ANAEROBIC BIOCONVERSION OF LIQUID AND SOLID WASTES FROM THE WINEMAKING PROCESS

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

South Africa is a developing country that relies on its agricultural sector as a main source of overall economic welfare. Development does not only give rise to new technology and new products but also results in increased amounts of liquid and solid waste.

Generally, the production of wine is considered an environmentally friendly process, but significant amounts of natural resources and organic amendments are necessary, while generating large amounts of liquid and solid wastes. Anaerobic digestion (AD) is an attractive and proven treatment option for both liquid and solid wastes as valuable products and depollution can be obtained. AD of liquid waste results in an effluent and biogas, while anaerobic composting of solid waste results in an organic amendment, leachate and biogas.

The overall objective of this study was to investigate the operational feasibility of the co-treatment of leachate produced during the anaerobic composting (AnC) of grape skins in an upflow anaerobic sludge blanket (UASB) reactor while treating winery wastewater. This first aim of this study was to investigate the efficiency of the anaerobic composting of grape skins. Laboratory-scaled digesters (1L) were utilised as anaerobic composting units. The most important operational parameters were identified (pH, moisture content and inoculum (size, ratio, composition)) in order to produce a pH stable, odour free compost in 21 days.

Experimental studies highlighted the importance of shredding waste as well as the addition of calcium oxide and green waste to increase the initial pH of the composting mixture. After optimising a 50% ($m.m^{-1}$) cow manure inoculum, lower inoculum concentrations (10, 15 and 25% ($m.m^{-1}$)) were investigated to make the process more economically viable. A 10% ($m.m^{-1}$) anaerobic compost (AC) inoculum was found to produce the most favourable results in terms of pH stabilisation and leachate generation. A 50% ($m.m^{-1}$) moisture level performed the best by attaining a pH > 6.5 on day 6 and having the highest end pH (7.65) on day 21, while white and red grape skins in an equal ratio were found to generate a higher end pH. With all these optimum parameters in place (shredded waste, green waste, CaO, inoculum, moisture, grape skins), a compost with a final pH (7.09), moisture (58%), nitrogen (2.25%), phosphorous (0.22%) and potassium content (1.7%) was obtained. The optimised parameters were scaled-up (1:10) by using polyvinyl chloride anaerobic digesters (20 L) to suit the operational requirements of the AnC process and also produced a stable compost within 21 days.

The second aim of this study was to investigate the combined anaerobic digestion of winery wastewater (WWW) and leachate obtained from the anaerobic composting of grape skins in an upflow anaerobic sludge blanket (UASB). This involved the operation of a 2.3 L laboratory-scale UASB reactor for 205 days. The reactor successfully co-treated WWW and leachate at *ca.* 8.5 $kgCOD.m^{-3}d^{-1}$ with a final chemical oxygen demand (COD) reduction of over 90%, a stable reactor effluent pH (7.61) and alkalinity (3 281 $CaCO_3$ $mg.L^{-1}$). This study showed the feasibility for the combined treatment of liquid and solid waste from the winemaking process. Although the

legal limits for reactor effluent disposal onto land was not met, significant reduction in COD concentrations were achieved, whilst producing a soil amendment that could potentially result in cost savings for chemical fertilisers. The benefits related to using anaerobic bioconversion as a treatment option for liquid and solid waste could possibly be advantageous to the wine industry as an environmental control technology, by converting liquid and solid waste into valuable resources.

UITTREKSEL

Suid-Afrika is 'n ontwikkelende land wat staatmaak op sy landbousektor as 'n hoofbron van algehele ekonomiese welstand. Ontwikkeling gee nie net aanleiding tot nuwe tegnologie en nuwe produkte nie, maar lei ook tot die verhoogde bydrae van vloeistof sowel as vaste afval.

Oor die algemeen, word die produksie van wyn beskou as 'n omgewingsvriendelike proses, maar aansienlike hoeveelhede natuurlike hulpbronne en organiese kunsbemesting word benodig, terwyl groot hoeveelhede vloeistof en vaste afval gegenereer word. Anaërobiese vertering (AV) is 'n aantreklike en bewese behandelingsopsie vir beide vloeistof en vaste afval aangesien waardevolle produkte en suiwering verkry kan word. AV van vloeistowwe lewer uitvloeisel sowel as biogas, terwyl anaërobiese kompostering van vaste afval 'n organiese kunsbemesting, loog en biogas lewer.

Die oorhoofse doel van hierdie studie was om die operasionele doeltreffendheid van die mede-behandeling van loog wat gegenereer word tydens die anaërobiese kompostering (AnK) van druiwe doppe in 'n opvloei-anaërobiese-slykkombers (OAS) reaktor terwyl kelderafvalwater behandel word, te ondersoek. Die eerste mikpunt van hierdie studie was om die doeltreffendheid van die anaërobiese komposteringsproses van druiwe doppe te ondersoek. Laboratorium-skaal verteerders (1L) is gebruik as anaërobiese komposteringseenhede. Die belangrikste operasionele parameters is geïdentifiseer (pH, voginhoud en inokulum (grootte, verhouding, samestelling)) om ‘n 'n pH-stabiele, reukvrye kompos te produseer in 21 dae.

Die belangrikheid van gesnipperde afval asook die byvoeging van kalsiumoksied en groen afval om die aanvanklike pH van die komposmengsel te verhoog, is deur eksperimentele studies beklemtoom. Na die optimering van 'n 50% ($m.m^{-1}$) koeimis inokulum, is laer inokulum konsentrasies (10, 15 en 25% ($m.m^{-1}$)) geondersoek om die proses meer ekonomies uitvoerbaar te maak. Daar is gevind dat ‘n 10% ($m.m^{-1}$) anaërobiese kompos (AK) inokulum die mees gunstige resultate lewer in terme van pH stabilisering en loog generering. ‘n 50% ($m.m^{-1}$) vloeistof vlak het die beste presteer deur 'n pH> 6.5 te bereik teen Dag 6 asook die hoogste eind pH (7.65) teen Dag 21, terwyl wit en rooi druiwe doppe in dieselfde verhouding gevind is om ‘n hoër eind pH te genereer. Met al hierdie optimum parameters in plek (gesnipperde afval, groen afval, kalsiumoksied, inokulum, vog, druiwe doppe) is 'n kompos met 'n finale pH (7.09), vog (58%), stikstof (2.25%), fosfor (0.22%) en kalium inhoud (1.7%) verkry. Die optimale parameters is opgeskaal (1:10) deur gebruik te maak van polivinielchloried anaërobiese verteerders (20 L) om aan die operasionele vereistes van die AnK proses te voldoen en ook om 'n stabiele kompos binne 21 dae te produseer.

Die tweede mikpunt van hierdie studie was om die gekombineerde anaërobiese vertering van kelderafvalwater en loog, verkry vanaf die anaërobiese kompos van druiwe doppe in 'n OAS reaktor, te ondersoek. Dit het die bedryf van 'n 2.3 L laboratorium-skaal OAS reaktor vir 205 dae ingesluit. Die reaktor het kelderafwater en loog suksesvol behandel by ongeveer 8.5 $kgCSV.m^{-3}d^{-1}$

met 'n finale chemiese suurstof vereiste (CSV) vermindering van meer as 90%, 'n stabiele reaktor uitvloeisel pH (7.61) en alkaliniteit (3 281 $CaCO_3 mg.L^{-1}$). Hierdie studie het die uitvoerbaarheid van die gekombineerde behandeling van vloeistof en vaste afval van die wynmaakproses getoon. Alhoewel die wetlike vereistes van die reaktor uitvloeisel vir storting op grond nie bereik is nie, is ‘n beduidende vermindering in CSV konsentrasies bereik, asook die vervaardiging van kunsbemesting wat die potensiële aankoopkoste van chemiese kunsmis kan verminder. Die voordele verbonde aan die gebruik van anaërobiese bio-omskakeling as 'n behandelingsopsie vir vloeistof en vaste afval kan moontlik voordelig wees vir die wynbedryf as 'n omgewingsbeheerende tegnologie deur om vloeistof en vaste afval om te skakel na waardevolle bronne.

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TABLE OF CONTENS

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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LIST OF ABBREVIATIONS USED

AC:	Anaerobic compost
AD:	Anaerobic digestion
AF:	Anaerobic filter
AFBR:	Anaerobic Fluidized Bed Reactor
AHR:	Anaerobic Hybrid Reactor
ANC:	Anaerobic Composting
AnSBR:	Anaerobic Sequence Batch Reactor
AOP:	Advanced Oxidation Processes
BFB:	Biofilm Fluidised Bed
BOD:	Biological Oxygen Demand
CM:	Cow manure
COD:	Chemical Oxygen Demand
CSTR:	Continuous Stirred Tank Reactor
E. coli:	*Escherichia coli*
EC:	Electrical conductivity
EGSB:	Expanded Granular Sludge Bed
ES:	Experimental study
ESP:	Exchangeable Sodium Percentage
FOG:	Fat, Oil and Grease
FW:	Food waste
GAC:	Granular Activated Carbon
GS:	Grape skins
HRT:	Hydraulic Retention Time
IC:	Internal Circulation Reactor
IR:	Incomplete reference
LW:	Liquid waste
M:	Moisture
ML:	Moisturising liquid
MPN:	Most Probable Number
OLR:	Organic Loading Rate
OPHA:	Obligate Hydrogen Producing Acetogens
ORP:	Oxidation Reduction Potential
PAC:	Powdered Activated Carbon
RBC:	Rotating Biological Contractor
SAR:	Sodium Absorption Rate
SBR:	Sequencing Batch Reactor

SW:	Solid waste
TAN:	Total Ammonia Concentration
TDS:	Total Dissolved Solids
TOC:	Total Organic Content
TSS:	Total Suspended Solids
UASB:	Upflow Anaerobic Sludge Blanket
UV:	Ultraviolet
VFA:	Volatile Fatty Acids
VSS:	Volatile Suspended Solids
WWW:	Winery Wastewater

CHAPTER 1

INTRODUCTION

Vinification is a significant agricultural activity in South Africa and wine is an established export product (SAWIS, 2010). Worldwide, agriculture is the largest user of water, with approximately 70% of freshwater withdrawal and up to 90% in developing countries (UNESCO, 2012). Due to the increased production of wine in South Africa, pressure on the usage of natural resources has intensified considerably (Van Schoor, 2005). Wine making requires a substantial amount of natural resources and organic-rich amendments whilst producing large quantities of liquid and solid wastes (Ruggieri *et al.,* 2009). The management and disposal of these residues are ecological problems, due to their seasonal and polluting characteristics (Bustamante *et al.,* 2008a)

Liquid waste is mainly produced by cleaning and washing operations during production, the rinsing of fermentation tanks, barrels, equipment and surfaces (Riaño *et al.,* 2011) and consists mostly of winery wastewater (WWW) which contains grape pomace, grape pips and yeast cells from the fermentation process (Devesa-Rey *et al*., 2011). These waste products can be a primary source of pollution, especially during the harvest season (Mace & Mata-Alvarez, 2002). Since most South African wineries are located in the Western Cape (Bruwer, 2003) and a number of them are found in the same water catchment area, contamination of downstream sources and water tables may occur (Marais, 2001). The generation of liquid waste is known to be approximately 1.2 times more than the volumes of wine produced (Vlyssides *et al.,* 2005).

Winery wastewater is characterised as a high strength organic waste, with low amounts of nitrogen and phosphorous (Toffelmire, 1972), a chemical oxygen demand (COD) of 0.8 - 12.8 $g.L^{-1}$ and a pH of 3 - 4 (Petruccioli *et al.,* 2000). Other compounds in winery effluent include alcohol, hexose sugars, carbon-based acids (Moosbrugger *et al.,* 1993), esters and polyphenolic compounds (Mosse *et al.,* 2011). The production of WWW is very inconsistent in terms of quality and discharge volume during the course of the year, but approximately 3.0 - 5.0 kL of wastewater is produced per tonne of grapes (Kumar *et al*., 2006). Immense pressure is placed on wine industries to comply with legal ecological requirements, whilst, upholding a competitive place in the international market. Rising costs have led the industry to seek sustainable management practices in terms of water demand and supply (Oliveira & Duarte, 2010).

Solid wastes generated during wine making include plant remains from de-stemmed grapes, bagasse from pressing, sediments from clarification and lees from the different decanting steps (Devesa-Rey *et al*., 2011), while the principal solid waste source generated during wine making is grape pomace (Diaz *et al.,* 2002). Winery solid wastes are generally characterised by an acidic pH, high polyphenol, organic and potassium content along with significant quantities of nitrogen and phosphate (Bustamante *et al*., 2008b). Difficulty arises in terms of the elimination, storage or conversion of these wastes as large amounts are produced (Arvanitoyannis *et al*., 2007)

especially during the harvest season. The improper disposal of grape pomace will cause ecological complications such as contamination of water sources and the generation of unpleasant odours (Brunetti *et al.*, 2011).

Several advantages exist in using a biological technology for the treatment of liquid and solid wastes. Anaerobic digestion (AD) of liquid waste has been reported as the most appropriate option for treating high strength organic wastewater (Rajeshwari *et al*., 2000) because depollution can be achieved (Chia *et al*., 2014) with the added benefit of low sludge production, low energy requirements and low maintenance costs (Pant & Adholeya, 2007). Anaerobic digestion also results in energy recovery (Chia *et al*., 2014) as a substantial amount (> 50%) of the chemical oxygen demand (COD) can be transformed into biogas (Pant & Adholeya, 2007) which can be utilised to substitute fossil fuels.

A drawback of the anaerobic digestion of organic waste is that the substrate to be treated often lacks certain nutrients essential to AD (Khalid *et al*., 2011). Winery wastewater is low in nitrogen and phosphorous (Moletta, 2005) which could require nutrient supplementation in order for AD to perform optimally. The introduction of another waste stream via co-digestion could provide the missing nutrients and balance the substrate composition (Kangle *et al*., 2012).

Large amounts of solid waste (grape skins) are produced by wineries that have the potential to be a valuable resource (Brunetti *et al.*, 2011) with which, currently very little is done. The generation of grape pomace has grown into an essential part of winemaking as more viticulturists and wine makers in South Africa recognise the benefits of using composted grape pomace on vineyards (Dillon, 2011). Anaerobic digestion of solid waste or anaerobic composting (AnC) produces an organic amendment a liquid effluent and biogas, that could be utilised as soil conditioner/plant nutrient in agriculture, and a renewable energy source (biogas), respectively (Pant & Adholeya, 2007; Khalid *et al*., 2011). AnC results in less environmental pollution and odour emissions as all liquids and solids generated are captured within a digester. An additional benefit of AnC is the fact that no aeration is needed, and therefore no bulking agents, which allows a considerable reduction in the volume of waste (O'Keefe *et al*., 1996). The liquid effluent (leachate) produced during the anaerobic composting of grape skins is a source of water, inoculum and nutrients that could supply winery wastewater with nutrients for optimum AD.

The objective of this study was to investigate the operational feasibility of the co-treatment of leachate produced during the anaerobic composting of grape skins in an UASB reactor treating winery wastewater. This will be accomplished by firstly investigating the efficiency of the anaerobic composting of grape skins and, secondly investigating the combined anaerobic digestion of winery wastewater with a co-substrate of leachate from the AnC of grape skins.

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CHAPTER 2

LITERATURE REVIEW

A. BACKGROUND

Globally, agriculture is the main consumer of water, with nearly 70% of water withdrawn from rivers, lakes and aquifers, and up to 90% water used in growing and developing economies (UNESCO, 2012). This makes the agricultural activity susceptible to water stress and scarcity (Croplife International, 2004; Pegram & Eaglin, 2011).

According to UNDESA (2013), 700 million people in about 43 countries are already experiencing water scarcity. It was further estimated that by 2025 approximately 1.8 billion people globally will be living in areas experiencing an absolute water scarcity. Water scarcity is typically defined as an inequity between the availability and demand as well as the detrimental effect of surface and groundwater quality (FAO, 2013). Statistics on world population growth show that the populace is expected to increase from 6.9 billion people in 2010 to 9.1 billion in 2050 (UNDESA, 2013) leading to additional food and water requirements (WEF, 2009). Demands for agricultural products are expected to increase by 70 - 90% by 2050 which adds further pressure on agricultural and water sources (WEF, 2009). If current water practices are continued, an increase in water stress could result in about 55% of the population to import food products by 2030 (WEF, 2009). The main challenge that the agricultural sector is facing, is not necessarily increasing food growth (70% increase by 2050) but producing 70% more food that are available on the plate (UNDESA, 2013). As the world economy growths, water requirements will increase and continue to outperform the population growth. Unlike energy, water has no substitutes or alternatives (WEF, 2009).

South Africa is a water scarce country with an irregular rainfall (DWAF, 2000a). The mean annual rainfall is approximately 500 mm which is far lower than the global average of 800 mm. Water scarcity in South Africa has been intensified due to restricted groundwater supplies and because 60% of streams arise from only 20% of the land (DWAF, 2000a). The National Water Resource Strategy estimates the available yield of freshwater in South Africa to be 13 227 million m^3 and as water demand was approximately 12 871 million m^3 in the year 2000, it means that 98% of the freshwater supply is used (Wassung, 2010). Groundwater is regarded as one of the most vital natural resources (Foster *et al.*, 2012) yet various human activities endanger freshwater systems directly (Kates *et al.,* 1990; Meybeck, 2003; Vörösmarty, 2010). Transportation, disposal of waste, human wellbeing (Gleick, 1993), climate, energy, food, financial growth and the human security challenges that the world will face over the next two decades are all related to water security (WEF, 2009). There is sufficient freshwater on earth to supply 7 billion people, but according to UNDESA (2014), too much water is being wasted, contaminated or polluted and not managed in a sustainable way.

Wine production is an agricultural activity of major importance to South Africa (Melamane *et al.,* 2007). Wine has been firmly established as a leading export product from the agricultural sector, being only second to minerals and motor cars (SAWIS, 2010). South Africa is ranked as the 8th largest producer of wine in the world, with 9.665 million hectolitres being produced per year (Eedes, 2013). This ranks South Africa behind Chile (seventh place), with 10.643 million hectolitres and before Germany (ninth place) with 9.611 million hectolitres (Eedes, 2013). During the wine production process, a considerable amount of liquid and solid wastes are generated (Gea *et al.,* 2005). These wastes include the carbon-based wastes (grape skins, pips, vine stalks and lees), winery effluent, greenhouse gasses and inorganic waste (diatomaceous earth, perlite) (Musee *et al*., 2007). Vineyards not only need a substantial amount of water for irrigation purposes, but water also forms an essential part within wine making for cleaning and sanitation (Gabzdylova *et al*., 2009). Historically, wine production has been considered an environmentally friendly process. Winemaking however, requires a significant amount of natural resources and carbon-rich amendments while producing a large amount of liquid and solid wastes. New solutions need to be considered to develop a sustainable industry (Ruggieri *et al.,* 2009).

THE WINE INDUSTRY

Winemaking is a biotechnology that is centuries old and that has become a worldwide enterprise affecting the economic wellbeing of several countries (Walker, 1999). The global production of wine in 2012 was 252 million hectolitres (OIV, 2013). Grapes are regarded as one of the most significant fruits over the world, with approximately 60 million metric tons being produced annually (Rockenbach, 2011). It is mainly cultivated as *Vitis vinifera* for the production of wine (Llobera & Cañellas, 2006). Environmental concerns associated with wineries are water pollution, soil degradation and damage to plant life due to poor disposal practices of liquid and solid wastes (EPA, 2004).

South African wine industry

Due to the increase in wine production over the past era in South Africa, pressure on the usage of natural resources such as water, soil and vegetation has increased drastically (Van Schoor, 2005). In 2012, an estimated harvest of 1095.1 million litres was produced (SAWIS, 2013) - 78% was used for wine production, 5.7% to wine for brandy production, 12.5 % for distilling wine and 3.6% to grape juice and grape juice concentrate (Eedes, 2013). The total vineyards in South Africa cover an area of approximately 101 016 ha with 378.5 million litres of wine exported (SAWIS, 2011). This represents nearly 48.5% of the wine production (SAWIS, 2011). The agricultural sector of South Africa plays a significant role in the economy of South Africa, providing work for approximately 940 000 people and generating 15% of the Gross Domestic Product (GDP) (Anon., 2009). During 2012, 915 711 tons of white and 428 188 tons of red varieties were harvested, and 1003 700 000 litres of wine produced (SAWIS, 2013). Previously, the Environmental Conservation

Act (Act 73 of 1989) of South Africa did not regard how waste products were produced, disposed of or recycled during the production process. The National Environmental Management Act (Act 107 of 1998) of South Africa however changed this and states that the full responsibility lies with landowners in the protecting and managing of the environment by means of sustainable processes (Dillon, 2011). The grape growing sector of the Western Cape puts immense pressure on the already scarce water resources of the province. The challenge is therefore to uphold an economically viable wine industry, while concurrently saving water (Waterwatch, 2013).

It is understood by historians that wine was produced in the Caucasus and Mesopotamia that dates back to 6000 B.C. (Pretorius, 2000; Bester, 2009). As the physical characteristics of grapes differ from vintage to vintage it is not possible to have a set production formula for winemaking (Jackson, 2008; Novo *et al.,* 2012). A simplified flow diagram of the wine production process is illustrated in Figure 2.1. The vinification process (Fig. 2.1) commences when grapes and juice reach the winery (Jackson, 2008). Wine is made by crushing and fermenting grapes, followed by straining of the grape skins and seeds where after it is stored and clarified and allowed to mature (NWQMS, 1998). According to Jackisch (1985), the winemaking process can be divided into four phases: (i) biological phase where the grapes grow and ripen (ii) microbiological/enzymatic phase also known as fermentation (iii) physical/clarification phase where minor particles in wine settle by gravity and (iv) the chemical and/or aging phase. Several differences exist between the white and red winemaking process. During the production of white wine, maceration is minimised and lasts only for a few hours (Jackson, 2008). For red wines however, this process is much longer and occurs together with alcoholic fermentation (Jackson, 2008). Malolactic fermentation (Fig. 2.1) is avoided for white and some sparkling wines but often encouraged for red wines and fuller, more complex white wines (Springham, 1999).

Waste produced during the winemaking includes both liquid and solid waste (Gea *et al.,* 2005). Solid waste generated by the winemaking process (Fig. 2.1) contains plant remains from de-stemmed grapes, sediments from clarification, bagasse from pressing and lees from various decanting steps (Devesa-Rey *et al*., 2011). Liquid waste generated from vinification is mainly wastewater which consists out of grape marc, grape pips and dead yeast cells from the alcoholic fermentation process (Devesa-Rey *et al*., 2011).

WINERY WASTE CHARACTERISTICS

Wineries produce large quantities of waste residues that could cause environmental problems due to their seasonal impact and polluting characteristics (FSA Consulting, 2006). Wastes that are produced during winemaking should be considered as part-and-parcel of the process. The choices and subsequent procedures that are decided upon in both the vineyard and cellar will directly control the sustainability of a farm, the wine industry and the agriculture of South Africa (Dillon, 2011).

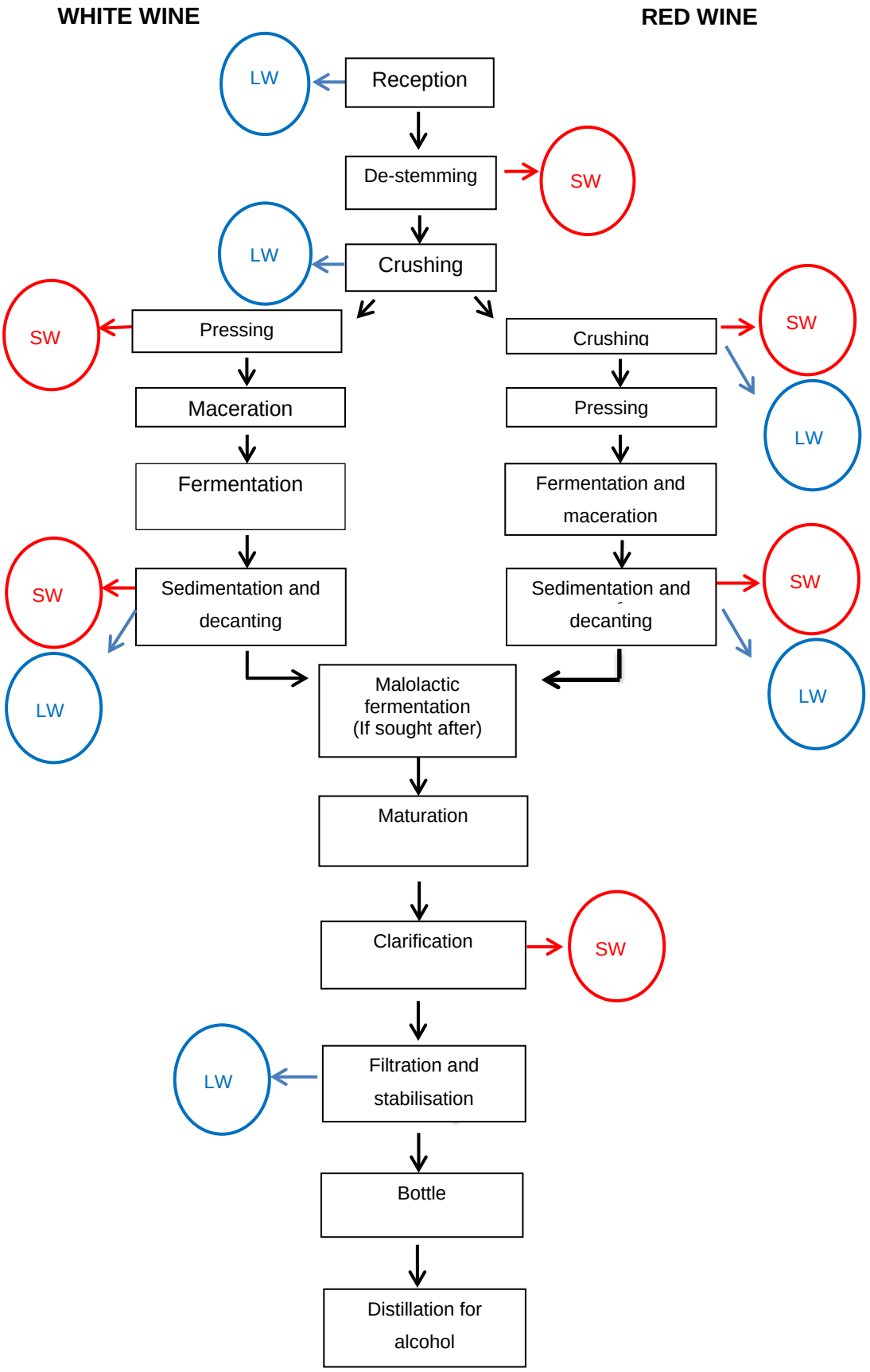

Figure 2.1 Schematic diagram of wine making and waste generation (Nogales *et al.,* 2005; Vlyssides *et al.,* 2005; Arvanitoyannis *et al.,* 2006; Jackson, 2008). LW= Liquid waste, SW= Solid waste.

Liquid waste

Currently, one of the main issues that the wine industry is facing is the management of large volumes of wastewater (Mosse *et al.,* 2011). The majority of wineries in South Africa are located in the Western Cape (Bruwer, 2003). Because several are found in the same water catchment area, contamination of downstream sources and water tables may occur (Marais, 2001). Wastewater in wineries is mainly generated by: various cleaning and washing operations during the production of wine; the rinsing of fermentation tanks, barrels, floors, equipment and surfaces (Riaño *et al.,* 2011); in addition to wastewater generated by bottling facilities; product losses; laboratory wastewater; and storm water that are captured in the wastewater management systems which also plays a polluting role (FSA Consulting, 2006). Wastewater so formed can serve as a primary source of ecological pollution, especially during the harvest season (Mace & Mata-Alvarez, 2002).

Characteristics of winery wastewater (WWW) differ in terms of the type of wine produced, the specific management practices applied (stage of production) and the volume of the tanks used (Vlyssides *et al.,* 2005). Typical quantities of winery effluent are shown in Table 2.1. The National Water Quality Management Systems (NWQMS) (1998) reported that wineries can generate up to five kilolitres of wastewater per ton of grapes processed. The amount is dependent on the degree of wash water recycling and if storm water is allowed to enter the effluent stream (NWQMS, 1998).

Table 2.1 Winery effluent amounts generated by different sized wineries (NWQMS, 1998)

Winery size	Crushed grape weight per vintage (ton)	Effluent generated per annum (kilolitres)
Large	≥ 20 000	40 000 - 240 000
Medium	5 000 - 20 000	5 000 - 10 000
Small	≤ 5 000	1 000 - 9 000

Wastewater generated by wineries is nearly 1.2 times more than that produced as wine (Vlyssides *et al.,* 2005) and although the wine industry does not have a reputation as a polluting industry, typical characteristics of wastewater can be an environmental threat (Ronquest & Britz, 1999; Brito *et al.,* 2007). The quality and volumes of winery effluent vary greatly during the year as they are dependable on different winery operations (Kumar *et al*., 2006). Winery effluent is typically described as a high strength organic waste, with a low nitrogen and phosphorous content (Toffelmire, 1972). Alcohol, hexose sugars (glucose and fructose), organic acids (acetic, propionic, tartaric) (Moosbrugger *et al.,* 1993; Keyser *et al.,* 2003), esters and polyphenolic compounds are components that are typically present in winery effluent (Mosse *et al.,* 2011). Winery wastewater is characterised by a chemical oxygen demand (COD) of 0.8 - 12.8 $g.L^{-1}$ and a pH of 3 - 4 (Petruccioli *et al.,* 2000). Literature reports that COD values can increase up to 25 $g.L^{-1}$, depending on the harvest capacity and the pressing activities in the wine cellar (Malandra *et al*., 2003; Strong, 2008).

In South Africa more than 95% of wineries dispose of winery wastewater by irrigation (Van Schoor, 2005). However, before wastewater can be discharged by means of irrigation, it has to comply with certain requirements as given in Table 2.2 (Republic of South Africa, 2004).

Table 2.2 Requirements for wastewater if land irrigation is intended for end use (Republic of South Africa, 2004)

Requirement	**Irrigation site size**		
	< 50 m^3	**< 500 m^3**	**< 2 000 m^3**
Faecal coliforms (per 100 mL)	< 100 000	< 100 000	< 1 000
COD[1] (mg.L^{-1})	< 5 000	< 400	< 75
pH	6 - 9	6 - 9	5.5 - 9.5
Ammonia (mg.L^{-1})			< 3
Nitrate/Nitrite (mg.L^{-1})			< 15
Chlorine (mg.L^{-1})			< 0.25
SS[2] (mg.L^{-1})			< 25
EC[4] (mS.m^{-1})	< 200	< 200	70 - 150
SAR[5]	< 5	< 5	
Ortho-phosphate (mg.L^{-1})			< 10
Fluoride (mg.L^{-1})			< 1
Soap, oil/grease (mg.L^{-1})			< 2.5

[1]Chemical Oxygen Demand, [2]Suspended Solids, [4]Electrical conductivity, [5]Sodium Absorption Rate

Uncontrolled discharge of untreated waste can have severe ecological, social and health risks and should therefore be minimised (Riaño *et al.,* 2011). Possible impacts from various liquid waste components are shown in Table 2.3. Winery wastewater can cause eutrophication of natural water resources, soil sodicity, salinity waterlogging and anaerobiosis (Van Schoor, 2005).

The wine industry often promotes itself as a “clean green image” but the management of waste can become a critical issue when polluting the environment. This matter is further aggravated by the fact that volumes of wastewater increases as the wine industry grows (Kumar *et al*., 2006). Section 39 of the National Water Act (1998) states that untreated winery effluent would infrequently qualify for release into natural water resources and should therefore either be treated prior to disposal or treated by alternative means (Van Schoor, 2005). Discarding of complex winery wastes signifies high costs to wine makers and therefore, identification of effective low cost treatment options is of high importance (Mosse *et al.,* 2011).

Table 2.3 Possible impacts of winery wastewater on the environment (EPA, 2004; Winewatch 2009)

Indicator	Component	Possible sources	Potential impact
pH, Calcium Carbonate ($CaCO_3$)	Alkalinity and/or acidity	Ion exchange processes which are acidic-pH ± 2 Production losses grape juice and wine is fairly acidic, pH 3.5 - 5.5 Breakdown of organic components during storage of wastewater further acidifies the wastewater	Death of water organisms at extreme pH Affects: Microbe activity during biological wastewater treatment Heavy metals solubility in the soil Growth of crops
Salinity	EC[1], TDS[2], chloride	Washing processes (Caustic Soda) By-products from ion exchange processes Salty groundwater used for cleaning purposes	Unpleasant taste to water Toxic to water organisms water uptake by crops are affected
Nutrients	Nitrogen, potassium, phosphorus and sulphur	Production losses: grape juice, wine and lees Proteins removed by fining are sources of nitrogen and phosphorous Phosphate cleansing agents and phosphoric acid	Eutrophication if stored in lagoons (unwanted odours) Poisonous to crops in large amounts. Potassium can cause decreased infiltration in soil
Organic material	TOC[3], COD[4], BOD[5]	Production losses: grape juice, wine and lees Residues from cleaning and diatomaceous earth waste Solid waste like skins and pips	Leads to oxygen depletion in water and consequently the death of water organisms Odour generation due to anaerobic decomposition
Metal contamination	Chromium, copper, mercury, nickel, zinc, cadmium and lead	Aluminium and copper, tanks and piping, lead from soldering as well as brass fittings	Toxic to both plant life and wildlife
Sodicity	SAR[6], ESP[7]	Washing processes (Caustic Soda) By-products from ion exchange processes Salty groundwater used for cleaning purposes	Affects the structure of soil Causes: Crusting of surface and inadequate aeration Low hydraulic conductivity and infiltration Subsoil becomes hard and dense

[1]Electrical Conductivity, [2]Total Dissolved Solids, [3]Total Organic Content, [4]Chemical Oxygen Demand, [5]Biological Oxygen Demand, [7]Sodium Absorption Rate, [8]Exchangable Sodium Percentage

Solid waste

Solid wastes that are generated by the winemaking process include stalk, grape pomace, wine lees and winery sludge (Bustamante *et al.,* 2008a). The primary solid source produced during the wine making process is grape pomace that contains seeds, stalks and peel (Diaz *et al.,* 2002).

Carbon-based by-products from the wine making process are characterised by an acidic pH, high polyphenol, organic and potassium content along with a substantial amount of nitrogen and phosphate (Bustamante *et al*., 2008b). Grape pomace is often disposed of in open areas, but could be used as animal feed (Sánchez *et al.,* 2002) or for extraction of tartaric acid (Nurgel & Canbas, 1998). Tartaric and malic acids are the main acid components present in a grape and the extraction thereof produces a valuable product (Nurgel & Canbas, 1998). Scarcity of grazing fields, especially during the dry season makes pomace as animal feeding a feasible option (Sánchez, *et al.,* 2002). Due to the low nutritional quality, the use of animal feed however, is limited (Mole *et al.,* 1993; Sánchez, *et al.,* 2002).

