polishing steps can also be applied to either the liquid or solid fractions of the digestate. Many of these treatments may also be applied preceding AD to reduce the pathogenic load in the feedstock, but may be more effective following AD and its associated initial decrease in pathogenic load associated with AD. This is particularly applicable as heat and chemical sterilisation efficiency are affected by the number of contaminants to be removed.

5.4.2.1 Solid digestate fraction

A suitable treatment option for the sludge fraction may be thermal composting shown to be efficient at removing pathogens such as *Salmonella* and *L. monocytogenes* from animal manure streams when directly applied to compost mixtures (Erickson et al., 2014). As mentioned in Section 5.4.1, thermophilic treatment is also efficient at inactivating helminth eggs with the efficacy of the treatment depending on the temperature and the duration of treatment (Aitken et al., 2005; Rojas Oropeza et al., 2001; Rubio-Loza and Noyola, 2010). Treatment of helminth eggs for periods exceeding three mins and temperatures of 60°C and above have been shown to result in the deactivation of the ova of *Ascaris* spp. (Naidoo et al., 2018; Naidoo and Foutch, 2018). Testing in the presence of sludge is required as sludge properties may change the heat transfer properties and offer protection for helminth eggs. For sludges heavily contaminated with parasitic eggs and pathogens, heat treatment may prove a viable option for pathogen reduction. The conditions of treatment would require optimisation for the thermal death characteristics of the specific pathogens present, the pathogen load and the sludge type.

5.4.2.2 Water from liquid fraction of digestate

The level of pathogen removal required is dependent on the water quality required for the application intended as set out by WHO in various publications, IRMA (IRMA, 2018) and in the South African Water Quality Guidelines (DWAF, 1996a) and summarised in Section 5.2. Treatments for the removal of pathogens from water sources include exposure to UV (De Nardi et al., 2011) or solar radiation (Martín-Domínguez et al., 2005; McGuigan et al., 2012), heat (Blanc et al., 2005; Clasen et al., 2008) or various chemical treatments including silver or copper ions (Blanc et al., 2005; Feng et al., 2000) possibly applied in the form of silver nanoparticles (Bao et al., 2011; Gangadharan et al., 2010), iodine (Backer et al., 2000), chlorine dioxide (López-Gálvez et al., 2018, 2017; Van Haute et al., 2017), hypochlorite (Zou and Wang, 2017), choramine (Furst et al., 2018) and sodium dichloroisocyanurate (Légaré-Julien et al., 2018; Naser et al., 2018). Certain chemical treatments, e.g., chlorination, result in the generation of undesirable by-products in the disinfected water and strict guidelines for the levels of these by-products in drinking water have been established (Alexandrou et al., 2018; Furst et al., 2018; Gorchev and Ozolins, 2011; Plewa et al., 2004; Postigo and Zonja, 2018; Savitz et al., 2006).

Most of the abovementioned treatment methods have been tested predominantly on bacteria, using the traditional indicator organisms, such as faecal coliforms, to assess the efficacy of the treatment. Should the feedstock contain large quantities of other microorganisms, helminths and possibly also other eukaryotic organisms such as fungi, methods may need to be adapted to ensure the removal of these organisms as well. Viruses are the most difficult to remove by physical processes such as filtration due to their small size. They also show differing sensitivities to disinfection methods. Although they may carry a low infective dose, they can survive in water for an extended time. High-power ultrasound was found to be ineffective for the inactivation of murine norovirus (MNV), whereas treatment with 80 mg/L peroxyacetic acid was successful at eliminating the virus (Sánchez et al., 2015)

Due to the possible complexity of the pathogenic organisms present within the AD digestate liquid, it may be required to perform a suite of microbiological and molecular techniques to identify and choose the correct set of reference pathogens for determining the safety of the AD digestate for both potable water usage and use as fertiliser for agricultural crops as well as the need for further processing prior

to re-purposing. It is proposed that an appropriate risk profile can be built up from knowledge of the feedstock and its analysis prior to use in the AD. Further, based on this risk profile, appropriate AD conditions can be developed to reduce the pathogen load, allowing the need for further processing to be assessed and appropriate processing approaches to be defined to facilitate appropriate re-purposing of both the water stream and associated products.

6 MODELLING OF AD

6.1 Introduction to modelling AD

As of 2013, there were approximately 2,200 large scale AD plants in operation worldwide (Lauwers et al., 2013). Performance of these plants can be unpredictable as significant variability in waste feedstocks may exist, leading to varying levels of digestibility while some may contain high inhibitor concentrations. Mathematical models that are representative of the AD mechanisms with well defined inputs can instil confidence in these processes (Lauwers et al., 2013). These models may be used to predict product yields, substrate consumption and operating conditions that can assist in designing and optimising these processes. From these, process parameters and costs can be determined to provide an improved understanding of the system with greatly reduced economic risk.

As outlined in the project proposal, it is desired to select or develop a model for the purpose of optimising the AD process with respect to CH_4 productivity. This chapter contains descriptions of the model development process and existing models, and finally their applicability to the optimisation of CH_4 productivity.

6.1.1 Model development in general

Characteristics of a good model are simplicity, description of the most relevant cause-effect relationships, the ability for parameters to be easily identified from experimental data, and the ability to predict performance under reasonably similar conditions accurately (Donoso-Bravo et al., 2011). According to Donoso-Bravo et al. (2011), the following procedure for building a model should be followed (assuming experimental data is available for the system being modelled):

1. Selection of parameters should start with bearing in mind the objectives for the model (i.e. the level of detail that will be required by the model)
2. Derivation of the mathematical expressions to be used
3. Implementation through coding the mathematical expressions into maths simulation software such as Fortran, C or Matlab
4. Sensitivity analyses can then be performed to determine the most and least influential parameters
5. Check the experimental data for errors and outliers
6. Calibration through formulating and minimising an objective cost function which serves as a measure of the disagreement between the experimental data and calculated values
7. Validation using the experimental data used to determine the parameters
8. Cross validation using different experimental data to test the prediction power of the model

6.1.2 Progression of modelling AD

Development of AD models began in the early 1970s to satisfy the need to improve process efficiency of AD (Donoso-Bravo et al., 2011) and to aid in the development of process control strategies (Lyberatos and Skiadas, 1999). These models mathematically translate biochemical mechanisms into linear and nonlinear equations that are solved to obtain concentration and energy profiles of the AD process (Angelidaki et al., 1993; Batstone et al., 2002; Ikumi, 2011; Yu et al., 2013). These frameworks have evolved from only describing the rate-limiting step, to identifying VFAs as a key intermediate and

describing their production, and finally towards more sophisticated model frameworks which incorporate more processes and detailed kinetics (Donoso-Bravo et al., 2011).

AD models are complicated by successive reactions occurring concurrently in the process that are mediated by multiple microbial populations. The degree of complexity of an AD model is determined by the outputs to be predicted (Yu et al., 2013). A multi-stage approach is often taken in development of models. However, determining kinetic constants, growth or death rates and other related parameters is often challenging and ultimately limits the accuracy of these approaches (Donoso-Bravo et al., 2011).

Leading models often adopted to predict AD performance include ADM-1 (Batstone et al., 2002), ADM-3P (Ikumi, 2011) and the comprehensive AD model proposed by Angelidaki et al. (1993). These models represent the industry standard and therefore form the basis of this review. The models described in the following sections are model frameworks in that they only describe the chemical species and processes to be included. Simulation software in which to implement the models varies. However, means to implement the differential equations that describe species concentrations (mass balance equations) and to perform the calibration and validation steps for their particular application are often universal and independent of the applied platform and, as such, will be discussed accordingly. In this sense, the models described in this chapter are described only in terms of the first step of the model development process described above.

6.2 ADM1

The development of ADM1 was initiated by the International Water Association (IWA) in 1997 when it established the Anaerobic Digestion Modelling Task Group. The aim was to introduce a standardised framework that could be used to model the bio-digestion of substrates to produce effluents, methane and other by-products (Batstone et al., 2002; Donoso-Bravo et al., 2011). In this model, the reactions are divided into biochemical and physico-chemical reactions in which complex substrates are broken down into carbohydrates, proteins, fats and inert components. These then undergo hydrolysis, acidogenesis, acetogenesis and methanogenesis according to the AD mechanism. A COD balance is used to track substrate degradation in the biochemical reactions making ADM1 versatile as most substrates can be characterised in terms of their soluble and insoluble COD.

In total, ADM1 describes 24 species and nineteen biological processes as illustrated in Figure 6-1 of which the yield, Monod half saturation coefficients and biomass growth terms are used to compute reaction rates and generate the material balances, thereby eliminating the need for stoichiometric reactions.

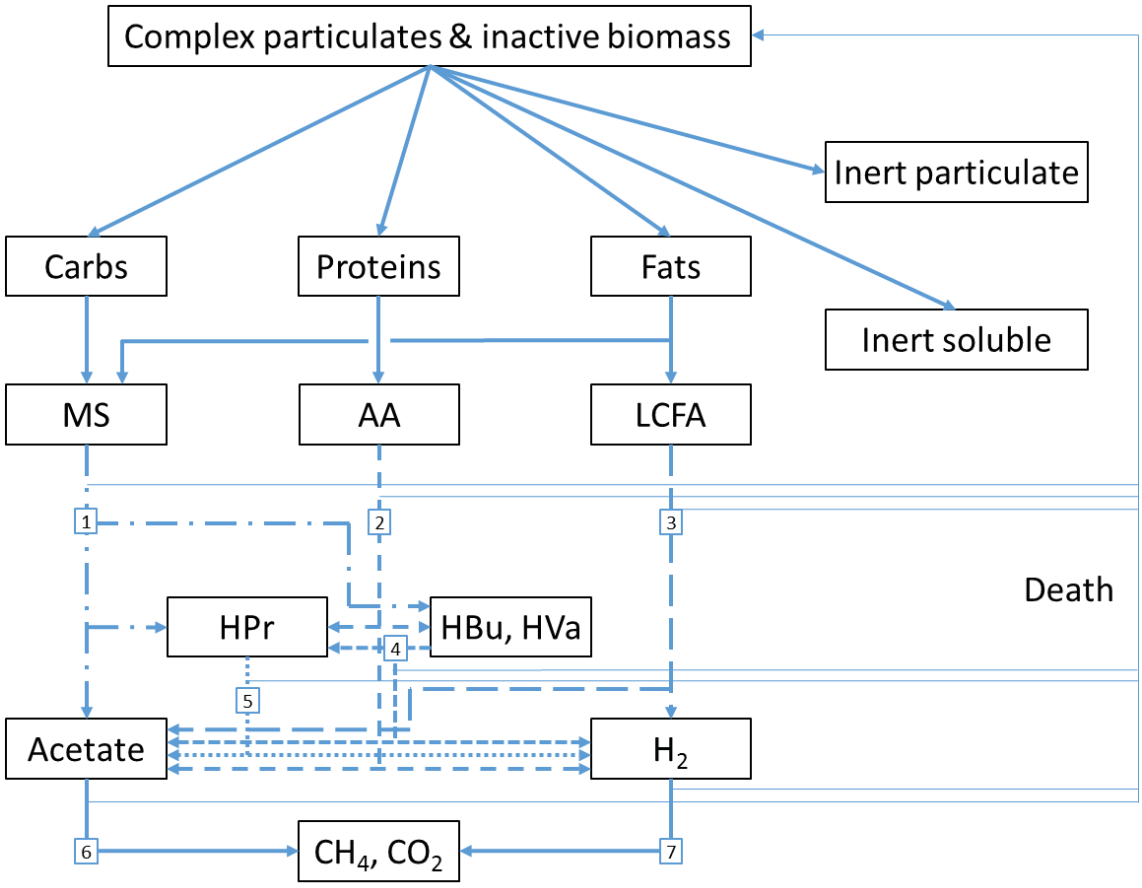


Figure 6-1 The conceptual model of the biochemical processes as implemented in ADM1: (1) acidogenesis of monosaccharides, (2) acidogenesis of amino acids, (3) acetogenesis of LCFA (4) acetogenesis of butyrate and valerate, (5) acetogenesis of propionate, (6) acetoclastic methanogenesis, (7) hydrogenotrophic methanogenesis

The model includes the initiation of AD by the disintegration of complex particulates to form carbohydrates, proteins, lipids and inerts (soluble and insoluble). Complex particulates also include dead whole-cell biomass that could include processes such as cell lysis in this initial step. Hydrolytic reactions then break down carbohydrates to form monosaccharides (MS); fats to form LCFA and proteins to form AA by hydrolysis. All these reactions are extracellular and are taken to proceed via first order kinetics (Equation 6.1) (Batstone et al., 2002).

$$r_h = k_i . X_i \qquad \text{Equation 6.1}$$

where
X_i [kg-COD.m^{-3}] is the COD concentration of the particulate component i
k_i [day^{-1}] is the associated constant for the rate of disintegration of component i
r_h [kg-COD.m^{-3}.day^{-1}] is the rate of hydrolysis

The monosaccharides and AA are then processed by two groups of acidogens to form a mixture of VFAs (acetic, propionic, butyric and valeric acids), hydrogen and carbon dioxide through a process termed acidogenesis. The mixed fatty acids, including LCFAs, valerate, butyrate and propionate are degraded by discrete groups of acetogens to form acetate in a process termed acetogenesis. Acetate and H_2 are then converted to CH_4 by acetoclastic and hydrogenotrophic methanogens respectively in a process termed methanogenesis (Batstone et al., 2002) (Section 2.3.1).

All intracellular conversions are taken to proceed via Monod-type kinetics that include substrate uptake and cell growth (Equation 6.2). Cell death proceeds via first order kinetics and dead cells are retained within the system as complex particulates (Batstone et al., 2002). Several threshold concentrations are

observed during AD before the system performance is challenged. Excess hydrogen concentrations, for example, inhibit the acetogenic activity while ammonia, a co-substrate providing nitrogen for growth and formed during acidogenesis of AA, impedes acetoclastic methanogenic activity as concentrations exceed metabolic requirements. Inhibition functions in ADM1 are therefore included for pH (Equation 6.3), hydrogen (Equation 6.4) and ammonia (Equation 6.5).

$$\rho_i = \mu_{max} . \frac{S_i}{K_{s,i} + \mathrm{S_i}} \cdot X_i \cdot I_{pH} I_{H_2} I_N \qquad \text{Equation 6.2}$$

$$I_{pH} = \frac{1 + 2 \times 10^{0.5(pH_{LL} - pH_{UL})}}{1 + 10^{(pH - pH_{UL})} + 10^{(pH_{LL} - pH)}} \qquad \text{Equation 6.3}$$

$$I_{H_2} = \frac{1}{1 + \frac{S_i}{K_i}} \qquad \text{Equation 6.4}$$

$$I_N = \frac{S_i}{S_i + K_i} \qquad \text{Equation 6.5}$$

where ρ [kg-COD.m^{-3}.day^{-1}] is the Monod-based reaction rate with a maximum growth rate μ_{max} [day^{-1}] as a function of soluble substrate S [kg-COD.m^{-3}] and particulate concentration X [kgCOD.m^{-3}] for component i. Inhibition I is incorporated by considering the aqueous pH (I_{pH}) within upper (pH_{UL}) and lower (pH_{LL}) limits and the concentrations of the inhibitory components S_i [kgCOD.m^{-3}] with associated constant of inhibition K_i [kgCOD.m^{-3}] for related hydrogen I_{H2} and ammonia I_N inhibitions.

The aforementioned physico-chemical equations are important to determine biologically inhibiting factors such as pH, dissolved gas concentrations and free acids and bases. Performance variables such as gas flow and alkalinity are also determined using this approach (Batstone et al., 2002).

ADM1 has been criticised for the implementation of a large number of processes that includes (bio)chemical species for which limited experimental datasets are available (Donoso-Bravo et al., 2011). The large number of processes can lead to calibration difficulties and model instabilities for increasingly complex feeds (Donoso-Bravo et al., 2011). (Bio)chemical species included in the model, namely proteins, carbohydrates, fats and their hydrolysis products (monosaccharides, AA, and LCFA respectively) are difficult to measure and are often not included in routine analyses, resulting in limited known input parameters for ADM1 (Sötemann et al., 2005).

6.3 Three-phase anaerobic digestion model (ADM3-P)

6.3.1 Model description

The UCT Water Research Group (WRG) developed a two-phase (aqueous-gas) AD model that was originally named UCTADM1 (Sötemann et al., 2005) as an extension of ADM1 aimed at modelling the digestion of sewage sludge. This model, similar to ADM1, is based upon a flowsheet developed by Gujer & Zehnder (1983) with some modifications (Sötemann et al., 2005):

1. The hydrolysis of complex materials act upon one group of compounds defined by the empirical formula $C_xH_yO_zN_a$ (e.g., for glucose x = 6, y = 12, z = 6 and z = 0). This is in contrast with the original flowsheet which describes three complex groups of compounds requiring hydrolysis, namely proteins, carbohydrates and lipids.
2. Because the hydrolysis process acts upon one generic compound $C_xH_yO_zN_a$, only one hydrolysis product is included in UCTADM1, namely glucose (as opposed to AA, monosaccharides and fatty acids). The choice of glucose is convenient due to the knowledge

existing around the acidogenesis of glucose to form VFA and is of little consequence due to the fact that acidogenesis is unlikely to be rate-limiting. Glucose therefore does not accumulate and acts as an intermediate between the complex generic molecule requiring hydrolysis and the VFAs.