As large quantities of solid waste are generated during winemaking it causes problems in terms of storage, elimination or conversion in both environmental and economic terms (Arvanitoyannis *et al*., 2007a). When this by-product is improperly disposed of and left unattended it could cause several environmental problems such as water contamination and foul odours (Brunetti *et al.*, 2011). Smith (2009) investigated the effect of the vine mealy bug surviving in unmanaged grape pomace piles. Her results showed that the bug could survive in these piles and when the pomace is spread into vineyards the bugs could consequently infest the vineyards. The author recommended that unattended piles should not be disposed of directly into vineyards, but rather be covered for at least a week with thick, clear plastic to prevent airflow and increased pile temperatures. It is also advised to avoid grape pomace and stems being in the same pile as “stemmy” piles generate less heat.

Possible sources and impacts of solid waste obtained from winemaking consist of: (i) production losses such as grape juice, wine and lees that leads to odour generation due to anaerobic decomposition; (ii) residues from citric, caustic soda and diatomaceous earth filter waste which causes smothering of habitats; and (iii) skins and pips that reach wastewater drains which reduces soil porosity, oxygen uptake and light transmission in water (EPA, 2004; Winewatch, 2009). Additionally, diseases are spread as decomposing masses host a variety of insects and pests (flies, mosquitoes, cockroaches, rats) that can act as carriers of illnesses leading to severe health complications (Sharholy *et al.,* 2008; Suthar, 2009).

The direct disposal of solid grape waste onto land, which is a common practice, also leads to severe problems due to the presence of degradation components such as tannins and polyphenols (Diaz *et al*., 2002). Oenocyanin (natural red pigment), reduces the disposal of this waste product onto land even further, apart from the attraction of insects, fermented odours and liquid release (Seenappa, 2012). It is thus essential that alternative solutions to current treatment options for solid grape waste are considered.

B. TREATMENT OPTIONS FOR LIQUID WASTE (WINERY WASTEWATER)

Liquid waste

Proof of successful water treatment dates back to ancient Egyptian inscriptions where a variety of water purification processes was described. This included the boiling and filtering of water as well as exposing it to sunlight. It was only realised at the beginning of the 20th century that direct disposal of wastewater caused ecological problems (Moharikar *et al.,* 2005).

Although various treatment options are available for the treatment of WW, all of aim to achieve the same- to lead to cause a significant reduction in the concentration of organic matter and solids that are present in the wastewater (Mosse *et al.,* 2011). The main factors for selecting a treatment option include the financial requirements and the skill that is required to manage the entire system (Mosse *et al.,* 2011). All wineries are unique in terms of wastewater production (from 0.5 -14 L per litre of wine) and their disposal practices (Oliveira & Duarte, 2010). Currently, wastewater treatment options include chemical, biological (Shivajirao, 2012) as well as physical technologies (Gie, 2007).

Physical methods

Disintegration, screening, grit removal, flow equalisation and chemical additions

The use of preliminary treatment is to protect the treatment process from build-up of debris, inorganic git, scum formation or reduced efficacy due to fat, oil and grease (FOG) build-up (WEDC, 2013).

Disintegrators (comminutors and macerators) have been used in the past at the inlet of wastewater systems to cut up solids (WEDC, 2013). These processes are no longer favoured in wastewater pre-treatment as it generates a poor quality sludge and cut up solids result in operational problems (EPA, 1995).

Most wastewater treatment facilities include screening as a first unit procedure for pre-treating wastewater. This process removes substances that could cause impairment and blockage to other equipment within the plant (USEPA, 2004). The screening of wastewater can be classified into (i) coarse screening and (ii) fine screening. Coarse screens (opening ≥10 mm) are often used as primary protection devices whereas fine screens (opening 3 – 10 mm) are used in systems that lack primary treatment to prevent operational and maintenance problems (GAH Global, 2010). Solids (seeds, skins, stem, leaves and grape marc) can be removed by either a basket strainer in the floor drain of a winery or by the installation of a screening/straining device in a winery directly upstream from a septic tank array (Storm,1997). A rotating drum screen needs less operational attention than an in-line screen or floor screener as these need regular cleaning and monitoring especially during the crushing season (Storm, 1997).

All wastewater treatment plants should be equipped with a grit removal facility (Anon., 2004). Grit can be defined as the heavier suspended material within wastewater that is typically made up of sand, cinders and gravel (USEPA, 1977). The removal of grit protects equipment from

blockage and abrasion. This process can be achieved by either conventional sedimentation (solid removal) or by mechanical sand and grit equipment. These include vortex, hydrocyclone and other units that operate similarly to spin out solids (AWWA, 2012).

Flow equalisation is a method used to combine wastewater in holding tanks to "equalise" it before releasing wastewater into downstream processes or right into municipal sewage systems (Olajire, 2012). The equalisation of fluctuating wastewater will help make hydraulic polluting rates more even and can improve the effectiveness of a treatment process (USEPA, 1977). Flow equalisation typically contains a holding tank and pumping equipment that lowers fluctuations of waste streams. The tank stores excessive hydraulic flow and stabilises the flow within 24 hours to a constant rate (Show, 2008). This process is frequently applied in the wine industry as preliminary treatment for wine/stillage (Kennedy & Jenks Consultants, 2013).

Chemical additions are often applied to wastewater to achieve pH neutrality or to assist with chemical flocculation of solids (Green & Kramer, 1979). To neutralise acidic winery effluent during the peak season, lime is added preceding secondary biological treatment. Lime used for dosage is the preferred chemical above that of sodium hydroxide as it causes ecological problems in terms of salinity and sodicity of lands (Dillon, 2011).

Sedimentation, coagulation and flocculation

Literature reports that about 25 - 50% of biological oxygen demand (BOD), 50 - 70% of total suspended solids (SS) and 65% of oils and greases are removed by pre-treatment (Pescod, 1992). Sedimentation or clarification of wastewater is a low-cost treatment for the separation of particles (Lekang, 2001). It is defined as the segment separation of suspended solid particles from a liquid by means of gravity settling. Sedimentation is influenced by the size of particles present, the viscosity and the density of the solid parts (Cancino-Madariaga & Aguirre, 2011). This process is achieved by reducing the velocity of water so that compounds will not remain in suspension to any further extent. When compounds are no longer supported by velocity, they can be removed by means of gravity (Nazaroff & Alvarez-Cohen, 2001). The main purpose of sedimentation is to enhance the filtration process by removing particles from the wastewater (Grecory & Zabel, 1990). This process can be applied before filtration as a pre-treatment process and is known as pre-sedimentation (plain sedimentation) (Yim *et al*., 2000). Sedimentation basins are available in rectangular, circular or square form (Hammer, 1975).

The solid separation of winery wastewater is desirable because it reduces the amount of work on the waste system (Toffelmire, 1972). Marais (2001) reported that dissolved and suspended matter in winery wastewater do not settle by gravity alone and thus need sedimentation agents. Because organic material in winery wastewater is present in soluble form, static sedimentation as a treatment option does not cause a significant concentration reduction (Brito *et al.,* 2007). Other disadvantages of sedimentation includes that it is time intensive and only partly eliminates turbidity and potential pathogens (Dangol & Spuhler, 2010).

Coagulation and flocculation are often referred to as the backbone of advanced water treatment processes as their main objective is to enhance separation of particulate compounds in processes like filtration and sedimentation (Shammas, 2005).

Beltran de Heredia *et al.* (2005) treated wine distillery wastewater by a Fenton-coagulation/flocculation process making use of calcium hydroxide $Ca(OH)_2$ as a base precipitant. The study showed that moderate COD reduction was obtained with the coagulation/flocculation and as expected the higher hydrogen peroxide dosages during the first Fenton's reaction led to better COD removals.

Due to the repulsion charges and sizes of colloidal and suspended particles they are easier removed by coagulation and flocculation than by gravity sedimentation (Mihelcic *et al.,* 2009). Coagulation is achieved by adding a chemical coagulant to wastewater to destabilize colloidal, dissolved and suspended particles (Mihelcic *et al.,* 2009). After the coagulation process these particles aggregate by flocculation and are removed by means of gravity settling or mechanical separation (Mihelcic *et al.,* 2009). Flocculation or conglomeration is a physical process where particles become enmeshed with each other. Dual tanks are normally used for these processes (Fig. 2.2). Within the first coagulant tank, the agitation rate is high when the destabiliser (coagulant) is added. The wastewater remains in this tank for only a limited amount of time. In the second flocculation tank gentle mixing of wastewater occurs for conglomeration and settling to ensue (Talty,1988).

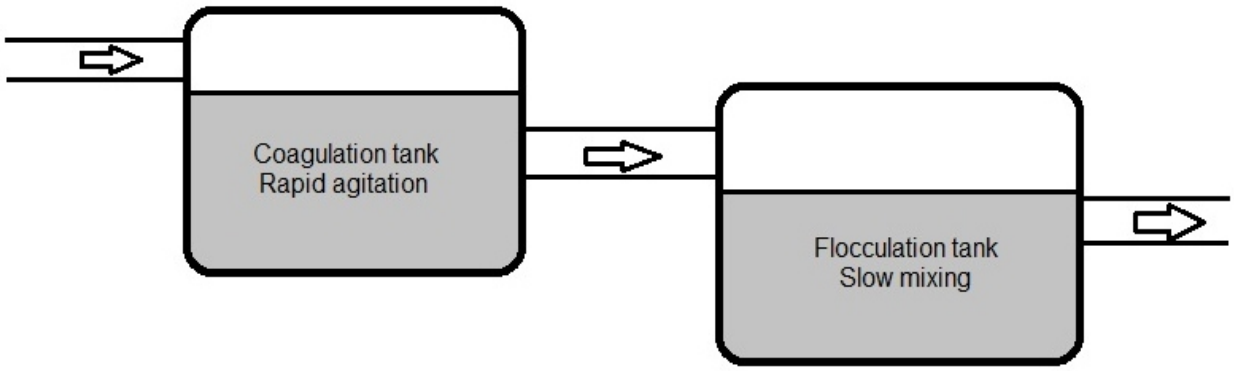

Figure 2.2 A schematic illustration of the coagulation-flocculation process (Talty, 1988).

The most common coagulants used in wastewater treatment include aluminium sulphate (alum), ferric chloride and ferric sulphate (Jiang &Lloyd, 2002; Bratby, 2006, Renault *et al.,* 2009). Alum is a common metal salt and a suitable coagulant for wastewater containing significant amounts of organic material. Iron coagulants can however, work over a wider pH range and are more effective in removing colour from wastewater (Rast, 2003). Zayas *et al*., (2007) investigated the effect of purifying vinasse which had been pre-treated biologically. The results showed that by using $FeCl_3$ as a coagulant at 20 $g.L^{-1}$ and vinasse effluent at a pH of 8.4, a COD removal of 84% could be achieved as well as sufficient colour and turbidity removal (99%). Braz *et al.* (2010) studied the effect of four different coagulants ($FeSO_4$, $Al_2(SO_4)_3$, $FeCl_3$ and $Ca(OH)_2$) on both red

and white winery wastewater. Results showed that coagulation and flocculation, within optimum and coagulant dosage ranges, lead to an effectual turbidity removal of 92,6% with aluminium sulphate in addition to a total suspended solids (TSS) removal of 95,4% with calcium hydroxide. The study also showed that coagulation and flocculation as a main treatment process had minor capability to remove COD in both the red and white winery wastewater. Coagulation/flocculation is a suitable full scale pre-treatment technology for the reduction of organic and suspended matter of winery wastewater (Braz *et al.,* 2010; Ioannou *et al*., 2013). Disadvantages of coagulation and flocculation include high operational expenses (chemical depletion) and excessive sludge formation that often limit the procedure as a main wastewater treatment option (Vesilind, 2003; Golob *et al.,* 2005; Kurniawan *et al.,* 2006).

Granular media filtration

Filtration is a common pre-treatment step in the management of treating winery wastewater. Solid separation (lees, stems and pomace) by means of filtration is essential as it contributes to waste reduction (Toffelmire, 1972). Supplementary removal of suspended material before biological and chemical treatment is commonly achieved by granular media filtration (Matsumoto *et al*., 1982). The removal of particles from water by means of granular media filtration plays an important role in potable water use, wastewater treatment and industrial water applications (Boller & Kavanaugh, 1995). This process has shown to effectively remove particles with low densities from a bacterial origin as well as high density inorganic solids such as titanium and ferric oxides (Boller & Kavanaugh, 1995). Filters can be classified in four different categories (Caliskaner *et al*., 1999):

1. Direction of the flow (up-flow, down-flow);
2. Type of media (multimedia, dual, single);
3. Flow driving force (pressure, gravity); and
4. the rate of the flow (rapid or slow granular media filtration).

The most widespread use of granular media filtration is the sand filters for the treatment of water and nowadays, wastewater treatment. Although sand filters have been used in water treatment for over four centuries it was only employed as a mass scale treatment at the beginning of the nineteenth century (Black *et al.,* 1984; Tien & Ramarao, 2007). Sand is usually used as filtration media, although other materials such as crushed magnetite, crushed anthracite (hard coal) and garnet could also be used (Droste, 2004). Filtration techniques are characterised by the mode of filtration and are classified as slow sand filtration or rapid sand filtration. The whole filtration process consists out of two phases, filtration and cleaning which is also known as regeneration (backwashing) (Hamoda *et al*., 2004a).

Slow sand filters

Slow sand filters are based on the slow movement of water through a porous sand media. The filtration unit contains two columns: one of water followed by one of sand, which has the ability to remove organic and inorganic material along with micro-organisms (Ari & Adin, 2006). Solids are typically removed by filtering through the media surface and accumulated matter called "Schmutzdecke" (Pizzi, 2010). The phrase "Schmutzdecke" is the German word meaning "dirty layer/skin" or "sludge blanket". This layer is regarded as a gelatinous mat where a mass of micro-organisms flourish and therefore the highest removal occurs here (Barrett *et al.,* 1991). Disadvantages of slow sand filters include: (i) the need for large surface areas and filtering media (ii) cleaning of filtering equipment is labour intensive (iii) microbial removal efficiency decreases in cold water due to the reduced biological activity of organisms and (iv) insufficient removal of fine clays unless a pre-treatment step is in place (USEPA, 2013). Sand filters are only applicable to wastewater that contains a low turbidity (Hammer, 1975).

Rapid sand filter

Created in North America as an alternative to the slow sand filter, the rapid sand filter was invented to use the entire depth filter bed as to ensure a higher quantity of water for a given surface area (Droste, 2004). Rapid sand or gravity filters, are the most common filters used in the treatment of wastewater to remove nonsettleable material (Hammer, 1975). The mechanisms of a rapid sand filter (also termed a gravity sand filter) are basically the same as those of the slow sand filter with the exception of the biological processes (Scholtz, 2006). In rapid sand filtration the biological activity is minimised, leading to a reduced filter run time in between cleaning procedures which restricts the formation of mature biological development. Rapid sand filtration functions at a tempo some ten times that of slow sand filtration (Scholtz, 2006). Even though rapid sand filtration is seen as an established technology for reducing suspended solids and requiring less land area and operation than slow sand filters, high capital costs and procedure costs are required (UNEP, 2013). Costs can be increased further if raw water needs to be pre-treated. Rapid filter technology also utilizes energy for pumping and high operational skills are needed (UNEP, 2013). Unless prechlorination or activated carbon adsorption has been applied as a pretreatment process, the rapid sand filter will not remove unpleasant odours and tastes. In terms of bacterial loadings sufficient chlorination should always follow the filtration process (Hardenbergh & Rodie, 1963).

Chemical treatment options

This process includes different chlorine varieties, ozone (O_3), oxygen (O_2) and permanganate (MnO_4^-). Chemical treatment of wastewater is mainly used as a tertiary treatment option and is suited for the removal of colour and odorous constituents as well as disinfection (Green & Kramer, 1979; Nazaroff & Alvarez-Cohen, 2001). The reduction of salt levels in winery wastewater is of high importance as salt levels cannot be lowered through commonly used treatment methods

(Winewatch, 2012). Technologies for salt removal include ion exchange (Mosse *et al.,* 2011) and membrane processes (Hamman *et al.,* 1990).

Chlorination

Wastewater treated ineffectively or left untreated could contain harmful pathogens (Okoh *et al.,* 2007). Chlorine usage in wastewater involves: (i) controlling odours and foul air, (ii) regulating activated sludge bulking, (iii) preventing septicity, (iv) obliteration of cyanide and (v) acting as a disinfecting agent (Black & Veatch Corporation, 2010). Chlorine is the most commonly applied disinfectant to wastewater. It damages the cellular components of micro-organisms and can be used to disinfect wastewater in either a solid, liquid or gas form (Okoh *et al.,* 2007). When added to wastewater chlorine can react with a range of compounds such as nitrogen, organic nitrogen, uric acid, cysteine, polyphenols, bacteria and viruses (Black & Veatch Corporation, 2010).

Chlorination can be also be used with sand filtration technology. It is often added before the filtering process (pre-chlorination) to kill algae that clogs filters and after filtration (post-chlorination) to effectively disinfect the wastewater (Hamoda *et al*., 2004b). Free chlorine has the ability to react with organics to form organochlorinated derivaties which are of great ecological concern and therefore any free chlorine remaining in wastewater has to be removed by dechlorination to protect aquatic life (Abarnou & Miossec, 1992; Okoh *et al.,* 2007). While chlorine is applied in water and wastewater as a disinfectant, the higher amount of impurities in wastewater leads to a higher chlorine dosage (Nazaroff & Alvarez-Cohen, 2001). Although chlorination is an effective disinfectant against bacteria and certain viruses, this technology is applied less frequently to wastewater due to the formation of toxic chlorinated by-products (Lazarova *et al*., 1999). The disadvantages of chlorination includes the poor inactivation of certain viruses and spores at low chlorine dosages when used for coliform elimination, the formation of lethal by-products and dechlorination cost which increases initial disinfection costs by approximately 20 % (Lazarova *et al*., 1999).

Advanced Oxidation Processes (AOP)

This technology relies on chemical initiators and energy to destroy contaminants found in water, wastewaters, soil and air. It includes UV radiation, ozonation, sonolysis, photocatalysis, wet air oxidation, electrochemical oxidation, the Fenton and photo-Fenton reagents and several combinations of the aforementioned (Mantzavinos *et al*., 2007). AOP generates reactive intermediates, with hydroxyl radicals (•OH) being the primary radical produced (Zwiener & Frimmel, 2000; Kraft *et al*., 2003). By being one of the strongest oxidising species, the hydroxyl radicals attack carbon-source compounds by either removing hydrogen ions or adding them to double bonds (Mourand *et al.,* 1995). These technologies are able to oxidise most carbon-source pollutants and reduce their concentrations in wastewater (Tabrizi & Mehrvar, 2004).

Lately, advanced oxidation processes have shown potential as an alternative treatment option for winery wastewater (Oller *et al*., 2011) and have been successfully applied in various

studies: (i) Agustina *et al.* (2008) studied the effect of a photocatalytic/photolytic reactor system on winery wastewater. It was found that the highest degree of photodegradation and COD removal to be at zero catalyst loading and 84%, respectively; (ii) Ioannou *et al.* (2013) investigated the purification of winery wastewater by reverse osmosis and oxidation of the concentrate by applying a solar photo-Fenton process. The authors found a COD removal rate of 97% along with a deduction rate of 67% for nitrogen and 76.2% for TSS. By applying the solar photo-Fenton oxidation process on the concentrate an additional COD reduction of 75% was achieved; and (iii) Lucas *et al.* (2010) investigated the effect of different advanced oxidation processes on winery wastewater. They found that the O_3/UV and O_3/UV/H_2O_2 advanced oxidation processes were the most feasible methods for the treatment of winery wastewater in a pilot-scale bubble column reactor as significant COD and TOC rates were observed. Disadvantages of AOP include long retention times for certain substances, the production of free radicals that can scavenge carbonate and bicarbonate ions and by-products generated from recalcitrant organic matter that may appear in the production water (Mourand *et al.,* 1995). Pretreatments are often necessary for the preferred oxidation reaction to take place when certain compounds in the wastewater compete for the oxidising agents (Hamman *et al*., 1990).

Adsorption

The adsorption process involves a mass transfer procedure where matter (adsorbate) is moved from an aqueous phase to a solid phase (adsorbent). The matter binds to the surface of the solid phase by chemical and/or physical interactions (Çeçen & Aktas, 2012). Activated carbons are the most frequently used adsorbents for the treatment of water as a variety of organic solutes can be removed from water and wastewater by means of adsorption (Çeçen & Aktas, 2012).

These porous adsorbents are mainly used in a powdered activated carbon (PAC) or granular activated carbon (GAC) forms (Cooney, 1998). Activated carbons can be manufactured from several raw organic materials and a variety of activation procedures (Worch, 2012). Traditionally, adsorption by means of activated carbon was applied to drinking water to remove components that cause odour and taste problems, but lately it is applied to wastewater to remove remaining organic material that cannot be biodegraded (Nazaroff & Alvarez-Cohen, 2001). The adsorption process represents one of the cheapest tertiary treatment technologies for the removal of recalcitrant organic compounds (Green & Kramer, 1979). For years it was mainly used as a treatment option for wastewater generated by chemical industries but due to upgraded equipment and reductions in operating costs it is also applied to municipal and industrial wastewater treatment plants (Green & Kramer, 1979).

Ion Exchange and membrane processes

The usage of sodium base cleaning agents (mostly NaOH) in wineries poses to be a major threat to the environment as the accumulation of sodium in the environment is a common problem (Mosse *et al*., 2013). This factor is of high importance if wastewater is disposed of onto land as

salt present in wastewater can have a significant effect on soil properties (Tillman & Surapaneni, 2002). Ion exchange is defined as the exchange of ions from a solution for other ions onto a surface. In wastewater treatment it is mainly used for the removal of metals that are toxic and for the retrieval of valuable metals (Droste, 2004). Although ion exchange is a common practice in winemaking, almost no literature is available on the application thereof on winery wastewater (Mosse *et al*., 2011).

Membrane processes have been applied in the treatment of water, seawater and brackish water for more than 30 years (Shivajirao, 2012). Membrane technologies are used for desalinisation and the removal of specific ions that are difficult to eliminate by means of other methods and is often applied to wastewater that is intended for reuse as it provides softening and eliminates organic material, viruses, bacteria and heavy metals (Hamman *et al.,* 1990). The process is grounded on the occurrence of semi-permeable membranes that work as filters (Shivajirao, 2012). Technologies include: electrodialysis, reverse osmosis, nanofiltration, ultrafiltration, and electrodialysis reversal (Hamman *et al.,* 1990; Taylor & Francis Group, 2010). Due to water scarcity conditions experienced worldwide, there is a strong motivation to recover unpolluted water from effluents for reuse (Melamane *et al.,* 2007). Nataraj *et al.,* (2006) studied the effect of a combination treatment (nanofiltration and reverse osmosis) on distillery spentwash. The authors achieved a significant removal rate of 99.80% TDS, 99.90% COD and 99.99% of potassium.

Although as mentioned above, a number of technologies are available for the removal of sodium from wastewater they have high capital costs (energy and maintenance) which make them impracticable for most wineries and specifically small wineries (Mosse *et al*., 2011). Tchobanoglous *et al*. (2003) also reported that a main limitation of these methods is the production of a concentrate that requires disposal.

Biological methods

Biological processes are based on microbes that use carbon and energy for growth in order to oxidise organic materials in wastewater (Erten-Unal, 2009). Microbial-based systems for organic material degradation have lately gained importance since biological treatment methods have a number of advantages above chemical or physical technologies. Biological treatments of wastewater are more effective due to the higher surface-to-volume ratio, they have less operational costs as systems can operate at ambient temperatures and they are more robust (Moharikar *et al.,* 2005). Biological treatment methods include aerobic and anaerobic treatments and have proven to be successful in treating polluted wastewaters (Genovesi *et al.,* 2000).

<u>Aerobic vs anaerobic treatment</u>

Previously, aerobic treatments were used continuously in industrial countries as energy costs involved in this treatment were low. Due to energy and disposal costs increasing drastically, industries were forced to consider other treatment options (Britz *et al*., 2002). Figure 2.3 illustrates

the implementation of anaerobic systems from 1981 - 2007. According to van Lier, (2008) up and until 2007, 2266 anaerobic full-scale systems were registered and operated worldwide. According to the author another 500 reactors could be added to the number which mainly consists of "home-made" reactors used by small local companies and industries. During 1981 – 2007, (Fig. 2.3, left) approximately 77% of reactors utilised were granular sludge bed based and only 28% expanded bed based. In 2002 - 2007, however (Fig. 2.3, right) the percentage of expanded based reactors increased to 57% and the granular sludge bed reactors to 89%. Ren, (2013) reported that nowadays China is the leader in retaining full-scale (300 m^3) anaerobic digesters, with an estimated 20 000 AD systems in operation at the end of 2012. This value represents over 30% of the total number of AD systems reported worldwide.

The main differences and advantages of anaerobic digestion over aerobic treatment are highlighted below (Fig. 2.4). Due to poor biological stabilisation in aerobic systems, a large amount of organic material is incorporated as microbial biomass causing excessive sludge production (Fig. 2.4) (de Lemos Chernicharo, 2007). In anaerobic systems only a small volume of biomass is produced as most of the biodegradable material is converted to biogas (de Lemos Chernicharo, 2007).

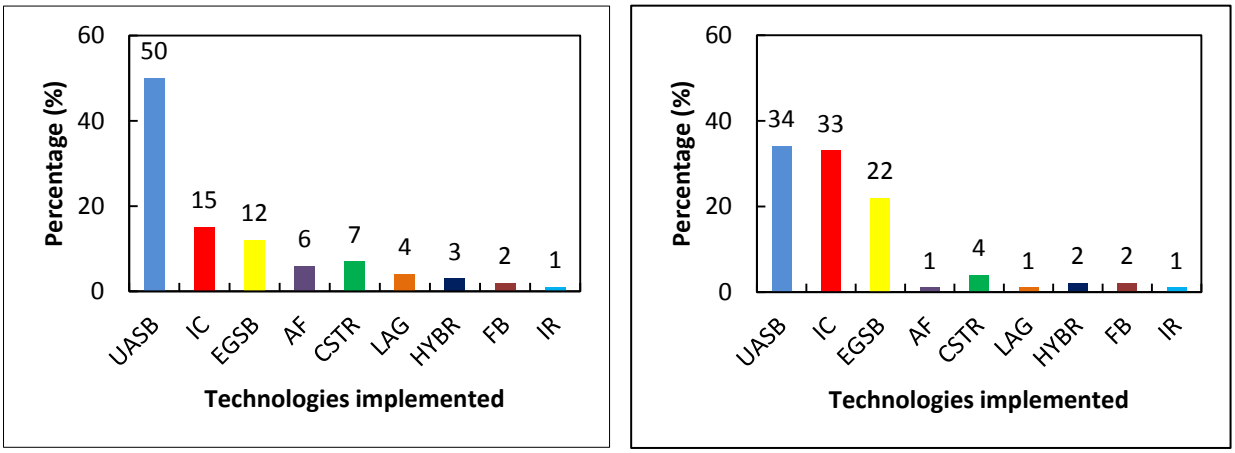

Figure 2.3 Schematic representation of anaerobic systems implemented from 1981 – 2007 (left) and 2002 – 2007 (right) (van Lier, 2008). UASB: Upflow Anaerobic Sludge Blanket, IC: Internal circulation reactor, EGSB: Expanded Granular Sludge Bed, AF: Anaerobic Filter, CSTR: Continuous Stirred Tank Reactor, Lag: Lagoons, HYBR: Hybrid, FB: Fluidised bed, IR: incomplete references.

Anaerobic treatment should not necessary be seen as a substitute to aerobic treatment, but rather as a treatment that can complement it. When these biological processes are combined, the advantages of both can be incorporated. The data in Table 2.4 shows the key differences and benefits of each treatment system.

Biogas generated by AD, consists of various gasses (CH_4, CO_2, CO, H_2, H_2S, NH_3, N_2, N_2O) (Gerardi, 2003) but methane (60 - 65%) and carbon dioxide (35 - 40%) are produced in larger quantities (Fillaudeau *et al*., 2008). Methane is a renewable energy source and a natural flammable gas (Gerardi, 2003). Methane produced during AD can be used as a fuel source for

boilers or function as a substitute for natural gas and fuel oils. It can also be utilised by engine-generators to generate electrical energy for on-farm usage or be sold to electrical companies (Kelleher *et al*., 2002).

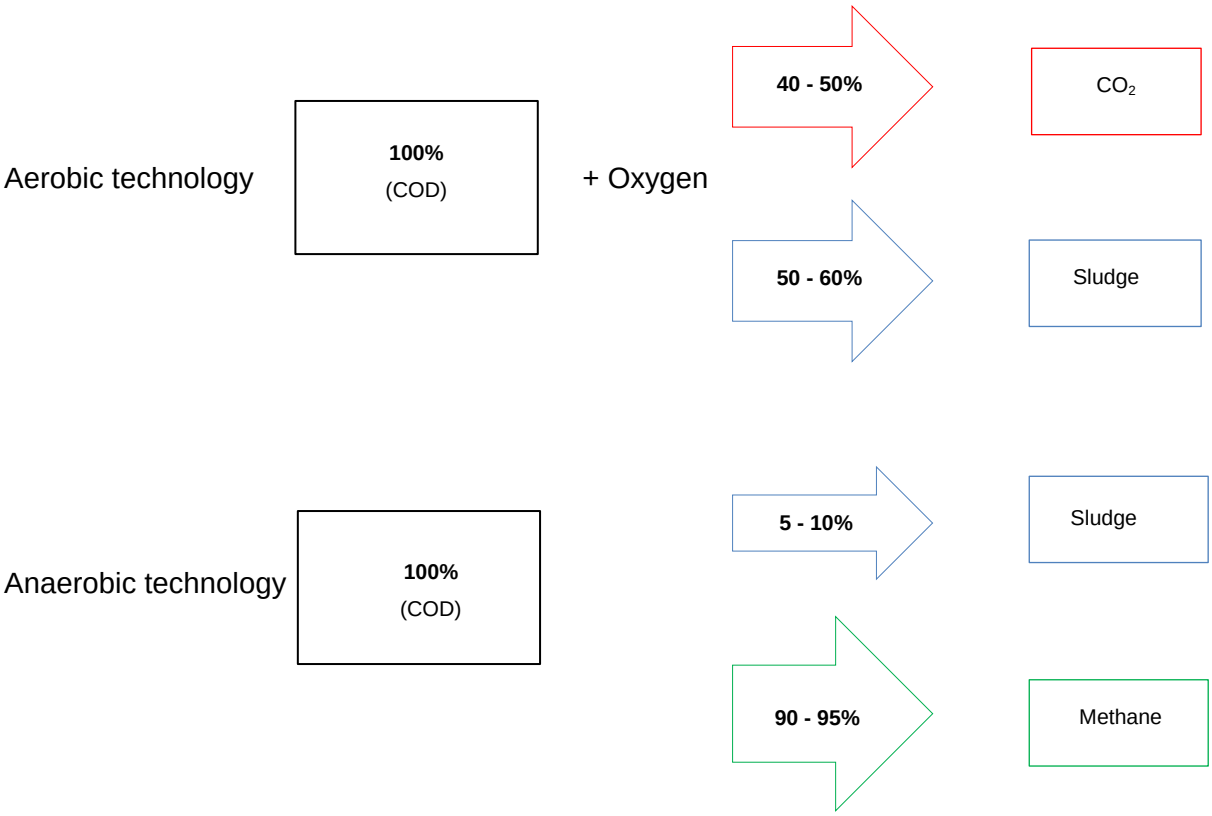

Figure 2.4 A schematic illustration highlighting the main differences between aerobic and anaerobic technologies (Parawira, 2004; Els *et al*., 2005).

Table 2.4 Main limitations and advantages between anaerobic and aerobic biological systems (Driessen & Vereijken, 2003; Fang, 2010)

	Aerobic	Anaerobic
Sludge production	high	low
Energy consumption	high	low
Energy (methane) production	no	yes
COD removal	90 - 98%	70 - 85%
Nitrogen/Phosphorous removal	high	low
Area requirement	high	low
Discontinues procedure	problematic	simple

Mosse *et al.* (2011) summarised that the most widespread used aerobic biological treatments in the wine industry includes: aerated lagoons; activated sludge; sequence batch reactor; and membrane bioreactor. Anaerobic treatment includes anaerobic sequence batch reactor, upflow anaerobic sludge blanket and anaerobic lagoons.

Aerobic treatment

Aerated Lagoons

Lagoons or ponds are shallow ground basins that are often used as an effective treatment option that needs minimal technology and maintenance (Nameche & Vasel, 1998). This technology involves the treatment of wastewater by means of natural processes that involve algae and aerobic micro-organisms (Stander & Theodore, 2008). This can be achieved by either aeration or constructing the lagoons shallow so that air and sunlight can reach it (Zhang *et al*., 2013). Oxygen and mixing requirements needed for the process to be optimal are obtained by either mechanical or diffused aeration (Stevenson, 2008).

Aerated lagoons can be defined as either aerated lagoons or aerobic-anaerobic/facultative lagoons (Bartsch & Randall, 1971). In aerated lagoons solids are kept in suspension due to mixing as supplied by the aeration equipment (Kormanik, 1972; Erten-Unal, 2009). The aerobic-anaerobic or facultative lagoons differ from the above mentioned as the mixing levels are low enough to allow the solids that are present to settle, while dissolved oxygen can still be dispersed through the water (Kormanik, 1972). Microbial reactions that occur in aerated lagoons are the same reactions found in activated sludge systems apart from the biological sludge (Stander & Theodore, 2008).

Aeration is used to enhance the organic removal efficiency of the treatment process (Moharikar *et al.,* 2005), shorten the treatment time and avoid organic overloading (Green & Kramer, 1979). Aerated conditions within a lagoon are sustained, unless the oxidation rate of organic matter exceeds the rate of reaerating (O'Connor *et al.,* 1960). Organic matter that is present in wastewater can be of such an extent that it causes the rate to be exceeded, leading to anaerobic conditions. In order to maintain an oxygen rich environment it is necessary to supply the lagoon with oxygen by artificial means (O'Connor *et al.,* 1960; Green & Kramer, 1979).

Facultative ponds, also termed oxidation or photosynthetic ponds are more common than aerobic ponds (Spellman, 2013). These ponds are approximately 0.9 - 2.4 m deep, containing an oxygen rich layer with an anaerobic layer beneath it (USEPA, 2011). According to literature retention times within these ponds may vary according to the temperature. In warmer climates 5 - 50 days have been reported, whereas in colder climates it was found to increase from 90 -180 days (USEPA, 2011)

Aerated lagoon technology has been applied on fish processing wastewater (Chowdhury *et al*., 2010), cane-sugar wastewater (Ramjeawon, 2000), domestic wastewater (Li *et al*., 2013) and tannery wastewater (Chandra *et al*., 2011). Montalvo *et al*. (2010) investigated the performance of pilot-scale aerated lagoons treating winery effluent. The effluent (18 700 $mg.L^{-1}$) was fed to an aerobic lagoon at a flow rate of 170 $L.d^{-1}$. The authors found a COD reduction of 91% after 21 days of treatment, where after the value was maintained almost constantly.

Aerated lagoons have several disadvantages; malodours tend to form if the lagoon is not properly designed and operated, the protection of groundwater needs to be taken into consideration, high mechanical aeration is very expensive (Barker, 1996), settled sludge needs regular removal, accumulation of sludge will increase in colder climates as microbial activity decrease and lagoons needs fairly large areas of land (USEPA, 2002).

Activated Sludge

The first activated sludge treatment plant was constructed in Worcester, England in 1916 (Nazaroff & Alvarez-Cohen, 2001). This process is the most common biological method for the wastewater treatment within industries and municipalities. (Ni *et al*., 2009). The utmost important component in this process is the use of an aeration tank, wherein micro-organisms are mixed with incoming wastewater (Moharikar *et al.,* 2005). The activated sludge process contains three elements (i) an aeration tank (reactor) where micro-organisms grow (ii) a clarifier, which is responsible for the liquid-solid separation and (iii) a recirculation system for transporting recovered sludge back to the aeration tank (Droste, 2004). Organic materials are biodegraded by being in contact with micro-organisms within an aerobic environment. Activated sludge treatment is regarded as a suspended growth process due to microbes being suspended in the water (Nazaroff & Alvarez-Cohen, 2001).

Brucculeri *et al*. (2005) investigated the co-treatment of municipal and winery wastewater in a traditional activated sludge process. Results obtained showed a 90% COD removal rate during vintage and 87% during non-vintage. Nitrogen removal was found to be 65% during both vintage and non-vintage. Petruccioli *et al*. (2000) studied the aerobic treatment of winery wastewater by means of a jet-loop activated sludge reactor. The reactor (15 dm^3) operated for more than a year and was fed winery wastewater collected at different times throughout the year. The COD of the wastewater ranged between 800 and 12 800 $g.m^{-3}$ and was fed to the reactor at an organic loading rate (OLR) of 0.8 and 12.8 $kg.m^{-3}.d^{-1}$. The authors found that the reactor responded well to variations in the wastewater and a COD reduction of 90% was obtained. Fumi *et al.* (1995) also treated winery wastewater by means of a long term, full scale activated sludge process. They found a significant COD reduction of 98%. The aerobic sludge produced, contained low levels of nitrogen, phosphorous and potassium as well as heavy metals and was therefore suitable for direct agricultural use or for the production of compost. Bolzonella *et al*. (2007) co-treated winery wastewater with municipal wastewater with a full scale activated sludge process for five years. They found a significant removal of COD, phosphorous and suspended solids. Sludge production during the vintage period was found to increase from 4.0 to 5.5 tons per day with a poor nitrogen removal of only 20%.

The main advantage of this technology is the almost complete oxidation of materials during short retention times, which leads to minimal space requirement (Green & Kramer, 1979). Limitations include high maintenance and capital costs, supervision by skilled operators are continuously needed (Green & Kramer, 1979) and aerobic sludge disposal is a main operating expenditure (Droste, 2004).

Rotating Biological Contractor (RBC) and Trickling Filters

The usage of RBC to treat wastewater dates back to the early 1900s where it consisted out of a cylinder with wooden slats. In the 1930s, it was replaced with metal discs due to clogging and bacteriological deterioration of the wood. Twenty years later it was replaced by expanded polystyrene discs which were eventually replaced with high density polyethylene (HDPE) discs (Mathure & Patwardhan, 2005).

RBC is a biological process used for the treatment of carbon-based *wastewater* and is characterised as an attached growth process (Show, 2008). It consists of a sequence of closely spaced circular plastic disks, which are partly submerged into a tank filled with untreated wastewater (Show, 2008). Discs usually consist of lightweight, styrofoam or high-density plastic materials (Droste, 2004). Microbial films develop on the surface of the circular disks which move through the wastewater as they rotate. Micro-organisms degrade organic material while being submerged in the wastewater and are provided with oxygen when the disks rotate into the air (Moharikar *et al.,* 2005). RBC has similarities to the activated sludge and trickling filter treatments but the biofilm process is the principal feature of this treatment option (Droste, 2004). Advantages of RBC over fixed film processes include less land area requirement, fewer complications with noise and odours, the process control is less complex and high removal rates of Biological oxygen demand (BOD) (Gray, 2010). Limitations, however, include the high capital costs, recurrent maintenance and unnecessary film-build after a power failure, which often can lead to impairment and failure of the motor on restart (Gray, 2010). Rotating biological contractors have been applied widely (Cortez *et al*., 2008) and successfully in a variety of applications including: bakers yeast wastewater (Nahid *et al*., 2001); food canning wastewater (Najafpour *et al*., 2006); metal contaminated wastewater (Costley & Wallis, 2001); and polyphenolic wastewater (Banerjee, 1997). Malandra *et al.* (2003) treated winery wastewater by means of a small scale RBC with 16 polystyrene discs. Winery wastewater was pumped through the system after excessive grape marc was removed and the hydraulic retention time was set between 0.35 and 1.4 hours. Due to the short hydraulic retention time the COD reduction rates were found to be only 43%. The authors mention that although the reduction is not as high as when compared to treatments such as anaerobic digestion or the activated sludge process, the RBC could be used as an effective pre-treatment option to lower the COD levels for further treatment by biological systems.

Trickling filters are an aerobic treatment system that is applied to wastewater to eliminate the organic material present therein (USEPA, 2000). This system operates by micro-organisms that attach to a medium to ensure the removal of organic matter. Trickling filters are also called attached-growth processes (USEPA, 2000). Filters contain fixed or rotating distributor arms that spray wastewater over media or rocks that are covered with a biological layer of slime. Due to the open spaces existing between the rock and other media, the process allows air to circulate through and consequently keep it oxygenated (Noyes, 1994; Moharikar *et al.,* 2005). The slime layer mainly consists of bacteria and algae but various other organisms (protozoa and metazoa) are also

present that have the ability to break down the organic matter (Nahid *et al.,* 2001; Moharikar *et al.,* 2005). Micro-organisms within the biofilm metabolise organic material into relatively harmless products (Vallero & Peirce, 2003). Although the trickling filter is easy to operate and manage, limitations include (Greiner & Timmons, 1998; Eding *et al*., 2006):

- Moderate removal rates;
- Biofilm detaching;
- Improperly designed filters could result in clogging; and
- Utilisation of low surface area media requires large volumes and floor space

The application of the trickling filter and activated sludge process on whey wastewater were investigated by Quirk & Hellman (1972). Other studies include the application of the trickling filter on brewery wastewater (Lemji & Eckstädt, 2013), domestic wastewater (Pal *et al*., 2010), sewage wastewater (Anon., 1961), food industry wastewater (El Defrawy & Shaalan, 2003), synthetic dairy wastewater (Raj & Murthy, 1999) and distillery wastes (Travieso *et al.,* 2006).

Wetlands

Wetlands, also termed constructed wetlands, are designed to eliminate impurities from contaminated water (Faulwetter *et al.,* 2009). This technology is an inexpensive alternative to conventional treatment options as it often functions without any mechanical or electrical equipment (Gray, 2010). Wetlands can be used as a depollution tool for both secondary and tertiary wastewater treatment, stormwater treatment and sludge stabilisation (Gray, 2010). Biodegradable organic compounds are decomposed by bacteria, fungi and actinomycetes that exist on exposed plants and soil in the wetland (USEPA, 1993). Wetlands are classified as either natural or constructed wetlands wherein large aquatic plants (macrophytes) like *Phragmites australis, Typha spp.* and *Scirpus spp.* occurs predominantly (Verhoeven & Meuleman, 1999; Kadlec *et al.,* 2000; Rousseau *et al.,* 2004). Macrophytes have several functions within wetlands (Brix, 1994):

- prevention of erosion;
- having a filtration effect on the water;
- supplying micro-organisms with a surface area to attach to;
- removal of nutrient rich materials;
- supplying oxygen to the system; and
- providing a habitation for wildlife

Natural wetlands include lake marginal, extensive fen systems as well as floodplain marshes (Verhoeven & Meuleman, 1999). Constructed wetlands include two different types of wetlands: (i) Infiltration wetlands, where wastewater runs vertically through permeable sediments and gathers within a drainage system; and (ii) Surface-flow wetlands where the wastewater runs parallel over sediments (Verhoeven & Meuleman, 1999).

Wetlands are well documented in literature and have been applied on a variety of wastes: dairy farm wastewater (Schaafsma *et al*., 1993); dairy parlour washings (Healy *et al*., 2007); leather processing wastewater (Calheiros *et al*., 2007); domestic wastewater and landfill leachate

(Vymazal, 2009); as well as winery wastewater (Shepherd *et al*., 2001). Another study on winery wastewater was investigated by Grismer *et al.* (2003) who evaluated the use of a constructed wetland to treat winery wastewater. The objective of the study was to determine the retention times of full-scale constructed wetlands and its treatment performance. The aforementioned was obtained by monitoring the water quality daily for pH, COD, TSS, total dissolved solids (TDS), ammonium, nitrogen, sulphates and sulphides. Two full-scale subsurface wetlands were evaluated during the harvest crush and spring season: 1) a wetland treating wastewater from a moderate-producing winery near Hopland, California; and 2) a smaller scale winery near Glen Ellen, California. At the Hopland wetland the authors found a COD reduction ranging from 49 - 79% and a tannin removal rate of 46 - 78%. They also found that the removal rates were greater during the non-crush period. At the smaller Glen Ellen wetland, the authors found an almost complete COD reduction rate of about 8 000 - 5 $mg.L^{-1}$. In this wetland however, they made use of a recirculation system, which suggests that when the wetland is properly managed and loaded it could be successful for the treatment of winery wastewater.

Disadvantages of wetlands include the requirement of large land areas and fairly level surfaces, possible occurrence and problems with pests and mosquitoes (Hammer & Bastian, 1989; Hammer, 1992), literature also states that wetlands could be a source of greenhouse gas emissions and that prolonged nutrient overloading could be fatal to biodiversity (Verhoeven *et al.,* 2006).

Sequencing batch reactor (SBR)

A SBR achieves equalisation, aeration and clarification in a scheduled sequence within one container that consists of five phases (Forbort, 2009): (i) reacting (aeration/mixing); (ii) filling; (iii) settling (sedimentation/clarification); (iv) draw (decanting); and (v) idling (Noyes, 1994; Torrijos *et al.,* 2001). SBRs are an example of a fill-and-draw activated sludge system and while the unit procedures in the SBR and activated sludge treatments are virtually the same there is one main difference. In conventional activated sludge systems, aeration and sedimentation occurs at the same time in separate tanks, whereas during SBR treatment the processes occur after one another in the same holding vessel (Tchobanoglous *et al*., 2003).

SBRs have been applied on municipal wastewater (Ni *et al.,* 2009; Monsalvo *et al*., 2012), soybean processing wastewater (Su & Hu, 2005), reject-wastewater (Wett *et al*., 1998), landfill leachate and domestic sewage (Diamadopoulos *et al.,* 1997), hypersaline wastewater (Woolard & Irvine, 1998), dairy wastewater (Torrijos *et al.,* 2001; Sirianuntapiboon *et al.,* 2005) and poultry wastewater (Pierson & Pavlostathis, 2000). Torrijos & Moletta (1996) investigated the treatment of winery wastewater by means of a SBR. The authors found that the SBR was an effective depollution tool for the treatment of winery wastewater as a total COD reduction of 90% was found. Other results also included a 95% soluble COD reduction, 97.5% BOD_5 reduction and a 50% and 88% reduction of nitrogen and phosphorous, respectively.

Although proven to be an effective depollution tool for the treatment of winery wastewater the following limitations are worth mentioning: (i) when compared to other conventional treatments, skilled operation for timing and control units are needed (Noyes, 1994); (ii) there is potential risk for settled or floating sludge to be discharged during the drawing or decanting phase; and (iii) equalisation is sometimes needed after treatment (Arvanitoyannis *et al.,* 2007b).

Anaerobic treatment

Anaerobic lagoons

Depending on the type of treatment option it is used for, this technology is also referred to as a polishing, stabilisation, or maturation lagoon. Anaerobic wastewater lagoons are constructed to treat wastewater before it is reused or released into natural watercourses (Mihelcic *et al.,* 2009). Anaerobic lagoons are deep non-aerated earth basins (Mihelcic *et al.,* 2009) with adequate volume to allow sedimentation of solids, digesting sludge and to degrade certain soluble carbon-rich material. Wastewater normally enters the lagoon at the bottom where it is mixed with active microbes within the sludge blanket (USEPA, 2002). Microbes degrade organic particles anaerobically, with a BOD_5 reduction rate of 50 - 80%. Anaerobic lagoons are mostly used for: (i) primary or secondary treatment of wastewaters having high organic loads (Gray, 2010); or (ii) for the treatment of sludge (Green & Kramer, 1979). This treatment is normally pursued by treatments such as the trickling filter or facultative lagoons (Green & Kramer, 1979). The anaerobic degradation of organic materials to carbon dioxide and methane is a complex biological and chemical process that includes four phases: hydrolysis; acidogenesis; acetogenesis; and methanogenesis (Tommaso, 2011). During the first phase complex compounds are broken down to smaller intermediates through hydrolysis (Banks & Wang, 2006). During phase two intermediates are broken down to simple compounds such as alcohol, lactic acid, volatile fatty acids (VFA) and CO_2. (van Lier *et al.*, 2008). In phase 3, acetogens use these smaller intermediates to produce organic acids that are used by methanogens (phase 4) to produce methane and carbon dioxide (Banks & Wang, 2006). Anaerobic lagoons have been employed to treat swine waste (Sharpe & Harper, 1999), dairy wastewater (Baena *et al*., 1998), sludge solids (Parker & Skerry, 1968) and distillery waste (Rao, 1972).

Although anaerobic lagoons are suited for treating high strength wastewaters, main limitations are the requirement of large land areas (Stubbart *et al*., 2006) and controlling odorous compounds (Heber *et al*., 2002).

Anaerobic filter (AF)

Anaerobic biological filters were first used at the end of 19th century for the treatment of sanitary sewage (Tommaso, 2011). The anaerobic contract filter is basically a non-aerated trickling filter with retention times of up to three days (Woodard & Curran, Inc., 2006). This process entails the fixing of biofilm onto a carrier that is a few hundred squares per cubic metre (Habouzit & Torrijos,

1998). Two types of systems exist: an upflow; or downflow filtering process. Typically, during the treatment process the wastewater is recirculated to have a homogenous distribution of wastewater (Habouzit & Torrijos, 1998). AFs have been used in treating confectionary wastewater, seafood-processing wastewater, fruit canning wastewater, winery wastewater and cheese dairy wastewater (Mendez *et al*., 1995; Di Berardino *et al*., 2000; Rajinikantha *et al*., 2009). Although AFs can be used as a main treatment option is more suitable as a post-treatment option (polishing step) where it adds operational safety and stability to the whole process (de Lemos Chernicharo, 2007).

A study done by Habouzit & Torrijos (1998), investigated the effect of treating winery wastewater by means of an acidogenic reactor followed by an AF and finally an aerobic post-treatment. Wastewater was obtained from a winery in France that produces approximately 35 000 hectolitres of wine per year. The COD of the wastewater generated varied between 8 - 16 $g.L^{-1}$. The authors found a COD removal rate of 24% during the acidogenic phase and a 70% removal rate during the anaerobic filter treatment. Another study done by Yu *et al*. (2006) investigated the efficacy of a lab-scale multi-fed upflow anaerobic filter process for the treatment of rice winery effluent. The reactor functioned at a temperature of 19 - 27°C, with an influent COD ranging from 8.34 - 25.76 $g.L^{-1}$. The authors found an 82% COD reduction rate with an OLR as high as 37.68 $gCOD.L^{-1}.d$ and a hydraulic retention time of 8 h.

The main drawback of upflow anaerobic filters is the difficulty in sustaining the necessary contact between the untreated wastewater and the sludge due to clogging (van Lier *et al*., 2008). In order to avoid filter clogging special care should be taken in removal of suspended solids (Tommaso, 2011). Literature also reports the following limitations: a large footprint with influent dispersion problems as well as a very long start-up period (Els *et al*., 2005).

ANAEROBIC DIGESTION (AD)

Anaerobic technology as a treatment option is acknowledged as one of the main advanced treatment options for ecological protection and when combined with other suitable procedures, it serves as a sustainable and suitable wastewater treatment option in developing countries (Seghezzo *et al*., 1998). One of these treatment options is anaerobic digestion (Britz *et al*., 2002). During the 1900's AD was already employed worldwide, mainly in the form of anaerobic ponds for treating sewage (Els *et al.,* 2005). It is defined as a fermentative process where organic materials are broken down and biogas is produced. This process will mainly occur when carbon-based materials are available and the redox potential is low (van Lier *et al.,* 2008). It is therefore often found in environments where no oxygen is present like waterways, sediments, marshlands and the gut of mammals (van Lier *et al.,* 2008).

AD serves as an attractive treatment option, as both depollution and energy recovery can be accomplished (Chen *et al*., 2008). It is the most suitable treatment option for high strength organic wastewater (Rajeshwari *et al*., 2000) and has been applied universally for the treatment of industrial wastewater (Moletta, 2005). Because the quality of the final effluent generated is not as good as obtained by aerobic treatment, AD is often used as a pre-treatment step where after

wastewater is released into municipal systems or undergoes an aerobic post-treatment step (Tchobanoglous *et al*., 2003). This process is a complex set of reactions wherein several groups of anaerobic and facultative organisms absorb and degrade organic material simultaneously (Cheremisinoff, 1996). Anaerobic digestion of winery wastewater is the most feasible treatment option as significant COD reductions have been reported (Toffelmire, 1972; Keyser *et al*., 2003; Moletta, 2005, Melamane *et al*., 2007; Ganesh *et al*., 2010).

Reactor types

Various types of anaerobic digesters exist: (i) bacterial growth can be classified as either suspended or fixed film; (ii) temperature is characterized as a psychrophilic, mesophilic and thermophilic system; and (iii) configuration which include a single stage phase or a two-stage phase (Gerardi, 2003). Recently, a considerate amount of time has been spent on developing anaerobic reactors for the treatment of wastes, to convert organic material into biogas (Rajeshwari *et al*., 2000).

Anaerobic expanded granular bed (EGSB) and anaerobic fluidised bed reactor systems (AFBR)

These systems involve the formation of active biofilms that developed on small, inert substrate material (McCarty & Smith, 1986). The expanded granular sludge bed reactor combines the features of both the Upflow Anaerobic Sludge Blanket (UASB) as well as the Biofilm Fluidised Bed (BFB) reactors. Like the UASB, biomass is present within the reactor in a granule form but the upflow velocities of the liquid (10 $m.h^{-1}$) and the gas (7 $m.h^{-1}$) are based on principles similar to the BFB reactor (Nicolella *et al.,* 2000a). By making use of effluent recirculation and a taller reactor, it led to the development of the EGSB (Seghezzo *et al*., 1998; Kato *et al*., 1999).

The EGSB reactor uses granular sludge that is known for having higher methane and good settling properties (van Lier *et al.*, 2008). The EGSB reactor has a cylindrical construction filled with supporting inert material that occupies 10% of the reactor volume (de Lemos Chernicharo, 2007). Various particles have been utilised as supporting materials including gravel, sand, coal and polyvinyl chloride. Biofilm becomes attached to the supporting material, which is expanded by the upward velocity of the liquescent (de Lemos Chernicharo, 2007). The EGSB has been used in the treatment of brewery wastewater, slaughterhouse wastewater, starch-containing wastewater, cold wastewater and malt-containing wastewater (Rebac *et al*., 1998; Kato *et al*., 1999; Núñez & Martínez, 1999; Guo *et al*., 2008a, Guo *et al*., 2008b). Advantages of these reactors are that the upflow velocity liquescent causes the sludge bed to expand which removes dead zones and allows better contact between the sludge and the wastewater. Another advantage is complete odour control due to the completely closed design of the EGSB (Zoutberg & de Been, 1997). Limitations include poor removal of SS and colloidal matter other than flocculent sludge washout which is a common occurrence (Seghezzo *et al*., 1998).

The working principles of the anaerobic fluidised bed reactor (AFBR) are very similar to those of the EGSB, excluding the size of the supporting materials and the expansion rate. The

upward velocity in this reactor should be high enough to fluidise the bed so that the gravity force equals the upward drag force (de Lemos Chernicharo, 2007). AFBR is one of the most suited methods for treating sewage at low temperatures as the reactor can sustain enough active microbes to overcome the limitation associated with slow growing anaerobic microbes (Sanz & Fdz-Polanco, 1990). The organisms responsible for hydrolysis and acidogenesis are associated with entrapped SS while the microbes responsible for methanogenesis are to be found in the film. This way, both methanogenesis and hydrolysis can be improved (Jewell, 1985). By utilising fluidised media, the reactor can maintain high biomass concentrations and consequently operate at lower HRT (Fernández *et al*., 2008). One of the biggest variables in using AFBR technology is selecting a suitable supporting material for microbial adhesion (Montalvo *et al*., 2008). AFBRs have been employed in phenolic wastewater (Bajaj *et al*., 2009), synthetic meat wastewater (Rudd *et al*., 1985), food processing wastewater (Wei *et al*., 2011), sewage (Sanz & Fdz-Polanco, 1990) and high-strength distillery wastewater (Fernández *et al*., 2008). Montalvo *et al*. (2008) treated red winery wastewater and tropical fruit wine in lab-scale mesophilic anaerobic fluidised bed reactors by using natural zeolite as supporting particles. Results found, showed that both reactors obtained a COD removal rate of more than 80 – 86% at an OLR up to 20 $gCOD.L^{-1}$ d^{-1}.

A serious limitation of the AFBR is when biofilm develops on the supporting carrier materials, as the density of the film covered particle decreases, resulting in washout. Full-scale applications of this technology are rare due to improperly sound design principles (Saravanan & Sreekrishnan, 2006) and monitoring biofilm attachment to supporting carrier particles (Zoutberg & de Been, 1997).

Internal circulation reactor (IC)

Due to washout-related problems experienced with conventional UASB reactors at start-up, more advanced anaerobic reactors have been acquired. One of these advanced technologies is the IC reactor (Liu & Tay, 2004). The first brewery utilising the IC system was the Heineken brewery in Den Bosch, The Nederlands in 1990 (Yspeert, 1993).

The IC reactor contains two upflow sludge blanket like reactor sections on top of each other with one highly-loaded and another low-loaded (Driessen & Yspeert, 1999). The first part has an EGSB where COD are mostly converted to biogas. The biogas is gathered by the bottom phase separator to generate a gas lift that transports water and sludge upwards to the gas/solid separator (Nicolella *et al*., 2000a). Biogas is separated here and the water-sludge combination is lead downwards, causing an internal circulation flow (Nicolella *et al*., 2000b). As most biogas is eliminated by the first separator the turbulence in the reactor is considerably reduced which allows the second separator to separate the anaerobic sludge efficiently (Driessen & Vereijken, 2003). IC reactors also use anaerobic granular sludge but tank heights often increases up to 24 meters (Brito *et al*., 2007).

The Organic Loading Rate (OLR) of IC reactors are often twofold that of UASB systems (15 - 30 $kgCOD.m^{-3}.d^{-1}$) (Pereboom & Vereijken, 1994; Driessen & Vereijken, 2003). The biogas

generation is the driving force for producing the internal circulation flow through an airlift action (Christi, 1998). IC reactors have been used to treat low-strength dairy, medium strength food processing and high strength brewery (Marín *et al.,* 1999), swine (Deng *et al*., 2006) and inuline wastewaters (Habets *et al*., 1997).

Anaerobic sequence batch reactor (AnSBR)

This technology is a promising technique consisting of a fill and draw process. The digester holds the anaerobic sludge where to wastewater is added for digestion to occur (Moletta, 2005). The AnSBR reactor process is characterized as a suspended growth process with reaction and liquid-solid separating that occurs within the same container (Tchobanoglous *et al*., 2003). Similar to aerobic SBR (Tchobanoglous *et al*., 2003), the AnSBR also involves four repetition phases: 1) feeding; 2) reaction; 3) settling; and 4) decant/liquid withdrawal (Ruiz *et al*., 2002). Zaiat *et al*. (2001) summarized important features of the four phases: (i) during phase one different feeding strategies can be used in discontinuous reactors (either batch or fed-batch); (ii) the type of agitation used is important as mechanical mixing and recycling of the gas can lead to increased liquid-solid contact; and (iii) phase three is dependent on the self-immobilising properties of the biomass. The biomass (as granules) should have good settleabilty properties as it can increase the separation of the liquid and solid phase and (iv) the liquid withdrawal phase should take place as quick as possible because oxygen could affect the anaerobic bacteria. The operation of this reactor has the capability to continue its mode of working until the satisfactory level of organic degradation has been achieved (Tommaso, 2011).

Several studies have investigated the AnSBR to treat cheese whey, brewery wastewater, fruit and vegetable wastes, palm oil mill effluent and dairy wastewater (Bouallagui *et* al., 2004; Mockaitis *et al*., 2006; O-Thong *et al.,* 2007; Xiangwen *et al*., 2008; Donoso-Bravo *et al*., 2009). Ruiz *et al*., (2002) investigated the treatment of winery wastewater by means of a lab-scale AnSBR. Wastewater was obtained from a winery in Narbonne, France with an average COD of 19.7 $g.L^{-1}$ and a TSS concentration of 1.4 $g.L^{-1}$. The experiment was carried out in a double walled 5L mesophilic reactor, using wastewater with an average OLR of approximately 8.6 $gCOD.L^{-1}.d^{-1}$. The results obtained by the authors showed a COD removal rate of greater than 98%.

Anaerobic hybrid reactor (AHR)

As anaerobic digestion is a common treatment option for high strength wastes, newer digesters are being established for treating both high and low strength wastewaters (Britz *et al*., 1990). Some technologies include more than one approach for instance a sludge bed with an anaerobic filter as in the hybrid digester (Büyükkamaci & Filibeli, 2002; Arvanitoyannis *et al*., 2007b).

In AHR technology, advantages of the sludge bed reactors and the fixed-bed reactors are combined into a reactor where 1) the support matrix is limited to the upper part and 2) the flocculant or granular sludge develops in the lower half. The upper half has a double function as it holds the suspended sludge and serves as a polishing step through biofilm development (Henry *et*

al., 1996). The lower part of the reactor functions as a UASB and contains granular sludge while the upper part contains a randomly-packed matrix to aid biomass retention and a surface for microbe attachment (McHugh *et al.,* 2003). Most of the organic material is converted to biogas in the lower UASB part of the reactor but any residual COD in the wastewater will be degraded by the AF part in the upper level (McHugh *et al.,* 2003).

A limitation of AHRs includes the difficulty in controlling biofilms (Tizghadam *et al.,* 2008) and that AHRs are more suited for the treatment of wastewater where granular sludge formation is problematic such as wastes from the chemical industry (Anon., 2013b). This technology has been applied in treating baker's yeast wastewater (a hybrid digester and an anaerobic filter), municipal landfill leachate (UASB and a fixed film reactor), synthetic pharmaceutical wastewater, slaughterhouse wastewater (sludge blanket and filter) and winery wastewater (UASB unit and a fixed bed anaerobic filter) (Britz *et al.,* 1990; Myburg & Britz, 1993; van der Merwe & Britz, 1993; Borja *et al*., 1998; Di Berardino *et al*., 2001).

Upflow Anaerobic Sludge Blanket (UASB)

The UASB is a well-established and proven technology for the treatment of high-strength organic wastewater due to the high biomass and microbial communities within the reactor (Liu *et al*., 2003). The UASB system is widely applied for treating wastewaters from the food industry, distilleries, tanneries and municipalities (Saleh & Mahmood, 2004).

The reactor was originally described by Ross and developed by Lettinga and his co-workers (Lettinga *et al*., 2001). Today, UASB reactors are one of the most broadly applied treatment options, and commonly applied for treating industrialised effluents (Li & Yu, 2011). This system is based on the main principal that micro-organisms can form dense granules by auto immobilisation (Fuentes *et al*., 2009). Anaerobic granular sludge is the main constituent of UASB technology (Liu *et al.,* 2002) and is therefore also referred to as anaerobic granular sludge bed reactors (Li & Yu, 2011). This anaerobic technology is characterised as a high-rate method that involves three stages: liquid; solid (biomass or sludge); and gas (Hung *et al.,* 2008). The UASB reactor contains a rectangular or circular container (van Lier *et al*., 2008) where wastewater enters at the bottom and flows upwards through a dense layer of microbes (sludge blanket) for the degradation of organic particles to CH_4 and CO_2. The resulting effluent exits the reactor through an outlet at the top (Droste, 2004; Mittal, 2006). The biogas that is generated causes hydraulic turbulence when it moves upwards through the reactor resulting in sufficient mixing within the system. This eliminates the need to add mechanical mixing (McHugh *et al.,* 2003). Advantages of the UASB include the ability to handle high organic loading rates at fairly low hydraulic retention times, a low energy demand (Tchobanoglous *et al*., 2003) and being able to endure discontinuous operation (Droste, 2004). Although the UASB reactor is reported to be more sensitive to waste constituents than other technologies and start-up is difficult as specific attention is needed for the development of the sludge (Droste, 2004) the UASB reactor is the most broadly and successfully

applied anaerobic system (van Lier *et al.,* 2008). The UASB reactor has been employed in varies types of wastewater and are shown in Table 2.5.

The UASB reactor has also been employed in various studies for treating winery wastewater. Kalyuzhnyi *et al.* (2000) treated diluted winery vinasse (1 - 17 gCOD.L^{-1}) by means of psychrophilic (9 - 10°C, 18 - 20°C) and mesophilic (20 - 35°C) reactors during four trials. Both the reactors were seeded with flocculant mesophilic sludge, but the psychrophilic reactor had an additional 30% adapted psychrophilic granular sludge. The COD reductions were higher than 85% for the higher temperature reactors and just above 60% for the 9 - 10°C psychrophilic reactor. Moosbrugger *et al.* (1993), treated grape wine distillery waste by means of a UASB reactor. The authors found a COD removal of 94% with a maximum OLR of 19 kgCOD.m^{-3}.d^{-1}. Keyser *et al*. (2003) evaluated three UASB reactors for treating winery wastewater. The control reactor was seeded with only sewage sludge. The pH during the trial varied (5.5 - 7.5) continuously clearly showing an unstable state. Even after 90 days the COD removal never reached 70%. The reactor showed problems commonly experienced with ordinary sludge seeding and had to be re-seeded constantly. Trial two consisted of an UASB reactor seeded with granular sludge which was enriched with *Enterobacter sakazakii.* The authors reported a COD removal of 90% in only 17 days with a HRT of 24 h. The last trial consisted of an UASB reactor that was seeded with brewery granules. Results showed a COD removal of 85% within 50 days. These results also indicated that granular seeding plays a significant role in reactor start-up and that UASB serves as an effective treatment option.

Table 2.5 Applications of UASB technology to treat various wastes

Waste	COD removal	Temperature	Reference
Leachate from food waste	96%	Mesophilic	Shin *et al.,* 2001
Canning and winery	53.0 - 98.9%	Mesophilic	Sigge *et al*., 2005
Bagasse-based wastewater	80 - 85%	Mesophilic	Chinnaraj & Rao, 2006
Cheese-producing wastewater	98%	Mesophilic	Gavala *et al.,* 1999
Black water and dairy parlour	> 80%	Psychrophilic	Luostarinen & Rintala, 2005
Dairy and domestic wastewater	69%	Psychrophilic	Tawfik *et al*., 2008
Tannery soak liquor	78%	Mesophilic	Lefebvre *et al*., 2006
Distillery wastewater	> 90%	Mesophilic	Wolmarans & de Villiers, 2002
Pot-ale from malt-whiskey	80%	Mesophilic	Goodwin *et al.,* 2001
Opaque brewery wastewater	57%	Mesophilic	Parawira *et al*., 2005
Distilled cane molasses	39 - 67%	Thermophilic	Harada *et al*., 1996

Lettinga (1995) stated that limitations of AD are not serious and can be overcome. Van Lier *et al*. (1992) illustrated that one of the main limitations of an UASB reactor namely the long start-up period could be significantly reduced by using granular sludge as a seeding material.

C. TREATMENT OPTIONS FOR SOLID WASTE

Solid waste

Managing solid waste in the food industry is often a problem, as it often represents 30% of incoming raw materials (Schaub & Leonrad, 1996). Presently the management of winery wastes are handled by external companies but this is a very expensive option for the wine industry in terms of disposal and transport costs (Ruggieri *et al*., 2009). In South Africa, the leading company for the processing of solid winery waste is Brenn-O-Kem located in both Wolseley and Worcester (Dillon, 2011; Anon., 2012). Brenn-O-Kem produces wine spirits, calcium tartrate, grape seed tannin, grape seed oil and grape skin by-products from grape pomace (Anon., 2013a). Wastetech is a registered waste and recycling company, that handles most solid waste removals within the South African wine industry (Dillon, 2011). Waste removal by means of external companies is costly and has problematic alternatives such as high transportation, high disposal and high environmental impacts (Ruggieri *et al*., 2009). Brenn-O-Kem processes approximately 25 – 30% of the total solid waste generated by the South African wine industry. Their contracts are with nearly 25% of all wineries, mostly allocated in the Breede River valley and the transportation costs are covered by the winery itself (Dillon, 2011).

Solid waste is disposed by means of landfilling, incineration, pyrolysis (Mariani *et al*., 1992; Encinar 1997; Di Blasi *et al.,* 1999; Kulcu & Yaldiz, 2005), aerobic composting (Manios, 2004; Flavel *et al.,* 2005) vermicomposting (Nogales *et al*., 2005) and anaerobic composting (O'Keefe *et al.,* 1993; O'Keefe *et al*., 1996; Mata-Alvarez *et al*., 2000; Griessel, 2002).

Landfilling

Possibly one of the oldest and most-common methods of solid waste disposal is by means of landfill-site dumping. This method of disposal is relatively simple, but careful selection needs to be considered for the site in order to avoid environmental problems. One of the most important features of landfill-site dumping is the ability to generate landfill leachate (Britz *et al.,* 1990). Liquid emissions are known as "leachate" (Shabiimam & Dikshit, 2011) or aqueous effluent that is commonly generated by rainwater trickling through waste.