3. Anaerobic oxidation, depicted as acetogenesis of LCFAs in ADM1, is not relevant due to UCTADM1 not recognising LCFAs.
4. The only VFAs included in UCTADM1 are acetate and propionate, with the distribution of acetate and propionate formed being regulated by the hydrogen partial pressure. Butyrate and valerate are not included due to these VFAs not being found in AD of sewage sludge, even during digester failure.

By characterising the feedstock into its elemental C, H, O and N content the elementally defined substrate ($C_xH_yO_zN_a$), together with the two identified VFAs, the UCTADM1 model describes only ten biological processes and fourteen species, representing a significantly simpler model than ADM1. This is mostly because the hydrolysis of sewerage sludge in the model is reduced to a single generically defined (idealised) substrate. However, because feedstocks are characterised only by the phase in which they are present (particulate/dissolved) and their empirical molecular formula, it is possible that a model developed under this conceptual framework would be less generically applicable than one developed using ADM1 (in which the feedstocks are described in greater detail).

This model was then extended to better cater for the context of the conventional domestic wastewater treatment plant, where the feedstocks to the AD include primary sludge (PS) and waste activated sludge (WAS) (Brouckaert et al., 2010). The model, now called ADM-3P, is documented online (Brouckaert et al., 2010; Haile et al., 2015; Ikumi et al., 2012) and has been implemented in the WEST wastewater treatment simulation software package. The major extensions are the inclusion of the element phosphorus, mineral precipitation, further classification of soluble and particulate components into biodegradable and non-biodegradable fractions, and species found in biological phosphorus removal systems (Brouckaert et al., 2010). The rationale for doing so revolves around the fact that the phosphate subsystem affects the digester pH, and that precipitation of the phosphate containing minerals $MgNH_4PO_4.6H_2O$ (struvite), $MgKPO_4.6H_2O$ (K-struvite) and $Ca_3(PO_4)_2$ affects the dissolved phosphate concentration (Brouckaert et al., 2010). The empirical formula used to define complex organics in this model was therefore extended to have the generic composition $C_xH_yO_zN_aP_b$. Figure 6-2 illustrates the conceptual model for this new three-phase model.

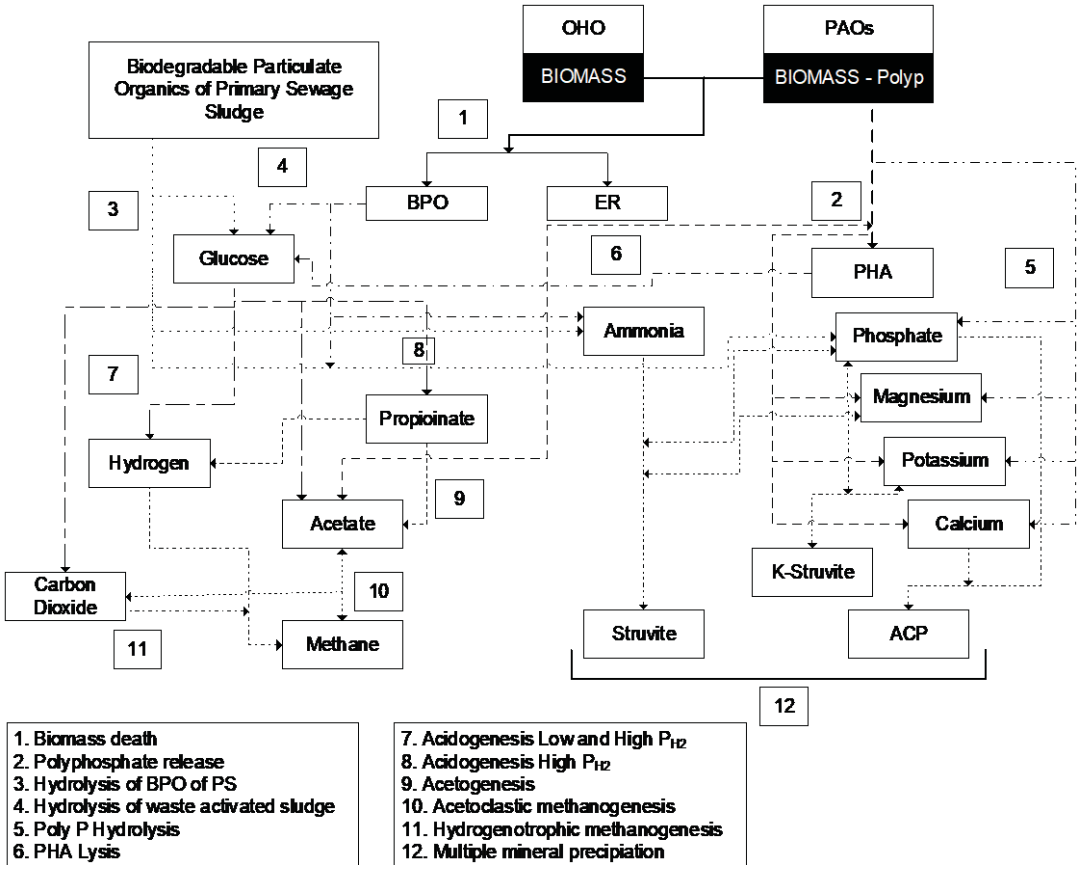


Figure 6-2 Illustration of the ADM-3P model adapted from Ikumi et al. (2012)

The ADM-3P model predicts the digester pH by formulating the weak acids/bases in terms of their ion concentrations (ion speciation) (Brouckaert et al., 2010). Although total species concentrations (e.g., for $CO_3 \leftrightarrows CO_3^{2-} + HCO_3^- + H_2CO_3$ + other CO_3 species in solution) are necessary for mass balance calculations, many reaction processes are dependent on the speciation of the ions in the solution (Brouckaert et al., 2010). Because the equilibrium reactions that determine the speciation of ions in solution proceed at a relatively instantaneous rate in comparison to the biological processes, they are assumed to be at equilibrium (Brouckaert et al., 2010). This results in increased numerical stability and faster simulation run times (Batstone et al., 2002; Haile et al., 2015). In addition to this, non-idealities in the aqueous phase are taken into account by correcting for the influence of the solution ionic strength (Haile et al., 2015). In the case of the ADM-3P, an external software program MINTEQA2, a speciation model used to determine equilibrium compositions of dilute aqueous solutions, is used to (re)speciate the ions after each time step in the process simulation based on the total species concentrations, temperature and pressure (Brouckaert et al., 2010; Ikumi et al., 2012).

6.3.2 Validation of SDM-3P

It is desired to investigate the potential of using the ADM-3P model for the purpose of optimising the CH_4 productivity. Most of the kinetic parameters have been well-calibrated for both sewage sludge and glucose digestion and it has been coded into the WEST wastewater treatment and MINTEQA2 simulation software package. It was developed with a focus on being able to predict digester failure (Haile et al., 2015), a matter of particular importance if the OLR is to be pushed to the point corresponding to maximum CH_4 productivity.

However, a number of potential problems exist with ADM-3P model as it is calibrated for the digestion of mixtures of PS and WAS (Ikumi et al., 2012). A large portion of the organics comprising the COD of these feedstocks are recalcitrant to biodegradation within AD systems (Haile et al., 2015). Since hydrolysis of these recalcitrant components in the feedstock is the rate-limiting step, it is expected that

the kinetic parameters within the ADM-3P model were calibrated most accurately for this step. It is therefore further expected that the kinetic parameters for the acidogenesis step are calibrated less accurately. While it is stated above that acidogenesis is unlikely to be rate-limiting for any AD system, the present study is exploring the potential for AD at increased OLR, with an interest in the effluent VFA concentration. This modelling objective is in contrast with the objective of predicting the performance of the AD process's capacity to treat sludge through removing COD, since the COD of the VFA leaving in the liquid phase is seen to be of value. It is therefore important that the acidogenesis step be modelled accurately. Given the aforementioned features of this model it is expected that the kinetic parameters for hydrolysis of substrates other than PS and WAS will require further calibration. Also, more specifically, it is possible that the simplification of modelling acidogenesis from glucose only may prove to be impractical for this step to be modelled accurately.

An issue that has been identified is that CH_4 is assumed to be completely insoluble in the liquid phase due to its low solubility in aqueous solutions (Brouckaert et al., 2010). While CH_4 is indeed sparingly soluble in water, at dilute feed concentrations an appreciable proportion of the influent COD can be lost in the effluent as dissolved CH_4 (Ferrer et al., 2012; Kim et al., 2011). Because a dilute feed is being used in the experimental investigation described in section 3.3, it is likely that the ADM-3P model would require modification to take dissolved methane into account. It is anticipated that the data from section 3.3 be used in the refinement of calibration of ADM-3P for its extended use; however, this is not within the project scope nor the timeline of the current project and does not meet current project requirements.

6.4 A simplified AD model

As mentioned, AD models vary in complexity from simple stoichiometric models based on the conversion of a generic representative substrate that excludes the formation of intermediates such as VFAs or the distribution of effluent products to complex models that extend the functionality of stoichiometric models (Angelidaki et al., 1993; Batstone et al., 2002; Ikumi et al., 2012). These complex models incorporate comprehensive substrate characteristics that are exposed to additional biochemical sub-processes to predict biogas and digestate compositions. By extension, the efficacy of a variety of waste streams can be explored. ADM1 and ADM-3P (WEST) are well established examples of these comprehensive approaches. However, inputs to these models may at times be over specified to component concentrations that are not easily measured in an industrial context (ADM1) that ultimately introduce error in simulation results (Ikumi et al., 2011) or, their current proven application is limited to a single source e.g., domestic wastewater (ADM-3P).

6.4.1 Development of a simplified AD model

Both ADM1 and ADM-3P require an extensive knowledge of high performance programming languages such as C++ and Java which decreases the ease of transferability between users. Using a platform such as Aspen Plus overcomes these challenges as inbuilt unit operations are inherent in this simulation package limiting the need for high level mathematical programming knowledge.

To assess the potential of a variety of waste streams for biogas production by AD and appraise valorisation prospects of co-product streams from these processes, complex models are not always necessary. A simplified approach with the overall predictive capabilities of more comprehensive models is favoured for rapid process and economic evaluation.

Substrates are elementally defined in ADM-3P which clearly simplifies model inputs compared to other solutions. However, ADM-3P presently has only been calibrated for domestic wastewater feeds limiting its applicability to already well defined feedstocks. Bounding ADM1 model inputs to feedstock characteristics that are common to most organic waste streams that can be easily measured or accessible is therefore preferred. As such, a model developed based on approaches proposed by Angelidaki et al. (Angelidaki et al., 1993) and Batstone et al. (2002) is preferred. ADM1 is an extension of the model by Angelidaki et al. (1993) with similar substrate utilisation approaches. Calibrated kinetic

parameters such as growth rates and half saturation constants that are readily available in the literature are shared in these models and are therefore appropriately transferable (Angelidaki et al., 1999; Barrera et al., 2015).

Table 6-1 Disintegration / hydrolysis constants (k_i in Equation 6.1), growth rates, half saturation (μ^{max} and K_s in Equation 6.2, respectively) and inhibition constants (K_i in Equation 6.4 & Equation 6.5) implemented in the developed model (Angelidaki et al., 1993; Batstone et al., 2002). GTO is the representative lipid glycerol trioleate in the feed, LCFA are degraded long chain fatty acids oleate from the hydrolysis of GTO and HAc is the VFA acetic acid arising from acetogenesis.

Kinetic constant	k_i (day^{-1})	μ_{max} (day^{-1})	K_s (g/L)	K_s (NH_3) (g/L)	K_i (g/L)
Hydrolysis					
Carbohydrates	10		-	-	0.33 (total VFA)
Fats (GTO)	10				
Protein	10		-	-	0.33 (total VFA)
Acidogenesis					
Glucose		30	0.26	0.05	-
Glycerol		6	0.01	0.05	-
Amino Acids		50	0.21	-	-
Acetogenesis					
Propionic acid		13	0.066	0.05	0.96 (HAc)
Butyric acid		20	0.11	0.05	0.72 (HAc)
LCFA		6	0.02	0.05	-
Valeric acid		30	0.175	0.05	0.4 (HAc)
Methanogenesis					
Acetic acid		8	0.14	0.05	0.26 (NH_3), 5.86 (K^+)

To maintain sufficient complexity to predict AD performance accurately and to remain within the ADM1 framework, organic substrate definitions for the model developed in this study were limited to their carbohydrate, lipid and protein components. Eleven key reactions that represented the four sub-processes of the AD mechanism sufficiently were incorporated and selected based on the abundance of the primary reactant (Table 6-1). Reactions considered in the model included initially hydrolysing the defined substrates to glucose, fats and AA respectively, which are then converted to VFAs by fermentative bacteria during acidogenesis. Following acetogenesis of the VFA products, acetic acid, CO_2 and H_2 are produced. Biogas, methane and CO_2 are thereafter formed via methanogenesis of the acetogenic products. VFAs, ammonia and light metal cations were also included in the initial substrate definition to determine the inhibitory contribution of these compounds on the overall digester performance.

6.4.2 Benchmarking of the simplified AD model

To evaluate the predictive capacity of the simplified modelling approach, the model developed in Aspen Plus was benchmarked against existing AD simulations. The purpose was to demonstrate the level of accuracy of the model relative to more comprehensive options and to evaluate whether the model could sufficiently represent AD performance for a range of substrates.

Four independent case studies were considered for benchmarking tests. These included laboratory and pilot scale municipal solid waste (MSW) operations as well as cow manure and pig manure bench scale AD substrates (Budiyono et al., 2011; Eliyan, 2007; Forgács et al., 2012; Fujita et al., 1980). The published experimental data and predicted simulation results for these varying substrates were used to

benchmark the simplified AD framework developed. Experimental data extracted from the published work included feed composition, operational conditions and product yields as presented in Table 6-2.

Table 6-2 Case studies used for validation (C – carbohydrates, L – lipids and P – proteins with remainder as ash. CH_4 yields in $m^3/(kgVS.day)$)

Substrate	Feed composition	Reactor size (L)	T (°C)	RT (days)	Loading rate	CH_4 Yields	Source
Cow manure	C: 70%, L: 2%, P: 2%	5	35°C	15	0.33 L/day	0.354	Budiyono et al. (2011)
MSW (Lab)	C: 61.5%, L: 10%, P: 16%	5	55°C	21	3 g VS/(L.day)	0.54	Forgács et al. (2012)
MSW (Pilot)	Not available	600	55°C	25	2 g VS/(L.day)	0.4	Eliyan and Penh (2007)
Pig manure	C: 44.06%, L: 4.9%, P: 23%	30	55°C	8	7.68 g VS/(L.day)	0.27m^3	Fujita et al. (1980)

For each of the substrates presented in Table 6-2, the corresponding process parameters were input into the model. Where data was unavailable, representative values for similar substrates in the public domain were used. The simulation results obtained from the model developed in this study were thereafter compared with corresponding literature simulation results relative to the steady state volumetric methane yield (m^3-CH_4/$kgVS_{added}$) (Figure 6-3).

The accuracy of the model was evaluated using regression analysis. Coefficients of determination (R^2) were used to provide a measure of precision for the model to estimate practical outcomes for the selected substrates. Coefficients of determination of 1 would indicate high precision, however, due to significant uncertainty in microbial behaviour during AD, R^2 values between 0.7 and 0.9 are often obtained (Donoso-Bravo et al., 2011).

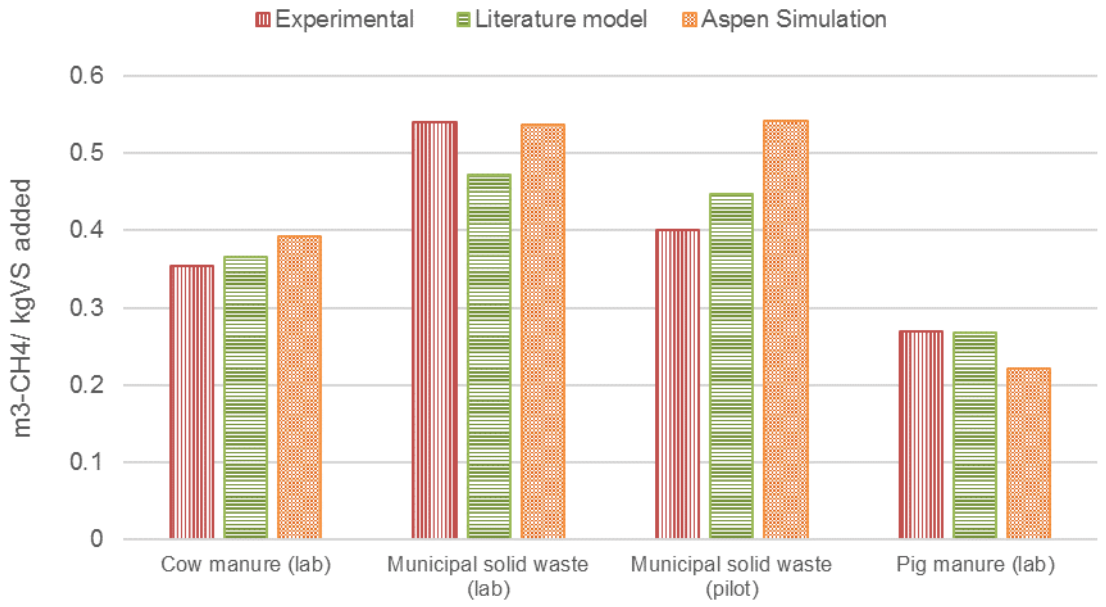


Figure 6-3 A comparison of the volumetric methane yields obtained from the AD model with experimental data and literature simulation results for the four case studies considered in Table 6-2.

For the cow and pig manure substrates, methane yields within a 10% margin of accuracy on average were predicted by the model while the experimental results for the laboratory-scale MSW system were

matched (Figure 6-3). However, a notable difference in methane yields between the pilot scale and the simulated results were observed. These contrasting results were likely due to the absence of published substrate compositions by Eliyan and Penh (2007). Consequently, as an estimate of the pilot scale composition generic data for industrial scale MSW AD processes investigated by Rajendran et al. (2014) were used. However, together with lack of data, changes in process hydrodynamics and potential unsteady state behaviour associated with scale-up operations, results from the model were significantly impacted. Consequently, a large margin of error was noted as these dynamic changes could not be replicated in the model. Regression analysis for this simulation yielded an R^2 value of 0.74.

Considering the assumptions and simplifications made in developing the AD model and the associated scale-up concerns, the coefficient of determination (R^2) was within acceptable margins of accuracy with methane predictions within 10-15% for varying waste streams. This showed that the adopted simplification of more comprehensive models is robust and may be used for diverse substrates. Benchmarking of the model further showed that it may be used with confidence as a rapid response tool to predict process performance that may be used to calibrate the WEST ADM-3P plant-wide model. Given the computational advantages and simplifications over more comprehensive approaches while still maintaining accuracy, the model may be employed comfortably in technoeconomic feasibility studies to investigate any process modifications that may be required to improve overall performance and overall profitability of the process, and in environmental assessments.

7 ENVIRONMENTAL, TECHNICAL AND ECONOMIC ASSESSMENT

7.1 Introduction to technoeconomic case study

This case study focuses on the AD of a vinasse waste stream from ethanol distilleries in the sugarcane growing areas of KwaZulu Natal, South Africa. Vinasse is a dark brown, acidic waste stream from the extraction processes of a sugar producing crop. The vinasse is approximately 90 % water and contains large amounts of organic compounds – polymers, sugars, fatty acids and proteins – as well as high levels of salts, primarily potassium. Traditionally, it has been applied to the sugarcane fields. The salts have value as replacements for synthetic fertilisers but can be a cause of long-term soil salinity increase while the organic content is either a usable source of energy or it is a source of greenhouse gas emissions and water body pollution when applied to sugarcane fields.

The technoeconomic analyses summarised in this chapter are based on a project which developed flowsheeting options and a new model of AD described elsewhere (Azegele, 2018). AD of the vinasse is the fundamental process, around which pre- and post- treatments were tested to improve process outcomes, profitability, and overall sustainability. The profitability provides a key part of the decision making process regarding the inclusion of energy, salt and water recovery technologies.

Conclusions are therefore largely drawn from relative cost-benefit analyses, deduced from comparisons of technoeconomics and profitability indicators, which would remain similar despite changes in the underlying assumptions.

7.2 Method of technoeconomic analysis

The process for determining costing and profitability is show in Figure 7-1. As Aspen's built in costing system uses proprietary algorithms and data which cannot be justified, capital and working capital costs were determined manually using costing curves, manufacturer quotes and heuristics along with OOM estimations (Davis et al., 2013; Turton et al., 2008). The fixed capital investment was then computed using a Lang factor approach which considered the installation, design and contingency fees involved in setting up the plant. A closer look at large scale biogas projects in Africa showed that the Lang factors associated with installation, piping, contingencies and fees were 60 % lower than the conventional values (summed value of 1.79 compared to 4.45) (Amigun and Blottnitz, 2007). Working capital was assumed to be 15 % of the fixed capital investment.

The fixed plant running costs (equipment, maintenance, wages, insurance, contingencies) and variable plant running costs (utilities and raw materials) were calculated and summed for each configuration. Labour costing assumed that there would be three shifts per day with an operator per processing unit, one chemical engineer and two laboratory staff with wages from PayscaleSA (2017).

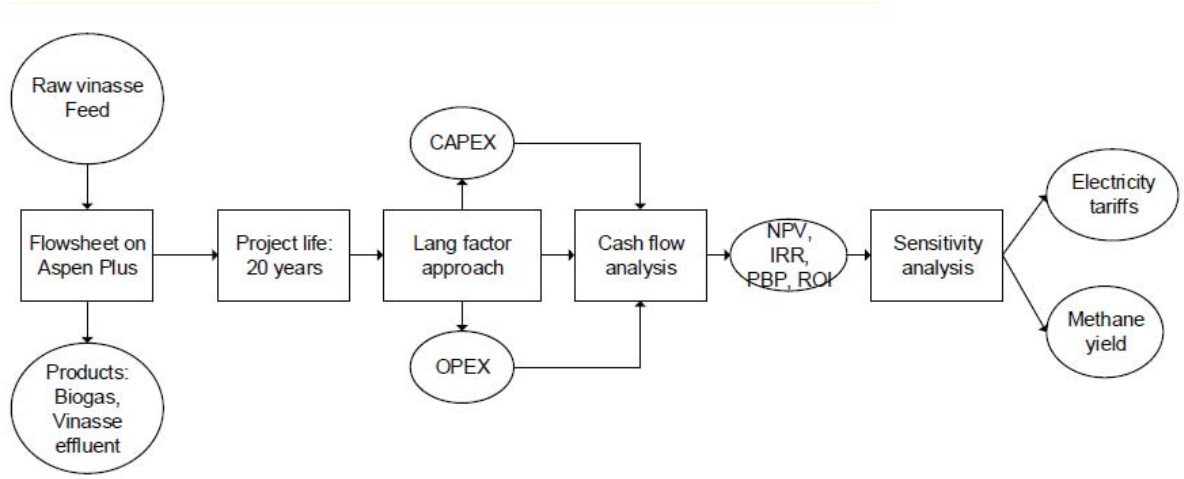


Figure 7-1 Technoeconomicanalysis methodology

The profitability indicators were then determined through a cash flow analysis done over a project lifespan of 20 years, with values in Table 7-3. Plant construction took place over the first two years with production commencing on the third. Revenues and expenses were escalated using the current inflation rate in South Africa. Depreciation was accounted for using the straight-line method where equipment values were decreased evenly throughout the project life time to their scrap value. Net profit before tax was computed by taking the difference between the gross profit and depreciation in each year. The sum of net profit after tax and depreciation formed the cash flow which was then discounted using the minimum acceptable return (15 %) to reflect its present value. Cumulative cash flows (discounted and normal) plots were used to obtain the profitability indicators such as payback period (PBP), net present value (NPV), return on investment (ROI), and internal rate of return (IRR).

These indicators provided a normalised profitability comparison of the different process configurations. However, it must be noted that the estimations concerning costs and revenues at this feasibility stage are only 25 - 40 % accurate due to diverse economic climates, regional differences and broad assumptions about cost savings on fertiliser through use of vinasse.

7.3 Base case for technoeconomics

The base case, presented in Figure 7-2, involves an anaerobic digester and a CHP plant to convert the biogas to usable electricity at the 44 % efficiency of Jenbacher spark ignition engine, with the ability to recover some the exhaust gas heat in the form of low pressure steam at 124 kPa. The points [A] and [B] in Figure 7-2 are the locations of pre- and post treatment respectively. The electricity generated was sold into the national grid at the recommended renewable energy feed in tariff of $0.1/kWh (NERSA, 2011). The AD digestate was applied to the sugar cane fields through fertirrigation or as a dewatered concentrate and was considered an indirect revenue stream in the form of a cost saving on the synthetic fertiliser required for the sugarcane plantations.

This AD-CHP process was selected as a base case as it is a common practice in industrial biogas plants which results in the production of a viable utility that can be used readily internally or sold off commercially.

The selected AD is an UASB digester, which allows the decoupling of hydraulic and sludge residence times suitable for the relatively dilute vinasse feed, operating at a temperature suitable for thermophiles (55°C), and 1 bar pressure. The vinasse from primary ethanol distillation (the analyser column) is at 90°C, so after ethanol plant heat integration, losses in pipes and the equaliser tank, it is quite likely to be approximately this temperature on flowing into the UASB. Part of the process is the dosing of NaOH

at 0.004 kg-NaOH/kgcod (Souza et al., 1992). Alkalisation is necessary to enable AD reactors to handle high OLR.

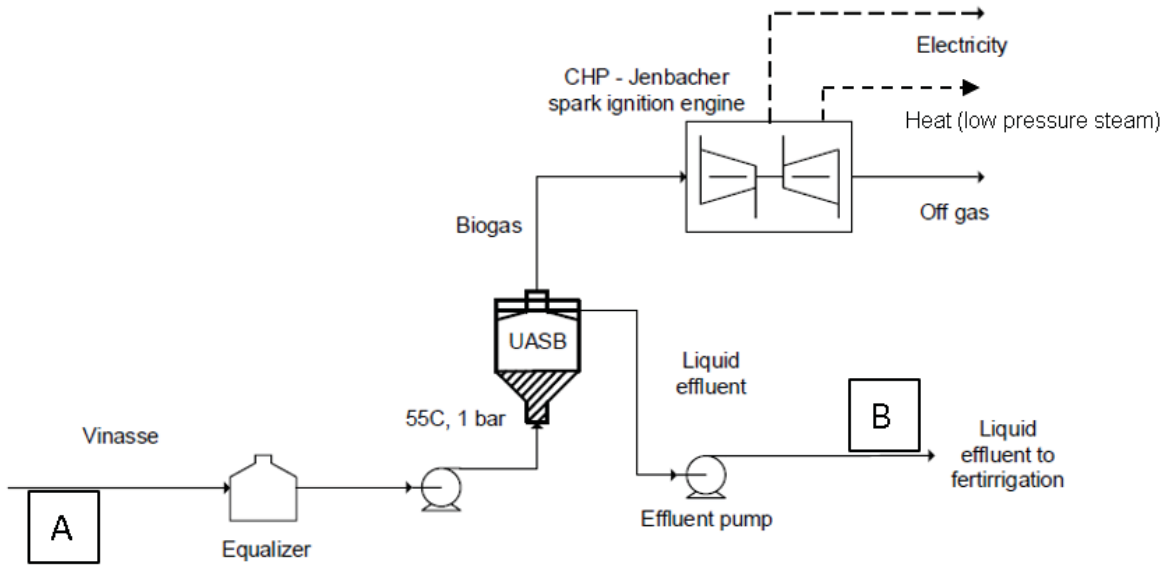


Figure 7-2 Process Flow Diagram of Base Case - AD and CHP

Biogas produced from the AD process is potentially an energy source, with literature values of 19 to 28 kJ/L at a 65:35 CH_4:CO_2 ratio and significant water and ammonia (Ryan et al., 2009; Wheeler et al., 1999). This energy can be exploited directly as heat from combustion or as electricity generated using CHP systems which also generate low pressure steam in a heat recovery system. Alternatively, the biogas may be upgraded to biomethane (95 % CH_4) by removing CO_2 through absorption or membrane processes.

7.3.1 Technoeconomic analysis of Base Case

The technoeconomic feasibility of the base case process provided information about the status of the project in terms of its profitability in a South African context.

The reactor capacity was taken as 2000 m^3 in a bid to simulate an industrial vinasse treatment plant and was assumed to be preceded by an equaliser tank at 60 % of its volume (Fuess et al., 2017). The raw vinasse mass flowrate was based on a constant OLR of 25 kgcod/m^3.day (consistent with Figure 3-8) and a mean residence time of 132 hours. Biogas was produced at a rate of 48.5 L-CH_4/kgvs, with a 53 % (v/v) CH_4 concentration and an energy density of 21.9 kJ/L. This was combusted to produce 410 kW of electrical work with an efficiency of 44 % (General Electric, 2008) and 90 kg/hr of 124 kPa low pressure steam, which was treated as a cost saving in the plant.

7.3.1.1 Capital costs

Table 7-1 Capital Costs, Lang Factors and Total Capital Investment for the Base Case

Equipment	Capacity	Construction	Costs USD (2016)	Source
UASB Reactor	2000 m^3	Reinforced Concrete	651 000	(Fuess and Zaiat, 2017)
Influent and Effluent Pumps	0.21 m^3/min	Carbon Steel	7 350	(Fuess and Zaiat, 2017)
Ancillary Equipment	-	-	11 180	(Fuess and Zaiat, 2017)
Equaliser Tank	1200 m^3	Reinforced Concrete	385 000	(Seider et al., 2003)
Jenbacher™ 620 CHP	-	-	1 770 000	(Darrow et al., 2015)
Total Cost (Purchase Cost of equipment- PCE)				**$ 2 820 400**
Lang Factor (Installation, electrical, site, instrumentation)				*1.6*
Physical Plant Cost (PPC)				$ 4 513 000
Lang Factor (Design, Engineering and Contingency)				*1.19*
Lang Factor (location)				*1*
Fixed Capital Investment (FCI)				$ 5 370 000
Working Capital (15 % of FCI)				*$ 805 500*
Total Capital Investment (TCI)				$ 6 176 000

The Jenbacher™ spark ignition engines are historically known to be expensive; however, they combine compression, fuel injection, combustion and heat recovery into a compact and highly specialised piece of equipment able to withstand impure biogas feeds, hence their high price (Darrow et al., 2015). Ancillary equipment included biogas flow metres, seals, pH monitors and gas analysers.

7.3.1.2 Operating costs and revenues

The total operating costs in Table 7-2 were calculated using a heuristic approach and industry benchmarks reported in the literature (Amigun and Von Blottnitz, 2010; Mohammed et al., 2016)

Table 7-2 Operating costs and revenues for the Base Case

	Base Case (AD-CHP) USD(2016)/yr	Source
NaOH (AD dosing)	38 690	(Alibaba, 2017)
Transport (fertirrigation) and utilities	16 000	
Labour	173 000	(PayscaleSA, 2017)
Maintenance: CHP system ($0.02/kWh)	68 400	(Darrow et al., 2015)
AD system (1.5 % of installed capital cost)	28 300	(Amigun and Von Blottnitz, 2010)
Total Operating Costs	323 000	
Indirect cost saving on fertiliser	92 600	(Alibaba, 2017)
Revenue	Electricity - 360 000	$0.10/kWh (NERSA, 2011)
	Steam - 7 000	

The labour costs were found to be significantly higher than the utility or raw material costs. AD plants are relatively cheap to operate and maintain, with costs estimated to be between 1 and 2 % of the PPC (Mohammed et al., 2016). CHP plants require specialised maintenance and service after every operating year (8000 hours), equivalent to $0.02 per kWh generated for the 1000 kW capacity Jenbacher™ 620 spark engine (Darrow et al., 2015). The primary source of revenue is electricity sold to the grid at the renewable energy feed in tariff (REFIT) for biogas that is set by the National Energy

Regulator, South Africa (NERSA, 2011). It was assumed that the effluent would be used for fertirrigation of agricultural land at an application rate of 350 m^3/ha, resulting in cost savings from the purchase of synthetic K-based fertiliser valued at $400/ton (Alibaba, 2017).

Factors relating to the South African economy, shown in Table 7-3, such as depreciation, scrap value, inflation rate and the average cost of capital were taken into consideration when developing the cumulative cash flow (Figure 7-3) and economic outcomes (Table 7-4). Sale of electricity to the grid may not be possible owing to government policy constraints on the minimum power generation capacity and volatility of the levelised cost of energy (EcoMetrix Africa, 2013; NERSA, 2011). Despite this, the revenues achieved from the sales may be considered as a cost savings on electricity purchase from the national distributor. Furthermore, biogas production and CHP are widely practised, making this a medium risk venture.