Primarily the landfilling of solid waste was done at the lowest expense possible; therefore waste was disposed of on nearby low-value lands, and often wetlands to generate a waste dump (Lee & Lee, 2004). Various processes in the waste (microbial, physical, and chemical) can transfer the impurities to the rain water that is percolating through. One of the main environmental challenges globally is the proper treatment and safe disposal of leachate (Shabiimam & Dikshit, 2011).

Leachate can be classified according to the age of the landfill. Leachate from landfills under the age of five years are termed "young leachate" with a pH under 6.5 and COD values as high as 10 000 $mg.L^{-1}$. Landfill leachates between the ages of five to ten years are referred to as

"intermediate leachate". Typical COD values in the ranges of 4 000 - 10 000 mg.L^{-1} have been reported for intermediate leachates with a pH of 7 (Alvarez-Vazquez *et al*., 2004). Leachates from landfills over 10 years old are classified as "stabilised leachate". The COD values are less than 2 000 mg.L^{-1} with a less significant biodegradable content and are also termed "old leachate" (Shabiimam & Dikshit, 2011).

The most important environmental impacts that are related to landfill-site dumping are the pollution of ground- and surface waters by landfill leachate (Kjeldsen *et al.,* 2002). Pollution of these sources is the most severe ecological effect caused by landfill run-off, because most landfills were traditionally built without engineered linings and leachate collection systems (Kjeldsen *et al.,* 2002). Particular attention should therefore be paid to leachate generated by landfills as various studies have shown that even an insignificant amount (less than 40 000m^3) of leachate could have an impact on groundwater (Bagchi, 1987).

Incineration

Incineration is defined as the thermal destruction of combustible waste in an enclosed device (DWA, 2013). The process takes place in a combustion chamber on fire grates, where after ash is transported with descending sloped floors (Ojovan & Lee, 2005).

Compounds commonly present in this waste include nitrogen, phosphorous, sulphur, halogens or metal (Lauridsen, 2008). Incinerator technologies that are currently being applied include rotary kiln, gasification, liquid injection and fluidised bed types. The temperatures used within these chambers ranges between 1 000 and 1 900°C (Lauridsen, 2008). Materials are burned at a controlled temperature which is high enough to destroy harmful chemicals. When materials are broken down, gas is produced that passes through equipment to remove remaining compounds such as metals, acids and ash particles (USEPA, 2012).

Air emissions from incinerators are one of the key issues that are crucial to control. Incinerator facilities in South Africa are mostly controlled by guidelines as stipulated in the South African Atmospheric Pollution Prevention Act, 1965 (Act 45 of 1965). The operational site and air emissions from incinerators should frequently be inspected by air contamination authorities (DWAF, 2000b). The collection, transportation and storage preceding the incineration process must also comply with specific conditions as prescribed in the South African Health Act, 1977 (Act 63 of 1977) (DWAF, 2000b).

Major disadvantages of this process are: (i) the high operating and capital costs; (ii) the presence of several metals, organic polluting substances; (iii) dioxins and furans in the air being released; (iv) remaining ash produced as an end product also needs to be disposed of in an appropriate manner as it may contain toxic materials; and (v) as also found with landfills, the siting of the facility could potentially be problematic (Chang, 2009; Herselman, 2009). Although a proven and well-established treatment option as well as the most-commonly used technology for treating nuclear waste, these advantages should always be weighed against the mentioned restrictions (Herselman, 2009).

Pyrolysis

Pyrolysis can be described as the thermal breakdown of biomass at high temperatures under anaerobic conditions to produce solid (char), liquid (bio oil) and gas (syngas) products (Bridgwater, 1999). The proportions of these products are dependent on the pyrolysis technique and the parameters chosen. Techniques, that involves slow heating over longer periods of time produces high char yields with adequate quantities of tar by-products (Onay & Kockar, 2003). Higher heating processes that are accompanied by a shorter reaction time produce higher liquid yields (Onay & Kockar, 2003).

Different pyrolysis techniques can therefore be divided into three subclasses: (i) conventional pyrolysis also termed carbonisation; (ii) fast pyrolysis; and (iii) flash pyrolysis (Demirbaş & Arin, 2002). Conventional pyrolysis is defined as a method that ensues under a slow heating rate to produce the highest char yield (Gheorghe *et al*., 2009) while occurring between temperatures of 300 - 700°C (Maschio *et al.,* 1992). During fast pyrolysis biomass is heated rapidly and generates aerosols, vapours and charcoal (Bridgwater, 1999). Fast pyrolysis is defined as a decomposition process at moderate heat temperatures with high heat transfer rates (Czernik & Bridgewater, 2004). The temperature range of this process slightly increases compared to conventional pyrolysis, with temperature reaching 600 – 1 000°C (Maschio *et al.,* 1992). Flash pyrolysis is accompanied with the smallest particle sizes and occurs at temperatures between 800 – 1 000°C (Maschio *et al.,* 1992).

During pyrolysis, solid waste is kept in a basket within the refrigeration sector of the reactor. A gas flow of nitrogen (200 $cm^3.min^{-1}$) is passed through for an hour to remove air from any part of the connection. Thereafter, the basket is fed through a heating zone and the process starts (Arvanitoyannis *et al.,* 2007b). The liquid fraction that is produced during pyrolysis consists of two phases: (i) a water soluble phase containing organic-oxygen material of a low molecular weight; and (ii) a water insoluble phase consisting out of insoluble carbon-rich material (bio oil) with a high molecular weight (Demçirbaş & Arin, 2002).

Encinar *et al.* (1997) studied the effect of pyrolysis on olive and grape bagasse to determine the most important characteristics of the charcoals formed as well as the quality and amount of gasses and liquids that were generated. The main gasses produced were hydrogen, carbon dioxide, carbon monoxide and methane. Amongst the liquids produced the authors found methanol, phenols, furfuryl alcohol, furfural and acetone. Di Blasi *et al.,* (1999) also investigated the pyrolysis characteristics of various agricultural residues on bench scale. These residues included wheat straw, olive husks, grape marc and rice husks with wood chips. The authors found that the devolatilization rates for grape residues were the slowest of all the residues and due to their higher lignin content, it was also found that olive and grape residues generated higher yields of ethylene (C_2H_4) and ethane (C_2H_6). Difficulties have been experienced with applying pyrolysis technology to solid waste. The temperatures in the pyrolysis chamber are adequate to keep ash and residues molten but difficulty arises in controlling the solidification of the molten particles as it exits from the reactor. Uneven cooling of molten ash causes unnecessary slagging which blocks

the reactor outlet and consequently prevents ash discharge. The slag must first be removed by shutting down the reactor in order for the process to continue (Noyes, 1994).

Composting

Approximately 80% of total waste generated by the wine making process is organic waste or organic by-products (Gea *et al*., 2005). In Uganda, landfilling and incineration was traditionally the most-common means of banana peel disposal, but the practices thereof have been proven to be unsustainable (Kalemelawa *et al*., 2012). Landfilling and incineration has also been a common method employed by food industries to dispose of solid food waste (Schaub and Leonard, 1996). Landfilling is often limited due to land scarcity (Kalemelawa *et al*., 2012) and incineration processes are energy consuming (Ke, 2010). Schaub and Leonard (1996), also reported environmental regulations and concern, in addition to the costs and closure of the landfill sites instigated other options to be considered.

An alternative option for the management of organic (winery) waste is composting (Nakata, 1994; Flavel *et al*., 2005; Ruggieri *et al*., 2009) as research has shown that due to the nature of its contents, pomace could be recycled as a soil conditioner (Diaz *et al.,* 2002; Flavel *et al.,* 2005; Brunetti *et al.*, 2011).

Three kinds of composting are known: aerobic composting; vermicomposting; and anaerobic composting (Horn, 1995).

Aerobic composting

Composting is defined as the biochemical degradation of organic materials to obtain a sanitary, soil-like end product (Kulcu & Yaldiz, 2005) which occurs in the presence of oxygen (Liang *et al.,* 2003; Zhu, 2007). Composting can be classified into four categories: windrow composting; aerated windrows; aerated static piles; and in-vessel composting. The aerated and in-vessel methods are commonly used when wastes are available in high concentrations, whereas windrow composting is more suited to farm operations (Lopez-Real, 1996).

The terms “stability” and “maturation” are often used interchangeably in composting even though they refer to different properties within the product (Said-Pullicino *et al*., 2007). These terms are generally used to describe the quality of compost (Som *et al*., 2009; Guo *et al*., 2012). Stability is defined as the degree to which organic material has been decomposed (Wu *et al*., 2000), whilst having resistance to additional diminishing (Wichuk & McCartney, 2010). Heat production, oxygen-uptake and carbon dioxide- release are parameters used to measure compost stability as it represents microbe activity (Ke, 2010). Mature compost will not cause unfavourable effects (phytotoxicity) to growing components or to the environment (Ke, 2010) and is typically defined as the degree to which phytotoxic organic materials have been degraded (Wu *et al*., 2000). Phytotoxicity to a plant can occur when organic material has only been partly decomposed (Said-Pullicino *et al*., 2007). Wichuk and McCartney (2010) describe mature compost as being “ready for a specific end use”. Maturity of compost is evaluated by plant development or by using sensory

activity (Iannotti *et al*., 1993). When immature and/or unstable compost is applied as an organic amendment it could inhibit plant growth by binding nitrogen and releasing noxious elements in the soil (Guo *et al*., 2012).

Aerobic composting is well documented and successfully applied on a variety of wastes: municipal solid waste (Pietro & Paola, 2004); biosolids from wastewater treatment (Liang *et al.,* 2003); olive tree branches, olive tree leaves, vine branches, pressed grape skins and pig manure (Manios, 2004); as well as green waste and biowaste (Som *et al*., 2009). Arvanitoyannis *et al.* (2007a), reported that the use of compost produced from winery wastes is increasing due to the overall state of soil, characterised by low levels of humus. Compost derived from pressed grape skins showed to produce one of the highest quality soil conditioners in terms of agronomic and physical features (Manios, 2004).

Composting as a treating option provides numerous advantages towards the food industry (Schaub & Leonard 1996). The volume of organic waste is reduced by 40%, major costs are saved as disposal of the compostable wastes are not necessary and the final end product can generate income (Schaub & Leonrad, 1996). When the end-product is applied as an organic amendment it improves the water holdings properties of the soil, reduces temperature variations and supplies nutrients (Flavel *et al*., 2005). Disadvantages of this process include: the high investment costs for the preparation of land and machinery like the construction of concrete floors and leachate collection systems; the stalk shredder as while as additional machinery needed for pile-turning (Ruggieri *et al*., 2009); and finally this process is labour intensive and time consuming (Horn, 1995).

Vermicomposting

During vermicomposting earthworms transform complex organic waste into a stable humus-like product (nutrient-rich bio-fertiliser) called vermicompost (Suthar, 2010) and earthworm biomass (Ndegwa & Thompson, 2000). The worms feed on the organic material present and emit the undigested materials as worm casts (Bansal & Kapoor, 2000) or vermicompost (Suthar, 2010). According to Munroe (2012), approximately 1800 species of earthworms exist globally. The *Eisenia fetida* is an extremely tough and adaptable worm (Munroe, 2012) known as the compost worm (Yadav & Garg, 2009) or the red wriggler (Ndegwa *et al*., 2000) which is native to most parts of the world.

Vermicomposting is categorised by both mechanical as well as biochemical procedures. Mechanical processes are characterised by the ventilation, mixing and grinding of the waste substrates (Ndegwa & Thompson, 2000). The biochemical process is represented by the decomposition of solid waste within the intestine of the earthworm (Nogales *et al*., 2005). Although the vermicomposting process is stabilised by earthworms and micro-organisms, the earthworms are the actual drivers of the process (Suthar, 2010). The microbes are responsible for the decomposition of the organic material while the worms improve the substratum and change the biological activity (Suthar, 2009).

Worms function as machine-driven blenders, changing the biological, physical and chemical form of the organic material. Through this process the C:N ratio of wastes are reduced and the uncovered surface area for microbes are increased which enhances the disintegration of waste (Domínguez, 2004). Vermicompost is a carbon-rich source with a high mineralisation rate that enhances the nutrient availability to plants (Domínguez, 2004) as the nutrient availability per kilogram weight is more than the carbon source from which it was originally produced (Garg *et al*., 2006)

Vermicomposting have been successfully applied in winery waste (Nogales *et al*., 2005), vegetable-solid waste (Suthar, 2009), tannery sludge mixed with cattle dung (Vig *et al*., 2011), sludges from paper mill and dairy (Elvira *et al*., 1998), kitchen waste, agro-residues, institutional and industrial wastes (Garg *et al*., 2006), and crop residues and cattle dung (Bansal & Kapoor, 2000).

One of the main disadvantages of this process is the maintaining of the temperature to below 35°C, as higher temperatures will kill the earthworms (Alidadi *et al*., 2005). The temperature is insufficient to destroy harmful pathogens and therefore the end product does not comply with the United States Environmental Protection Agency (USEPA) for the destruction of pathogens (Alidadi *et al*., 2005). Other disadvantages of vermicomposting over traditional composting includes: a larger space requirement as worms are surface feeders; and more resources are needed for the start-up process by either the need to acquire worms or the time and labour to grow them (Munroe, 2012).

Anaerobic composting (AnC) or Anaerobic digestion (AD) of solid waste

Anaerobic composting (AnC) or anaerobic digestion (AD) of solid waste is a natural occurring process in which micro-organisms degrade organic matter to nutrients in a simpler form in an anoxic environment (Liang *et al*., 2003). AD of solid waste is the main degradation process found in a landfill (Rapport *et al*., 2008). The main advantages of this process is the production of biogas an organic amendment (Khalid *et al*., 2011) and a liquid effluent, which can be used as a renewable energy source and a valuable soil conditioner, respectively (Mata-Alvarez *et al*., 1992). Anaerobic treatment of solid wastes is classified according to certain parameters: (i) the continuousness of the system; (ii) the temperature at which the system operates; (iii) the design of the anaerobic reactor; and (iv) the solid content of the waste (Li *et al*., 2011). AnC as a treatment option is used increasingly by farms and agro-industrial corporations to produce methane (Lesteur *et al*., 2010).

Greenhouse gasses like carbon dioxide (CO_2) and methane (CH_4) are produced during landfill-site dumping. These gasses are released into the earth's atmosphere and cause severe ecological pollution (Khalid *et al*., 2011). If the same principles are applied in a controlled AnC surrounding, favourable results are obtained: (i) decomposition in a sealed environment prevents methane from escaping to the atmosphere; and (ii) if not used as a renewable energy source burning of methane will release carbon-neutral CO_2 (Ward *et al*., 2008).

Even though various gasses are produced within a digester, the only gas of economic value is biogas, which consists out of approximately 60 - 65% methane and 35 - 40% carbon dioxide (Gerardi, 2003). Biogas can serve as a renewable source of energy which can replace the use of fossil fuels in the production of power, heat and vehicle fuel (del Real Olvera & Lopez-Lopez, 2012).

Anaerobic composting as a treating option has been applied to a variety of wastes: crab-picking wastes (O'Keefe *et al.,* 1996); municipal solid waste (O'Keefe *et al*., 1993); fruit and vegetables waste (Bouallagui *et al*., 2005); and industrial wastes (Khalid *et al*., 2011).

Although AnC is not as widely recognised as aerobic composting, (Fernández *et al*., 2008) this process should be the preferred method for the treatment of solid waste as: (i) It is a net producer rather than a consumer of energy (O'Keefe *et al*., 1993); (ii) a higher organic loading rate (Bouallagui *et al*., 2005); and lower biomass are obtained (Ward *et al.,* 2008); (iii) less environmental pollution and odour emissions are obtained as all liquids and solids produced during this process are captured within a digester; and (iv) because no aeration is required for the process and therefore no bulking agents, a substantial reduction in the volume of waste is allowed (O'Keefe *et al*., 1996).

D. THEORY OF ANAEROBIC DIGESTION

AD is a proven method for treating liquid, solid and semi-solid carbon-based wastes, offering benefits above conventional (aerobic) methods, particularly from an energetic and ecological point. Sludge produced is minimal and stable, and contains nutrients that could be utilised as soil enrichers (Marín *et al*., 1999). According to Fang *et al*. (1994) the success of AD by means of a UASB reactor depends on the development of active and settleable sludge granules. The authors also stated that the microstructure of a UASB granule is subjected to the type of substrate used. Els *et al*. (2005) summarised the microstructure of a UASB granule and digestion (Fig. 2.5).

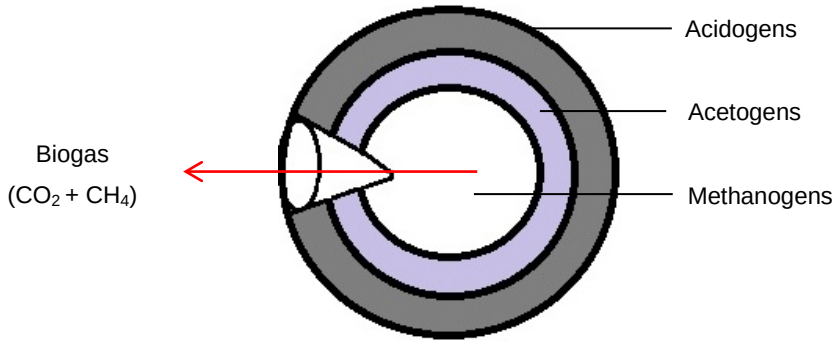

Figure 2.5 Illustration of the UASB granule on microbial level (Fang *et al*., 1994; Els *et al*. 2005).

Acidogenic microbes appear to be on the outer layer of the granule followed by the acetogenic species and finally the methanogens (Fig. 2.5) (Els *et al*., 2005). Digestion can be divided into four phases: hydrolysis (liquefaction); acidogenesis; acetogenesis; and methanogenesis (Fillaudeau *et al*., 2008; Ponsá *et al*., 2008). No individual organism can perform

these reactions individually and therefore AD consists of a complex ecosystem of various microbial groups that all work together in a coordinated way to generate methane and carbon dioxide (Anderson *et al.,* 2003). The organisms depend on one another for providing appropriate nutritional substrates and maintaining a proper environment (correct redox potential, ionic balance and hydrogen pressure) for optimal digestion (Ditchfield, 1986).

Hydrolysis

Due to the inability of acetogens and methanogens to utilize complex polymeric molecules directly these substances must first be degraded to smaller soluble monomers before methanogenesis can proceed (de Lemos Chernicharo, 2007). Consequently, hydrolysis (Fig. 2.6) involves the degradation or hydrolysing of complex compounds such as cellulose, proteins, fats and carbohydrates into soluble monomers like amino acids, glucose and fatty acids (Iannotti *et al*., 1982; Enders & Siebert-Raths, 2011).

These actions are performed by extracellular enzymes excreted by microbes from fermentative group one (Fig. 2.7) (Khanal, 2008). Since enzymes e.g. cellulose, protease, lipase, amylase, chitinase and pectinase are extracellular, they are capable of degrading large substrate polymers that cannot cross the bacterial cell wall to soluble monomers (Anderson *et al*., 2003). The bacteria who implement the first stage of the anaerobic process belongs to family of *Enterobacteriaceae* and *Streptococcaceae* as well as the genus *Bacteroides, Clostridium, Butyrivibrio, Eubacterium, Bifidobacterium and Lactobacillus* (Novaes, 1986).

Acidogenesis and Acetogenesis

Soluble products from hydrolysis are broken down further by facultative anaerobes and anaerobes (group one) to intermediate products (Khanal, 2008). Acidogenesis results in the production of hydrogen gas, carbon dioxide, organic acids, organic-nitrogen and organic-sulphur compounds. Table 2.6 shows the principal compounds (acids, alcohol and organic-nitrogen) produced by fermentation during anaerobic digestion. It is also indicated whether these compounds can be used directly as substrates by methanogens or indirectly after being degraded to acetate by fermentative bacteria (Gerardi, 2003). The most significant VFA or organic acid that is produced is acetate (Table 2.6) responsible for nearly two-thirds of methane generated in mesophilic and thermophilic reactors (Zinder, 1990). Carbon dioxide and hydrogen can also be converted directed to methane or acetate (Fig. 2.7) (Gerardi, 2003).

The acidogenic phase consists of various fermentative species including *Lactobacillus, Escherichia Coli, Bacillus, Clostridium, Ruminococcus, Propionibacterium, Micrococcus, Streptococcus, Eubacterium and Butyribacterium* (Anderson *et al.,* 2003). By consuming extra oxygen that enters the feeding, these facultative organisms also protect oxygen sensitive methanogens (Anderson *et al.,* 2003). During the production of acetic and propionic acid, large amounts of hydrogen is also formed which decreases the pH medium (de Lemos Chernicharo, 2007). There are however, two ways possible for hydrogen to be consumed in the medium: (i)

methanogens utilize hydrogen and carbon dioxide to generate methane; and (ii) through the formation of organic acids (de Lemos Chernicharo, 2007). The fastest conversion process in the

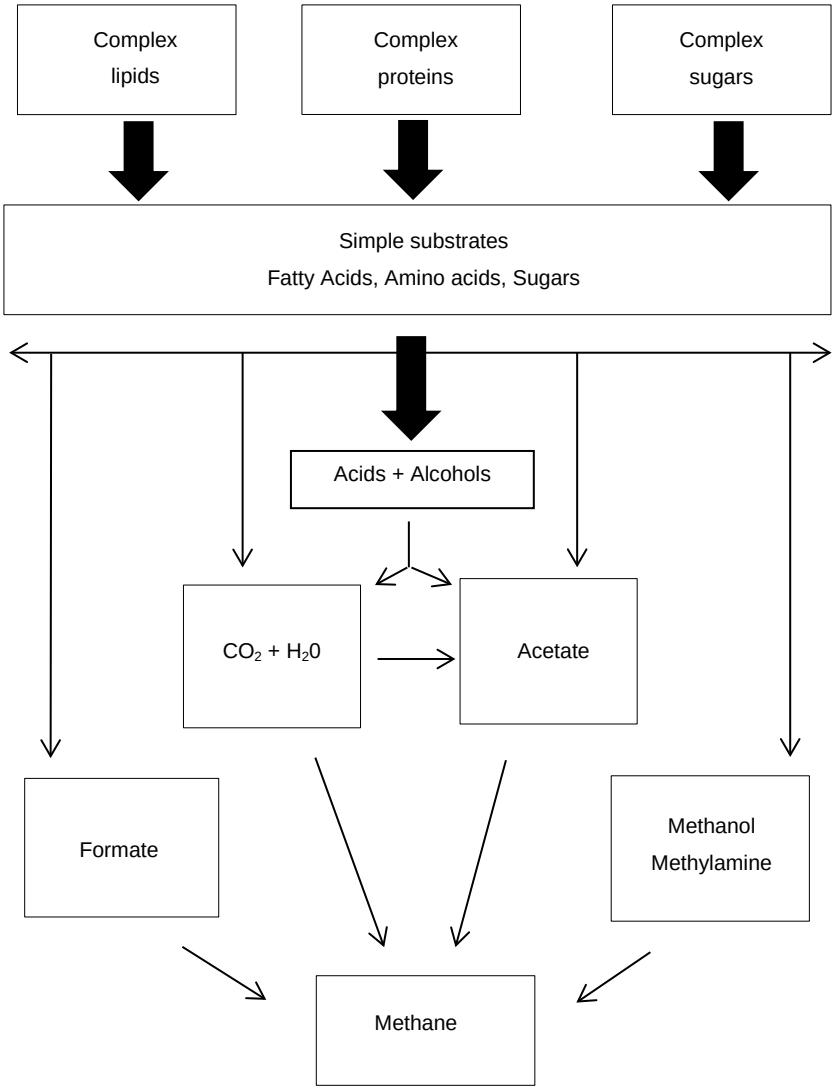

Figure 2.6 A model of the biochemical reactions which proceeds through the hydrolysis of waste during anaerobic digestion (Christ *et al*. 2000; Gerardi, 2003).

food chain is acidogenesis, that results in ten to twenty times higher microbial growth rates and five times higher microbial yields and conversion rates when compared to methanogens (Van Lier *et al*., 2008). Acetate is not only generated via the acid-forming phase (acidogenesis) but also through acetogenesis. Here, VFA combines with alcohol to be converted to acetic acid, hydrogen and carbon dioxide by hydrogen-producing acetic microbes group two (Fig. 2.7) (Khanal, 2008). During these conversions it is essential that the hydrogen partial pressure is maintained at a very low level for thermodynamically favoured conditions (Speece, 1983).

The oxidation of reduced substances to acetate, carbon dioxide and hydrogen are performed by obligate hydrogen producing acetogens (OHPA) (Aresta, 2012). The β-oxidation (Fig. 2.7) of even-numbered fatty (to acetate) and uneven-numbered fatty acids (acetate, propionate and hydrogen) are also performed by OHPA's (McInerney *et al*., 1981).

Table 2.6 Most significant compounds generated by fermentation during anaerobic digestion (Gerardi, 2003)

Compound name	Chemical formulation
Acetate[1]	$C_2H_4O_2$
Butanol	$C_4H_{10}O$
Butyric acid[2]	$C_4H_8O_2$
Caproic acid (hexanoic)	$C_6H_{12}O_2$
Formic acid[1]	CH_2O_2
Ethanol[2]	C_2H_6O
Lactic acid	$C_3H_6O_3$
Methanol[1]	CH_4O
Methylamine[1]	CH_5N
Propyl alcohol	C_3H_8O
Propionic acid[2]	$C_3H_6O_2$
Succinic acid	$C_4H_6O_4$

[1]directly used by methanogens, [2]indirectly used by methanogens

Several OHPA's have been identified: (i) *Syntrophomonas wolfei*, an anaerobic, nonphototrophic bacterium (McInerney *et al*., 1981; Wofford, 1986; Beaty & McInerney, 1987); *Syntrophobacter wolinii*, a nonmotile Gram-negative rod (Boone & Bryant, 1980); *Syntrophus buswellii*, a motile, Gram-negative, anaerobic rod-shaped organism (Mountfort *et al*., 1984); and *Methanothermobacter thermoautotrophicus* (previously *Methanobacterium thermoautotrophicum*) (Luo *et al*., 2002).

The activities of OHPA's serves as the link between the initial fermentation and final methanogenesis stage and are dependent on the activity of hydrogen utilising species (such as hydrogenophilic methanogens) (O'Flaherty & Lens, 2003). These species maintain very low levels of hydrogen partial pressure to keep the OPHA's reactions exergonic and are therefore referred to as synotrophs (O'Flaherty & Lens, 2003). Synotroph plainly means "eating together" and this refers to the connection between the hydrogen-producing and hydrogen-consuming methanogenic species (Parawira, 2004).

Acetogens are responsible for oxidising the products from the acidogenic phase to a suitable substrate for the methanogens to use (de Lemos Chernicharo, 2007). Thus, these metabolic processes produce the main substrates (acetic acid, hydrogen and carbon dioxide) that are utilised by the methanogens to generate methane (Ditchfield, 1986).

Methanogenesis

During the last phase of anaerobic digestion, methanogens convert the end-products of previous stages to methane (Gray, 2010). Three major metabolic pathways exist for the production of methane (Khanal, 2008):

1. Carbon dioxide reducing or hydrogenotrophic pathway;
2. Acetoclastic or acetotrophic pathway; and
3. Methyltrophic pathway

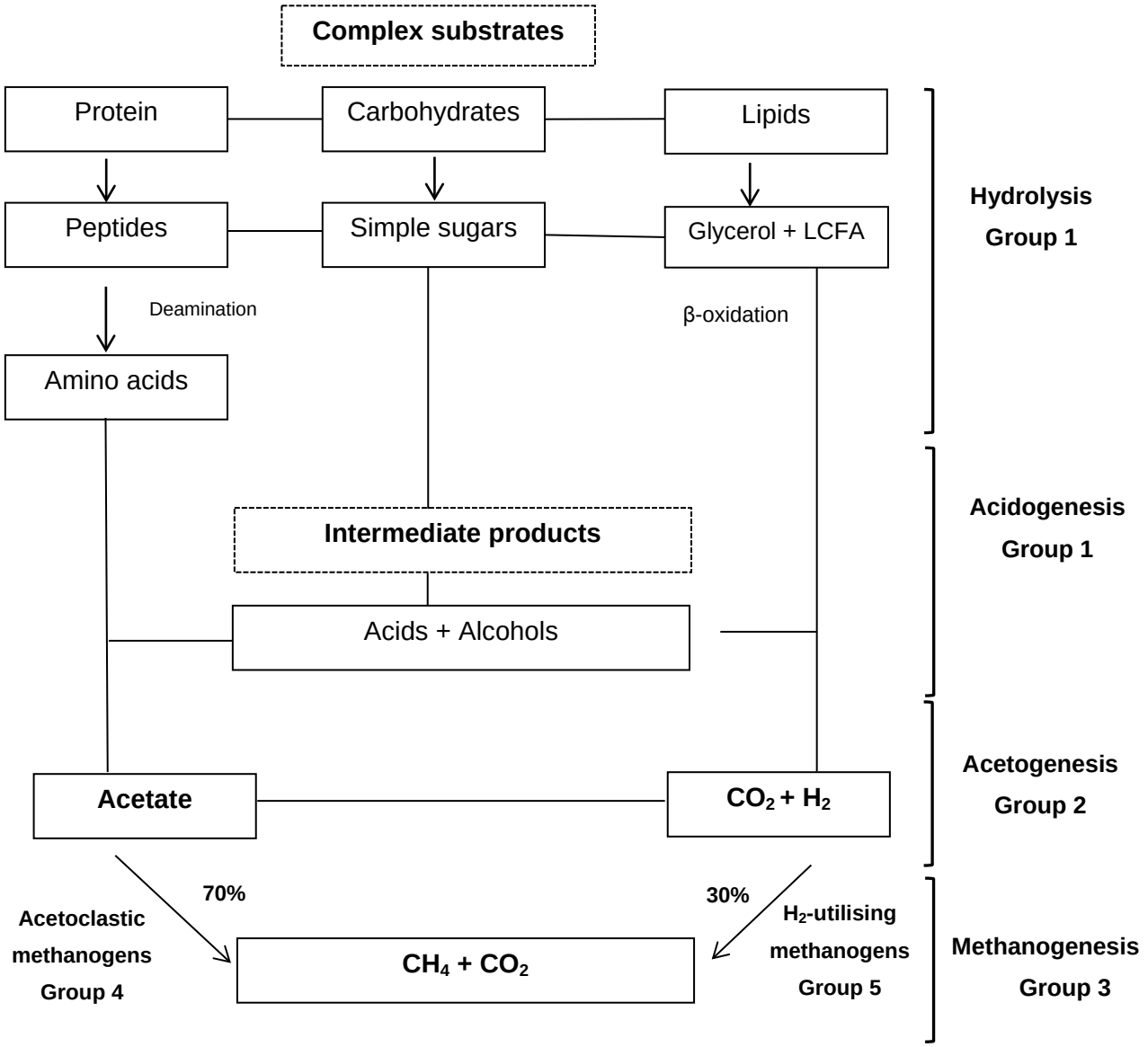

Figure 2.7 Metabolic route of AD at molecular level (Ditchfield, 1986; Anderson *et al*., 2003; Gerardi, 2003; Kashyap *et al*., 2003; Kumar, 2006).

When taking COD in consideration (Fig. 2.7), approximately 70 - 72% comes from the decarboxylation of acetate (eq. 1) via the acetoclastic methanogens (Fig. 2.7) while the rest is from the reduction of carbon dioxide (eq. 2) via the hydrogen-utilising methanogens (Fig. 2.7) (McCarty, 1964; Ditchfield, 1986; del Real Olvera & Lopez-Lopez, 2012). Methylotrophic methanogens develop on substrates that contain the methyl group. Illustrations of these substrates include methanol (eq. 3) and methylamine (eq. 4) (Gerardi, 2003). Although most methane is generated by the acetoclastic methanogens, the significance of the hydrogen-utilising methanogens should not be underestimated as they remove hydrogen molecules (eq. 2) from the system (Ditchfield, 1986).

Splitting of acetic acid

$$C^*H3COOH \rightarrow C^*H_4 + C0_2 \quad (1)$$

Reduction of carbon dioxide

$$C0_2 + 8H \rightarrow CH_4 + 2H_20 \quad (2)$$

$$3CH_4O + 6H \rightarrow 3CH_4 + 3H_2O \quad (3)$$

$$4(CH_3)_3\text{-}N + 6H_2O \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \quad (4)$$

The removal of these molecules has a dual function: (i) it promotes the conversion of butyric and propionic acid to acetic acid; and (ii) it prevents organic acid build-up so that the digestion process can continue under stable, steady state conditions (Ditchfield, 1986). The two most important genera of acetotrophic methanogens (Grop 4 are the *Methanosarcina* and *Methanosaeta* species (Gerardi, 2003; de Lemos Chernicharo, 2007) (formerly Methan-othix) (Khanal, 2008). *Methanosarcina* organisms are coccid (spherical cells) that uses several methanogenic substrates including methanol, methylamines (Table 2.6) and sometimes H_2/CO_2. The doubling time of these organisms on acetate is 1 – 2 days. The *Methanosaeta* species are bacillus (rod shaped) cells that only develop on acetate with a doubling time of 4 – 9 days (Khanal, 2008). The most commonly isolated hydrogen-utilising methanogens from anaerobic systems includes *Methanobacterium, Methanospirillum and Methanobrevibacter* (de Lemos Chernicharo, 2007).

The generation of gaseous methane as an end product is the actual process responsible for the organic matter removal measured in terms of COD (Lawrence & McCarty, 1967). The biodegradation of organic material to acetate is often the rate-limiting step in the degradation of organic molecules and anaerobic conversions whereas with poorly biodegradable materials, the hydrolysis phase may turn out to be the rate-limiting phase (Gerardi, 2003). The overall rate-limiting phase in anaerobic digestion is the conversion of VFA towards CH_4 as methanogens function slower than acetogens (Nazaroff & Alvarez-Cohen, 2001). The slow growth of methanogens explains why a long start-up period is needed with unadapted seeding materials (van Lier, 2008).

Factors affecting AD of liquid and solid waste

As methane organisms are strict anaerobes, they are prone to sensitivity against changes in pH, temperature and alkalinity and therefore various operational conditions should be checked and sustained for optimum methanogen activity (Gerardi, 2003). Because methanogenesis are often regarded as the rate-limiting step in anaerobic treatment, systems are monitored for biogas production (del Real Olvera & Lopez-Lopez, 2012). For AD of liquid wastes physical factors such as solid retention time and loading rate, hydraulic retention time, temperature, mixing, and oxygen affects the process. Chemical factors include pH, alkalinity, VFA content, nutrients and toxic substances (Junge, 1980). Factors affecting AD of solid wastes include pH, alkalinity, moisture, carbon source and nitrogen (Khalid *et al*., 2011).

Temperature

Temperature is one of the principal AD parameters as it controls the rate of anaerobic degradation and more specifically hydrolysis and methanogenesis (Nayono, 2010). Several reports in literature exist on the significant effect that temperature has on the process kinetics and biogas production of AD (Bouallagui *et al.,* 2009b; Riau *et al*., 2010). The hydrolysis and acidogenesis stages are not affected by temperature as much as the acetogenic and methanogenic groups due to the fact that there are generally some organisms among the mixed population who have their optimum temperature in the range wherein the digester is functioning (Parawira, 2004). As acetogenesis and methanogenesis are performed by more specific species they are prone to be more sensitive to temperature (Rajeshwari *et al*., 2000). Lower temperatures during AD are known to cause a decrease in the microbe growth, substrate utilisation and biogas production (Trzcinski & Stuckey, 2010). According to Lettinga *et al.* (2001) chemical and biological reactions under psychrophilic conditions occur a lot slower than mesophilic conditions and therefore require more energy for the degradation of organic materials. Although methane production can take place a wide over range of temperatures (Table 2.7) (Gerardi, 2003), AD mostly takes place at mesophilic or thermophilic temperatures with their prime temperature being 35°C and 55°C, respectively (Angelidaki & Ahrino, 1994; Ward *et al.,* 2008).

Table 2.7 Ideal temperature ranges for optimum methane production (Gerardi, 2003)

Microbes	Range (°C)
Psychrophilic	5 - 25
Mesophilic	30 - 35
Thermophilic	50 - 60
Hyperthermophilic/Stearothermophilic	> 65

Even though thermophilic temperatures could lead to an increase in the reaction rate, the methane yield attained at a specific organic amount stays the same regardless of the temperature (del Real Olvera & Lopez-Lopez, 2012). Thermophilic conditions are more prone to toxic sensitivity, operational costs are higher and temperatures are more difficult to control (Gerardi, 2003). Mesophilic reactors are more stable with less energy requirements when compared to thermophilic reactors (El-Mashad *et al*., 2003; Fernández *et al.,* 2008). Organisms operating in the mesophilic range (Table 2.7) are known to be more robust and tolerable to changes in ecological parameters. Systems that are smaller, poorly insulated, or in colder environments can all benefit from using mesophilic reactors to minimise system crashing. Due to the stability of this process, mesophilic AD systems are the preferred anaerobic treatment over thermophilic temperatures (Zaher *et al.,* 2007).

pH and alkalinity

According to del Real Olvera & Lopez-Lopez (2012) pH and alkalinity are related and together causes an appropriate surrounding for methanogenesis to occur. Sufficient alkalinity is needed in any anaerobic digestion system to sustain a stable pH and optimal biological activity (Lee *et al*.,

2009). Various pH ranges for anaerobic digestion has been reported by researchers (Khalid *et al*., 2011) but according to Ward *et al*. (2008) the ideal range is 6.8 – 7.2. Anderson *et al*. (2003) also stated that although the pH for AD is reported to be 7, the optimal is thought to be 6.5 – 7.8. McCarty (1964) also stated that digestion can proceed normally between a pH of 6.6 - 7.6 with optimum being between 7.0 – 7.2. The suggested pH range ensures an acceptable environment for methanogens to work and it also helps sufficient buffering capacity or alkalinity (Gerardi, 2003). The organisms responsible for acidogenesis have an optimal pH 5 – 6 (Droste, 2004) while methanogens work well in pH ranging from 6.8 – 7.2 (Gerardi, 2003).

Below a pH of 6.6, the development rate of methanogens is slowed (McCarty, 1964; Mosey & Fernandes, 1989) while a too high alkalinity surrounding could lead to granule disintegration and consequently digester failure (Sandberg & Ahring, 1992). Alkalinity in digesters is a result from the degradation of organic-nitrogen molecules as well as the production of carbon dioxide from degrading organic molecules (Gerardi, 2003). Alkalinity plays a vital role in regulating the pH in the digester by buffering the acidity (VFA) derived by the acidogenesis stage (Gerardi, 2003). Researchers have reported digester failure during treatment due to the accumulation of VFA which inhibits the works of methanogens (Parawira *et al*., 2006). The buffering capacity is typically referred to as the alkalinity of an anaerobic digestion system. The buffering action is the equilibrium of the CO_2 and the bicarbonate ions which supplies resistance to pH changes and are therefore proportional to the concentration of the aforementioned (Ward *et al.,* 2008). A more dependable method of measuring imbalances in the digester is the measuring of the buffer capacity rather than direct pH measurement, because an accumulation of fatty acids will lower the buffer capacity before decreasing the pH (Ward *et al.,* 2008). According to Gerardi, (2003) an optimum buffering capacity of about 1 500 – 3 000 $CaCO_3$ $mg.L^{-1}$ is needed for a stable and maintained digestion process.

Factors impacting liquid waste degradation

Nutrients

Nutritional requirements by organisms are very important as nutrients supply: (i) cellular building blocks that are used for growth; and (ii) ensures that the cell are able to synthesize enzymes and co-factors responsible for driving metabolic and biochemical reactions (Anderson *et al*., 2003). The occurrence of ions is a very important parameter in a reactor as it can affect the granulation development and stability of the UASB (Rajeshwari *et al*., 2000). For AD to be optimal various organic and inorganic substances are needed. This includes macronutrients such as nitrogen and phosphorous as well as sulphur, vitamins and trace elements (iron, nickel, magnesium, selenium, copper, cobalt) known as micronutrients (Mata-Alvarez, 2003). Even though these nutrients are required in very low amounts, the lack of them causes a significant effect on the growth and performance of microbes (Rajeshwari *et al*., 2000). These elements can often be missing in waste streams arising from only one source (Rajeshwari *et al*., 2000) and should be supplemented before

treatment (Pol, 1995). Winery wastewater is known as a high-strength organic waste with a low nitrogen and phosphorous content (Toffelmire, 1972) with C:N:P of 81:1:1.35 (Ronquest & Britz, 1999). According to Gerardi, (2003) a C:N:P ratio of 1000:7:1 is needed for high strength wastewaters so that the digester can perform optimally.

Retention times

Hydraulic retention time (HRT) can be defined as the amount of time that waste remains in the digester and in contact with the biomass. For easily biodegradable compounds such as sugar, the HRT is low whereas more complex compounds may need longer HRTs (Khanal, 2008). The solid retention time (SRT) on the other hand, is a main parameter for controlling waste stabilisation of organic compounds in anaerobic digesters (Kuscu & Sponza, 2007). The SRT indicates the mean residence time of the microbes and is related to the growth thereof (Clara *et al*., 2005). Higher SRT values are more beneficial as it leads to higher removal capacities, reduces the digester volume and it provides buffering capacity. HRT values influences the rate and extent of and methane generation and is one of the most significant factors affecting the transformation of volatile substrates into gaseous products (Gerardi, 2003). For AD to be optimal a low HRT/SRT ratio has been reported by Alphenaar *et al*. (1993).

Toxicity and inhibition

A significant variety in literature exists on the inhibition or toxicity levels of materials on anaerobic digestion. The most important explanation for this is due to the fact that AD is such a complex process that includes several mechanisms such as antagonism, synergism and acclimation (Chen *et al*., 2008). Toxicity can either be classified as (i) acute or (ii) chronic. Acute toxicity is when an unacclimated population are exposed to a sudden high concentration of hazardous waste while chronic toxicity involves gradual exposure over a long amount of time (Gerardi, 2003). The digestion process can be inhibited by materials arising from the effluent stream such as ammonia, heavy metals and halogenated compounds or from metabolic by-products from microbes such as ammonia, sulphide and VFA (Khanal, 2008) that can either slow down the digestion process (toxicity) or lead to process failure (inhibition) (Anderson *et al*., 2003). Although various compounds at different thresholds levels affect AD, generally VFA, pH, free ammonia and hydrogen sulphur are the most common (Mata-Alvarez, 2003). Free ammonia (NH_3) is more toxic than ionized NH_4, because free ammonia can passively transport across the cell membrane and dissociate which leads to intracellular pH changes (Nishio & Nakashimada, 2013).

Mixing

Apart from the type of reactor design, most anaerobic reactors are mixed to supply organic substrates to active microbes, to release trapped biogas bubble and to ensure that sedimentation of denser material does not occur (Ward *et al*., 2008). Mixing can be accomplished in the following ways (i) mechanical drivers such as turbines or propellers, (ii) hydraulic sheer force by recirculating

the feed and (iii) recurrent gas circulation (Anderson *et al*., 2003*).* Slow, gentle mixing ensures that the metabolic activities of both acetogens and methanogens remain in close contact with one another. Mixing is also advantageous to acetogens as it supplies efficient hydrolysis of waste products and generates acids and alcohols (Gerardi, 2003). If mixing is not sufficient it could lead to pockets of material in the digester which are not at the same temperature, pH and stages of digestion that will affect the overall performance of the digestion process (Stafford, 1981). A system therefore only requires adequate mixing (Khanal, 2008) as excessive mixing could also lead to a decrease in biogas generation (Ward *et al*., 2008).

Factors impacting solid waste degradation

Moisture

Higher moisture contents or humidity (60 – 80%) leads to a higher methane generation (Hernández-Berriel *et al*., 2008) although the moisture initially added will drop to a lower level as the digestion process continues (Khalid *et al*., 2011). Water is essential for (i) methane generation as nutrients need to be dissolved before being utilised by microbes (ii) it aids with the diffusion of substrates towards organism sites and (iii) high moisture contents dilutes the concentration of carboxylic acid that adds to the buffering capacity (Lay *et al*., 1997). Hernández-Berriel *et al*. (2008) studied the effect of two different moisture contents (70%, 80%) on the digestion of municipal solid waste. The reactor containing 70% moisture generated a stronger leachate and therefore a higher methane percentage was produced. Lay *et al*. (1997) studied the combined effect of pH and moisture content on the digestion of high-solid sludge. The authors found that moisture content was an essential ecological parameter that can increase methane generation as the results obtained, showed at an optimum pH the activity of the methanogens decreased from a 100 - 53% when the moisture contents were lowered from 96 – 90%.

Nutrients (Carbon source/substrate, Nitrogen, C/N ratio)

The microbiological ecosystem of a digester will depend on the substrate type, substrate amount and other factors such as pH and temperature (Ghaniyari-Benis *et al*., 2009). As certain types of carbon sources support the working activity of certain microbes, this forms the basic foundation for good digester performance (Zhao *et al*., 2010). Fernández *et al*. (2008) investigated the effect of substrate concentration on anaerobic digestion and reported that the initial concentration of the substrate can significantly influence the AD process. Nitrogen is the inorganic macronutrient that is utilised in large concentrations by microbes for growth (de Lemos Chernicharo, 2007). Nitrogen is vital for protein synthesis (Kayhanian & Rich, 1995) and due to the fact that micro-organisms grow more in carbohydrate rich waste than waste containing proteins and VFA, the amount of nitrogen needed for waste containing carbohydrates are six times larger (de Lemos Chernicharo, 2007). Ammonia is produced by the degradation of nitrogenous materials present in the waste generally in a protein form (Kayhanian, 1999). Nitrogen is absorbed in ammonia form which also helps with

stabilising the digester pH. According to Fricke *et al*. (2007) a nutrient ratio of C:N:P:S in 600:15:15:3 is adequate for methanization to take place. Although a too high ammonia concentration can inhibit digestion (Chen *et al*., 2008) a total ammonia concentration (TAN) of about 200 $mg.L^{-1}$ could be beneficial to AD (Liu & Sung, 2002). The carbon to nitrogen ratio (C/N) is an important parameter in a biological process (Lin & Lay, 2004). Bouallagui *et al*. (2009a) advised a C/N ratio of 22 – 25 for digestion of fruit and vegetable waste, while an optimal ratio of the degradation of the organic fraction of municipal solid waste was suggested to be 20 – 35 (Guermond *et al*., 2009),

E. CO-DIGESTION

Co-digestion can be defined as the combined treatment of one or more wastes with balancing characteristics (Ağdağ & Sponza, 2007). AD serves as a potential treatment option to decrease ecological burdens and supply biogas for energy (Alvarez & Lidén, 2008). A main factor limiting AD of organic wastes is an unbalanced supply of nutrients (Khalid *et al*., 2011). Winery wastewater is known to have a low nitrogen and phosphorous content (Toffelmire, 1972) which needs to be supplemented for AD to be optimal (Ronquest & Britz, 1999; McLachlan, 2004). Co-digestion is an alternative solution as co-substrates can supply the missing nutrients and balance the substrate composition (Mata-Alvarez *et al*., 2000; Umetsu *et al.,* 2006; Jagadabhi *et al*., 2008; Pagés-Díaz *et al*., 2014) and is a known method to increase methane yields of AD. Advantages of co-treatment includes: (i) the dilution of hazardous components such as ammonia; (ii) a better nutrient balance; (iii) increased biodegradation of organic matter; (iv) an improved biogas yield (Mata-Alvarez *et al*., 2000; Khalid *et al*., 2011); and (v) increased digestion rates as well as hygienic stabilisation (Sosnowski *et al*., 2003;).

The produced leachate is a source of water, inoculum and nutrients that is desirable for optimum AD. Another advantage of co-treatment is that VFA and related fermentative products, formed during start-up are removed and carried over to the aged reactor to convert into methane. This helps eliminating digester instability that is commonly found in single-stage digesters (O'Keefe *et al*., 1993). Co-digestion is well documented in literature and has been applied widely:

- olive mill wastewater and wine-grape residues with slaughterhouse wastewater (Fountoulakis *et al*., 2008);
- Sewage sludge together with grease trap sludge obtained from a meat processing plant (Luostarinen *et al*., 2009);
- Municipal solid waste (organic fraction) with FOG waste acquired from sewage treatment plant (Martín-González *et al*., 2010);
- Chicken manure with agricultural wastes (Abouelenien *et al*., 2014);
- Food waste (FW) and fruit/vegetable residue with dewatered activated sludge (Guo *et al*., 2014);
- Solid slaughterhouse wastes and agro-residues (Pagés-Díaz *et al*., 2014);
- Disposable diapers with waste activated sludge (Torrijos *et al.,* 2014)

Rodríguez *et al*. (2007) evaluated the operational performances of ABR co-treating winery wastewater and waste activated sludge. Results obtained showed that the percentages of methane generation were lower with wastewater alone in comparison with sludge and wastewater together. The maximum biogas produced was found to be at a 50:50 ratio of wastewater and sludge together with a 60% COD reduction. Riaño *et al*. (2011) studied the effect of anaerobic co-digestion of swine manure with winery wastewater. The authors found that co-treatment with 40% winery wastewater lead to improved removal efficiencies of approximately 52% for total COD and 61% of volatile suspended solids (VSS) compared to digesting the swine manure alone. The study showed that the co-treatment of swine manure and winery wastewater is capable of generating methane gas efficiently.

F. GENERAL CONCLUSIONS

Winemaking is a centuries old biotechnology that has become a worldwide initiative affecting the economic wellbeing of several countries (Walker, 1999). Winemaking however, needs a significant amount of natural resources and carbon-rich amendments whilst producing a large amount of liquid and solid wastes (Ruggieri *et al.,* 2009). These wastes could cause environmental problems due to their polluting characteristics (FSA Consulting, 2006). Ecological concerns often associated with wineries are water pollution, soil degradation and damage to plant life due to poor disposal practices of liquid and solid wastes (EPA, 2004).

The preceding review evidently indicates that biological treatment of liquid and solid wastes is an effective treatment option with several advantages. Anaerobic digestion of liquid waste (winery wastewater) is an attractive treatment option as both depollution and energy recovery can be accomplished (Chen *et al*., 2008) while anaerobic composting of solid waste (grape skins) generates biogas, an organic amendment (Khalid *et al*., 2011) and a liquid effluent, which can be used as a renewable energy source and a valuable soil conditioner, respectively (Mata-Alvarez *et al*., 1992).

A main feature that limits anaerobic digestion, is the unbalanced supply of nutrients within waste substrates (Khalid *et al*., 2011). Winery wastewater that is known to have low nitrogen and phosphorous content (Toffelmire, 1972) needs to be supplemented for digestion to be optimal (Ronquest & Britz, 1999; McLachlan, 2004). Co-digestion or co-treatment is an alternative solution as co-substrates can supply the missing nutrients and balance the substrate composition (Mata-Alvarez *et* al., 2000; Umetsu *et al.,* 2006; Jagadabhi *et al*., 2008; Pagés-Díaz *et al*., 2014). Co-digestion is also a known method to increase methane yields of AD. To date, very little literature is available on co-treating leachate from anaerobic composting within a UASB reactor treating winery wastewater. These combined treatment options have enormous potential in future application within the wine industry in order to create sustainable water and waste utilisation practices.

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CHAPTER 3

DETERMINING OPTIMUM OPERATIONAL PARAMETERS FOR ANAEROBIC COMPOSTING OF GRAPE SKINS

Summary

Wine production is of major importance to South Africa and requires significant amounts of natural resources and carbon-rich amendments while generating large volumes of liquid and solid wastes. Anaerobic composting of solid waste generates an organic amendment, a liquid effluent (leachate) and biogas. These products can be used as a valuable soil conditioner and a renewable energy source (biogas). In order to create a functional anaerobic composting system, the objective must be to identify which operational parameters to optimise.

During this study eight experimental studies were performed to optimise operational parameters to achieve the above objective. It was found that strict control of pH, moisture, inoculum composition and the initial inoculum size were necessary to produce a quality end-product. After finding that grape skins cannot be composted alone due to its high carbon content and low pH, green waste and CaO were added and the mixture soaked overnight at 37°C. It was also essential to shred all waste to speed up the composting process so as to reach a final composting period of 21 days. The removal of leachate generated during the digestion process was important in avoiding acidification. It was also found that the alkalinity of the anaerobic wastewater reactor effluent, used as a moisturising liquid, played a significant role in buffering the volatile fatty acids (VFA) formed during the initial stages of composting. With all optimum operational parameters in place (6 g CaO, 50% ($m.m^{-1}$) moisture, 20% ($m.m^{-1}$) green waste, 150 g white and red grape skins in an equal ratio (50:50), and 15% ($m.m^{-1}$) cow manure as inoculum, a stable end-compost was produced. The composting process described was scaled-up (1:10) and also produced a stable compost within 21 days.

Introduction

The generation of solid waste is a growing worldwide issue due to increases in production and thus its management needs to be improved (Sinan Bilgili *et al*., 2007). Solid waste produced during winemaking includes grape pomace (seeds, stalk and skins), wine lees and winery sludge (Bustamante *et al.,* 2008a). The primary solid waste portion generated during the wine making process is the pomace (Diaz *et al.,* 2002). These wastes are characterised by an acidic pH, high polyphenol, organic and potassium contents along with considerable amounts of nitrogen and phosphate (Bustamante *et al*., 2008b).

Research has shown that due to the nature of its composition, grape pomace can be recycled as a soil conditioner (Diaz *et al.,* 2002; Flavel *et al.,* 2005; Brunetti *et al.*, 2011). Anaerobic digestion (AD) of solid waste or anaerobic composting (AC) is a well-known method for

the treatment of liquid, solid and semi-solid carbon-based wastes. It offers several benefits over conventional (aerobic) treatments, mainly from an energetic and environmental point of view (Marín *et al*., 1999). Products generated by the anaerobic composting of solid waste (grape skins) include: (i) an organic amendment (Khalid *et al*., 2011); (ii) a liquid fraction; and (iii) biogas which can be used as valuable soil conditioners and a renewable energy source (biogas), respectively (Mata-Alvarez *et al*., 1992).

According to literature the main factors influencing anaerobic composting include the nutrient content, moisture levels, pH, alkalinity, temperature (Khalid *et al*., 2011) and the initial inoculum source (Forster-Carneiro *et al*., 2007). Grape pomace consists of 8% seeds, 10% stems, 25% skins, 57% pulp and is high in nitrogen, potassium and calcium (Westover, 2006). However, due to the chemical characteristics and high carbon content of winery waste it cannot be composted alone (Kulcu & Yaldiz, 2005). Westover (2006) reported that grape pomace alone degrades slowly due to the low pH (3.5 – 3.8) and therefore lime or other feedstocks are often added to increase the pH.

Currently, with the trend in organic farming, the use of nitrogen rich organic wastes is favoured as a substitute to the addition of chemical nitrogen sources. Cow and poultry manure are examples of organic nitrogen wastes that can be used to substitute chemical nitrogen sources (Kalemelawa *et al*., 2012). AnC systems have successfully been operated with cow manure (CM) as inoculum in other composting trials (Griessel, 2002). The advantages of using cow manure are that it is a plentiful resource (Ward *et al*., 2008), the high alkalinity in cow manure serves as a buffering capacity for the accumulation of VFA's formed and nutrients (macro and micro) present are essential for microbial growth (Astals *et al.,* 2012). In another study peach pulp and apple pomace were successfully composted by using anaerobic granules as inoculum (Griessel, 2002).

Literature warns that sufficient moisture is important for: (i) methane production as nutrients need to be dissolved before being utilised by the microbes; (ii) it helps with the diffusion of substrates to organism sites; and (iii) a higher moisture content dilutes the concentration of carboxylic acid which then enhances the buffering capacity (Lay *et al.,* 1997). Alkalinity is required in any biological system to sustain a stable pH and optimal biological activity (Lee *et al.,* 2009). Although different pH ranges have been reported for optimum AD (Khalid *et al*., 2011), the ideal range is between 6.5 and 7.8 (Anderson *et al*., 2003), while the optimum is considered to be 6.8 – 7.2 (Ward *et al*., 2008). This pH range ensures an acceptable working environment for methanogens and helps with sufficient buffering capacity (Gerardi, 2003).

The aim of this study was thus to optimise the operational parameters for the anaerobic composting of grape skins, to produce stable compost. This will be done by identifying and optimising operational parameters in terms of inoculum (size, ratio and composition), pH, moisture content, green waste addition and grape skin (carbon source) ratios.

Materials and Methods

Laboratory-scale anaerobic compost digesters

During this study, modified Schott bottles (1 L) were used as laboratory-scale anaerobic compost digesters (Fig. 3.1). Synthetic pot scourers (diameter = 80 mm) were placed at the bottom of the digesters to prevent clogging of the leachate outlet (Fig. 3.1). Moisture was added to the digesters through the neck of the bottle. A biogas outlet allowed gas production and composition to be measured, while another outlet was used to flush the digesters with nitrogen prior to the start of the experimental study (ES) (Fig. 3.1). The leachate formed was drained from the digester via an extension at the bottom of the unit.

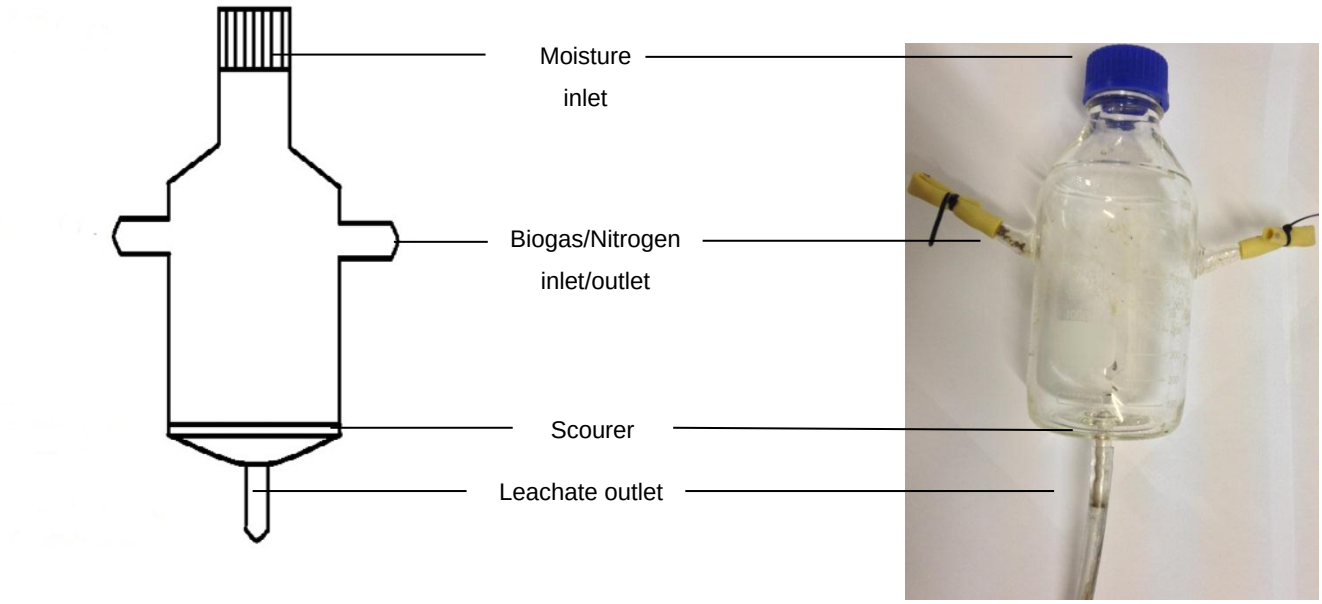

Figure 3.1 Schematic illustration and photograph of the modified Schott bottles (1 L) that were used as laboratory-scale anaerobic compost digesters (Griessel, 2002).

Scaled-up digesters

For the up-scaling (1:10) study, 20 L poly vinyl chloride (PVC) containers (500 x 400 mm) were used as anaerobic composting units (Fig. 3.2) (Griessel, 2002). Synthetic pot scourers were placed at the bottom of the units to prevent the clogging of the leachate outlet. Inlets/outlets (Fig. 3.2) allowed moisturising liquid to be added and biogas production to be measured.

Substrate

Red and white grape skins were obtained during the harvest season of 2012 and 2013 from Muratie Wine Estate, Stellenbosch, South Africa. Grape skins were vacuum packed in plastic bags and stored at -18°C until utilised. Fresh green waste (grass) was obtained the day before each ES commenced from Langverwacht Landscaping, Stellenbosch, South Africa, and was kept in a sealed container at 4°C until needed. The pH adapted effluent that was used throughout this study as a moisturising liquid had a dual purpose: (i) to provide micro-organisms and substrate with

moisture; and (ii) to help increase the pH of the system by washing out VFA's formed during the initial stages of composting.

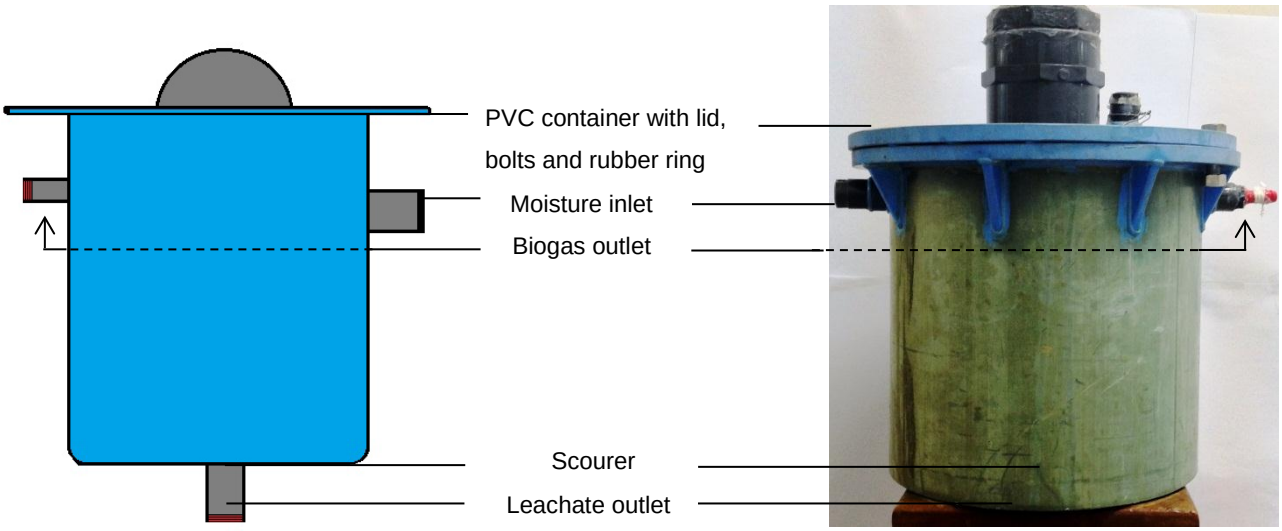

Figure 3.2 Schematic illustration and photograph of the PVC digesters that were used to up-scale the anaerobic compost digesters (Griessel, 2002).

Inoculum

Anaerobic granules were obtained from a full-scale UASB reactor treating distillery wastewater in Wellington, South Africa (The James Sedgwick Distillery, Wellington, South Africa). Fresh cow manure or cattle dung was acquired the day before each ES commenced and was kept in a sealed container at 4°C until needed. The cow manure was obtained from Welgevallen Research Farm, Stellenbosch University, South Africa. Anaerobic compost used as inoculum during Experimental Study (ES) 4 – 7 was obtained from the end-product compost from ES2.

Preliminary experimental studies

After identifying the operational parameters that showed control possibilities (inoculum (size, ratio and composition), pH, moisture content, green waste addition and grape skin (carbon source) ratios), preliminary experimental studies were conducted to determine the impact of different operating conditions. The following factors were investigated: degree of shredding of the grape skins and other solids; calcium oxide (CaO)/lime solutions; green waste inclusion; pH of moisturising liquid; as well as the frequency of moisturising the compost. The moisture content of the solid waste in the digesters was kept constant (50% $m.m^{-1}$) by the addition of pH adjusted (pH = 10) effluent from a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor treating grain distillery wastewater (GDWW) (Robertson, 2013).

Experimental Studies 1-8

The experimental set-up of ES 1 - 8 is given in Table 3.1. Moisturising liquid was added to the digesters every 24 h. The volume of UASB reactor effluent that was added to the digesters depended on the volume of leachate generated by each individual unit (i.e. volume leachate produced that was removed and the same volume of UASB effluent that was added). Laboratory grade calcium oxide (CaO)/lime (Merck, Germany) dissolved in tap water was added to the grape skins and green waste of both the laboratory-scale digester (6 g in 150 mL) and to the up-scaled digester (60 g in 1500 mL). The grape skins mixtures were allowed to soak for 24 h at 37°C.

The volumes of discharged leachate for each composting reactor unit was measured daily (ES 1 – 8) and stored at 4°C until needed for analysis (ES 7 - 8). The polyphenol, nitrogen and phosphorous contents of the leachate were determined at the beginning of ES7. For both ES7 and ES8 the following parameters were monitored daily: pH; leachate volumes; and alkalinity. The chemical oxygen demand (COD) and biogas composition were analysed three times a week while total suspended solids (TSS) and volatile suspended solids (VSS) were determined once a week. Microbial analyses were done on the compost and leachate before and after the 21 day duration.

Physico-chemical analyses were determined by an external analytical laboratory, Bemlab (Strand, South Africa). As the ideal pH for digestion has been reported as 6.5 – 7.8, with an optimum of 6.8 – 7.2, a pH of 6.5 was chosen as a reference pH (i.e. to be reached as quickly as possible for optimum digestion and methane generation). Composting digesters were flushed with nitrogen for 20 s, sealed and kept in an incubator room at 37°C. All studies, as shown in Table 3.1, were conducted in triplicate.

Experimental study 1: Effect of inoculum composition and ratio

Three laboratory-scale anaerobic compost digesters were used during ES1 and consisted of: shredded white and red grape skins (150 g each) (1:1); moisture content of 50% ($m.m^{-1}$); shredded green waste (20% $m.m^{-1}$) and inoculum (50% $m.m^{-1}$). The inoculum consisted of anaerobic granules and fresh cow manure (1:1). The three digesters differed only in terms of their inoculum ratio content. Digester 1 contained anaerobic granules and cow manure in a 50:50 ratio, Digester 2 in a 25:75 ratio and Digester 3 in a 0:100 ratio (Table 3.1). The effluent obtained from a UASB reactor treating GDWW (moisturising liquid) was set at pH 10 with 2M potassium hydroxide (KOH).

Experimental study 2: Effect of inoculum size

The purpose of this study was to determine the effect of a lower inoculum concentration on the digestion process. The contents of the three digesters used consisted of: shredded white and red grape skins (150 g each) (1:1); moisture content of 50% ($m.m^{-1}$); shredded green waste (20% $m.m^{-1}$) and inoculum (10, 15 and 25% $m.m^{-1}$). The inoculum consisted of only cow manure (CM). The three digesters differed only in terms of their inoculum size. Digester 1 contained 10% ($m.m^{-1}$) CM, Digester 2 contained 15% ($m.m^{-1}$) CM and Digester 3 had 25% ($m.m^{-1}$) CM (Table 3.1). Adapted GDWW set at pH 10 was used as moisturising liquid.

Table 3.1 Experimental set-up used during ES 1 to 8

	Lab-scale							Up-scale
	ES1	ES2	ES3	ES4	ES5	ES6	ES7	ES 8
Inoculum	**anaerobic granules + cow manure**	**cow manure**	AC from ES2	AC from ES2	AC from ES4	AC from ES4	cow manure	cow manure
Moisturising liquid	GDWWW	GDWWW	WWW (Alkalinity)	WWW	WWW	WWW	WWW	WWW
Inoculum size per digester ($m.m^{-1}$)	50	**10, 15, 25**	**10, 15, 25**	**10**	10	10	**15**	**15**
Inoculum ratio	**50:50; 25:75; 0:100**	-	-	-	-	-	-	-
Moisture content ($m.m^{-1}$)	50	50	50	50	**35, 40, 45, 50, 55, 60**	50	**50**	**50**
Green waste ($m.m^{-1}$)	20	20	20	20	20	20	**20**	**20**
Grape skin ratio (white:red)	50:50	50:50	50:50	50:50	50:50	**25:75; 50:50; 75:25**	**50:50**	**50:50**

***Parameter investigated is highlighted**

Experimental study 3: Effect of anaerobic compost (AC) as inoculum

The third study was done to investigate the effect of anaerobic compost and a lower inoculum size on the digestion of red and white grape skins. Three digesters containing shredded white and red grape skins (150 g each) (1:1), moisture content of 50% ($m.m^{-1}$), shredded green waste (20% $m.m^{-1}$) and inoculum (10, 15 and 25% $m.m^{-1}$), were used. The inoculum consisted of AC obtained from the Experimental study 2 (ES2). Digester 1 contained 10% ($m.m^{-1}$) AC, Digester 2 15% ($m.m^{-1}$) AC and Digester 3 contained 25% ($m.m^{-1}$) AC (Table 3.1). Throughout this ES, moisturising liquid (reactor effluent from an UASB reactor treating winery wastewater) was used. Due to the fact that the UASB reactor was still in a start-up phase, the winery effluent generated had a very low alkalinity (±800 $mgCaCO_3.L^{-1}$) compared to the alkalinity from the UASB treating GDWW (±4 000 $mgCaCO_3.L^{-1}$), used in the preliminary experimental studies as well as ES1 - ES2. Therefore, di-potassium hydrogen orthophosphate (K_2HPO_4) and potassium hydrogen carbonate ($KHCO_3$) were used in equal masses to adjust the alkalinity of the winery effluent from day 10 onwards to a desired level (3 500 $mgCaCO_3.L^{-1}$).