Table 7-3 Operating Costs, Revenue, Capital cost and economic factors used in cash flow analysis

	USD (2016)
Operating Costs	336 000
Cost Savings (fertiliser, steam)	99 000
Revenue (electricity)	360 000
Total Capital Investment (fixed and working capital)	**6 176 000**
Taxation	28
Escalation	6.2
Years of depreciation (linear)	10
Scrap value	5
Weighted Average Cost of Capital (WACC / discount rate)	15

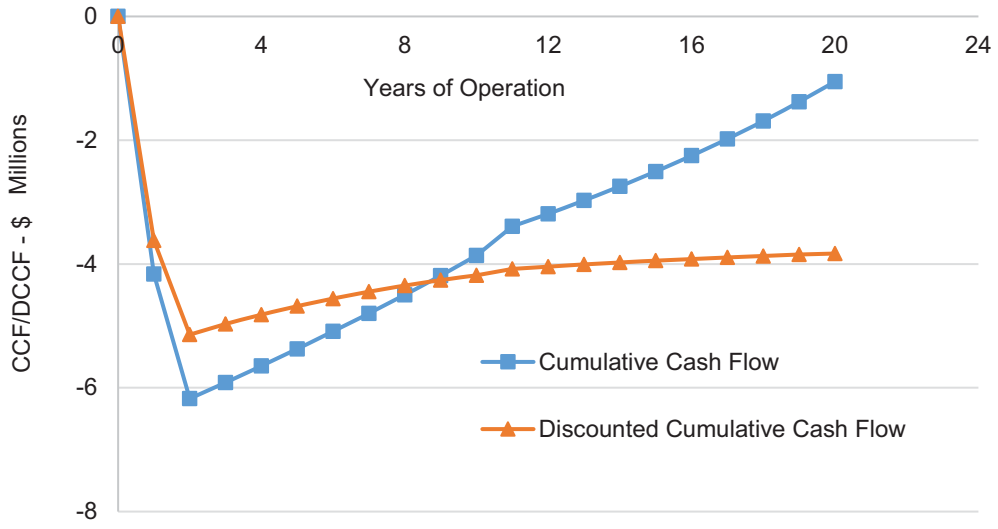


Figure 7-3 Cumulative cash Flow (USD, 2016) for the base case for a 20 year lifespan

Table 7-4 Economic outcomes for the Base Case

PBP (fixed capital)	18.9 years
ROI	0 %
IRR	-1.8 %
NPV after 20 years	- $4 million

The base case cumulative cash flow (Figure 7-3) and economic outcomes (Table 7-4) show the AD-CHP process to be unprofitable. However, the cost of 'doing nothing' can be regarded as the disposal of the raw vinasse (305 kL/day) into the municipality wastewater treatment facility at an annual cost of approximately $80 700, calculated using the average industrial effluent disposal tariffs (R9/kL) in South Africa (eThekwini Water and Sanitation, 2018). Taking this into account improves the viability of the AD-CHP process.

Sensitivity analysis of the process inflows in the model were used to identify trends and opportunities for process optimisations through potential revenue generating opportunities or cost savings through incorporation of additional processing units, which are presented in the following sections.

- Potassium ion removal reduces inhibition of the AD process, increasing methane production and creates a K_2SO_4 revenue stream, but at the cost of additional process units.
- Alternate uses of the biogas, considering that the CHP plant is 63 % of the CAPEX and 33 % of OPEX.
- Recovery and re-use of water, which also reduces transport costs of the now concentrated digestate to the fields.

7.3.2 Technoeconomic analysis of pre-treatment

Various substances present in the vinasse are inhibitory to the AD process, such as ammonia, inorganic salts, acetic acid and phenolic compounds (Chen et al., 2008). Removal of these compounds at the process step [A] in Figure 7-2 has the potential to improve the profitability of the AD process.

High levels of potassium are inhibitory to the methanogenesis step of the AD process due to osmotic imbalances (Chen et al., 2008), and potassium is a valuable component of fertiliser. Kugelman and McCarty (1965) post a 50 % reduction in methane production above a potassium concentration of 11.6 g/L. This is supported by unpublished data from the CeBER labs at UCT. The Aspen model shows a 12 % increase in CH_4 yields from 48 to 55 L-CH_4/kgvs and 16 % increase in the electrical output from the CHP plant after a 70 % reduction in vinasse potassium concentration down to 7 g/L.

Several pre-treatment options were considered (ozonation, electrodialysis, ion exchange and RO), of which only strong acid-cation exchange was suitable in terms of potassium ion removal, process parameters and operating costs (Zhang et al., 2012). A 70 % recovery of potassium was viable using Amberlite resin, with elution of absorbed ions using sulphuric acid followed by crystallisation of K_2SO_4. The running costs include resin replacement, sulphuric acid, and crystallisation utilities (heating, cooling and pumping). Higher recoveries are possible but with greater capital and running costs, hence 70 % was selected.

At the proposed plant capacity, an annual potassium sulphate production of 1 500 tons was achieved, valued at $400 per ton (Alibaba, 2017).

The major additional equipment required for ion exchange were vertical columns for potassium adsorption and regeneration of resin, pumps and a heating crystallisation system to recover K_2SO_4 crystals.

Table 7-5 Capital costs comparing Base Case and pre-treated (IEX) using Lang Factors in Table 7-1

Equipment	Base Case (AD-CHP) USD (2016)	Pre - Treatment (IEX-AD-CHP) USD (2016)
Ion Exchange (Columns, Pumps, Crystallisation Unit)	-	2 710 000
UASB Reactor, Equaliser tank, pumps and ancillary equipment	1 055 000	1 055 000
Jenbacher^{TM} 620 CHP	1 770 000	1 770 000
Total Cost (PCE)	2 820 400	5 532 000
Total Capital Investment (TCI)	6 176 000	12 112 000

Notably, the cost of ion exchange (IEX) equipment was significantly higher than either AD or CHP. This resulted in a 100 % increase in the initial capital investment of the IEX-AD-CHP route relative to the base case (AD-CHP).

Table 7-6 Operating costs comparing Base Case and pre-treated (IEX)

	Base Case (AD-CHP) USD (2016) / yr	With Pre-treatment (IEX-AD-CHP) USD (2016) / yr
NaOH (AD dosing)	38 690	38 690
H_2SO_4 eluent	-	27 670
Utilities (electricity, water)	80	(included in O&M)
Transport (fertirrigation)	15 000	14 500
Labour	173 000	185 000
Operation & Maintenance		
CHP system ($0.02/kWh)	68 400	76 000
AD system (1.5 % of capital cost)	28 300	28 300
Ion Exchange (10 % of Capital cost)	-	272 000
Total Operating Costs	**324 000**	**643 000**
Indirect cost saving on fertiliser	92 600	73 200
Revenue (Electricity and steam)	367 000	426 000
Revenue (potassium sulphate)		600 000

The 150 % increase in operating costs was mainly due to the IEX maintenance costs (117 %), sulphuric acid purchase, increased labour with the additional process units and increased CHP maintenance due to greater electricity generation. Smaller indirect cost savings on fertiliser were realised due to the reduced potassium levels, thus fertilising a smaller area.

Table 7-7 Economic outcomes for pre-treatment compared to the Base Case

	Base Case (AD-CHP)	With Pre-treatment (IEX-AD-CHP)
PBP (fixed capital)	18.9 years	12.7 years
ROI	0 %	3.2 %
IRR	-1.8 %	2.0 %
NPV after 20 years	- $ 4 million	- $ 6.5 million

Addition of the pre-treatment doubled the capital cost, but also significantly increased revenue with the addition of a K_2SO_4 stream. While this was enough to decrease the PBP and increase the ROI and IRR values, it was still well below the desired return, with a significant negative NPV after 20 years. Again, the waste treatment costs remaining on not implementing these technologies must be considered. In

sustainability terms, the reduction of potassium salt concentrations to below 5 g/L in the digested vinasse is expected to reduce the risk of soil salinisation with increased fertirrigation over the long-term (Christofoletti et al., 2013).

An alternative would involve the ion exchange but without the crystallisation, creating a potassium rich liquid stream. This would reduce capital costs but would increase operational costs as there is no regeneration of H_2SO_4.

7.3.3 Technoeconomic analysis of varying biogas usage

The vinasse AD process in the base case produces 65 % methane biogas with a calorific value of 22 kJ/L, comparable to published results (Barrera et al., 2015; Fuess et al., 2017; Walsh et al., 1989). Upgrading of this biogas is an alternative to CHP and does away with the large capital and maintenance cost of the Jenbacher™ engine. Biogas upgrading involves removing CO_2 as well as other undesired components such as H_2S, NH_3 and water vapour to produce 95 - 97 % pure biomethane which can be compressed and sold for domestic use or injected into a gas grid (Axelsson et al., 2012).

High pressure water scrubbing (HPWS) was preferred over amine scrubbing and gas permeation through membranes due to its simplicity and sustainability in terms of harsh chemicals, high pressures and water recycling (Bick et al., 2012). Additionally, HPWS has a 5 - 10 % lower unit cost compared to amine scrubbing and membrane separation (Leme and Seabra, 2017), as well as lower operating costs without the need for high heat energy requirements for amine solvent regeneration or frequent membrane replacement (Axelsson et al., 2012).

The HPWS was modelled as a counter current flow of biogas and water at 10 bar through an absorber column packed with plastic pall rings that had sixsix theoretical stages (Cozma et al., 2014, 2013). The process takes advantage of the fact that carbon dioxide is 25 times more soluble than methane in water. The process achieves 93 % recovery of CH_4 and 99 % removal of CO_2, H_2O (g) and NH_3, with a biomethane purity of 95 % and a calorific value of 38 kJ/L.

The biomethane can be sold commercially as a substitute to natural gas at $19/GJ (EcoMetrix Africa, 2013). Recycling of one of the off-gas streams can improve biomethane recovery by 5 % but increased the capital costs by 18 %, with increases in operating costs, making it not worthwhile. The process water is mostly recycled, but some is purged to prevent solute build-up, requiring a fresh water makeup stream.

Table 7-8 Capital costs for Base Case and biogas upgrade using Lang Factors in Table 7-1

Equipment	Base case (AD-CHP) USD (2016)	Biogas upgrade (AD- HPWS) USD (2016)
UASB Reactor, Equaliser tank, pumps and ancillary equipment	1 055 000	1 055 000
Jenbacher™ 620 CHP	1 770 000	-
HPWS (Columns, compressors, pumps)	-	116 000
Total cost (PCE)	2 820 400	1 171 000
Total Capital Investment (TCI)	6 176 000	2 560 500

Table 7-9 Operating Costs of Base Case and Biogas Upgrade

	Base case (AD-CHP) USD (2016) / yr	Biogas upgrade (AD- HPWS) USD (2016) / yr
NaOH (AD dosing)	38 690	38 690
Utilities (electricity, water)	80	65 000
Transport (fertirrigation)	15 000	15 000
Labour	173 000	173 000
Operation & maintenance CHP system ($0.02/kWh)	68 400	-
AD system (1.5 % of capital cost)	28 300	28 300
HPWS system (10 % of capital cost)	-	12 000
Total operating costs	323 000	331 000
Indirect cost saving on fertiliser	92 600	92 600
Revenue	Electricity, steam - 367 000	Biomethane - 572 000

The capital costs (Table 7-8) are much lower for the biogas upgrade than the base case as the CHP plant was 2.5 times more expensive than the HPWS system. The overall operating costs (Table 7-9) are similar as the reduced maintenance cost of the HPWS counters its increased water (makeup stream) and electricity (pumping) costs. The HPWS has no effect on the digestate, which is transported to the sugarcane fields and used for fertirrigation as in the base case.

Table 7-10 Economic outcomes for Biogas Upgrade compared to the Base Case

	Base case AD-CHP	Bio Gas upgrade AD-HPWS
PBP (fixed capital)	18.9 years	3.7 years
ROI	0 %	19 %
IRR	-1.8 %	16.3 %
NPV after 20 years	- $4 million	196 000

Table 7-10 shows that replacing the CHP system with a biogas upgrading process resulted in a significant increase in the profitability owing to a 50 % reduction in equipment costs and 59 % increase in revenue at similar operating costs. The PBPPBP is significantly shorter, IRR is greater than the cost of capital (15 %) and thus the 20 year NPV is positive, with the project likely to return a small net profit.

7.3.4 Technoeconomic analysis of post treatment

The liquid digestate from the AD has a relatively high moisture content ranging between 85 and 90 % (Wilkie et al., 2000). In the preceding cases, this is directly applied to the sugarcane fields and results in cost savings on synthetic fertiliser purchase. This section explores water recovery strategies, at position [B] in Figure 7-2, to increase sustainability of the AD process with regards to water, alternate uses of the digestate and the potential cost savings. The nutrient rich concentrate can be directly applied to farmland as fertiliser with lower transport costs than vinasse or used as an animal feed supplement, depending on potassium concentration (Christofoletti et al., 2013).

Dewatering processes such as multi-effect evaporation (MEE) and RO have often been practiced in industry and have varying characteristics which affect their economics and applicability in the context of vinasse treatment (Carvalho and Luiz da Silva, 2011; Nataraj et al., 2006).

The RO process is preceded by a filter at 20 bar to remove large suspended solids before a nanofiltration system to decolourise the digestate by removing colloidal compounds and heavy polymers responsible for the dark brown pigment (Nataraj et al., 2006). The pressure of the filtrate stream is increased to 50 - 55 bar, its osmotic pressure, using a series of centrifugal pumps before the RO modules which reduce the water content of the vinasse from 90 % to ± 60 % to form a concentrate.

Water and VFAs are recovered in the permeate stream. Thereafter, an energy recovery device in the form of a turbine is used to reduce the permeate pressure to 1 bar and recover its energy simultaneously.

The MEE process consists of five evaporators in series with intermediate condensers, based on a Brazilian vinasse evaporation case study (Carvalho and Luiz da Silva, 2011). Concentrated vinasse from MEE ('concentrated molasses solids' CMS) is drawn from the last effect with a water content of approximately 40 % (Rein et al., 2011), with further water reductions not worth the elevated equipment costs (Rein, 2007).

The heat requirement for MEE was calculated to require 10 % of the biogas produced as fuel to generate the steam for the first MEE evaporator stage. Considering boiler or combustion inefficiency, the fraction of biogas required may increase to 15 %. This was up to nine times the energy of the RO's high pressure pumping requirements, translating to increased operating costs.

Table 7-11 Capital costs for Base Case and Post Treatment using Lang Factors in Table 7-1

Equipment	**Base Case (AD-CHP) USD (2016)**	**RO (AD- CHP-RO) USD (2016)**	**Evaporation (AD- CHP-MEE) USD (2016)**
UASB Reactor, Equaliser tank, pumps and ancillary equipment	1 055 000	1 055 000	1 055 000
Jenbacher^{TM} 620 CHP	1 766 000	1 766 000	1 766 000
RO system	-	64 000	-
MEE system	-	-	181 000
Total Cost (Purchase Cost of equipment PCE)	**2 821 000**	**2 895 000**	**3 001 000**
Total capital investment (TCI)	**6 176 000**	**6 310 000**	**6 571 000**

The MEE capital costs were 140 % higher than the RO, but the capital costs for both downstream processes were insignificant (4 % and 2 % respectively) compared to the base case AD-CHP costs.

Table 7-12 Operating Costs of Base Case and Biogas Upgrade

	Base Case (AD-CHP) USD (2016)/yr	**RO (AD-CHP-RO) USD (2016) / yr**	**Evaporation (AD-CHP-MEE) USD (2016)/ yr**
NaOH (AD dosing)	38 690	38 690	38 690
Utilities (electricity, water)	80	47 000	80
Transport (fertirrigation)	15 000	15 000	15 000
Labour	173 000	173 000	173 000
Operation & maintenance CHP system ($0.02/kWh)	68 400	68 400	68 400
AD system (1.5 % of capital cost)	28 300	28 300	28 300
RO (10 % of Capital cost)	-	25 600	-
RO membrane replacement	-	40 000	-
MEE (10 % of Capital cost)	-	-	32 000
Total operating costs	323 000	436 000	362 000
Indirect cost saving on fertiliser	92 600	250 000	250 000
Revenue (Electricity and steam)	367 000	367 000	367 000

The addition of RO or MEE to the base case resulted in a 34 % and 12 % increase of the total operating costs respectively. The RO process used electricity for high pressure pumping while a 5 year lifespan of the spiral wound RO membranes was assumed due to fouling and general wear (Bick et al., 2012). This introduced a cost of membrane replacement that made up 9 % of the total operating costs for the AD-CHP-RO process. The high pressure steam required for the MEE process was obtained from excess steam from upstream processes and the CHP heat recovery system and therefore not included

in the cost analyses. This resulted in cost savings for the MEE process which kept the operating costs lower compared to the RO process.