Experimental study 4: Effect of a 10% ($m.m^{-1}$) AC inoculum

Due to the apparent importance of the alkalinity of the moisturising liquid (ML), ES3 was repeated with winery UASB effluent that had a high inherent alkalinity. A 10% ($m.m^{-1}$) AC inoculum was used during this experimental study. The three digesters used consisted of the following: shredded white and red grape skins (150 g each) (1:1); moisture content of 50% ($m.m^{-1}$); shredded green waste (20% ($m.m^{-1}$)); and inoculum (10% ($m.m^{-1}$)). The final end-product compost from ES2 was used again as AC inoculum. Winery UASB reactor effluent was used as moisturising liquid for the remainder of the experimental studies (ES4 - ES8).

Experimental study 5: Effect of different moisture levels

After optimising the inoculum size, composition and ratio it was decided to determine the impact of different moisture levels on the efficacy of the composting process. Six digesters were used during this study and contained: shredded white and red grape skins (150 g each) (1:1); shredded green waste (20% ($m.m^{-1}$)); AC inoculum (10% ($m.m^{-1}$)) and different moisture (M) contents (35, 40, 45, 50, 55 and 60% ($m.m^{-1}$)) (Table 3.1).

Experimental study 6: Effect of different grape skin (carbon source) ratios

The purpose of ES6 was to determine if any differences on the efficacy of the composting process exist when using different white and red grape skin (GS) ratios. Three digesters were used each containing: shredded green waste (20% $m.m^{-1}$); AC inoculum (10% ($m.m^{-1}$)) and moisture (50% $m.m^{-1}$). The three digesters differed only in terms of grape skin ratio content. Digester 1 contained white (WGS) and red grape skins (RGS) in a 25:75 ratio, Digester 2 in a 75:25 ratio and Digester 3 in a 50:50 ratio (Table 3.1).

Experimental study 7: Effect of the optimised parameters

The final laboratory-scale study included all the optimum parameters (inoculum (ratio, size, type), moisture content, grape skin ratio) that had been identified in the previous studies. Three digesters were used and consisted of 6 g CaO, 50% ($m.m^{-1}$) moisture, 20% ($m.m^{-1}$) green waste, 150 g white and red grape skins (50:50) and 15% ($m.m^{-1}$) cow manure. Cow manure was chosen as inoculum as in "industrial" applications a "first batch" would always require an inoculum other than anaerobic compost. An inoculum size of 15% was chosen to ensure an efficient "start-up/first batch".

Experimental study 8: Effect of optimised parameters on the up-scaled composting process

This study included all the optimum parameters in terms of inoculum (ratio, size, type), moisture content and grape skin ratio as used in ES7. It was also decided to use 15% ($m.m^{-1}$) inoculum during the up-scale study (Table 3.1). Three up-scaled digesters (total digester volume of 5 550 g) were used and consisted of 60 g CaO, 50% ($m.m^{-1}$) moisture, 20% ($m.m^{-1}$) green waste, 1 500 g white and red grape skins in an equal ratio (50:50) and 15% ($m.m^{-1}$) cow manure as inoculum.

Analytical methods

pH and Alkalinity

The pH and alkalinity of the composting leachate were measured using a digital pH meter (WTW) and electrode (Xylem Inc., Germany) and a titration method, respectively (APHA, 1998). The alkalinity is expressed as mg $CaCO_3.mL^{-1}$.

COD

The leachate samples were digested with a COD digestion reactor (Hach Co. Loveland, U.S.A), cooled and colorimetrically measured using a DR2000 spectrophotometer (Hach Co. Loveland, CO) set at 585 nm, and standardised procedures (APHA, 1998). COD Solution A and Solution B (Merck, Germany) for the measuring range 500 – 10 000 $mg.L^{-1}$ were used. The COD of leachate produced on day 1 (used for co-treatment) was also confirmed by using a Spectraquant® COD Cell Test (5 000 – 90 000 $mg.L^{-1}$) (Merck, Germany). All analyses were performed in duplicate. The COD concentration was used to simulate the carbon value in the determination of the C:N:P ratio.

TSS and VSS

Analyses of leachate were performed once a week according to standard methods (APHA, 1998).

Biogas

A Varian 3300 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a thermal conductivity detector and a 2.0 x 3.0 mm i.d. Hayecep Q (Supelco, Bellefonte, PA) 80/100 mesh packed column was used to determine the biogas composition. The oven temperature was set to

55°C, helium was used as the carrier gas at a flow rate of 30 mL.min^{-1}. A sample volume of 0.2 mL was used (Sigge, 2005) and all analyses were done in duplicate.

Nitrogen, phosphate and polyphenols

The content of both these nutrients (nitrogen and phosphorous) were confirmed by means of Spectroquant® Nitrogen (0.5 – 15.0 mg.L^{-1} N) and Spectroquant® Phosphate Cell tests (0.05 – 5.0 mg.L^{-1} PO_4-P) (Merck, Germany) using a Merck Spectroquant® Nova 60 spectrophotometer. Polyphenol content within compost leachate was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). Analyses were performed in duplicate and a dilution of 1:50 was used.

Coliforms and Escherichia Coli

Microbial analysis on both the compost leachate and compost from ES7 and ES8 were determined on days 1 and 21 according to the SANS 9308 (SANS, 2012) method using a Colilert-18 kit (IDEXX, USA). A 10 g compost sample was placed in a stomacher bag, and 90 mL of sterile physiological saline solution (PSS) was added. The sample was stomached for approximately 2 min, after which the liquid content was used to prepare a dilution series (10^{-1} to 10^{-10}). For the compost leachate, 10 mL was added to 90 mL saline solution, where after a dilution series (10^{-1} - 10^{-10}) was prepared. Duplicates of each dilution series were prepared as to ensure an end sample volume of 100 mL. Colilert-18 reagent indicator (4-methylumbelliferyl-β-D-glucuronide) (MUG) was added to each of the duplicate Schott bottles. Each dilution was poured into a Quanti-Tray/2000 (IDEXX, USA) and sealed with a Quanti-Tray® Sealer Model 2X (IDEXX, USA). The trays were incubated at 37°C for 18 h. After the 18 h incubation period, total coliforms were determined by counting the wells within the Quanti-Tray that developed a yellow colour. The presence of *E. coli* was determined by counting the wells that fluoresced under ultra violet light (365 nm) (Spectroline® Model CM-10 Fluorescence Analysis Cabinet). The positive counts for both total coliforms and *E. coli* were used to determine the corresponding loads by using an IDEXX Quanti-Tray®/2000 most probable number (MPN) table (IDEXX, USA; SANS, 2012). Microbial counts were expressed as either MPN.100 mL^{-1} or MPN.100 g^{-1}.

Physico-chemical analyses

The pH (KCl), electrical resistance and total extractable cations (Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na)) were all determined according to the methods described by The Non-affiliated Soil Analyses Work Committee (1990) and standard operating procedures (SOP) from Bemlab Pty Ltd (Strand, South Africa). The method for pH determination in soil as described by The Non-affiliated Soil Analyses Work Committee (1990), was adapted. Samples were dried overnight at 105°C, after which a paste was made (5 g sample and 25 mL 1N KCl) and the pH was measured by placing the pH electrodes in the mixture. Results are reported in pH (KCL). For electrical resistance a saturated soil paste was prepared by mixing air-dried samples with de-

ionised water. The electrical resistance of the paste is expressed as ohms. During this study the electrical conductivity (dS.m^{-1}) (inverse of the resistivity) was used for discussion purposes.

For the total extractable cations (Potassium (K^+), Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Sodium (Na^+)) and Phosphorous (P) as well as micro-nutrient analysis (Boron (B), Copper (Cu), Manganese (Mn), Zinc (Zn), Iron (Fe) and Cobalt (Co)) compost samples were first prepared by drying overnight at 70°C, where after they were milled to approximately 40 micron and ashed at 480°C. The samples were then extracted through filter paper by adding 50:50 HCl (32%) solution. The extracted solutions were analysed against suitable standards with a Varian Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) Optical Emission Spectrometer. The results were expressed as mg.kg^{-1}.

The total Carbon (C) and Nitrogen (N) were determined directly using total combustion on a Leco Truspec® CN N analyser. Both C and N were expressed as %C and %N. Ammonium (NH_4) and Nitrates (NO_2) were extracted with a 1M KCl solution and analysed with an Auto Analyser at Bemlab Pty Ltd (Strand, South Africa). The results were reported as mg.kg^{-1}. All analyses were conducted in triplicate.

The moisture, bulk density and ash were also determined according to SOP from Bemlab (Strand, South Africa). The gravimetric moisture content was done on a mass/mass basis by drying compost samples over night at 70°C. Bulk density of compost samples was determined by weighing 60 cm^3 of compost at 20°C. The results were given as kg.m^{-3}. The ash content was performed by weighing 2 g of dried (70°C), sieved (40 micron) compost and ashing it overnight in a muffle furnace at 480°C. Results were expressed as the ash percentage of the dried compost sample. In order to lower the moisture content after the 21 day period the composts was dried on open trays in an incubator room for 24 h at 37°C.

Results and Discussion

Preliminary experimental studies

It was found during the preliminary studies that the composting process can be sped up by using shredded waste as smaller pieces most probably provide more available surface areas for micro-organisms. Results obtained showed that the laboratory-grade CaO resulted in better solubility than the industrial CaO and this was therefore used in the subsequent studies. Moisturising liquid (pH adapted effluent from an UASB reactor treating GDWW) was added to the digesters every 24 hours, depending on the volume of leachate generated within each individual unit. It was noted that in order to improve the digestion process, the leachate formed would have to be drained continuously as this led to the accumulation of VFA's and caused the pH to drop to below 6.5. The pH drop probably led to growth inhibition of the microbes responsible for the digestion and subsequently resulted in process failure. During the preliminary study, colour changes were observed; red-purple to brownish-green for the grape skins and dark green to yellow for the leachate. By the end of the 21 day study the grape skins changed into a uniform soil-like texture.

Experimental study 1: Effect of inoculum composition and ratio

Inoculum during this study consisted of 50% ($m.m^{-1}$) anaerobic granules and fresh cow manure in ratios of 50:50, 25:75 and 0:100. The results, in terms of leachate volume and pH are shown in Figure 3.3. It can be seen that Digester 2 achieved a pH of 6.68 by day 5 and a final pH of 7.81 by day 21 (Fig. 3.3). Digester 1 surpassed a pH of 6.5 by day 7, reaching a final pH of 7.6. Digester 3 reached a pH above 6.5 by day 15 and a final end pH of 7.79. In terms of the stabilisation of the pH, Digester 1 and Digester 3 performed the best with a neutral pH (Fig. 3.3) being reached by day 16, while Digester 2 (25:75) only reached a pH above 7 by day 18. The overall pH stability (in terms of pH being above 6.5) of Digester 1 was seen to be better than Digester 2 and Digester 3, with only four pH measurements after day 6 being below 6.5. The stabilisation of the pH as seen for both Digester 1 and Digester 3 from day 16 onwards (Fig. 3.3) can probably be linked to the stabilisation and the microbial activity. In general, an initial increase in pH was seen for all 3 digesters, after which the pH decreased slightly and stabilised only to increase and stabilise again. A possible explanation for the initial pH increase, after which a slight decrease was observed (Fig. 3.3) could be due to the high alkalinity in the cow manure serving as a buffering capacity for the accumulation of simple VFA's formed during the early composting stages (Astals *et al.,* 2012). The second increase in pH can possibly be contributed to the microbial breakdown of these acids and the release of alkali and alkali earth metals that were connected to carbon matter (Smith & Hughes, 2002). Another explanation could be the high amount of CaO present in the digester during the early stages of composting. The CaO could possibly have led to a higher alkalinity and therefore a higher pH. As the CaO "washed out" of the system (through the daily "moisturising action") the alkalinity decreased, possibly leading to a decrease in pH. Over time the digesting system started to generate its own alkalinity, possibly allowing the pH to rise and eventually stabilise around neutral.

Digester 1 produced the most leachate (1 071 mL) over the 21 day ES compared to Digester 2 (759 mL) and Digester 3 (728 mL) (Fig. 3.3). A possible explanation for this is the high moisture content of manure and granules. The moisture content of both the granules and manure were determined to be 92% and 80%, respectively. A higher ratio of granule:manure inoculum content could therefore possibly lead to a higher moisture content and consequently a higher volume of leachate generation. In terms of volume reduction Digester 1 performed the best with a 21% mass reduction followed by the Digester 2 (18%) and Digester 3 (17%).

Overall the digesters followed a similar trend (Fig. 3.3) as pH increased over the ES period and leachate volume decreased. Although Digesters 1 and 2 reached a pH of 6.5 earlier, the pH of Digester 3 (containing only cow manure) was found to be very close to the "lower limit" around the same time and all three digesters reached the same end pH (Fig. 3.3). Due to the fact

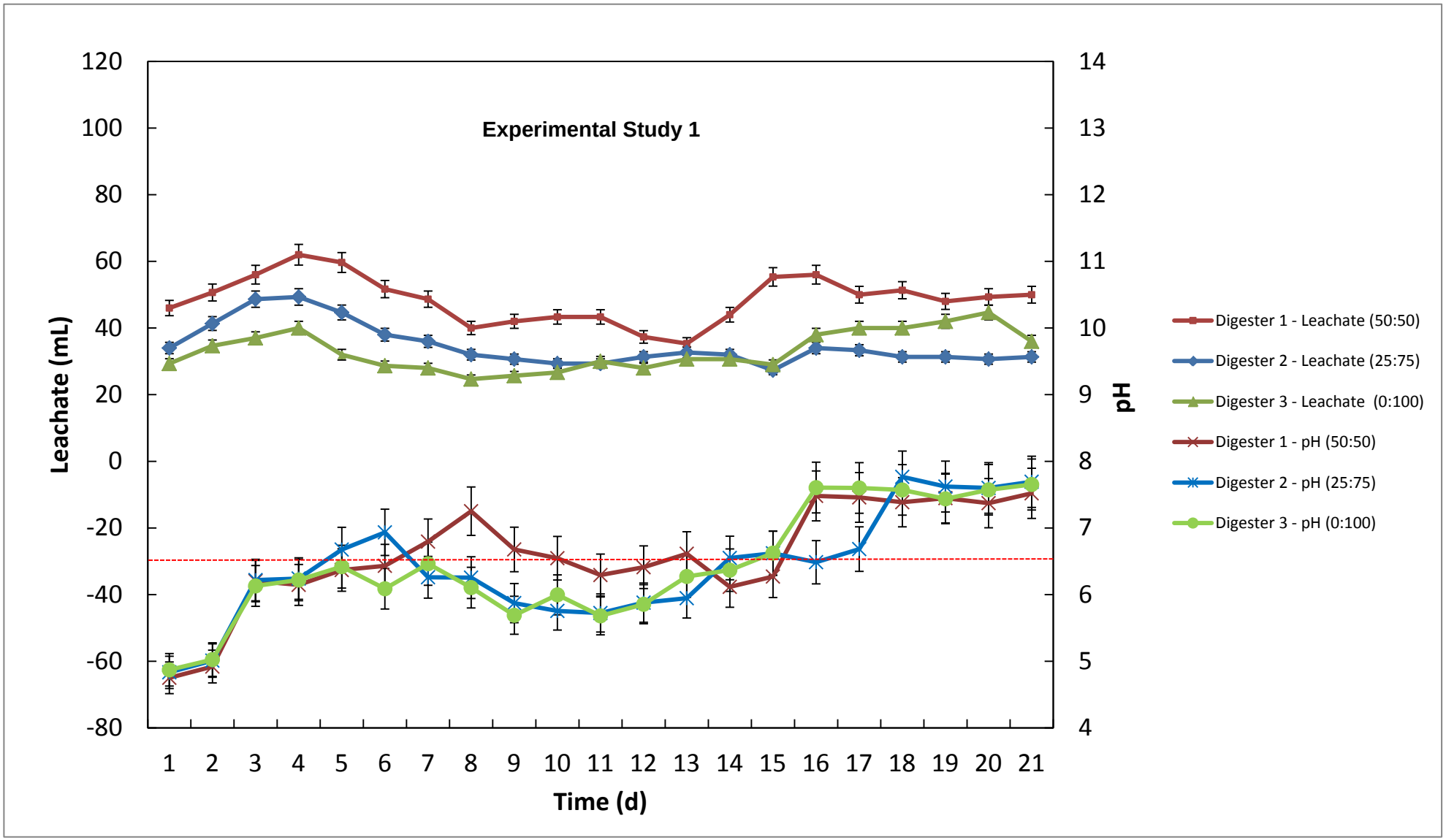

Figure 3.3 pH and leachate volumes generated with the different inoculum compositions and ratios over the ES1 period (n=3) (Red line indicates pH of6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

that the highest pH difference between cow manure and anaerobic granules as inoculum (Digester 1) and cow manure alone (Digester 3) were approximately one (Fig. 3.3) as well as the fact that anaerobic granules are expensive and availability is limited (Liu & Tay, 2004) the use of anaerobic granules for inoculation purposes was stopped in further experimental studies.

Experimental study 2: Investigating the effect of inoculum size

Based on the successful application of a 50% ($m.m^{-1}$) inoculum as found during ES1, it was decided to investigate the effect of lower inoculum concentrations (10, 15, 25% ($m.m^{-1}$)) on the composting process. The advantage would be that a lower inoculum concentration could allow a higher amount of grape waste to be treated making the process more feasible and economical. A similar pH trend for that observed (Fig. 3.4) in the previous study where 50% ($m.m^{-1}$) inoculums of anaerobic granules and cow manure in different ratios were used. The volume of leachate generated in this case decreased and the pH increased during the 21 day period. It was expected that the inoculum concentration would be correlated to the size of the microbial community, and therefore that the higher inoculum content would result in better process stability in terms of pH and degradation. This however, was not the case as Digester 1, with the lower inoculum (10% ($m.m^{-1}$)) slightly outperformed Digester 3 containing 25% ($m.m^{-1}$) inoculum. Digester 1 surpassed a pH of 6.5 by day 6, which could favour methane production. Digesters 2 and 3 reached a pH above 6.5 only by days 11 and 13, respectively (Fig. 3.4). The pH of Digester 1 reached neutral by day 12 and stabilised to an end pH of 8.01 by day 21 (Fig. 3.4). Digester 1 also generated the most leachate throughout the study (791 mL) with Digester 3 producing 747 mL followed by Digester 2 producing the least (712). Leachate volumes gave a good indication of digester activity, as it appeared that higher volumes of leachate benefitted the pH of the composting process (i.e. higher volumes leachate produced, generated compost with a higher end pH).

Digester 3 had the highest volume reduction (27%), compared to Digester 2 with a 21% reduction. It was also noted during this experimental study that not all of the moisturising liquid seeped through the grape skins. This resulted in a water layer forming within the digester that consequently added to the final moisture content of the compost. After drying the compost for 24 h at 37°C in an incubator room, the volume reduction of the compost increased significantly to 67%, 75% and 76% for Digesters 1, 2 and 3, respectively.

Although Digester 1 reached a pH > 6.5 first, the pH of Digester 3 was very close to the “lower limit” and pH stabilisation was seen for all 3 digesters (Fig. 3.4) from day 11. Due to the similar results obtained, it was decided that using the lowest possible concentration of inoculum (10% ($m.m^{-1}$)) would be most beneficial. The lower inoculum concentration would allow more grape waste to be treated making the process more feasible.

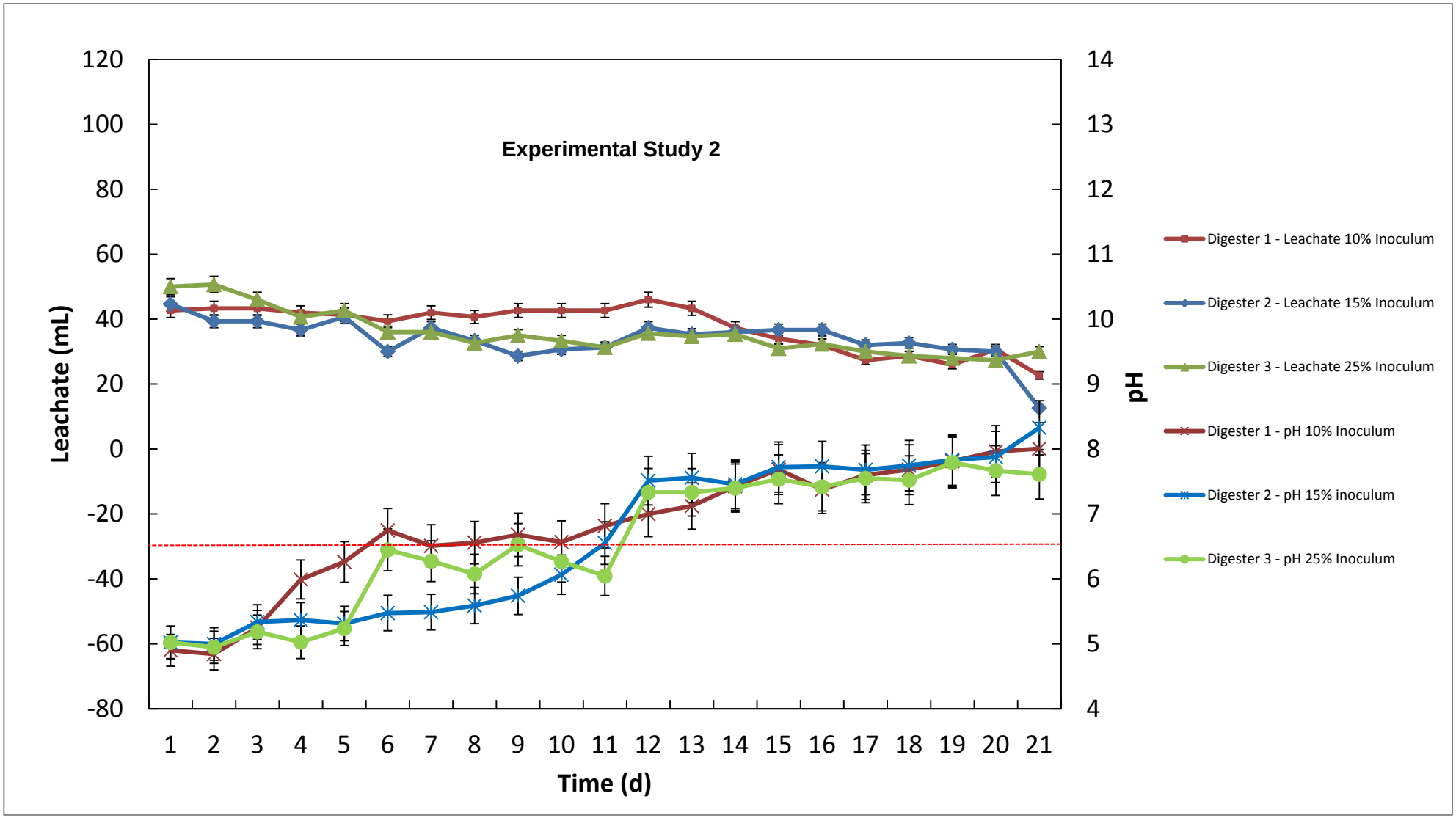

Figure 3.4 pH and leachate volumes generated during the investigation of the inoculum size on the efficacy of the composting process over the ES2 period (n=3) (Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

Experimental study 3: Effect of anaerobic compost (AC) as inoculum

After optimising the CM inoculum size (10% ($m.m^{-1}$)) in ES2, it was decided to use AC as inoculum during this experimental study. AC as an inoculum could possibly be more beneficial than CM in attaining a pH > 6.5 before day 6, as the micro-organisms responsible for degradation of the solid waste have already acclimatised to the anaerobic conditions. The aim was thus to reach a pH > 6.5 as early as possible as this would favour methane production and reduce unpleasant odours due to the accumulation of VFA's.

The results, in terms of leachate volume and pH, are shown in Figure 3.5. Unlike the previous experimental studies, none of the digesters had reached a pH of 6.5 by day 10 (Fig. 3.5) as the highest pH at this point was observed in Digester 2 (4.74). Therefore, the UASB winery effluent used as moisturising liquid, was adjusted from day 10 onwards to approximately 3 500 $mgCaCO_3.L^{-1}$. A slight increase (Fig. 3.5) in pH was seen hereafter as Digesters 1 and 2 reached a pH above 6.5 by day 15 (Fig. 3.5). Digester 3 containing 25% ($m.m^{-1}$) inoculum, failed to reach a pH above 6.5 throughout the 21 day ES period. Digester 3 generated the lowest volume of leachate (498 mL), compared to Digester 1 (655 mL) and Digester 2 (643 mL). The leachate from Digester 3 also had an unpleasant acidic odour due to the very low pH, indicating digester instability.

Although results obtained were not as expected, Digester 1, with the lower inoculum concentration (10% ($m.m^{-1}$)) performed the "best" during this experimental study by maintaining a neutral pH from day 17 and reaching a final pH of 7.78 (day 20). This ES highlighted the important role of alkalinity during pH stabilisation.

Experimental study 4: Effect of a 10% ($m.m^{-1}$) AC inoculum

From the results obtained in ES3 it was evident that alkalinity is an important parameter in the AnC of grape skins and thus, ES3 was repeated with winery UASB reactor effluent as a ML with a high inherent alkalinity (3 500 $mgCaCO_3.L^{-1}$).

The digesters reached a pH > 6.5 by day 7 (Fig. 3.6) while pH stabilisation (pH 7 – 8) was seen from day 8 onwards, reaching a final pH of 7.60. The total volume of leachate produced by the digesters over 21 days was found to be 1 009 mL (Fig. 3.6). Again the same trend can be seen in terms of leachate generation and pH evolution for all the digesters as the pH increased, where after it slightly stabilised, and increased again (Fig. 3.6). To monitor the activity within the digesters the leachate generated on day 11 and day 16 was analysed for alkalinity content and chemical oxygen demand (COD) concentration. Results indicated an increase in alkalinity from *ca.* 6 103 to 6 308 $mgCaCO_3.L^{-1}$ and decrease in COD from *ca.* 74 196 to 29 403 $mg.L^{-1}$. An increase in the alkalinity content and decrease in the COD concentration was expected as this was taken as a clear indication that degradation had taken place in the digesters. The overall pH results from ES4 (Fig. 3.6) showed to be more stable than results obtained from ES3 (Fig. 3.5) indicating the significant importance of the high inherent alkalinity of the moisturising liquid during pH evolution.

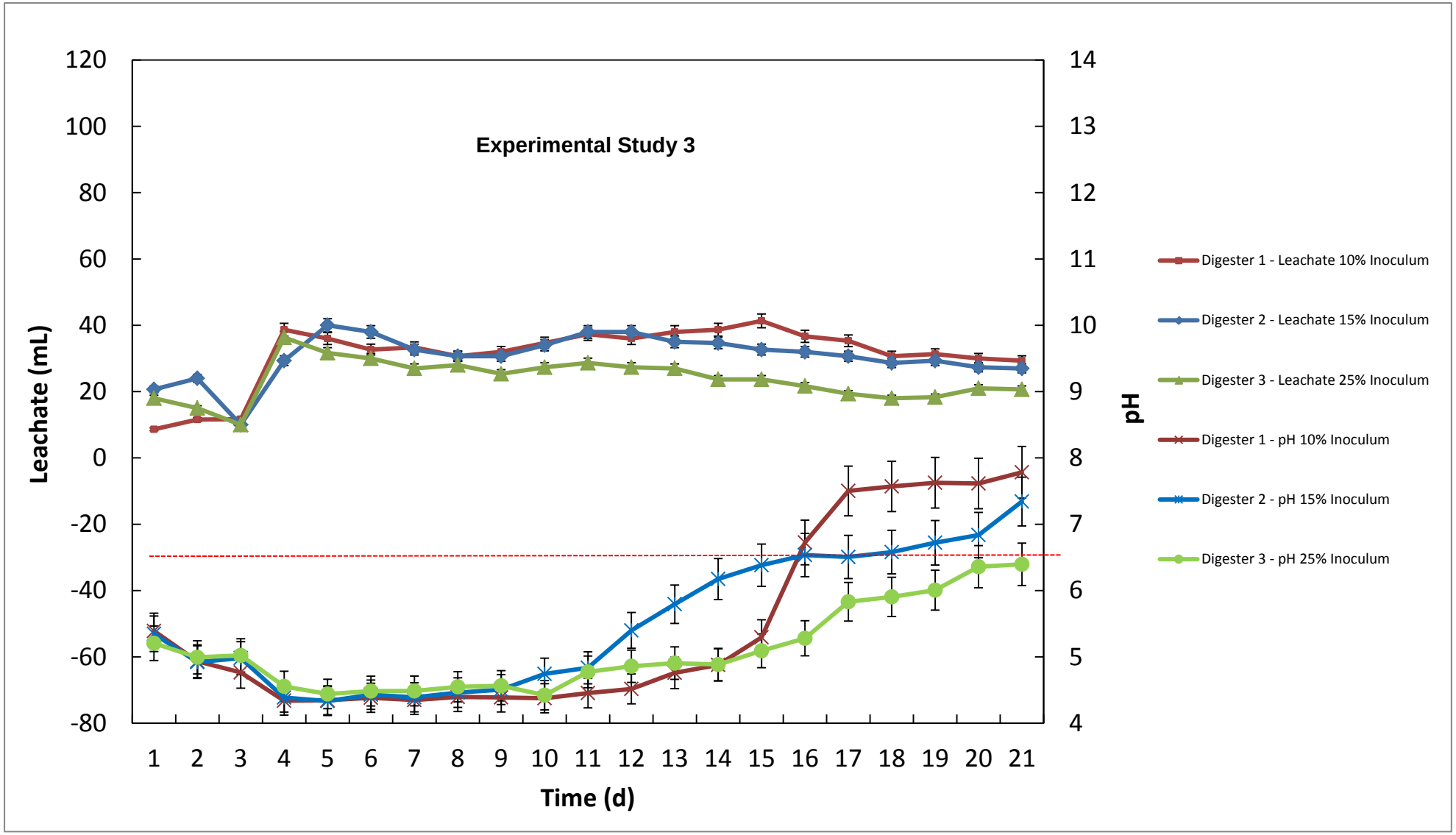

Figure 3.5 pH and leachate volumes generated during the investigation of anaerobic compost (AC) as an inoculum source during the composting process over the ES 3 period (n=3)(Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

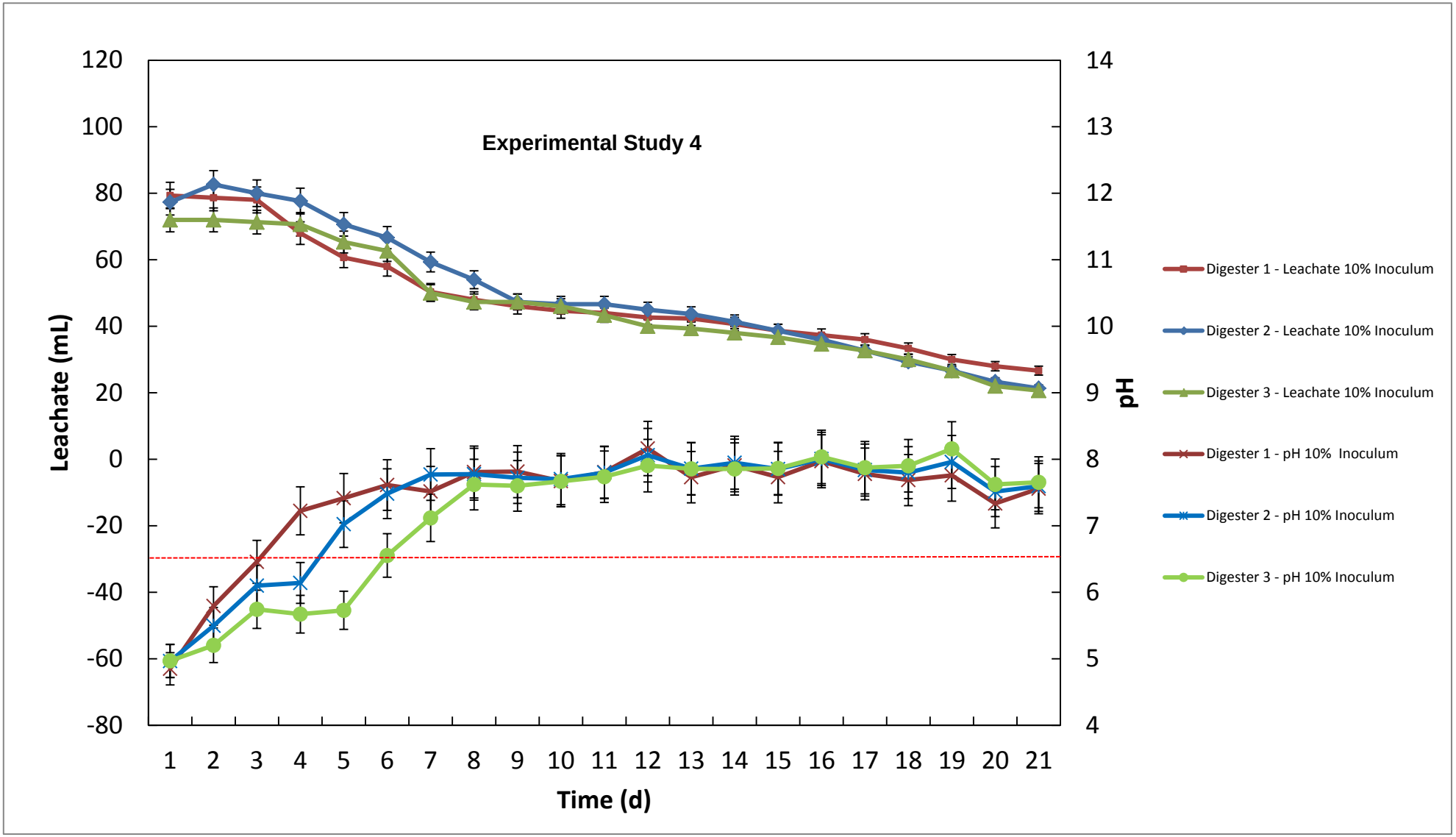

Figure 3.6 pH and leachate volumes generated during the investigation of anaerobic compost (AC) as an inoculum source during the composting process over the ES4 period (n=3) Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

Experimental study 5: Effect of different moisture levels

After establishing the optimum inoculum size (10% ($m.m^{-1}$)), during ES1 and ES2, and inoculum type (AC in ES3), it was decided to also investigate the effect of various moisture (M) levels (35, 40, 45, 50, 55 and 60% ($m.m^{-1}$)) on the anaerobic composting process. Literature reports that although it is difficult to maintain constant moisture levels during digestion, higher levels facilitate the degradation process (Khalid *et al*., 2011). This agrees with results obtained in this study as the digesters operated at lower moisture levels (35 and 40% ($m.m^{-1}$)) failed to reach a pH > 6.5 (Fig. 3.7) and although Digester 3 (45% ($m.m^{-1}$)) reached 6.5 by day 17, it decreased again, only reaching a pH of 7.18 by day 21 (Fig. 3.7). The digesters operated at lower moisture contents produced less leachate over the ES period (Table 3.2) and showed a lower final pH (Fig. 3.7). Digesters 5 and 6 showed pH stabilisation from day 16 onwards, but only reached a neutral pH around day 14 and day 16, respectively. Digester 4 performed the best and was the first digester to reach a pH > 6.5 (day 6). It also had the highest final pH (7.65) (Fig. 3.7). Therefore, it was clear from these results that a moisture content of 50% ($m.m^{-1}$) was the optimum

Table 3.2 Total leachate volumes produced by the digesters during ES6 over the 21 day period

Moisture content % ($m.m^{-1}$)	35	40	45	50	55	60
Leachate volume (mL)*	550	597	805	969	1062	627

***Total leachate volume (mL) generated over the 21 day period**

Experimental study 6: Effect of different grape skin (carbon source) ratios.