Notably, there were significant increases in the cost savings from fertiliser application with the addition of either water recovery process. A much larger area was fertilised with CMS due to differing assumptions used in determining application rates (300 m^3/ha for the liquid digestate compared to 3 tons/ha for CMS).

Table 7-13 Economic outcomes for Post Treatment compared to the Base Case

	Base Case AD-CHP	RO AD-CHP-RO	MEE AD-CHP-MEE
PBP (fixed capital)	18.9 years	12.7 years	12.9 years
ROI	0 %	3.2 %	3.1 %
IRR	-1.8 %	2.0 %	1.9 %
NPV after 20 years	- $4 million	- $ 3.4 million	-$ 3.5 million

Both post treatment options showed increased profitability to the base case, mainly due to the change in assumptions about the value as fertiliser replacement. The RO and MEE economic outcomes were quite similar, with the higher RO operating costs countering the higher capital cost of MEE. However, both processes are still far from profitable.

The long-term sustainability of an AD process may well involve recovery of water. Due to the presence of volatile acids, the recovered water (68 700 m^3/yr) is categorised as fit-for-purpose and was re-integrated upstream, such as a water make up stream for the cooling systems, saving $27 700/yr at a municipal water tariff of R5/kL, or as the fresh water requirement (22 000 m^3/yr) in the HPWS process. The water can be treated to remove volatile compounds, but this cost of this is prohibitive.

7.4 Environmental assessment

The AD of organic rich wastewater with simultaneous recovery of VFAs or methane or both delivers environmental value in terms of enhancing resource efficiency of water and in providing a source of bioenergy. Through simultaneous 'fit-for-purpose' water recovery and energy recovery, the environmental burden of the wastewater 'as is' can be reduced. To optimise the environmental burden reduction, it is necessary to maximise the energy and water recovery with the minimal introduction of unit processes with associated burden. To achieve this, it is necessary to ensure, amongst others, optimal operation of the AD unit operation. It is also necessary to weigh up the additional recovery of value achievable with environmental burden required to achieve this.

From the analysis of the simultaneous production of methane and VFA and the subsequent recovery or upgrading of VFAs, it is found that this approach is not favourable environmentally, owing to both the reduced efficiency of the AD and the technical challenges of the recovery, requiring multiple (energy-requiring) steps for its required concentration. Furthermore, the environmental benefit of CHP compared with use of methane for steam generation preferred the latter from an environmental perspective (Cohen, 2006). Further analysis of energy recovery units is required to assess biogas upgrading.

The flow sheet development undertaken in sections 7.3 seeks to present enhanced economic and environmental value. By introducing ion exchange prior to the AD, we seek to both control the salinity of soils resulting from the fertigation with salts-rich, vinasse-based AD digestate. We also seek to enhance resource efficiency of K present and to maximise AD operation and methane recovery. By integrating AD with biogas processing via biogas upgrading via HPWS or through CHP, we seek to minimise methane emissions and associated GHG and to maximise energy recovery. By post AD treatment of the digestate, we seek to recover fit-for-purpose water for re-use and to concentrate key nutrients for re-use, thereby improving resource efficiency and minimising associated transport costs and energy required for transport simultaneously.

Consideration of environmental assessment is required alongside technoeconomic assessment to inform selection of appropriate process flowsheets.

7.5 Conclusions

A summary of the tested changes to the base case of AD and CHP is shown in Figure 7-4.

Removal of inhibitory potassium by ion exchange resulted in a 30 % increase in methane yield during AD, increasing revenues from electricity sales. Consequently, this increased the base case profitability (ROI and IRR from 0 % and -1.8 % to 2 % and 3.2, respectively) as well as environmental performance of the process.

Substituting HPWS in place of the CHP in the base case flowsheet reduced the capital costs significantly while selling biomethane rather than electricity increased revenue. The biogas upgrade process greatly increased profitability (ROI: 19.6 %, IRR: 16.3 %), most notably due to comparatively lower capital cost of HPWS equipment relative to the Jenbacher™ engine for CHP. Additional assessment of this selection through LCA is desirable.

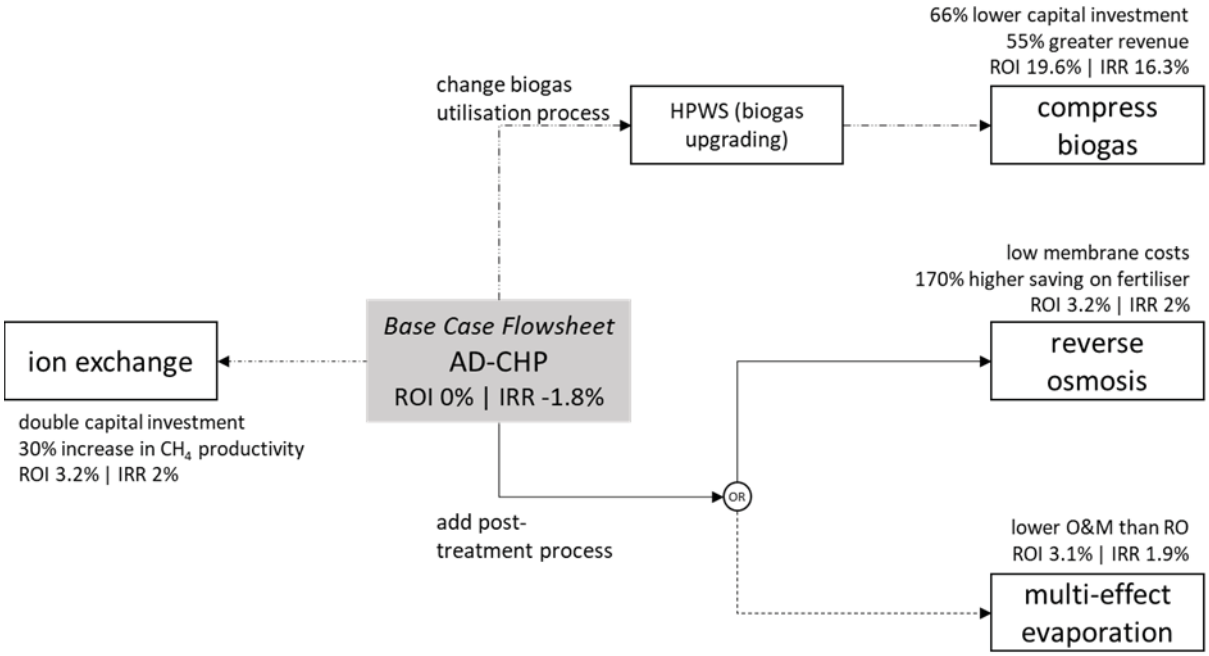


Figure 7-4 Summary of the sensitivity of the base case to addition of pre- and post treatment unit operations in terms of performance and technoeconomic feasibility

Addition of dewatering processes through RO or MEE increased the profitability of the base case (ROI and IRR at ±3 % and ±2 %), but both remain below the weighted average cost of capital (15 %) which suggests that the routes were unlikely to return any profits. There are both sustainability and cost saving benefits to water re-use; further elucidation of these is expected to enhance process decision making and help to identify the key components of the process for further refinement to maximise economic benefit and minimise environmental burden. Furthermore, the cost of “doing nothing” needs to be included realistically. The minimal cost here is the standard wastewater treatment cost imposed by the municipal treatment works; however, it may be expected that penalty tariffs are also incurred. Further, the generation of bioenergy from waste resources has potential to activate carbon credits, due to be implemented in South Africa in 2020, demonstrating its positive impact with respect to reduced environmental burden.

Efficacy of the AD process is determined by a series of design decisions, with a suitable combination of units for the waste stream type, location, local needs and shortages providing the best economic, environmental and sustainable outcome.

8 CONCLUSION

While AD is a well-researched topic, focus on it has fulfilled two different needs: the first being the upgrading of wastewater by removal of residual organics to biogas and the second the generation of biogas from renewal resources. The former has focused on resultant water quality, with treatment typified by long treatment times and considering biogas as a convenient byproduct. The latter has focused on maximising methane yield from sludges and solid waste with limited consideration of water. In this project, we sought to bring these together to focus on maximising the resource efficiency of wastewater through maximising production of bioenergy or higher value products or both while optimising resultant water quality and recovery. The project set out to explore the combined optimisation of energy recovery from wastewater while maximising the rate of biogas formation and hence space time utilisation within a system in which the recovery of fit-for-purpose water is a requirement. In addition, we have sought opportunities for enhanced value recovery and potential for higher value organic products, in particular VFAs for use as platform chemicals, in addition to or instead of biogas, while minimising the environmental footprint.

Acknowledging that the water quality standards for drinking water, irrigation water, recreational water, water for aquaculture and aquatic uses all differ, it is recognised that the final water quality recovered post-AD can be designed as fit-for-purpose. Further, reviewing water quality across South African wastewaters in terms of organic carbon loading, it is recognised that substantial potential value is carried in the organic carbon loading. This value is supplemented by the N and P loading.

In spite of the quantity of research performed, there is still little evidence of the consideration of operating AD for optimised CH_4 productivity or of the potential to form and use higher value products such as VFAs with platform chemical potential, or to ensure beneficiation of the N and P nutrients. The trade-offs that exist between the conventional use of AD as a treatment process characterised by high COD removal and CH_4 yields, and its use as a wastewater valorisation process, based on the wastewater biorefinery concept, are visible on a macroscopic level. In this project, experimental and modelling research has been carried out to gain a better understanding of the influence of the OLR and wastewater composition on the trade-offs between these approaches to wastewater valorisation using AD.

The potential to maximise the volumetric methane productivity was explored using an UASB reactor. The reactor was designed to enable a varying fresh feed rate while using recycle to maintain a constant upflow linear velocity to ensure consistent mixing of the bed and avoid formation of zones within the reactor for ease of analysis and modelling. On increasing the OLR to the reactor, both increasing volumetric methane productivity and volumetric organic degradation rate were observed up to an OLR of in excess of 28 g-COD/L/day. At higher rates the system became less robust with a tendency to acidify, associated with a decreasing organic degradation rate and a sharp reduction in methane productivity. Based on these findings, a stable operating window for organic degradation to methane was proposed, associated with robust performance and good conversion of organics to methane.

Owing to the lack of robustness of the dual product AD process, it was decided that it is preferable to select either methane or VFA as the desired product of AD, rather than mixed products. Should VFAs be the desired product, these should be produced by acidogenic fermentation under conditions inhibiting methanogenesis. This decision is supported by the negative impact of VFAs on methanogenesis with reduced robustness of the process as well as the difficulty in recovering VFAs from low concentration streams owing to their partitioning between both product streams. Owing to this difficulty in separation and concentration, the valorisation of VFA streams of a minimum concentration of some 30 g VFA/L is preferred, supporting the single product approach.

AD has been the topic of copious research attention as a wastewater treatment biotechnology with the potential to contribute towards energy needs (through CH_4 production). Recently, its added potential to contribute to downstream biochemical processes through VFA production has been recognised. This research has opened up new ideas with regards to the waste streams that can be processed, and the ways in which the AD process is operated. Applications of particular interest are the AD of raw domestic wastewater as well as agricultural and industrial wastewater streams, although post treatment steps are required to produce clean water and a nutrient rich stream.

While the generation of the energy or platform chemical product forms a key output of the process, recovery of water and additional components of value is of equal importance, requiring further processing of the digestate. Typically, solid – liquid separation such as sedimentation or filtration is required for particulate removal. The subsequent removal of solutes is typically carried out by adsorption, ion exchange, ultrafiltration, RO, distillation or evaporative processes to to deliver fit-for-purpose water. Here micro-, ultra-, nano- filtration and RO, precipitation (for struvite) and adsorption are preferred, depending on the water quality. The nutrients N and P, as ionic species, typically report to the concentrate stream on nanofiltration or RO, facilitating preparation for further use. The short chain VFAs partition across both outgoing streams from these nanofiltration and RO, requiring a subsequent adsorption step to deliver compliant water and further illustrating why the production of VFAs is preferred only where high concentrations can be delivered. The role of these unit operations in the overall process has been presented.

Further to this, microbiological quality of both water and products must be assured. The pathogen load entering the anaerobic digestor depends on both the source of the AD feedstock and how it was stored. It is particularly impacted by the presence of human or animal faeces in the AD reactor feed. Organisms of concern include coliforms and zoonotic pathogens which cause disease in humans and animals, as well as viruses and helminths, The degree of reduction in the pathogenic load leaving the digestor is governed by the conditions within the digestor, particularly the operating temperature (mesophilic or thermophilic) and the residence time. If required, pathogen removal may be undertaken by, for example, heat treatment. In summary, good process control is essential for appropriate microbiological water quality, requiring three steps: (1) awareness of pathogenic load of source, (2) availability of rigorous and rapid monitoring techniques and (3) effective treatment methods either within or in addition to the AD. While monitoring methods are traditionally culture based, increasing motivation for rapid approaches based on molecular biology and immunology approaches are growing. Further, it is noted that the water-upgrading steps reported above also impact the pathogen load positively.

Design, operation and monitoring and control of the digestion process is benefitted by the availability of a representative model of the process. To interrogate both the operation of the process as well as the development of the flow sheet in which the AD process sits in order to maximise both value from and resource productivity of the wastewater, the existing models were first considered. ADM1 was developed as a comprehensive model of AD processes in the 1990s. However, it evaluates the digestion of three broad categories of feedstock components: carbohydrates, proteins, and fats and lipids. Further it considers digestion via the four VFAs valeric acid, butyric acid, propionate and acetate as well as acetoclastic and hydrogenic methanogenesis, considering 24 chemical species and nineteen process steps. As the level of detail required is seldom available for either feed stock or intermediates, the detailed use of this model in early stage design is not feasible. ADM1-3P was developed from this model through introducing simplifications to consider a single ‘substrate’ in terms of its elemental composition, hydrolysis to glucose and only the VFAs propionate and acetate. This was achieved by tailoring the calibration of the model to primary sewage sludge and weakly activated sludge, thereby reducing the species and sub-processes considered. However, ADM1-3P is not calibrated for the range of feedstocks addressed for AD in this project. To overcome this, a simplified stoichiometric model was built in Aspen and validated across a range of feedstocks, demonstrating its ability to predict performance within 10%. This model was used to investigate process parameters and technoeconomic feasibility of the flow sheets interrogated for valorisation of wastewaters. It can be extended to detailed environmental analysis using life cycle analysis.

To demonstrate the potential for value addition from a complex wastewater, AD of the vinasse stream produced as waste product of ethanol fermentation of molasses is considered. The base case flow sheet of AD of vinasse with CHP of the methane to electricity and steam and disposal of the AD digestate to irrigation showed that the environmentally beneficial process does not yet deliver a net positive return. The technoeconomic feasibility was improved by recovery of potassium as a fertiliser product with accompanying improvement of the methane yield and productivity; however, a positive NPV was still not found. Replacement of the CHP process by biogas upgrading reported the best economic case. In all cases, it is necessary to compare the outcome to the cost of “doing nothing” i.e. the cost of waste treatment; this increases the economic prospects of these treatment approaches for maximising resource efficiency and valorisation. In each flowsheet addressed, environmental benefit was established through the effective treatment of the recalcitrant wastewater, removing burden, as well as the improved resource efficiency through the creation of an energy product. In addition, the development of a fit-for-purpose water stream was created for return to the upstream process, thereby reducing fresh water requirements.

In conclusion, the study has delivered an integrated assessment of the simultaneous remediation of wastewater, recovery of energy and delivery of fit-for-purpose water, while enhancing resource productivity through additional potential products. Monitoring and intervention to ensure pathogen-free fit-for-purpose water is considered. The simplified AD model, embedded in the flow sheet framework, sets the scene for evaluation of varied wastewater streams for their valorisation within the context of environmental burden reduction.