After identifying optimal values for the following parameters- inoculum ratio, size and type (10% ($m.m^{-1}$) AC) as well as optimal moisture conditions (50% $m.m^{-1}$), it was decided to investigate the effect of different white and red grape skins (carbon source) ratios (25:75, 50:50, 75:25) on the composting process (Fig. 3.8). The results, in terms of leachate volume and pH are shown in Figure 3.8. Digester 3 (50:50), containing equal volumes of WGS and RGS was found to perform the best with a pH > 6.5 being reached by day 15 and a final pH of 7.18. Digester 1 (25:75) reached a pH > 6.5 (7.03) by day 16, but failed to stabilise, only reaching a pH of 5.72 by day 21 (Fig. 3.8). Digester 2 (75:25) failed to reach a pH of 6.5 within 15 days and stopped producing leachate. As a result the pH could not be recorded for the rest of the study. Digester 3 generated the most leachate during the ES (total 805 mL), followed by Digester 1 with 626 mL and the lowest for Digester 2 with a total of 170 mL. The low volumes of leachate generated by Digester 2 correlated with the low pH values obtained (Fig. 3.8), indicating overall composting failure.

The poor performance of Digester 3 (75:25) can possibly be explained in terms of the winemaking process. It was expected that the white grape skins (WGS) would perform better as during the production of white wine, the maceration process only lasts a few hours (Jackson, 2008). For the production of red wine it is a much longer process and occurs together with alcoholic fermentation (Jackson, 2008). Taking the abovementioned into consideration it was expected that the nutritional content and pH of white grape skins to be more favourable.

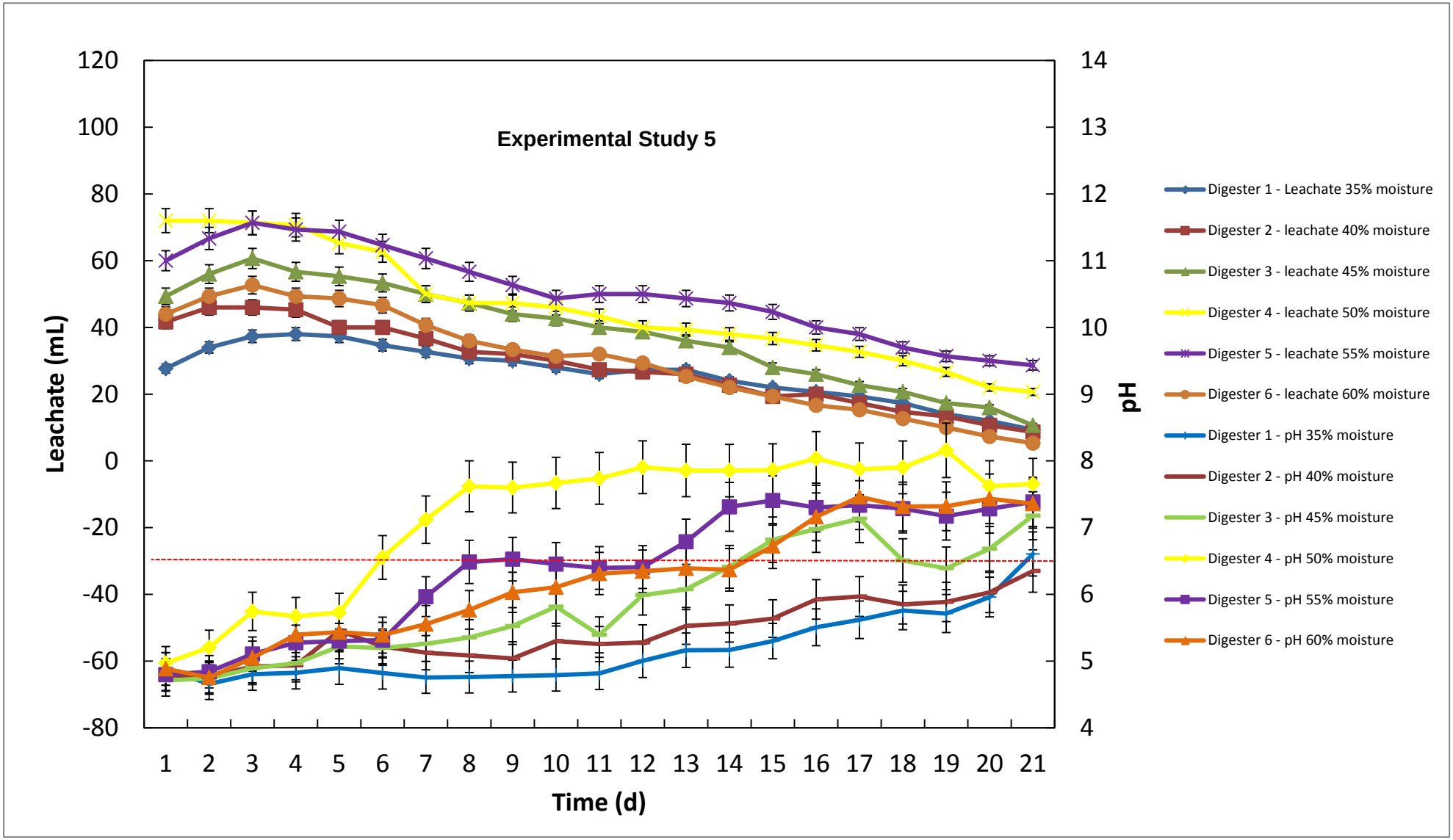

Figure 3.7 pH and leachate volumes generated during the investigation of different moisture levels on the composting process over the ES5 period (n=6) (Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

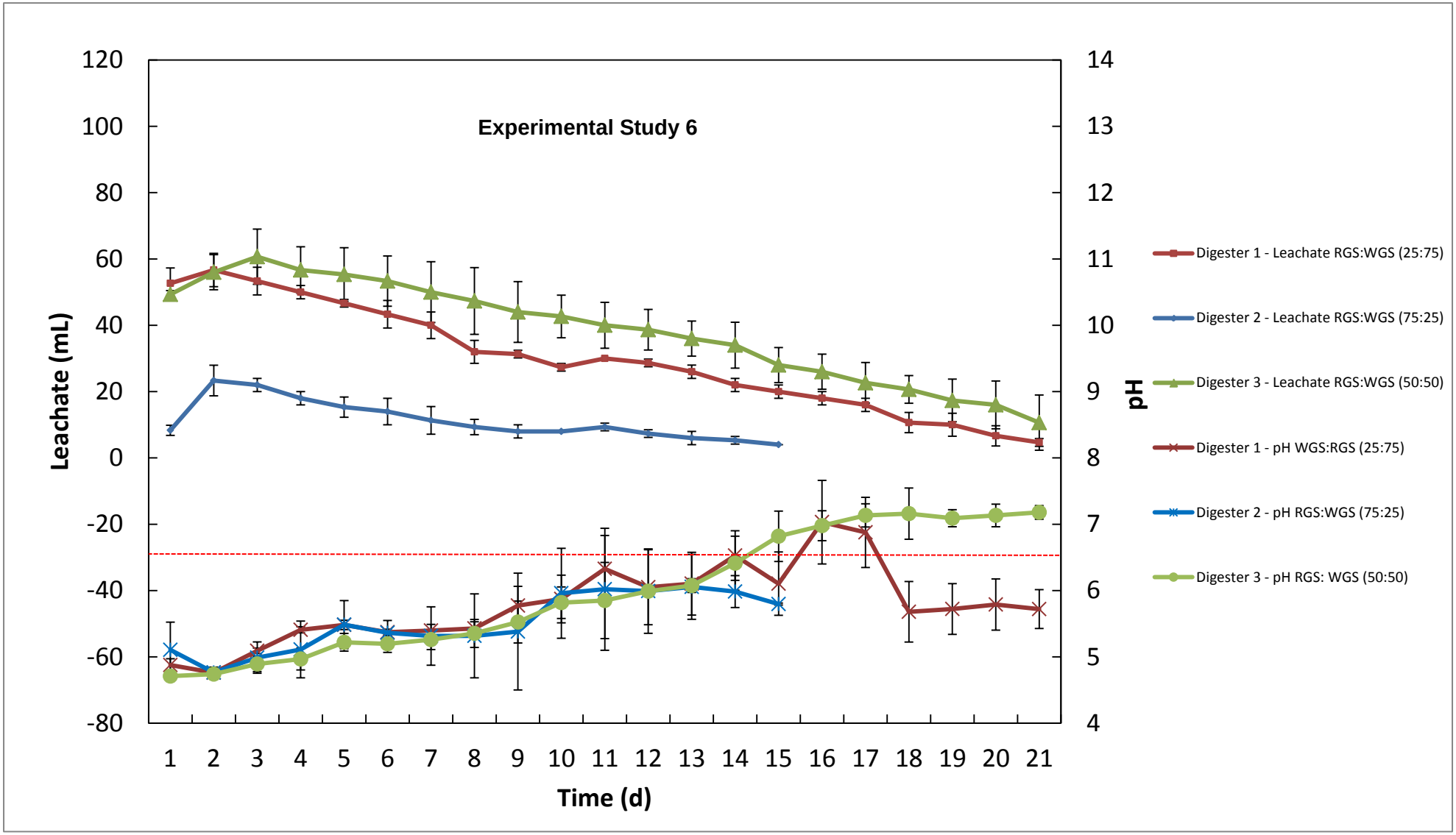

Figure 3.8 pH and leachate volumes generated during the investigation of different grape skin (carbon source) ratios on the composting process over the ES6 period (n=3) (Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

Although the moisture content for both types of grape skins were found to be 70%, the pH of the white grape skins (3.81) was found to be higher than the pH of red grape skins (RGS) (3.21). In Fig. 3.8 it is clear that the 50:50 ratio of grape skins (Digester 3) performed the best in terms of pH stabilisation and leachate generation and this parameter was therefore taken as the optimum grape skin (carbon source) ratio for the anaerobic composting process.

Experimental studies 7 and 8: Effect of optimised parameters on the composting process

The last lab-scale study (ES7) as well as the up-scale (ES8) was done to investigate the combined effect of all the optimised parameters as identified during the previous experimental studies. It was decided to use a 15% ($m.m^{-1}$) CM inoculum in both studies, instead of a 10% ($m.m^{-1}$) AC inoculum because in "industrial" applications a "first batch" would always require an inoculum other than AC. An inoculum size of 15% ($m.m^{-1}$) was chosen to ensure an efficient "start-up/first batch" as previous studies showed that the activity of the microbial community is related the inoculum size (Griessel, 2002). The optimum moisture content (ES5, 50% $m.m^{-1}$) and grape skin ratio (ES6, 50:50) were applied during both the ES7 and ES8.

Results obtained during experimental studies 7 and 8 are shown for both the lab-scale (Fig. 3.9, Table 3.3) and the up-scale digesters (Fig. 3.10, Table 3.4). The results for the physico-chemical analyses of the composts from both the lab-scale and the up-scale are shown in Table 3.5. Several photo images taken before and after the composting process are shown in Figure 3.11.

As very little literature exists on the quality and composition of anaerobic compost, it was decided to compare results obtained from the physico-chemical analysis of the anaerobic compost with guidelines and regulations regarding aerobic compost. Regulations regarding the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act no. 36 of 1947) of South Africa contains very little information regarding compost requirements and according to Malherbe (2014) there is a lack in South Africa for guidelines on composting (Malherbe S. 2014, Senior Research Agronomist, ZZ2 Laboratories, Mooketsi, South Africa, personal communication, 28 August). In contrast there are clear guidelines in Europe and in the USA. According to Brinton (2000), it is difficult to summarise international compost quality standards as various regulations and guidelines exist. In Table 3.6 the guidelines from different countries are summarised (Alexander, 1994; Brinton, 2000; Allen, 2014; ECN, 2014, Watson, 2014).

pH, leachate volume, alkalinity and COD

The pH changes and leachate generation during ES7 followed the same trend (Fig. 3.9) as seen during the previous studies with the pH increasing and leachate production decreasing over the 21 day study period. The lab-scale digesters (ES7) surpassed a pH of 6.5 on day 10 and reached a final end pH of 7.09. The up-scale study (ES8) also followed the general pH and leachate trend with digesters reaching a pH > 6.5 on day 6 with a final pH of 7.20 (Fig. 3.10). According to results obtained from the pH (KCl) analysis, the end pH values (Table 3.5) for the compost, with the

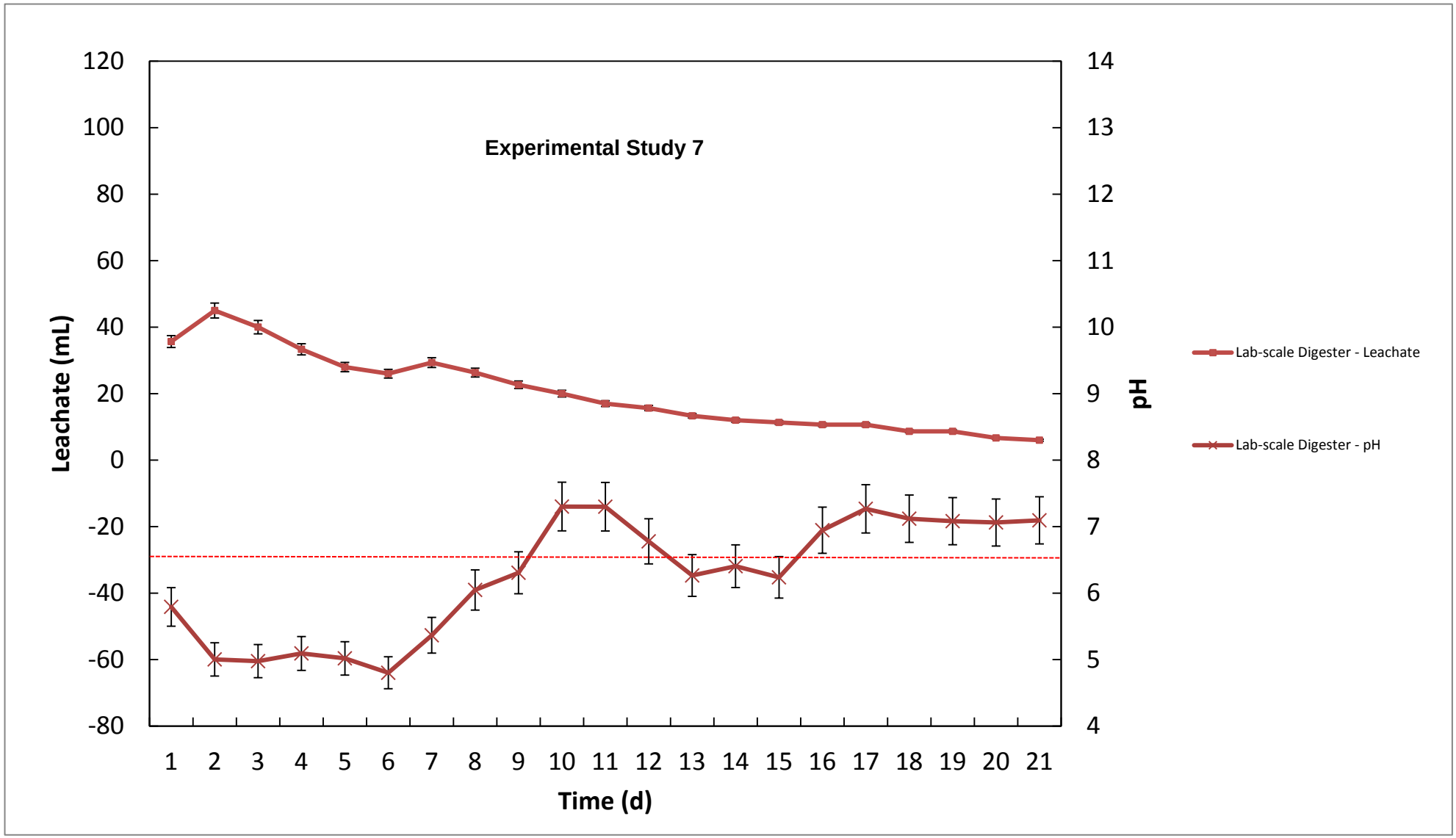

Figure 3.9 pH and leachate volumes generated during the investigation of the effect of optimised parameters on the lab-scale composting process over the ES7 period (n = 3) (Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

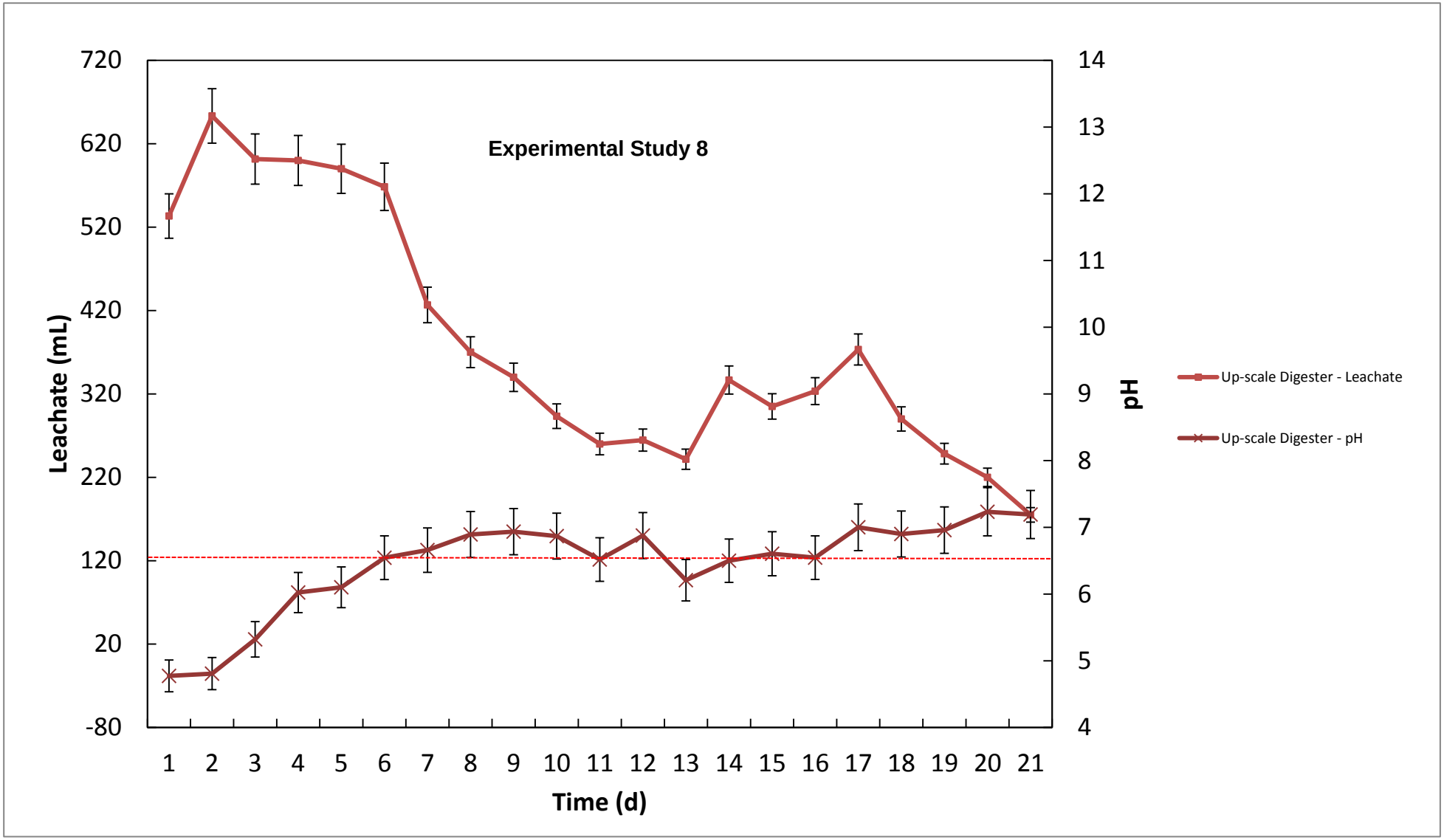

Figure 3.10 pH and leachate volumes generated during the investigation of the effect of optimised parameters on the up-scale composting process over the ES 8 period (n=3) (Red line indicates pH of 6.5 which was taken as the lower limit for optimum operation) (Error bars represent error at 95% confidence interval).

Table 3.3 Summary of results obtained from the composting leachate of the lab-scale digesters during anaerobic composting of grape skins in Experimental Study 7 (n=3)

	Time (d)								
	1	4	7	10	14	16	19	20	21
Alkalinity[1]	4 942	2 725	5 983	10 383	11 450	12 625	19 300	20 750	23 000
COD[2]	13 2795	-	76 458	-	59 353	57 645	-	53 910	-
TSS, VSS[3]		2.005;1.500			1.625;1.265			1.200;1.040	
Biogas[4]	0.004	0.00	0.003	0.00	0.00	0.00	0.00	0.00	0.00
Methane[5]	No methane detected								

1 Alkalinity ($mgCaCO_3.L^{-1}$), 2 ($mg.L^{-1}$), 3 ($g.L^{-1}$), 4 Biogas ($L.day^{-1}$), 5 Methane (%)

Table 3.4 Summary of results obtained from the composting leachate of the up-scale digesters during anaerobic composting of grape skins in Experimental Study 8 (n=3)

	Time (d)								
	1	4	7	10	14	16	19	20	21
Alkalinity[1]	2 751	6 233	7 800	8 100	7 892	8 992	14 658	17 800	19 067
COD[2]	13 9815	60 975	46 800	46 170	37 778	-	13 662	12 771	-
TSS, VSS[3]		5.335;4.325			1.645;1.395			1.220;1.080	
Biogas[4]	0.002	0.003	0.002	0.002	0.006	0.002	0.002	0.002	0.003
Methane[5]	No methane detected								

1 Alkalinity ($mgCaCO_3.L^{-1}$), 2 ($mg.L^{-1}$), 3 ($g.L^{-1}$), 4 Biogas ($L.day^{-1}$), 5 Methane (%)

Table 3.5 Chemical and physical characterisation of the composts obtained during the investigation of the effect of the optimised parameters on the composting process

Scale	Sample	pH (KCl)	EC ($dS.m^{-1}$)	Moisture (%)	Density ($kg.m^{-3}$)	Ash (%)	NH_4-N ($mg.kg^{-1}$)	NH_3-N ($mg.kg^{-1}$)	Nitrogen (%)	Carbon (%)
Lab	1	7.0	0.143	59	491.10	11.80	152.32	3.68	2.27	38.74
	2	6.7	0.167	59	485.70	12.15	310.44	3.08	2.17	37.36
	3	7.1	0.167	55	395.00	13.40	163.72	3.32	2.32	38.96
Up	1	6.5	0.167	48	434.50	13.90	384.40	4.08	2.18	38.30
	2	6.4	0.167	47	388.00	13.45	430.00	6.44	2.04	35.76
	3	6.6	0.167	48	410.50	13.60	415.82	5.67	2.30	36.38

	Sample	Phosphorous (%)	Potassium (%)	Calcium (%)	Magnesium (%)	Sodium ($mg.kg^{-1}$)	Manganese ($mg.kg^{-1}$)	Copper ($mg.kg^{-1}$)	Iron ($mg.kg^{-1}$)	Zinc ($mg.kg^{-1}$)	Boron ($mg.kg^{-1}$)
Lab	1	0.24	1.65	1.91	0.10	307	32.43	20.85	180.80	36.79	11.33
	2	0.22	1.74	2.05	0.11	301	34.39	31.31	133.66	36.94	8.53
	3	0.21	1.71	2.09	0.11	354	33.66	18.44	138.32	37.28	6.32
Up	1	0.21	1.78	2.67	0.13	364	38.84	11.30	119.94	40.90	6.79
	2	0.21	1.72	2.56	0.13	356	37.08	11.31	116.08	45.01	7.69
	3	0.23	1.76	2.71	0.12	362	36.75	11.36	117.09	43.80	7.43

Figure 3.11 Images taken of the waste before and after the anaerobic composting process **(a** = before shredding; **b** = after shredding**)** of green grass and grape skins, **(c)** shredded grape skins and grass before the addition of CaO, **(d)** compost obtained after the 21 day period before drying and **(e),** the final compost (dried for 24 h at 37° C and milled).

Table 3.6 Summary of guidelines from different countries regarding compost quality

	Local South Africa SOP*	USA	Europe and Germany
pH	6.5 – 7.5	6.8 – 7.3	declared
Nitrogen (%)	-	1.0 – 2.0	-
NH_4 ($mg.kg^{-1}$)	< 500	-	-
NH_3 ($mg.kg^{-1}$)	200 – 500	-	-
Phosphorous	-	0.6 – 0.9%	< 1 200 $mg.L^{-1}$
Organic matter (%)	25 – 60	> 30	> 20%
Electrical conductivity ($dS.m^{-1}$)	1.5 – 3	< 4.0	-
Bulk density ($kg.m^{-3}$)	-	534 – 593	-
Potassium	-	0.2 - 0.5%	< 2 000 $mg.L^{-1}$
Moisture (%)	20 – 40	45 – 50	< 75
Ash (%)	< 37.5	-	-
Calcium	-	-	-
Magnesium	-	-	-
Sodium ($g.L^{-1}$)	-	-	< 2.5
Manganese	-	-	-
Copper ($mg.kg^{-1}$)	-	1 500	100
Iron	-		-
Zinc	-	2 800	400
Boron	-	-	-
C:N	12 – 15	15 - 20	18
Pathogens	.		Salmonella non-detected in 25 g *E.coli* < 1 000 $MPN.g^{-1}$
Other	No offensive odours, glass, wire or other unacceptable fragments		

***Standard Operating Procedure**

exception of the sample from Digester 2 (up-scale), are all within limits (6.5 – 7.3) (Table 3.6). The pH (KCl) method (5 g sample and 25 mL (1N) KCl) gives a better indication than the pH (H_2O) of the hydrogen ion activity because potassium chloride is used to mask variations in salt concentrations that could be caused by fertiliser residues, irrigation water or the microbial degradation of organic matter (The Non-Affiliated Soil Analyses Work Committee, 1990). The pH of compost is of importance as the compost can alter the pH of the soil which could directly affect the availability of nutrients to the plant (Tester, 1990). Although a pH of 6.4 is slighty below the value recommended by a local South African composting company (Table 3.6), Darlington, (2012) reported that most types of compost have a between pH of 6 and 8. He states that it depends on the substrate from which the compost was derived.

The alkalinity for the lab-scale digesters was found to increase (Table 3.3) from 4 942 $mgCaCO_3.L^{-1}$ on day 1 to 23 000 $mgCaCO_3.L^{-1}$ by the end of the 21 day study. The up-scale digesters also showed an increase from 2 751 $mgCaCO_3.L^{-1}$ to 19 067 $mgCaCO_3.L^{-1}$ by the end of the ES (Table 3.4). Both lab-scale and up-scale digesters showed a decrease in alkalinity after Day 1 which increased gradually from day 6 for the lab-scale (Table 3.4) and Day 3 for the up-scale digesters (Table 3.5), respectively. Possible explanations for this include: (i) the high alkalinity of the cow manure used could have served as additional buffering capacity for the accumulating VFA's formed (Astals *et al.,* 2012) during the initial stages of composting or; (ii) resulting from the grape skin mixture to soak with CaO overnight at 37°C. Literature states that due to the low pH of grape pomace (3.5 – 3.8) it degrades slowly on its own and therefore lime or feedstock may be added to increase the pH (Westover, 2006). The pH results from this study are in accordance with literature as the pH of the WGS and RGS before composting was found to be 3.81 and 3.20. The lime/CaO mixture was added to lift the initial pH and it is possible that the residual CaO washed out with the leachate by day 1 and led to a higher alkalinity measurement. It was expected to see an increase in alkalinity as the pH and alkalinity are important for the composting process and together result in a suitable environment for methanogenesis to occur (Del Real Olvera & Lopez-Lopez, 2012). It is well known that adequate alkalinity is required in any anaerobic digestion system to sustain a stable pH and optimal biological activity (Lee *et al*., 2009). Sufficient alkalinity is required for proper pH control (Gerardi, 2003) and thus explains why the alkalinity increased as the pH stabilised.

The COD of the leachate was found to decrease over the 21 days for both the lab-scale (Table 3.3) and up-scale (Table 3.4) from 132 795 – 53 910 $mg.L^{-1}$ and 13 9815 – 12 771 $mg.L^{-1}$, respectively. A decrease in COD concentration was expected and taken as an indication of successful biodegradation of the organic matter present in the substrate (Fernández *et al*., 2008).

Microbial analysis

Cow manure is a known source of coliforms and *E. coli* (Himathongkham *et al*., 1999) and thus it was expected that the results obtained show the presence of these organisms. Coliforms counts for both the leachate and compost on day 1 were 26.2×10^6 MPN.100 mL^{-1} and 85.878×10^6 MPN.100 g^{-1}, respectively. The *E. coli* counts for the leachate were 65.7×10^4 MPN.100 mL^{-1} and 58×10^6 MPN.100 g^{-1} for the compost. The Coliform and *E. coli* count of the leachate after the 21 day study period were found to be 248×10^4 MPN.100 mL^{-1} and 178×10^4 MPN.100 mL^{-1}, respectively. The *E. coli* counts for the leachate obtained from experimental studies 7 and 8 were higher (178×10^4 MPN.100mL^{-1}) than the counts from the final compost for both the lab-scale digesters (980 MPN.100 g^{-1}) (ES7) and up-scale digesters (970 MPN.100 g^{-1}) (ES8). A possible reason for the *E. coli* loads being higher in the leachate could be due to the daily wetting of the compost. The wetting liquid was added from the top of the digester and trickled through the compost allowing the *E. coli* to be "washed out" from the compost and transferred into the leachate. However, the *E. coli* loads in the compost from both the lab-scale digesters and the up-

scale digesters were within recommended limits (< 1 000 MPN.g^{-1}) (Table 3.6), but the loads in the leachate were too high (> 1 000 MPN.mL^{-1}). Although *E. coli* are normally destroyed in a hot compost pile (Garrett *et al*., 2012) the digesters in this study were kept at 37°C, where they could easily multiply as this temperature is the optimum growth temperature for *E. coli* (Glass, 1982).

Pasteurisation of compost is highly recommended and is often applied in the composting industry (Cotter, 2014). Additionionally it eliminates insects, other microbes and pathogens. Heat treatment can also help reduce high ammonia levels by convertion to nitrogenous compounds. In the mushroom industry a recommended temperature of 54° - 60°C is applied for 3 – 6 h (Cotter, 2014).

Electrical conductivity (EC), moisture, bulk density and ash in compost

The electrical conductivity is an indication of the total dissolved salts in the sample (The Non-Affiliated Soil Analyses Work Committee, 1990). When soluble salt levels are present in a too high concentration it could affect germinating seeds and plant growth (Watson, 2014). All crops differ in their salt tolerance levels and EC does not indicate the type of salts that are present in the sample (Sullivan & Miller, 2001). The EC of the compost from the lab-scale and up-scale digesters (Table 3.5) are within the limits (Table 3.6) although according to Allen (2014), acceptable salt levels are based on the intended use of the compost. Watson (2014) state that an EC range of 0.13 – 0.34 dS.m^{-1} is low risk and that it is a suitable range for seedlings and sensitive plants.

The moisture content of compost is easily determined but may generally vary due to different storage conditions, feedstocks and processing (Sullivan & Miller, 2001). The moisture content of all compost samples obtained in ES7 and ES8 were within international recommended limits, although the digesters had moisture contents above that of the recommended value applied by a local South African composting company (Table 3.6). Darlington (2012) stated that compost should have a moisture content between 35 – 60% as the moisture can affect the bulk density of the product and consequently transportation costs. Compost with a moisture content under 35% could indicate poor stabilisation or the product may have been stored for an exceptionally long time and moisture loss may have taken place. Composts with a too low moisture content (< 35%) are often dusty and unpleasant to handle (Sullivan & Miller, 2001), whereas extremely wet compost can be heavy and problematic to apply uniformly (Darlington, 2012). According to section 35 of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, (Act 36 Of 1947) compost may only be sold if the moisture content does not exceed 40%. Griessel (2002), also reported very high moisture contents (82 – 84%) of compost samples after anaerobic digestion. Possible solutions for reducing the moisture content of compost further include: (i) spreading compost samples in the sun or; (ii) placing samples in open containers in an incubator room at 37°C for 24 h.

Bulk density (weight per unit volume) of compost, is affected by several factors including: the moisture content; particle size; ash content and; the degree of composition (Sullivan & Miller, 2001). By determining the bulk density of compost, a conversion of nutrient data to volume basis

is possible. Due to the fact that volume basis is the form in which compost is handled (Allen, 2014), this test can be used to provide data on the water and air retaining properties of the samples (Watson, 2014). This test together with moisture analyses is used by compost users to determine volume-based application rates (Sullivan & Miller, 2001). The bulk density values for the compost are lower than the recommended values from the USA (Table 3.6) but according to Sullivan & Miller (2001), compost obtained from bigger piles, or those that were badly packed in a truck could have higher bulk density values.