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Appendix A WRC reports on AD

Table: A-1 The 24 entries found on the WRC Knowledge Hub to 2016 when "AD" was selected to be searched as a whole word

No	Report no.	Year	Authors	Title	Document type
1	KV 350/16	2016	Chaúque EFC; Zvimba JN; Ngila JC; Musee N; Mboyi A; Momba MNB	Fate and behaviour of engineered nanoparticles in simulated wastewater and their effect on microorganisms	Research Report
2	TT 661/16	2016	Sikosana M; Randall DG; Petrie DJ; Oelofse M; Russo V; von Blottnitz H	Nutrient and energy recovery from sewage: towards an integrated approach	Research Report
3	2131/1/15	2015	Tesfamariam EH; Annandale JG; de Jager PC; Ogbazghi Z; Malobane ME; Mbetse CKA	Quantifying the fertiliser value of wastewater sludges for Agriculture	Research Report
4	1822/1/14	2015	Ikumi DS; Harding TH; Vogts M; Lakay MT; Mafungwa H; Brouckaert CJ; Ekama GA	Mass balances modelling over wastewater treatment plants III	Research Report
5	2110/1/14	2014	Harrison STL; Van Hille RP; Mokone T; Motleleng L; Smart M; Legrand C; Marais T	Addressing the Challenges Facing BSR as a Strategy for AMD Treatment: Reactor stage - raw materials, products and process kinetics	Research Report
6	1620/1/11	2011	Ekama GA; Mebrahtu MK; Brink IC; Wentzel MC	Mass balances and modelling over wastewater treatment plants	Research Report
7	1538/1/09	2009	Buckley CA; Brouckaert CJ	A feasibility study in eThekwini municipality on AD for the treatment of toxic and High Strength organic wastes: A study of the business case of treating high strength Industrial Wastes	Research Report
8		2009	Machnicka A; Grübel K; Suschka J	The use of hydrodynamic disintegration as a means to improve AD of activated sludge	Water SA Manuscript
9	K5/455	2007	Strydom JP; Mostert JF; Britz TJ	AD of dairy factory effluents	Research Report
10		2007	Wang J; Zhang Z-j; Zhang Z-f; Zheng P; Li C-J	The influence and mechanism of influent pH on anaerobic co-digestion of sewage sludge and printing and dyeing wastewater	Water SA Manuscript
11	1074/1/06	2006	Remigi EU; Buckley CA	Co-digestion of high strength/toxic organic effluents in anaerobic digestors at wastewater treatment works	Research Report
12		2005	Sötemann SW; Ristow NE; Wentzel MC; Ekama GA	A steady state model for AD of sewage sludges	Water SA Manuscript
13		2005	Sötemann SW; Musvoto EV; Wentzel MC; Ekama GA	Integrated biological, chemical and physical processes kinetic modelling Part 2 – AD of sewage sludges	Water SA Manuscript
14	762/1/04	2004	Sacks J; Buckley CA	AD of high strength or toxic organic effluents in available digester capacity	Research Report
15		2000	Lahav O; Loewenthal RE	Rapid communication: Measurement of VFA in AD: The five point titration method revisited	Water SA Manuscript
16		1997	Strydom JP; Britz TJ; Mostert JF	Two-phase AD of three different diary effluents using a hybrid bioreactor	Water SA Manuscript

No	Report no.	Year	Authors	Title	Document type
17		1996	Carliell CM; Barclay SJ; Buckley CA	Treatment of exhausted reactive dyebath effluent using AD: Laboratory and full-scale trials	Water SA Manuscript
18	365/1/95	1995	Nell JH; Kafaar A	The evaluation and improvement of the anaerobic digestion ultrafilteration (ADUF) effluent treatment process	Research Report
19	459/1/93	1993	Strohwald NKH	Laboratory scale treatment of acetic acid effluent by the anaerobic digestion ultrafiltration (ADUF) process	Research Report
20	460/1/93	1993	Strohwald NKH	An investigation into the application of the anaerobic digestion ultrafiltration (ADUF) process to fruit processing effluent	Research Report
21	TT 55/92	1992	Ross WR; Novella PH; Pitt AJ	AD of wastewater sludge: Operating guide	Research Report
22		1991	Lin CY	AD of landfill leachate	Water SA Manuscript
23		1990	Ross WR; Barnard JP; le Roux J; de Villiers HA	Application of ultrafiltration membranes for solids-liquid separation in anaerobic digestion systems: The ADUF process	Water SA Manuscript
24		1987	Ross WR; Louw LM	Monitoring and control of AD	Water SA Manuscript

Table: A-2 All WRC Research Documents to 2016 (Research Reports, Technical Briefs, Water SA Articles) on AD categorised by main subtopic (section 2.1.2)

No	Cat	Year	Title	Authors	Report number
1	A1	2009	Guideline for the inspection of wastewater treatment works	Boyd LA; Mbelu AM	Research Report No.TT 375/08
2	A1	2009	Guidelines for the utilisation and disposal of wastewater sludge Volume 3 of 5: Requirements for the on-site and off-site disposal of sludge	Herselman JE; Snyman HG	Research Report No.TT 349/09
3	A1	2009	Guidelines for the utilisation and disposal of wastewater sludge Volume 4 of 5: Requirements for the beneficial use of sludge at high loading rates	Herselman JE; Moodley P	Research Report No.TT 350/09
4	A1	2009	Guidelines for the utilisation and disposal of wastewater sludge Volume 5 of 5: Requirements for thermal sludge management practices and for commercial products containing sludge	Herselman JE; Burger LW; Moodley P	Research Report No.TT 351/09
5	A1	2009	Improved inspection of wastewater treatment works		Brief - Technical Brief
6	A1	2008	Guidelines for the utilisation and disposal of wastewater sludge Volumes 1-5: Impact assessment		Research Report No.TT370/08
7	A1	2006	Guidelines for the utilisation and disposal of wastewater sludge. Volume 1 of 5: Selection of management options	Snyman HG; Herselman JE	Research Report No.TT 261/06
8	A1	2005	Preliminary design guidelines for the development of a granulating bioreactor	Els ER; Lorenzen L; Van Zyl PJ; Britz TJ	Research Report No.1239/1/05
9	A1	1999	Guidelines for the design and operation of sewage sludge drying beds	Ceronio AD; Van Vuuren LRJ; Warner APC	Research Report No.TT107/99

No	Cat	Year	Title	Authors	Report number
10	A1	1997	Operation manual for biological nutrient removal wastewater treatment works	Lilley ID; Pybus PJ; Power SPB	Research Report No.TT 83/97
11	A1	1996	A manual on mine water treatment and management practices in South Africa (Vol 1): Literature reviews	Pulles W; Howie D; Otto D; Easton J	Research Report No.527/1/96
12	A1	1996	A manual on mine water treatment and management practices in South Africa (Vol 2): Coal mine site visit reports	Pulles W; Howie D; Otto D; Easton J	Research Report No.527/2/96
13	A1	1993	A South African design guide for dissolved air flotation conditioning of low and medium salinity water	Haarhoff J; Van Vuuren L	Research Report No.TT 60/93
14	A1	1992	AD of wastewater sludge: Operating guide	Ross WR; Novella PH; Pitt AJ	Research Report No. TT 55/92
15	A1	1992	The education and training needs of watercare operators and operations managers in the RSA	Ernst EA; Greeff A	Research Report No.KV 35/92
16	A1	1991	National industrial water and wastewater survey		Research Report No.145/1/91
17	A2	2005	An evaluation of dedicated land disposal practices for sewage sludge	Herselman JE; Wade PW; Steyn CE; Snyman HG	Research Report No.1209/1/05
18	A3	2015	Improving industrial wastewater treatment for production of biogas		Brief - Technical Brief
19	A3	2013	REVIEW: Long-term sustainability in the management of acid mine drainage wastewaters – development of the Rhodes BioSURE Process	Rose P	Water SA Manuscript
20	A3	2009	A feasibility study in eThekwini municipality on AD for the treatment of toxic and High Strength organic wastes: A study of the business case of treating high strength Industrial Wastes	Buckley CA; Brouckaert CJ	Research report no. 1538/1/09
21	A4	2009	Market analysis for UASB seeding granules: local and international markets	Musee N; Lorenzen L	Research Report No.KV 224/09
22	B1	2006	The assessment and classification of inorganic manganese containing wastes	Banister S; Zhao B; Coetser SE; Pulles W	Research Report No.1344/1/06
23	B1	2004	The Rhodes BioSure process Part 1: Biodesalination of mine drainage wastewaters	Rose PD; Corbett CJ; Whittington-Jones K; Hart OO	Research Report No.TT195/04
24	B1	2004	The regional treatment of textile and industrial effluents	Barclay S; Buckley CA	Research Report No.456/1/04
25	B1	2003	Survey of pesticide wastes in South Africa and review of treatment options	Naidoo V; Buckley CA	Research Report No.1128/1/03
26	B1	1996	Treatment of exhausted reactive dyebath effluent using AD: Laboratory and full-scale trials	Carliell CM; Barclay SJ; Buckley CA	Water SA Manuscript
27	B1	1989	Water and wastewater management in the tanning and leather finishing industry : NATSURV 10		Research Report No.TT 44/90
28	B1	1987	Investigations into water management and effluent treatment in the pharmaceutical industry		Research Report No.106/3/87
29	B1	1987	Investigations into water management and effluent treatment in the processing of pulp and paper		Research Report No.104/1/86
30	B1	1987	Investigations into water management and effluent treatment in the South African metal finishing industry		Research Report No.106/1/87
31	B2	2015	Sustainable beneficiation of brewery effluent		Brief - Technical Brief

No	Cat	Year	Title	Authors	Report number
32	B2	2007	AD of dairy factory effluents	Strydom JP; Mostert JF; Britz TJ	Research Report No. /455
33	B2	2007	UASB treatment of a highly alkaline fruit-cannery lye-peeling wastewater	Sigge GO; Britz TJ	Water SA Manuscript
34	B2	2006	Co-digestion of high strength/toxic organic effluents in anaerobic digestors at wastewater treatment works	Remigi EU; Buckley CA	Research Report No.1074/1/06
35	B2	2006	Treatment of apple and wine processing wastewaters using combined UASB technology and ozonation scenarios	Sigge GO; Britz TJ; McLachlan T	Research Report No.1364/1/06
36	B2	2006	A customised bioreactor for beneficiation and bioremediation of effluents containing high value organic chemicals	Burton SG; Cowan DA; Garcin C; Werner C	Research Report No.1361/1/06
37	B2	2005	Treatment of dairy wastewater in UASB reactors inoculated with flocculent biomass	Nadais H; Capela I; Arroja L; Duarte A	Water SA Manuscript
38	B2	2005	An assessment of the quality of liquid effluents from opaque beer-brewing plants in Bulawayo, Zimbabwe	Ikhu-Omoregbe DIO; Kuipa PK; Hove M	Water SA Manuscript
39	B2	2004	AD of high strength or toxic organic effluents in available digester capacity	Sacks J; Buckley CA	Research Report No.762/1/04
40	B2	2003	Treatment of wastewaters with high nutrient (N and P) but low organic (COD) contents	Musvoto EV; Ubisi MF; Sneyders MJ; Lakay MT; Wentzel MC; Loewenthal RE; Ekama GA	Research Report No.692/1/02
41	B2	2002	IAPS and the treatment of domestic and industrial wastewaters Part 2: Abattoir wastewaters	Rose S; Hart OO; Shipin OV; Muller JR	Research Report No.TT191/02
42	B2	1998	Influence of OLR and HRTHRT on the efficiency of a UASB bioreactor treating a canning factory effluent	Trnovec W; Britz TJ	Water SA Manuscript
43	B2	1993	Laboratory scale treatment of acetic acid effluent by the anaerobic digestion ultrafiltration (ADUF) process	Strohwald NKH	Research Report No.459/1/93
44	B2	1993	An investigation into the application of the anaerobic digestion ultrafiltration (ADUF) process to fruit processing effluent	Strohwald NKH	Research Report No.460/1/93
45	B2	1991	AD of landfill leachate	Lin CY	Water SA Manuscript
46	B2	1990	Water and wastewater management in the paper and pulp industry: NATSURV 12		Research Report No.TT 49/90
47	B2	1990	Water and wastewater management in the sugar industry: NATSURV 11		Research Report No.TT 47/90
48	B2	1989	Water and wastewater management in the dairy industry : NATSURV 4		Research Report No.TT 38/89
49	B2	1989	Water and wastewater management in the edible oil industry : NATSURV 6		Research Report No.TT 40/89
50	B2	1989	Two-year study on the enhancement of biological phosphate removal by altering process feed composition: Metabolic control mechanisms	Lotter LH	Research Report No.137/3/89
51	B2	1987	A guide to water and wastewater management in the fruit and vegetable processing industry		Research Report No.TT 30/87
52	C1	2007	Methodology and survey of organic pollutants in South African sewage sludges: Volume 1	Jaganyi D	Research Report No.1339/1/07

No	Cat	Year	Title	Authors	Report number
53	C1	2007	Guidelines for the utilisation and disposal of wastewater sludge. Volume 2 of 5: Requirements for the agricultural use of wastewater sludge	Snyman HG; Herselman JE	Research Report No.TT 262/06
54	C1	2004	Development of biological treatment technology for the remediation of edible oil effluent	Suruijal S; Tivchev G; Kasan HC; Bux F	Research Report No.1084/1/04
55	C1	2003	The use of algal and yeast biomass to accumulate toxic and valuable heavy metals from wastewater	Duncan JR; Stoll A; Wilhelmi B; Xhao M; Van Hille R	Research Report No.616/1/03
56	C1	2003	Development of integrated biosorption systems for the removal and/or recovery of heavy metals from mining and other industrial wastewaters and determination of the toxicity of metals to bioremediation processes	Van Hille RP; Antunes APM; Sanyahumbi D; Nightingale L; Duncan JR	Research Report No.1243/1/03
57	C1	2002	Addendum to permissible utilisation and disposal of sewage sludge		Research Report No.TT 154/01
58	C1	1995	Bioremediation technology for the treatment of contaminated soil in South Africa	Pearce J; Snyman HG; Van Heerden H; Greben H; Oellermann RA	Research Report No.543/1/95
59	C1	1994	The use of yeast biomass and yeast products to accumulate toxic and valuable heavy metals from wastewater	Duncan JR; Brady D; Stoll AD	Research Report No.464/1/94
60	C1	1994	Effect of pollutants on the physiology of fish in the Olifants River (Eastern Transvaal)	Van Vuren JHJ; Du Preez HH; Deacon AR	Research Report No.350/1/94
61	C1	1992	The use of yeast biomass and yeast products to accumulate toxic and valuable heavy metals from wastewater	Duncan JR; Brady D	Research Report No.392/1/93
62	C1	1989	The effective use of water by means of an algal aquaculture system	Mitchell SA	Research Report No.182/1/89
63	C2	2016	Nutrient and energy recovery from sewage: An integrated approach	Sikosana M; Randall DG; Petrie DJ; Oelofse M; Russo V; von Blottnitz H	Brief - Technical Brief
64	C2	2010	Energy from wastewater– a feasibility study	Burton S; Harrison S; Pather-Elias S; Stafford W; Van Hille R; Von Blottnitz H; Cohen B	Research Report No.TT 399-09
65	C2	2010	Energy from wastewater-a feasibility study	Harrison S; Pather-Elias S; Burton S; Cohen B	Research Report No.TT 400/09
66	C2	2009	En+A70:E73ergy from wastewater - A feasibility study technical report	Burton S; Cohen B; Harrison S (Prof); Pather-Elias S; Stafford W; Van Hille R; Von Blottnitz H	Research Report No.1732/1/09
67	C2	1998	Biological excess phosphate removal (1984 - 1987)	Wentzel MC; Ekama GA; Dold PL; Loewenthal RE; Marais GvR	Research Report No.148/1/88
68	C3	2015	Quantifying the fertiliser value of wastewater sludges for Agriculture	Tesfamariam EH; Annandale JG; de Jager PC; Ogbazghi Z; Malobane ME; Mbetse CKA	Research report No.2131/01/15

No	Cat	Year	Title	Authors	Report number
69	C3	1985	Removal of phosphate and nitrogen compounds from biological filter effluents	Brodisch KEU	Research Report No.58/1/85
70	D1	2016	Influence of phase separator design on the performance of UASB reactors treating municipal wastewater	Dos Santos SL; Chaves S.R.M.; Van Haandel AC	Water SA Manuscript
71	D1	2011	Comparison between a two-stage and single stage digesters when treating a synthetic wastewater contaminated with phenol	Hernandez JE; Edyvean RGJ	Water SA Manuscript
72	D1	2008	Reactor design for metal precipitation in mine-water treatment	Lewis AE; Mokone T; Nathoo J	Research Report No.1729/1/08
73	D1	2007	Comparison of accelerated anaerobic granulation obtained with a bench scale rotating bioreactor vs. a stationary container for three different substrates	Els ER; Keet K	Water SA Manuscript
74	D1	2007	Dual stage ceramic membrane bioreactors for the treatment of high strength industrial wastewaters	Edwards W; Leukes WD; Bezuidenhout CC; Linkov VM; Johannes P; van Rensburg J; Neomagus HWJ; Burgess JB	Research Report No.1371/1/07
75	D1	2007	Integrated research for use in constructed wetlands for treatment of winery wastewater	Burton S; Sheridan C; Law-Brown J; Le Roes M; Cowan D; Rohr L; Mashaphu N	Research Report No.1544/1/07
76	D1	2007	Treatment of high strength and toxic organic industrial effluents in the anaerobic baffled reactor	Bell J; Dama P; Mudunge R; Buckley CA	Research Report No.853/1/07
77	D1	2007	UASB treatment of a highly alkaline fruit-cannery lye-peeling wastewater	Sigge GO; Britz TJ	Water SA Manuscript
78	D1	2006	A customised bioreactor for beneficiation and bioremediation of effluents containing high value organic chemicals	Burton SG; Cowan DA; Garcin C; Werner C	Research Report No.1361/1/06
79	D1	2006	The evaluation of the anaerobic baffled reactor for sanitation in dense peri-urban settlements (ABR)	Foxon KM; Buckley CA; Brouckaert CJ; Dama P; Mtembu Z; Rodda N; Smith M; Pillay S; Arjun N; Lalbahadur T; Bux F	Research Report No.1248/1/06
80	D1	1998	Performance and biomass characterisation of an UASB reactor treating domestic wastewater at ambient temperature	Ruiz I; Soto M; Veiga MC; Ligero P; Vega A; Blázquez R	Water SA Manuscript
81	D1	1997	Two-phase AD of three different diary effluents using a hybrid bioreactor	Strydom JP; Britz TJ; Mostert JF	Water SA Manuscript
82	D1	1993	An investigation into the application of the anaerobic digestion ultrafiltration (ADUF) process to fruit processing effluent	Strohwald NKH	Research Report No.460/1/93
83	D1	1993	Laboratory scale treatment of acetic acid effluent by the anaerobic digestion ultrafiltration (ADUF) process	Strohwald NKH	Research Report No.459/1/93
84	D1	1992	Pelletisation in UASB systems	Moosbrugger RE; Sam-Soon PALNS; Wentzel MC; Ekama GA; Loewenthal RE; Marais GvR	Research Report No.249/1/96

No	Cat	Year	Title	Authors	Report number
85	D2	2015	Mass balances modelling over wastewater treatment plants III	Ikumi DS; Harding TH; Vogts M; Lakay MT; Mafungwa H; Brouckaert CJ; Ekama GA	Research report No. 1822/1/14
86	D2	2014	Addressing the Challenges Facing BSR as a Strategy for AMD Treatment: Reactor stage - raw materials, products and process kinetics	Harrison STL; Van Hille RP; Mokone T; Motleleng L; Smart M; Legrand C; Marais T	Research report no.2110/01/14
87	D2	2011	Mass balances and modelling over wastewater treatment plants	Ekama GA; Mebrahtu MK; Brink IC; Wentzel MC	Research report No.1620/1/11
88	D2	2010	BSR with primary sewage sludge in an UASB reactor – Part 5: Steady state model	Poinapen J; Ekama GA	Water SA Manuscript
89	D2	2010	BSR with primary sewage sludge in an UASB reactor – Part 5: Steady state model	Poinapen J; Ekama GA	Water SA Manuscript
90	D2	2010	Biological sulphate reduction with primary sewage sludge in an UASB reactor – Part 6: Development of a kinetic model for BSR	Poinapen J; Ekama GA	Water SA Manuscript
91	D2	2010	Biological sulphate reduction with primary sewage sludge in an UASB reactor – Part 6: Development of a kinetic model for BSR	Poinapen J; Ekama GA	Water SA Manuscript
92	D2	2007	An extended investigation of the mechanism and kinetics of bacterial sulphate reduction	Gopal H; Harrison S (Prof); Van Hille R (Dr); Icgen B (Dr); Jacobs T; Lewis A (Prof); Moosa S (Dr); Pillay V	Research Report No.1251/1/07
93	D2	2006	Development of a kinetic model for BSR with primary sewage sludge as substrate	Jansen van Vuuren A; Pretorius E; Benadé N	Water SA Manuscript
94	D2	2006	Mass balance-based plant-wide wastewater treatment plant models – Part 1: Biodegradability of wastewater organics under anaerobic conditions	Wentzel MC; Ekama GA; Sötemann SW	Water SA Manuscript
95	D2	2006	Mass balance-based plant-wide wastewater treatment plant models – Part 2: Tracking the influent inorganic suspended solids	Wentzel MC; Ekama GA; Sötemann SW	Water SA Manuscript
96	D2	2006	Mass balance-based plant-wide wastewater treatment plant models – Part 3: Biodegradability of activated sludge organics under anaerobic conditions	Wentzel MC; Ekama GA; Sötemann SW	Water SA Manuscript
97	D2	2006	Mass balance-based plant-wide wastewater treatment plant models – Part 4: Aerobic digestion of primary and waste activated sludges	Wentzel MC; Ekama GA; Sötemann SW	Water SA Manuscript
98	D2	2005	A steady state model for AD of sewage sludges	Sötemann SW; Ristow NE; Wentzel MC; Ekama GA	Water SA Manuscript
99	D2	2005	Integrated biological, chemical and physical processes kinetic modelling Part 1 – Anoxic-aerobic C and N removal in the activated sludge system	Sötemann SW; Musvoto EV; Wentzel MC; Ekama GA	Water SA Manuscript
100	D2	2005	Integrated biological, chemical and physical processes kinetic modelling Part 2 – AD of sewage sludges	Sötemann SW; Musvoto EV; Wentzel MC; Ekama GA	Water SA Manuscript

No	Cat	Year	Title	Authors	Report number
101	D2	2004	The mechanisms and kinetics of biological treatment of metal-containing effluent	Hansford GS; Harrison STL; Lewis AE; Moosa S (Dr); Knobel A; Ristow NE	Research Report No.1080/1/04
102	D2	2002	IAPS and the treatment of domestic and industrial wastewaters Part 1: The AIWPS Model	Rose PD; Hart OO; Shipin OV; Ellis PJ	Research Report No.TT190/02
103	D2	1998	Biological sludge stabilisation Part 1: Kinetics of aerobic sludge digestion	Van Haandel AC; Catunda PFC; de Souza Araújo L	Water SA Manuscript
104	D3	2009	The Rhodes BioSURE process: Part 3 - Sulphur removal unit operations	Molwantwa JB; Bowker M; Gilfillan J; Rein N; Dorrington RA; Hart OO; Rose PD	Research Report No.TT411/08
105	D3	2008	Membrane-related research impact assessment		Research Report No.TT366/08
106	D3	2007	Application of cyclodextrin polymers in the removal of organic pollutants from water	Sithole SP; Nxumalo EN; Mhlanga SD; Salipira KL; Mamba BB; Krause RM; Malefetse TJ	Research Report No.KV194/07
107	D3	2003	Cleaning and pre-treatment techniques for ultrafiltration membranes fouled by pulp and paper effluent	Swart P; Domingo GS; Jacobs EP; Burch G	Research Report No.1035/1/01
108	D3	2002	Salinity, sanitation and sustainability Vol 4: The Rhodes BioSURE® Process (Part 2)	Whittington-Jones KJ; Corbett CJ; Rose S	Research Report No.TT 196/02
109	D3	1998	Development of a cross-flow microfilter for rural water supply	Pillay VL	Research Report No.386/1/98
110	D3	1995	Development of electro-osmotic sludge dewatering technology	Smollen M; Kafaar A	Research Report No.427/1/95
111	D3	1995	Industrial applications of membranes	Malherbe GF; Morkel CE; Bezuidenhout D; Jacobs EP; Hurndall MJ; Sanderson RD	Research Report No.362/1/95
112	D3	1995	The deveolpment of characteristics and cleaning techniques to classify foulants and remove them from ultra- and microfiltration membranes by biochemical means	Swart P; Maartens A; Swart AC; Jacobs EP	Research Report No.531/1/96
113	D3	1995	The evaluation and improvement of the anaerobic digestion ultrafilteration (ADUF) effluent treatment process	Nell JH; Kafaar A	Research Report No.365/1/95
114	D3	1995	The evaluation and improvement of the anaerobic digestion ultrafilteration (ADUF) effluent treatment process	Nell JH; Kafaar A	Research Report No.365/1/95
115	D3	1990	Development of wastewater pre-treatment technologies: Crossflow microfiltration & the development of support systems for crossflow microfiltration and technical performance evaluation thereof on industrial water and wastewater		Research Report No.146/1/92
116	D3	1990	Application of ultrafiltration membranes for solids-liquid separation in anaerobic digestion systems: The ADUF process	Ross WR; Barnard JP; le Roux J; de Villiers HA	Water SA Manuscript
117	D3	1989	An exploratory investigation of crossflow microftration for solid/liquid separation in biological wastewater treatment	Bailey AD; Dold PL	Research Report No.242/1/90

No	Cat	Year	Title	Authors	Report number
118	D4	2016	Fate and behaviour of engineered nanoparticles in simulated wastewater and their effect on microorganisms	Chaúque EFC; Zvimba JN; Ngila JC; Musee N; Mboyi A; Momba MNB	Research Report No.KV 350/16
119	E1	2013	Evaluating 5 and 8 pH-point titrations for measuring VFA in full-scale PS hydrolysate	Hey T; Sandström D; Ibrahim V; Jönsson K	Water SA Manuscript
120	E1	2009	Practical application and statistical analysis of titrimetric monitoring of water and sludge samples	Van Hulle SWH; de Meyer S; Vermeiren TJL; Vergote A; Hogie J; Dejans P	Water SA Manuscript
121	E1	2005	Sulphate measurement in organic rich solutions: Carbonate fusion pre-treatment to remove colour interference	Ristow NE; Sötemann SW; Wentzel MC; Loewenthal RE; Ekama GA	Water SA Manuscript
122	E1	2004	A metal content survey of South African sewage sludge and an evaluation of analytical methods for their determination in sludge	Snyman HG; Herselman JE; Kasselman G	Research Report No.1283/1/04
123	E1	2000	Rapid communication: Measurement of VFA in AD: The five point titration method revisited	Lahav O; Loewenthal RE	Water SA Manuscript
124	E1	1998	Biological sludge stabilisation Part 2: Influence of the composition of WAS on anaerobic stabilisation	de Souza Araújo L; Catunda PFC; Van Haandel AC	Water SA Manuscript
125	E1	1995	Fingerprinting of activated sludge systems using PAGE analysis of total protein extractions for the optimisation of biological phosphorus removal	Casey T; Erasmus A; Cloete TE	Research Report No.776/1/98
126	E1	1992	Simple titration procedures to determine H2CO3* alkalinity and short chain fatty acids in aqueous solutions	Moosbrugger RE; Wentzel MC; Ekama GA	Research Report No.TT 57/92
127	E2	2007	The influence and mechanism of influent pH on anaerobic co-digestion of sewage sludge and printing and dyeing wastewater	Wang J; Zhang Z-j; Zhang Z-f; Zheng P; Li C-j	Water SA Manuscript
128	E2	2005	Hydrolysis of primary sewage sludge under methanogenic, acidogenic and sulphate-reducing conditions	Loewenthal RE; Ristow NE; Soteman SW; Wentzel MC; Ekama GA	Research Report No.1216/1/05
129	E2	1998	Influence of OLR and HRT on the efficiency of a UASB bioreactor treating a canning factory effluent	Trnovec W; Britz TJ	Water SA Manuscript
130	E2	1989	Two-year study on the enhancement of biological phosphate removal by altering process feed composition: Plant and laboratory studies		Research Report No.137/2/89
131	E2	1986	Enhancement of biological phosphate removal by altering process feed composition		Research Report No.137/1/86
132	E3	2011	Identification of arsenic resistance genes in microorganisms from maturing fly ash-acid mine drainage neutralised solids	Cowan D; Musingarimi W; Tuffin M I	Research Report No.1655/1/10
133	E3	2008	Hydrolytic enzymes in sewage sludge treatment: A mini-review	Burgess JE; Pletschke BI	Water SA Manuscript
134	E3	2007	Characterisation of planktonik microbial populations in paper-mill water systems	Wolfaardt F; Grant RM; Kock MM	Research Report No.1459/1/07
135	E3	2007	The microbiology of fly ash - AMD neutralisation systems	Cowan DA; Shitandi A; Van IJperen C; Kuhn E	Research Report No.1549/1/07
136	E3	2006	Online real-time enzyme diagnostic system for the detection and monitoring of faecal contamination of water intended for drinking purposes	Pletscke B; Togo C; Wutor V	Research Report No.1446/1/06

No	Cat	Year	Title	Authors	Report number
137	E3	2006	Pathogen destruction in urine diversion sanitation systems: Vol 1	Austin LM; Phasha MC; Cloete TE	Research Report No.1439/1/06
138	E3	2005	Measurement of heterotrophic active biomass in activated sludge systems	Lee BJ; Lakay MT; Wentzel MC	Research Report No.KV 163/05
139	E3	2005	Activity of heterotrophic and autotrophic biomass in BNR activated sludge	Cronje GL; Beeharry AO; Lakay MT; Wentzel MC; Ekama GA	Research Report No.1179/1/05
140	E3	2005	An evaluation of different commercial microbial or microbially-derived products for the treatment of organic waste in pit latrines	Taljaard L; Venter IS; Gorton D	Research Report No.1377/1/05
141	E3	2004	Investigation into the enzymology of accelerated primary sewage sludge solubilisation and digestion in sulphate reducing systems	Whiteley CG; Pletschke BI; Burgess JE; Tshivhunge AS; Ngesi N; Whittington-Jones K; Enongene G; van Jaarsveld F; Heron P; Rashamuse; Rose S	Research Report No.1170/1/04
142	E3	2004	Laboratory and field scale evaluation of agricultural use of sewage sludge	Snyman HG; van der Waals JH	Research Report No.1210/1/04
143	E3	1999	Marine pollution pathogenic microorganisms in shellfish	Grabew WOK; van der Veen A; de Villiers JC	Research Report No.411/1/99
144	E3	1996	Research on human viruses in diffuse effluents and related water environments	Grabow WOK; Taylor MB; Wolfaardt M	Research Report No.496/1/96
145	E4	2013	Market dynamics as a driver towards the evolution of research needs: the case of up flow anaerobic sludge blanket seeding granules	Musee N; Lorenzen L	Water SA Manuscript
146	E4	2010	Monitoring, evaluation and verification of long-term performance of the passive water treatment plant at Vryheid coronation colliery	Molwantwa JB; Coetser SE; Heath R; Pulles W; Bowker M	Research Report No.1623-1-10
147	E4	2009	Process design manual for small wastewater works	Freese SD; Nozaic DJ	Research Report No.TT 389-09
148	E4	1999	Septic tank systems in the South African coastal zone	Wright A	Research Report No.597/1/99
149	E4	1998	The solubilisation of N and P during PS acid fermentation and precipitation of the resultant P	Banister SS; Pitman AR; Pretorius WA	Water SA Manuscript
150	E4	1998	Operation and maintenance of solids-free sewer (SFS) systems in South Africa: Guidelines for engineers	du Pisani JE	Research Report No.TT 97/98
151	E4	1998	The operation and maintenance of settled sewerage (SS) systems in South Africa	du Pisani JE	Research Report No.708/1/98
152	E4	1997	Consolidation of activated sludge research II (January 1991 to December 1994)	Wentzel MC; Ekama GA; Marais GvR	Research Report No.356/1/97
153	E4	1997	Aspects of sewage sludge handling and disposal	Lotter LH; Pitman AR	Research Report No.316/1/97
154	E4	1994	Occupational competencies for the occupation of watercare operator and watercare manager	Ernst EA	Research Report No.KV 55/94
155	E4	1992	Evaluation and optimisation of dual digestion of sewage sludge (Executive summary)	de Villiers HA; Messenger JR; Kenmuir K; Laubscher SJA; Ekama GA	Research Report No.189/1/92
156	E4	1987	Monitoring and control of anaerobic digestion	Ross WR; Louw LM	Water SA Manuscript

No	Cat	Year	Title	Authors	Report number
157	E5	2009	The use of hydrodynamic disintegration as a means to improve AD of activated sludge	Machnicka A; Grübel K; Suschka J	Water SA Manuscript
158	E5	2007	Part 4: Process scale-up in the treatment of mine drainage wastewaters and the disposal of sewage sludge	Neba A; Whittington-Jones KJ; Rose S	Research Report No.TT 198/07
159	E5	2007	Transforming the Petro Process to Biological Nutrient Removal	Shipin OV; Meiring PGJ	Research Report No.971/1/07
160	E5	2007	Microwave enhanced digestion of aerobic SBR sludge	Kennedy KJ; Thibault G; Droste RL	Water SA Manuscript
161	E5	1998	Optimisation of PS acidogenic fermentation for biological nutrient removal	Banister SS; Pretorius WA	Water SA Manuscript
162	E5	1998	The transfer of wastewater management technology to the meat processing industry	Cowan JAC	Research Report No.239/1/98
163	E5	1992	Evaluation and optimisation of dual digestion of sewage sludge Part 3: Evaluation of the technology for practical implementation	de Villiers HA; Messenger JR; Kenmuir K; Laubscher SJA; Ekama GA	Research Report No.189/4/92

Appendix B Previous studies where the effects of changes in the OLR were documented

Waste Type	Process Type	HRT	SRT	OLR		VS Reduction	CH4		VFA Concentration	VFA		Alkalinity	pH	Reference
		days	days	Value	Unit	%	Yield $Nm^3/kgVS_{ad\ ded}$	Productivity $Nm^3/m^3.day$	As COD kg/m3	Yield $kg/kgVS_{ad\ ded}$	Productivity $kg/m^3.day$	$gCaCO_3/l$	-	
Poultry solid (diluted with de-ionised water)	Mesophilic CSTR	100	100	0.8	kgVS/m^3.d	76	0.52	0.42	6.64	0.08	0.066	-	-	Salminen and Rintala (2002)
		50	50	0.8		74	0.55	0.44	3.04	0.08	0.061	-	-	
		25	25	2.1		63	0.31	0.65	17.60	0.34	0.704	-	-	
		13	13	2.1		31	0.09	0.19	11.30	0.41	0.869	-	-	
Sugar-beet sap wastewater	Mesophilic UASB for VFA Production	0.79	-	7	kgCOD/m^3.d	-	-	-	4.6	0.83	5.823	-	-	Lettinga et al. (1980)
		0.5	-	10.2		-	-	-	3.96	0.78	7.920	-	-	
		0.3	-	18.6		-	-	-	4.72	0.85	15.733	-	-	
		0.23	-	31.7		-	-	-	4.2	0.58	18.261	-	-	
		0.21	-	24.3		-	-	-	3.8	0.74	18.095	-	-	
		0.2	-	34.6		-	-	-	4.3	0.62	21.500	-	-	
		0.18	-	46.8		-	-	-	6.19	0.73	34.389	-	-	
		0.13	-	67.3		-	-	-	6.5	0.74	50.000	-	-	
		0.12	-	83.5		-	-	-	5.72	0.57	47.667	-	-	
	UASB (30 C)	24-48 hrs	-	4-5	kgCOD/m^3.d	95	-	-	-	-	-	-	-	
Excess secondary sludge	Batch tests	8	-	10.8	kg/m^3	-	0.015	-	-	0.075	-	-	-	Yuan et al. (2006)
Dewatered sewage sludge	Mesophilic CSTR (37 C)	35	35	1.7	kgVS/m^3.d	55	0.30	0.51	0.045	0.0008	0.001	-	-	Nges and Liu (2010)
		30	30	1.6		59	0.39	0.62	0.12	0.0025	0.004	-	-	
		25	25	2.5		53	0.36	0.90	0.1	0.0016	0.004	-	-	
		20	20	3		49	0.33	0.99	0.15	0.0025	0.008	-	-	
		15	15	4		50	0.32	1.29	0.186	0.0031	0.012	-	-	
		12	12	5.7		45	0.31	1.78	0.478	0.0070	0.040	-	-	
		9	9	6.8		35	0.27	1.85	0.48	0.0078	0.053	-	-	
		5	5	13.6		27	0.21	2.87	1.287	0.0189	0.257	-	-	
		3	3	20.6		22	0.07	1.44	2.933	0.0475	0.978	-	-	
	Thermophilic CSTR (50 C)	35	35	1.7	kgVS/m^3.d	56	0.32	0.54	0.105	0.0018	0.003	-	-	
		30	30	1.6		54	0.39	0.62	0.13	0.0027	0.004	-	-	
		25	25	2.5		52	0.35	0.87	0.125	0.0020	0.005	-	-	
		20	20	3		48	0.32	0.97	0.15	0.0025	0.008	-	-	
		15	15	4		52	0.33	1.33	0.196	0.0033	0.013	-	-	
		12	12	5.7		47	0.32	1.82	0.516	0.0075	0.043	-	-	
		9	9	6.8		36	0.28	1.88	0.786	0.0128	0.087	-	-	
		5	5	13.6		33	0.21	2.80	2.796	0.0411	0.559	-	-	
		3	3	20.6		30	0.11	2.25	4.784	0.0774	1.595	-	-	
		5.11	420	1.1		92	0.220	0.2424	0.35	0.062	0.068			

Waste Type	Process Type	HRT	SRT	OLR		VS Reduction	CH4		VFA Concentration	VFA		Alkalinity	pH	Reference
		days	days	Value	Unit	%	Yield $Nm^3/kgVS_{added}$	Productivity $Nm^3/m^3.day$	As COD kg/m3	Yield $kg/kgVS_{added}$	Productivity $kg/m^3.day$	$gCaCO_3/l$	-	
Potato-chips processing wastewater	Thermophilic contact reactor	3.71	254	1.5	$kg_{COD}/m^3.d$	91	0.273	0.4091	0.42	0.075	0.113			Şentürk et al. (2010)
		2.13	144	2.5		89	0.279	0.6970	0.55	0.103	0.258			
		1.64	81	3.35		86	0.262	0.8788	0.7	0.127	0.427			
		1.15	57	4.5		79	0.273	1.2273	1	0.193	0.870			
		1.06	52	5		76	0.273	1.3636	0.79	0.149	0.745			
Beet molasses alcoholic fermentation wastewater (untreated)	Mesophilic mixed reactor with support for biomass immobilisation (35 C)	53.5	-	1.5	$kg_{COD}/m^3.d$	93.7	0.197	0.296	0.3	0.004	0.006	7.5	8	Jiménez et al. (2003)
		32	-	2.5		91.6	0.198	0.495	0.65	0.008	0.020	15.4	8.4	
		22.7	-	3.5		88.7	0.2	0.7	0.99	0.012	0.044	10.2	7.8	
		17.8	-	4.5		87.7	0.186	0.835	0.6	0.007	0.034	14.8	8.3	
		14.7	-	5.5		85	0.168	0.925	4.7	0.058	0.320	17.9	8.2	
		12.3	-	6.5		73.9	0.167	0.761	5.01	0.063	0.407	18.7	8.1	
		10.6	-	7.5		68.6	0.101	0.755	9.3	0.117	0.877	18.9	8.3	
Palm oil mill effluent	Upflow fixed film	1.5	4.68	23.15	$kg_{COD}/m^3.day$	89.5	0.28	6.5	0.158	0.005	0.105	-	7.9	Najafpour et al. (2006)
Domestic wastewater	UASB (20 C)	14-17 hrs	-	~1	kgCOD/m³.day	85-65	NA	NA	NA	NA	NA	NA	NA	Seghezzo et al. (1998)
	UASB (13-17 C)	14-17 hrs	-	~1		70-55	NA	NA	NA	NA	NA	NA	NA	
Pharma-ceutical wastewater	4 x 2.75 l UASBs in series	4	-	0.43	kgCOD/m³.day	75	0.360	0.155	0.199	0.1157	0.0498		7.83	Chelliapan et al. (2011)
		4	-	0.86		75	0.246	0.211	0.065	0.0189	0.0163		7.57	
		4	-	1.23		70	0.246	0.302	0.25	0.0508	0.0625		7.78	
		4	-	1.53		70	0.245	0.375	0.374	0.0611	0.0935		7.84	
		4	-	1.86		70	0.256	0.476	0.25	0.0336	0.0625		7.84	
		3	-	2.48		55	0.145	0.361	0.949	0.1276	0.3163		7.77	
		2.5	-	2.98		50	0.127	0.378	1.127	0.1513	0.4508		7.41	
		2	-	3.73		45	0.099	0.371	1.468	0.1968	0.7340		6.94	
		4	-	1.86		70	0.269	0.500	0.28	0.0376	0.0700		7.80	
Synthetic sucrose, milk powder and nutrient medium	3 x 8.5 ℓ UASB reactors	2.8	-	54	kgCOD/m³.day	0.78	0.185	10	0.070	0.0110	0.5960	-	7.5	Fang and Chui (1993)
		1.8	-	84		0.69	0.190	16	0.20	0.0325	2.7312	-	7.5	
		1.8	-	83		0.7	0.193	16	0.22	0.0347	2.8802	-	7.1	
		2.8	-	100		0.74	0.160	16	0.48	0.0409	4.0940	-	7.4	
		2.2	-	130		0.77	0.196	25.5	0.40	0.0337	4.3857	-	7.3	
		1.8	-	160		0.75	0.147	23.5	0.67	0.0556	8.8981	-	7.4	
		3	-	160		0.37	0.084	13.5	5.44	0.2722	43.5462	-	7.1	
		2.3	-	210		0.33	0.083	17.5	4.74	0.2358	49.5096	-	7.1	
		1.8	-	260		0.26	0.069	18	5.49	0.2813	73.1343	-	7.0	

Appendix C Reactor dimensions

Specification of reactor dimensions

To specify the reactor dimensions, it was decided that a cylindrical reactor geometry would be used, and the working volume (total reactor volume minus the GLSS volume) was set at 2 L. This volume was decided upon as being large enough for samples to be taken from the sludge bed for total suspended solids analysis and to minimise errors associated with measurement and operational equipment (e.g., gas meters, pumps), while not requiring unreasonable volumes of feed to be prepared and stored.

To make the data comparable to digestion of a high strength raw sewage, a research objective outside of this investigation, the initial feed COD was set at 1.5 g/L until an OLR of 22 g-COD/L.day (a safe estimate for the maximum OLR at which a raw sewage AD study would likely run) was reached. These two constraints allowed calculation of the minimum HRT to be 1.64 hours.

Using these specifications as starting points, the remaining reactor dimensions were calculated using the following relationships:

$$A = \frac{\dot{v}_{Feed} + \dot{v}_{Recycle}}{u_{Upflow}}$$ Equation: C-1

Substituting the numerator with Equation 3-5 after multiplying both sides by $\dot{v}_{Feed}$

$$A = \frac{\dot{v}_{Feed} \cdot Recycle\ ratio}{u_{Upflow}}$$ Equation: C-2

Further substituting Equation 2-6 for $\dot{v}_{Feed}$

$$A = \frac{V_{Reactor} \cdot Recycle\ ratio}{u_{Upflow} \cdot HRT}$$ Equation: C-3

where
A is the reactor cross-sectional area in dm^2
u_{Upflow} is the specified upflow velocity in dm/h
$V_{Reactor}$ is the reactor volume in L

As can be seen from Equation 8, to determine the cross-sectional area it is necessary to specify the recycle ratio and upflow velocity in addition to the reactor volume and HRT. The upflow velocity was specified at an arbitrary value between 0.5-1.0 m/h, a desirable range for UASB reactors (Chernicharo et al., 2015; Saravanan and Sreekrishnan, 2006). The dimensions were then set to be designed for the conditions occurring at the minimum HRT according to Equation: C-4. This is because, for a given cross-sectional area, there is a maximum feed flow rate (corresponding to the minimum HRT) above which the recycle flow rate cannot be further decreased to maintain a constant upflow velocity. It was decided that the recycle ratio should be equal to two at this minimum HRT to ensure that benefits of recycling mentioned previously still occur under these conditions.

$$A = \frac{V_{Reactor} \cdot Recycle\ ratio_{min}}{u_{Upflow} \cdot HRT_{min}}$$ Equation: C-4

where
HRT_{min} is the minimum value of the HRT (=1.64 hours)
Recycle $ratio_{min}$ is the minimum value of the recycle ratio, corresponding to the minimum HRT

However, the value calculated for the cross-sectional area did not correspond to an available standard diameter of PVC piping. The diameter was adjusted to an available standard size of PVC piping using the MS Excel Solver plugin, which was specified to vary the design upflow velocity. This scaled the upflow velocity to a value of 6.77 dm/h to obtain the correct size diameter. A summary of the most relevant reactor dimensions is included in Table C-1, and a side view illustration of the final reactor design, including dimensions, is presented as Figure: C-1.

Table C-1 Important reactor dimensions

Reactor body	Inner diameter	67.8 mm
	Height	554 mm
	Diameter of constriction	57.8 mm
GLSS	Inner diameter	106 mm
	Height	226 mm
	Gas collection hood diameter	65 mm

Finally, temperature control systems were installed on both reactors. These systems maintained the reactor temperatures at 37°C using external electrical heating coils which were operated in response to the difference in the measured and set point temperatures. The temperature in each reactor was measured using a thermocouple inserted into a purpose-built port 80% up the height of the reactor body (between the top two sludge sampling ports) on the opposite side to the sludge sampling ports.

Ancillary equipment

Substrate was prepared and autoclaved in 10 L Schott bottles with modified lids that allowed for the connection of feed tubing and an air filter.

Substrate was pumped into both reactors using a Masterflex L/S variable speed drive peristaltic pump (Cole Parmer, Item # EW-77521-47) fitted with two Masterflex pump heads (Cole Parmer, Model 7013-52). Substrate was recycled within each reactor with a pump of the same type fitted with two larger model Masterflex pump heads (Cole Parmer, Model 7014-52).

Volumetric biogas production was measured using Wet Tip Gas Meters, which work using a simple mechanism to measure the biogas produced. More information on these gas meters can be found at http://wettipgasmeter.com/.

Each reactor effluent port was connected to a 500 mL filter flask raised on a retort stand. These flasks served as a buffer between air and the reactor effluent, allowed for the retention of washed-out sludge, and maintained the head of pressure required in the reactors for the biogas produced to overcome flowing into the bottom of the gas meters. Pipes were attached to the effluent nozzle of each filter flask, allowing the overflow from the flasks to flow into an effluent drum.

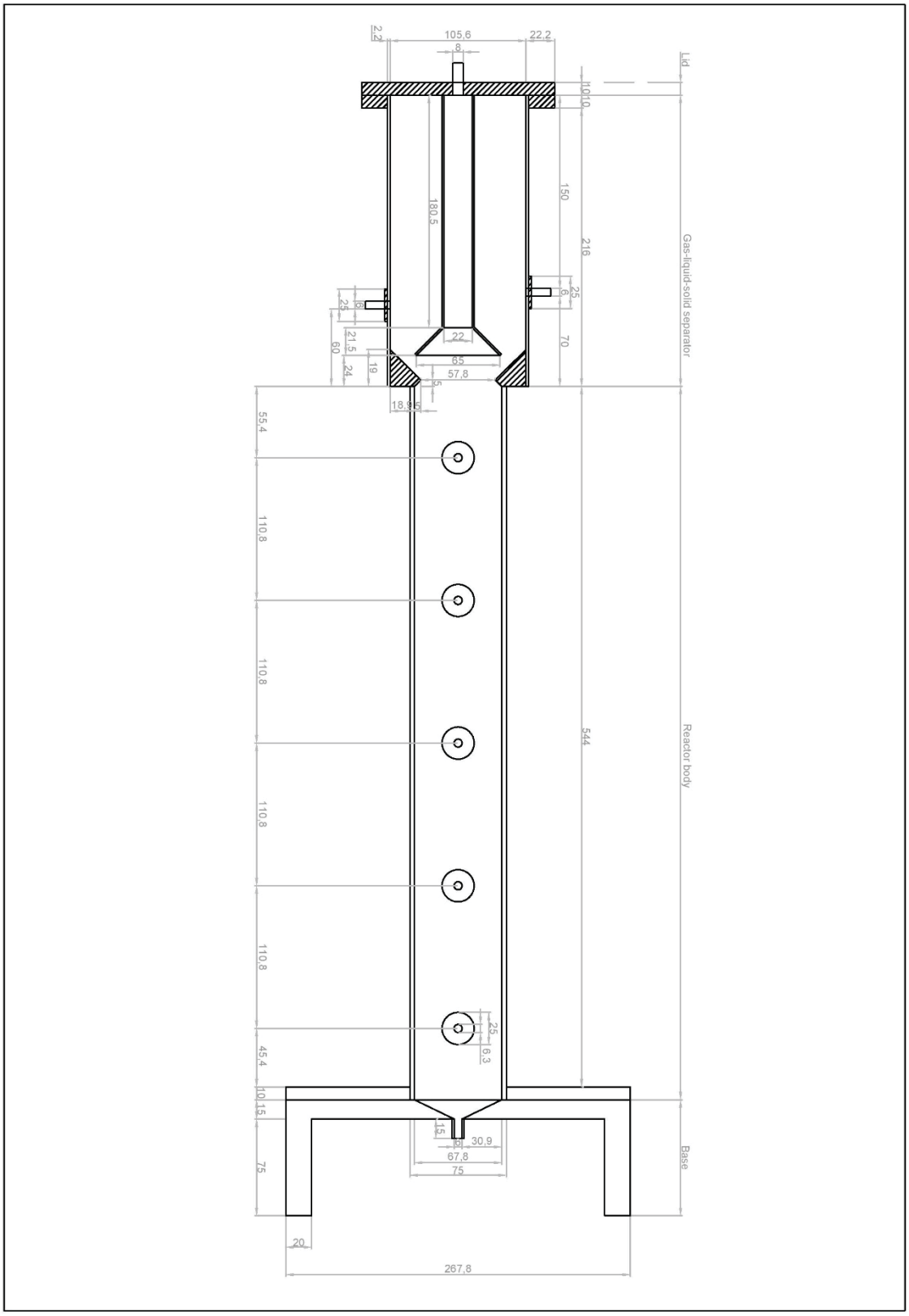

Figure: C-1 Side view of the UASB reactors including dimensions