The ash content of compost is an indication of the inorganic elemental content (Board, 2004). The ash content (Table 3.5) of the compost from both the lab and up-scale digesters (ES7 and ES8) were within requirements as stipulated in Table 3.6. Section 35 of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, (Act 36 Of 1947) states that compost may only be sold if the ash content does not exceed 67%. Based on results obtained, it can be assumed that the end-products from these experimental studies are suitable to be used as compost in terms of EC, bulk density and ash.

Total N (nitrates and ammonium) P, K Ca, Mg, Na, Cu, B, Fe, Zn in compost

The above nutrients are all important to plant growth but the macronutrients are usually the most important. Macronutrients include nitrogen, phosphorous, potassium, calcium, magnesium and sulphur whereas iron, zinc, copper, boron and manganese are classified as micronutrients (Watson, 2014). Nitrogen, phosphorous and potassium are classified as primary nutrients needed for vine growth and calcium, magnesium and sulphur as secondary nutrients. These primary and secondary nutrients needed for optimal vine growth are absorbed by the plant roots from the soil (Raath, 2006).

According to Raath & Schutte (2001), a total nitrogen percentage of < 0.7% is considered low and a value of > 2.0% as high. The nitrogen results (Table 3.5) of the compost from both the lab-scale and up-scale studies (ES7 and ES8) can therefore be classified as high. Raath & Schutte (2001), also mention that total inorganic nitrogen (NH_4^+ and NH_3^-) indicates the amount of nitrogen that is immediately available to the plant. When the inorganic nitrogen is too high (> 7 000 $mg.kg^{-1}$) it could cause burning of the plant roots, whereas a too low value (< 70 $mg.kg^{-1}$) could indicate that the compost lost nutrients through leaching. According to the recommended guidelines (Table 3.6) the NH_4 values are all within limits, but the NH_3 values were very low.

Only a partial amount of the total P, Mg and Ca content is available to the plants whereas all of the total compost K is available (Sullivan & Miller, 2001). Phosphorous has a very low mobility in soil and should therefore be applied during the preparation of the soil to correct the P content in the subsoil. After this most vineyards need very little P fertilisation as the P requirements for vines are approximately one sixth of that of K and N (Raath & Diedericks, 2006). Raath & Schutte (2001) stated that a P concentration of > 1.2% is considered high and < 0.2% as low. The P content of composts from experimental studies 7 and 8 (Table 3.5) were both found to be in the lower category. However, only when the amount of compost to be applied to a field has

been determined, the correct amount of P to be applied can also be calculated. If the P content is still too low the soil can be supplemented with rock phosphate (Raath & Schutte, 2001). Knowledge of reaction kinetics between different plant nutrients is of vital importance as over-fertilisation of K can happen easily that could affect the availability of other nutrients. A K concentration of > 0.5% is regarded as high, whereas < 0.1% can be seen as low (Raath & Schutte, 2001). Results from this study (Table 3.5) showed that the K content of the ES7 and ES8 compost is in the higher category. Ca is usually the main cation (Ca^{2+}) found on soil particles and bound tighter than Mg^{2+} and K^{+} (Raath & Diedericks, 2006). Antoine & Junod (2004) stated that the main mineral components found in grapes are Ca, K, Mg, Na and Fe. Similar results were found during this study except that the compost had a higher Ca than K content. A possible explanation for this could be due to the CaO used during the CaO soaking to increase the initial pH of the grape skins. Although the Na is relatively high compared to other nutrients (Table 3.5), the results showed that the EC was within limits for the compost.

According to Raath & Schutte (2001) Mn, B, Cu and Zn in concentrations of > 200 $mg.kg^{-1}$, > 50 $mg.kg^{-1}$, > 200 $mg.kg^{-1}$ and > 350 $mg.kg^{-1}$, respectively is considered as high. Although micronutrients and heavy metals can build up over time all of the above mentioned in the ES7 and ES8 compost were in the normal range.

Biogas and methane

The biogas values obtained during these studies were found to be low or even absent at times for the lab-scale (Table 3.3) and even for the up-scale digesters (Table 3.4). Possible explanations for this could be: (i) the low nutritional content of the grape skins (GS) and; (ii) the unfavourable oxidation reduction potential (ORP) within the digester.

According to Antoine & Junod (2004), GS mainly consist of cellulose, hemi-cellulose, pectin, insoluble proteins, tartaric as well as malic acids. Cellulose is one of the most abundant carbohydrate polymers in nature and is very resistant to microbial degradation as it is insoluble (Kim, 2011). According to Chen *et al*. (2008), agricultural wastes that are associated with a low gas yield during AD have high lignocellulosic contents or a high C:N ratio. Grape pomace is also characterised by a high content of polyphenols (Arvanitoyannis *et al*., 2007). This is a known organic compound that can be toxic at high concentrations to the consortium in anaerobic digesters (Gerardi, 2003;). Although good compost were obtained in ES7 and ES8 the polyphenol content of composting leachate was found to be > 200 $mgGAE.L^{-1}$. This could have led to the inhibition of the methanogen part of the consortium.

Optimum ranges for anaerobes to degrade substrates are when their surroundings are between -200 and -400 millivolts. Any dissolved oxygen that enters the AD system can raise the ORP which leads to the inhibition of anaerobic activity (Gerardi, 2003). In order to avoid acidification of the compost, moisturising liquid was added every 24 h to the digesters through the cap opening. Any oxygen that centered the system could possibly have led to a higher ORP and consequently inhibited or slowed the methanogen activity.

Conclusions

During this study it was found that the strict control of specific operational parameters (pH, moisture, inoculum size and ratio, and composition) was essential to produce a good compost product within 21 days. During experimental studies 7 and 8, the digester parameters reached stable-state conditions. Stable-state is defined as a state which can be maintained indefinitely without system failure (Cobb & Hills, 1990), during which the variation in digester operational parameters is less than 10%. Volume reductions of 59% and 67% were achieved for the lab-scale (ES7) and up-scale digesters (ES8), respectively. Cow manure was found to be the best inoculum to use for start-up in terms of availability and economic feasibility as well as leading to good quality compost with a stable end pH. A lower inoculum size was also optimised with anaerobic compost as the most favourable inoculum. A lower inoculum size has the advantage of allowing more grape waste to be treated and is more economically feasible while anaerobic compost as an inoculum could possibly result in a system where the pH reaches 6.5 earlier quicker due to the fact that the microbial consortium have already acclimatised to the anaerobic environment.

It was found that a high alkalinity of the moisturising liquid as obtained from an UASB reactor was crucial in terms of pH stabilisation. With optimum parameters in place, a pH-stable, odour-free compost was obtained for both lab-scale and up-scale digesters with nutritional characteristics complying with guidelines (pH, ash, N%, K%, P%, Ca%, Mg%, Na%, Cu%, Fe%, Zn%, B%, EC).

Due to the fact that biogas production and methane generation (%) was found to be low or even absent, future research should investigate the effect of co-digesting grape skins with a carbohydrate rich waste source such as green kitchen waste or apple pomace. These wastes could possibly provide enough nutrients to facilitate the generation of increased volumes of biogas and methane. This study showed that the AnC of grape skins on lab-scale is a possible solution in terms of solid waste recycling. However, when the AnC of grape skins on an industrial scale is considered various factors (CaO addition, shredding of waste, leachate removal, temperature control) still need to be investigated in order to create a more sustainable wine industry.

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CHAPTER 4

CO-TREATMENT OF LEACHATE PRODUCED DURING THE ANAEROBIC COMPOSTING OF GRAPE SKINS IN AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR TREATING WINERY WASTEWATER

Summary

Liquid waste generated during the winemaking process consists mainly of wastewater which contains grape pips and dead yeast cells from the fermentation, while solid waste includes grape pomace (seeds, stems, skins, pulp) and filter waste. The uncontrolled discharge of untreated waste can have severe ecological, social and health risks and must be minimised. Anaerobic biological treatment is recognised as one of the primary advanced treatment options for environmental protection and when combined with other appropriate procedures, it can serve as a sustainable and suitable wastewater treatment option in developing countries. Anaerobic digestion (AD) of liquid waste results in both depollution and energy recovery, while anaerobic composting (AnC) of solid waste generates a soil amendment (compost), leachate and biogas. Leachate can serve as a liquid fertiliser or can be utilised as a nutrient source during co-treatment in anaerobic digestion.

This study presents the results obtained from monitoring the co-digestion of anaerobic composting leachate and winery wastewater (WWW) in a laboratory-scale UASB reactor. The UASB had an operational volume of 2.3 L and a hydraulic retention time of 24 h. The reactor was monitored and operated in four different phases to reach an organic loading rate of *ca*. 8.5 kgCOD.$m^{-3}d^{-1}$ by day 205. A final COD reduction (> 90%), effluent pH (7.61), alkalinity (3 281 $CaCO_3$ mg.L^{-1}) and methane (67%) was achieved even when the volume of leachate to be co-treated was doubled from its “calculated maximum value”. The combination of waste streams (co-treatment) has enormous potential in future application within the wine industry in order to create sustainable water and waste utilisation practices.

Introduction

Oenology is an old worldwide technology affecting the economic wellbeing of many countries (Walker, 1999). The production of wine in 2012 was approximately 252 million hectolitres (OIV, 2013). Winery wastewater volumes are almost 1.2 times more than the wine produced (Vlyssides *et al.,* 2005) and, although the wine industry does not have a reputation as a polluting industry, typical characteristics of it can be an ecological threat (Ronquest & Britz, 1999; Brito *et al.,* 2007).

One of the main concerns the wine industry is facing, is the management of large volumes of wastewater (Mosse *et al.,* 2011). Winery wastewater is defined as a high strength organic waste, with a low nitrogen and phosphorous content (Toffelmire, 1972). Alcohol, hexose sugars, organic acids (Moosbrugger *et al.,* 1993; Keyser *et al.,* 2003), esters and polyphenolic compounds

are constituents that are typically present in winery effluent (Mosse *et al.,* 2011). Winery effluent is characterised by a chemical oxygen demand (COD) of between 0.8 - 12.8 $g.L^{-1}$ and a pH of 3 - 4 (Petruccioli *et al.,* 2000). Depending on the harvest capacity and activities in the wine cellar, the COD of WWW can reach 25 $g.L^{-1}$ (Malandra *et al*., 2003; Strong, 2008). The uncontrolled disposal of untreated WWW may result in severe environmental, social and health risks and should therefore be minimised (Riaño *et al.,* 2011).

Anaerobic digestion (AD) of liquid waste is an attractive treatment option as both depollution and energy recovery can be achieved (Chen *et al*., 2008). Anaerobic composting of solid waste generates an organic amendment (Khalid *et al*., 2011), a liquid effluent (leachate) (Mata-Alvarez *et al*., 1992) and biogas. The leachate generated during AnC is a source of water, inoculum and nutrients that is desirable for optimum AD (O'Keefe *et al*., 1993). The main limitation of the AD process is the unbalanced level of nutrients in the waste substrate (Khalid *et al*., 2011). WWW that is known to be low in nitrogen and phosphorous (Toffelmire, 1972) requires to be supplemented for the digestion process to be optimal (Ronquest & Britz, 1999; McLachlan, 2004). Co-treatment is an alternative solution to supplementation as co-substrates can supply the missing nutrients and optimise the substrate composition (Mata-Alvarez *et al*., 2000; Umetsu *et al.,* 2006; Jagadabhi *et al*., 2008; Pagés-Díaz *et al*., 2014). According to Kangle *et al*. (2012) co-substrates improve the biogas yields during AD due to positive synergisms that are established as well as supplying the system with missing nutrients.

The aim of this study was to investigate the co-treatment of leachate from the anaerobic composting of grape skins in an upflow anaerobic sludge blanket (UASB) treating winery wastewater. This will be achieved by using a lab-scale UASB reactor that is treating winery wastewater. The optimum leachate volume that can be co-treated will be determined, as well as monitoring the efficacy of the treatment process.

Materials and Methods

UASB reactor design and set-up

The UASB reactor (Fig. 4.1) treating WWW was set up as described by Trnovec & Britz (1998), Ronquest & Britz (1999) and McLachlan (2004). The reactor had an operational volume of 2.3 L with a hydraulic retention time (HRT) of 24 h. Substrate was fed semi-continuously to the bottom of the reactor (Fig. 4.1) by means of a peristaltic pump (Watson-Marlow 501) and an electronic timer. Recirculation was made possible with the aid of a peristaltic pump (Watson-Marlow 302S) at an upflow velocity of 2.4 $m.h^{-1}$. The temperature of the reactor was maintained at 35°C by an electronic control unit and heating tape (Meyer *et al*., 1993). A temperature probe was also inserted to measure temperature variation in the middle of the cylinder (Fig. 4.1). In order to prevent excess heat loss, the reactor was covered with shock-absorbent plastic (Meyer *et al*., 1993). Biogas exited through the top of the reactor and the volume of gas was measured by using a manometric unit equipped with an electronically counter (Fig. 4.1). The overflow of the reactor

emptied through a U-shaped tube (Fig. 4.1) that prevented atmospheric oxygen from entering the system.

Substrate (WWW and leachate)

Winery wastewater was obtained during the harvest season from Distell Ltd, Stellenbosch, South Africa (February - April 2013 and 2014). The WWW was stored in 25 L drums and kept at -18°C. When WWW was required, drums were defrosted and kept at 4°C until needed. Leachate removed from the anaerobic composting of grape skins was obtained from experimental studies as described in Chapter 3 of this study and stored at 4°C until utilised. WWW was supplemented with 500 $mg.L^{-1}$ K_2HPO_4, 500 $mg.L^{-1}$ potassium hydrogen carbonate ($KHCO_3$), 250 $mg.L^{-1}$ ($(NH_2)_2CO$) (urea) and diluted with tap water to the required COD. When needed, the substrate or influent was adjusted to a pH of 7.0 with 2M potassium hydroxide (KOH).

UASB operation

The UASB reactor was seeded with 350 g anaerobic granules (sludge bed height = 32.5 cm) (volatile suspended solids (VSS) = 0.082 $g.L^{-1}$) obtained from a full-scale UASB reactor treating distillery wastewater in Wellington, South Africa (The James Sedgwick Distillery, Wellington, South Africa). To reactivate the granules, the UASB was fed pH adjusted tap water (7.00) containing Di-Potassium hydrogen orthophosphate (K_2HPO_4) and urea ($(NH_2)_2CO$) (500 $mg.L^{-1}$ each) for 24 h. The reactor was then fed a mixture of a synthetic glucose substrate (SGS) (Show *et al.*, 2004) and WWW (10% $v.v^{-1}$) diluted to a chemical oxygen demand (COD) of approximately 1 000 $mg.L^{-1}$. Additionally, trace element solution (1 $mL.L^{-1}$) as described by Nel *et al*. (1985) was added weekly to the substrate. The SGS:WWW ratio was steadily decreased until the reactor substrate only consisted of diluted WWW. The COD of the WWW was then increased stepwise to a COD of *ca.* 4 000 $mg.L^{-1}$. As the volume of leachate (Chapter 3 of this thesis) added to the winery wastewater was steadily increased, the volume of tap water was reduced by the same volume to reach a substrate COD of *ca.* 8 500 $mg.L^{-1}$. The operating period (205 days) was divided into four phases consisting of different feeding regimes.

Volume of Leachate to be co-treated

The amount of leachate to be co-treated was calculated by using data (tonnes of grapes processed per season) provided by two local wineries (Winery X, Winery Y) situated in Stellenbosch, South Africa during the harvest season of 2013. According to literature 700 – 3 800 L wastewater is produced per tonne of grapes processed (Cohen *et al.,* 2013). Approximately 250 – 300 kg grape skins (GS) are obtained per tonne of grapes (Jordaan, P. 2014, Assistant Winemaker, Paardeberg, Malmesbury, South Africa, personal communication, 14 April 2014). The total volume of leachate generated during the anaerobic composting of grape skins was calculated from Experimental Study 5 in Chapter 3 of this thesis.

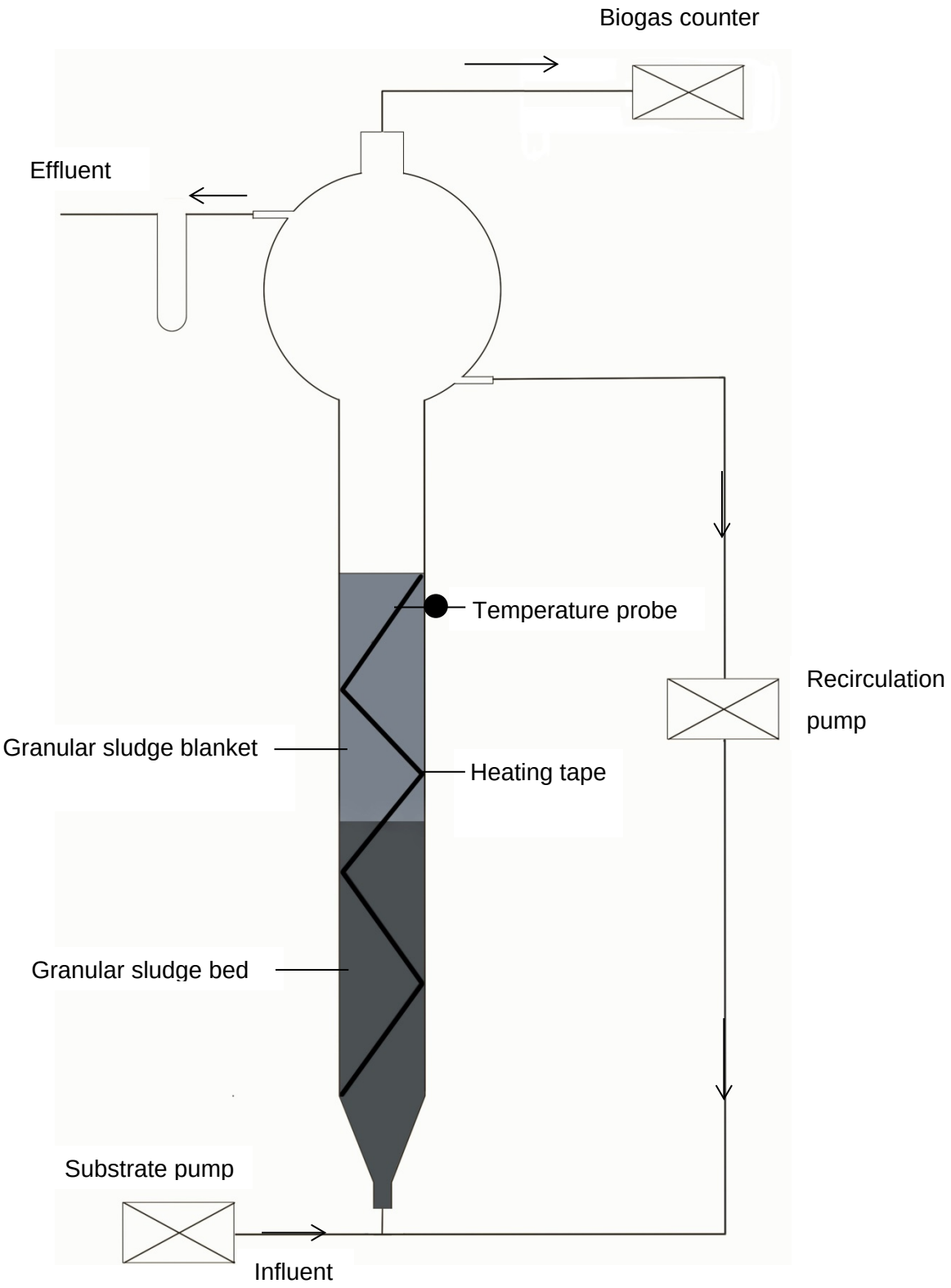

Figure 4.1 Schematic diagram of the UASB used to co-treat WWW and leachate.

Winery X

- Total of 300 tonnes grapes processed during harvest season (Average of 275 kg grape skins produced per ton of grapes. Therefore, 83 tonnes of GS produced per season)
- Average amount of WWW produced per tonne of grapes = 2 250 L

∴ 300 tonnes × 2 250 L = 657 000 L wastewater produced during harvest season

∴ 48 mL leachate produced per day for 300 g grape skins (Chapter 3 of this thesis, ES5)

∴ 160 mL leachate produced per day per kg of grape skins

∴ 160 L leachate produced per day per tonne of grape skins

∴ 83 tons grape skins × 160 L leachate = 13 280 L leachate

657 000 L WWW : 13 280 L leachate

49.5 L WWW : 1 L leachate

→If 2 L of substrate is fed to the reactor every 24 h, 40 mL of leachate is needed to be co-treated

Winery Y

- 850 tons grapes processed during harvest season (average of 275 kg grape skins produced per ton of grapes. Therefore, 234 tonnes of GS produced per season)
- Average amount of WWW produced per tonne of grapes = 2 250 L

∴ 850 tonnes × 2 250 L = 1 912 5000 L wastewater produced during harvest season

∴ 48 mL leachate produced per day for 300 g grape skins (Chapter 3 of this thesis, ES5)

∴ 160 mL leachate produced per day per kg of grape skins

∴ 160 L leachate produced per day per tonne of grape skins

∴ 234 tons grape skins × 160 L leachate = 37 400 L leachate

1 91 2000 L WWW : 37 400 L leachate

51.0 L WWW: 1 L leachate

→If 2 L of substrate is fed to the reactor every 24 h, 40 mL of leachate is needed to be co-treated

Analytical methods

Parameters used to monitor the WWW substrate and effluent during this study included: pH; alkalinity [as calcium carbonate (mg $CaCO_3.mL^{-1}$)]; total suspended solids (TSS); volatile suspended solids (VSS); nitrogen; phosphate; COD; and polyphenols. Sludge bed height (Fig. 4.1) before and after the co-treatment of leachate and WWW was also measured.

For COD analysis the influent, effluent and leachate samples were digested with a COD reactor (Hach Co. Loveland, U.S.A), cooled and colorimetrically measured using a DR2000 spectrophotometer (Hach Co. Loveland, CO) set at 585 nm, and standardised procedures (APHA, 1998). The COD of leachate produced on day 1 (used for co-treatment) were also confirmed by using a Spectraquant® COD Cell Test (5 000 – 90 000 $mg.L^{-1}$) (Merck, Germany). The COD reduction was calculated from the soluble COD in the effluent (after treatment) and the total COD in the substrate (before treatment). All analyses were performed in duplicate. The COD concentration were used for the carbon value in the determination of the C:N:P ratio.

Analyses for TSS and VSS on leachate and effluent were performed once a week according to Standard Methods (APHA, 1998). A Varian 3300 gas chromatograph (Varian Inc., Palo Alto, CA) and a Varian 4290 integrator was used to determine biogas composition. The Gas Chromatograph was equipped with a thermal conductivity detector and a 2.0 x 3.0 mm i.d. Hayecep Q (Supelco, Bellefonte, PA) 80/100 mesh packed column. The oven temperature was set to 55°C, helium was used as the carrier gas at a flow rate of 30 $mL.min^{-1}$. A sample volume of 0.2 mL was used (Sigge, 2005) and all analyses were done in duplicate.

The nitrogen and phosphate content of the WWW were confirmed by using Spectroquant® Nitrogen and Spectroquant® Phosphate Cell tests (Merck, Germany). The nitrogen (0.5 – 15.0 $mg.L^{-1}$ N) and phosphate (0.05 – 5.0 $mg.L^{-1}$ PO_4-P) values were measured using a Merck Spectroquant® Nova 60 spectrophotometer. Polyphenol content of raw WWW was determined using the Folin-Ciocalteau method (Singleton & Rossi, 1965). Analyses were performed in duplicate.

In the previous chapter of this thesis (Chapter 3) it was confirmed that *E. coli* was present in the composting leachate, and thus microbial analysis on the effluent obtained from the UASB co-treating WWW and composting leachate was determined at the end of Phase D. Analysis was performed according to the SANS 9308 (SANS, 2012) method using a Colilert-18 kit (IDEXX, USA). Effluent (10 mL) was added to 90 mL saline solution, where after a dilution series (10^{-1} - 10^{-10}) was prepared. Duplicates of each dilution series were prepared as to ensure an end sample volume of 100 mL. Colilert-18 reagent indicator (4-methylumbelliferyl-β-D-glucuronide) (MUG) was added to each of the duplicate Schott bottles. Each dilution was poured into a Quanti-Tray/2000 (IDEXX, USA) and sealed with a Quanti-Tray® Sealer Model 2X (IDEXX, USA). The trays were incubated at 37°C for 18 h. After the 18 h incubation period, total coliforms were determined by counting the wells that showed a yellow colour. The presence of *E. coli* was determined by counting the wells that fluoresced under ultra violet light (365 nm) (Spectroline® Model CM-10 Fluorescence Analysis Cabinet). The positive counts for both total coliforms and *E. coli* were used to determine the corresponding loads by using an IDEXX Quanti-Tray®/2000 most probable number (MPN) table (IDEXX, USA; SANS, 2012). Microbial counts were expressed as MPN.100 mL^{-1}.

Results and Discussion

The performance of the UASB reactor was monitored, by assessing different operational parameters of the substrate and the UASB effluent during the trial period. Different Phases (A - D) were used to increase the organic loading rate (OLR) to *ca*. 8.5 kgCOD.$m^{-3}d^{-1}$ (day 205).

Phase A (Day 0 – 100)

Phase A involved the start-up and stabilisation of the reactor over a 100 day trial period. The aim was to reach and maintain an OLR of *ca.* 4.1kgCOD.$m^{-3}d^{-1}$ while the reactor effluent generated in this phase was also used as a moisturising liquid for compost from Experimental Study 3 (Chapter 3 of this thesis). The operational efficiency parameters that were monitored are shown in Table 4.1. The substrate COD was increased stepwise from day 0 to day 100 from *ca.* 1 000 – 4 100 mg.L^{-1} and OLR 1.0 - 4.1kgCOD.$m^{-3}d^{-1}$. The substrate consisted of WWW and SGS, diluted with tap water to the desired COD. As the pH and COD reduction of the system increased, the SGS was systematically reduced in 10% (v.v^{-1}) increments and replaced with WWW until the reactor influent consisted of only WWW.

Table 4.1 Operational parameter ranges for the UASB reactor during Phase A of the treatment of WWW

Parameter	Day 0 – 100
Alkalinity (mg$CaCO_3$.L^{-1})	150 – 1 625
COD of substrate (mg.L^{-1})	1 000 – 4 100
COD reduction (%)	32 – 87
OLR (kgCOD.$m^{-3}d^{-1}$)	1.0 – 4.1
Biogas (L.d^{-1})	0.54 – 4.59
Substrate pH	7.0 – 7.5
Effluent pH	6.7 – 7.5
TSS (g.L^{-1})	0.230 - 0.305
VSS (g.L^{-1})	0.115 - 0.190

Initially, the reactor effluent pH was below (pH 6.6; Table 4.1) the recommended level (6.8 – 7.2) (Anderson *et al*., 2003; Gerardi, 2003, Ward *et al*., 2008) for optimum AD. In order to increase the pH of the system, the pH of the substrate was increased to 7.5 (Table 4.1). After the pH of the system had a pH between 7.0 – 7.10, the substrate pH was again lowered to pH 7.0. Thereafter the reactor pH remained stable between 7.3 – 7.5 until the end of Phase A (day 100).

The alkalinity of the system at the start (< 1 000 mg$CaCO_3$.L^{-1}) was lower than the recommended range (1 500 – 3 000 $CaCO_3$ mg.L^{-1}) (Anderson *et al.*, 2003; Gerardi, 2003. The alkalinity was found to increase to *ca.* 1 625 mg$CaCO_3$.L^{-1} by day 100 (Table 4.1). The COD reduction and biogas production was initially very low but steadily increased to reach *ca.* 87% and 4.59 L.d^{-1}, respectively (Table 4.1).

Phase B (Day 101 – 142)

The purpose of Phase B was to maintain the desired substrate COD (± 4200 $mg.L^{-1}$) as found during the harvest season of 2013 and to ensure that the reactor was at a stable state before the co-treatment with leachate was started. Stable state is defined as a state which can be sustained indefinitely without reactor system failure, with a variation in reactor performance of < 10% (Cobb & Hill, 1990).

The substrate COD was kept constant between 4 000 and 4 300 $mg.L^{-1}$ until day 144 (Fig. 4.2). The small variation in COD levels was ascribed to the characteristics of WWW which are known to differ in terms of the type of wine produced and the specific management practices (Vlyssides *et al.,* 2005). An increase in COD reduction was seen from day 101 until day 116, after which a decrease in COD reduction (%) was observed that was ascribed to the increase in substrate COD (Fig. 4.2). From day 121 until day 132 a stable COD reduction was seen (92 - 93%), after which a slight increase in COD reduction was found until the end of Phase B, where COD reduction was 96%.

The pH of the reactor effluent increased from day 108 to day 118 (Fig. 4.3) and while it was higher than the recommended pH value for methanogens (Anderson *et al.*, 2003) it decreased after day 120 to between pH 7.0 – 7.5. Due to a drop in alkalinity (< 2 000 $CaCO_3$ $mg.L^{-1}$) by day 128 (Fig. 4.3) the pH of the substrate was increased that led to a gradual increase in the effluent pH until day 140 after which the effluent pH decreased again to 7.3. Although the alkalinity content varied during Phase B (Fig. 4.3), it was within the recommended value of 1 500 – 3 000 $CaCO_3$ $mg.L^{-1}$ (Gerardi, 2003). Adequate alkalinity (buffer capacity) is required in any AD system to sustain a stable pH and optimal biological activity (Lee *et al*., 2009). Determination of the buffer capacity is a more reliable method of measuring imbalance in the digester, because an accumulation of fatty acids will lower the buffer capacity before decreasing the pH (Ward *et al.,* 2008). This statement agrees with results obtained during this study as the alkalinity and pH decreased and increased (Fig. 4.3) concurrently. Due to the fact that significant variations in alkalinity and pH can be introduced into a reactor by the substrate feed (Gerardi, 2003), a possible explanation for the fluctuation in alkalinity as seen in Figure 4.3 could be due to the variation in chemical composition of the winery wastewater.

Biogas production was initially low (4.9 $L.d^{-1}$) (Fig. 4.4), possibly due to a rapid increase in the OLR, but increased to *ca*. 8.3 $L.d^{-1}$ towards the end of Phase B. The methane (%) varied between 63 – 70% during Phase B (Fig. 4.4). Biogas can consist of various gasses (CH_4, CO_2, CO, H_2, H_2S, NH_3, N_2, N_2O) (Gerardi, 2003) but methane (60 - 65%) and carbon dioxide (35 - 40%) are the major ones (Fillaudeau *et al*., 2008). An increase above 30% in the CO_2 concentration could possibly indicate reactor instability as an increase in the CO_2 concentration is associated with

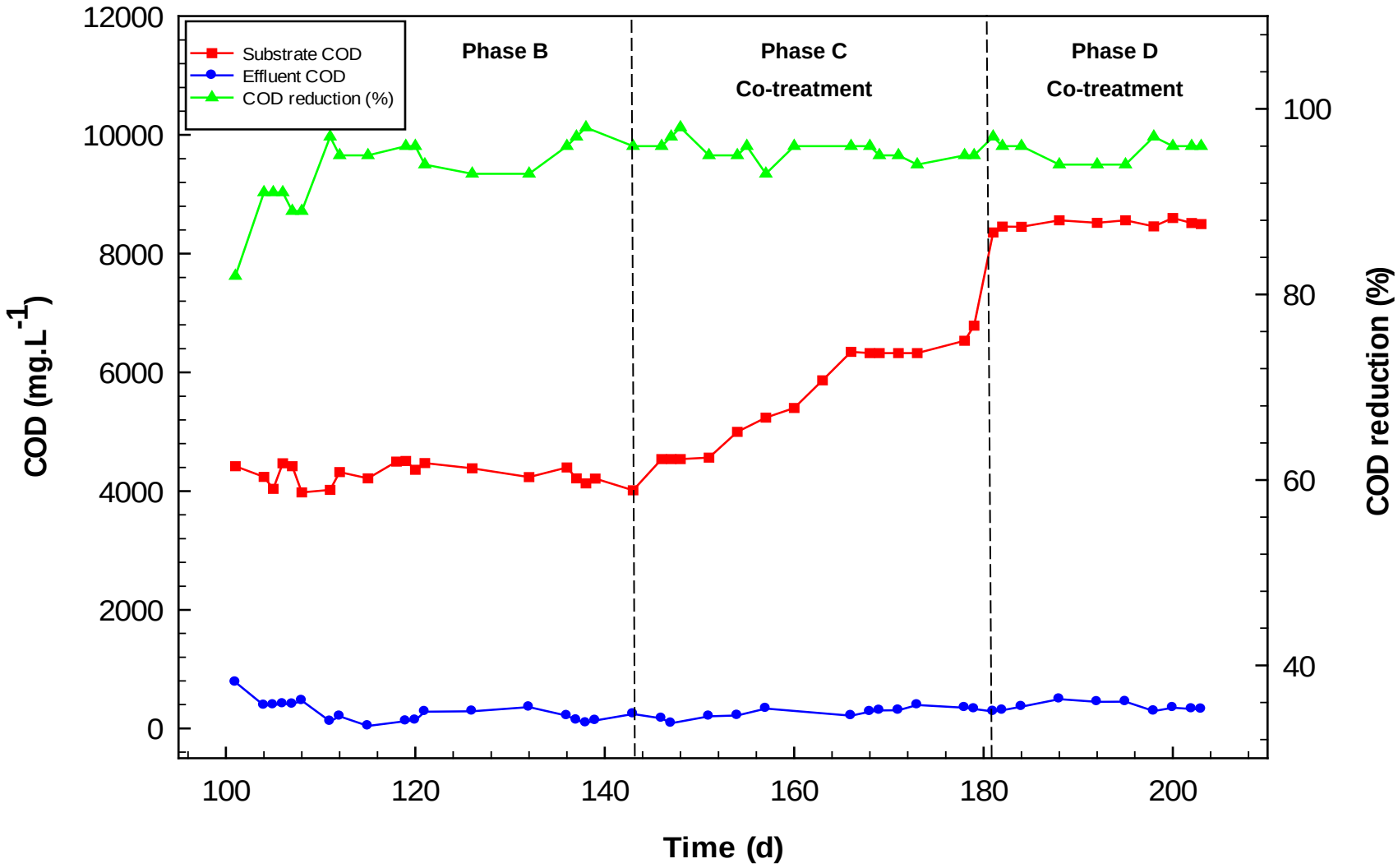

Figure 4.2 Substrate COD, effluent COD and COD reduction of the UASB reactor co-treating WWW and composting leachate.

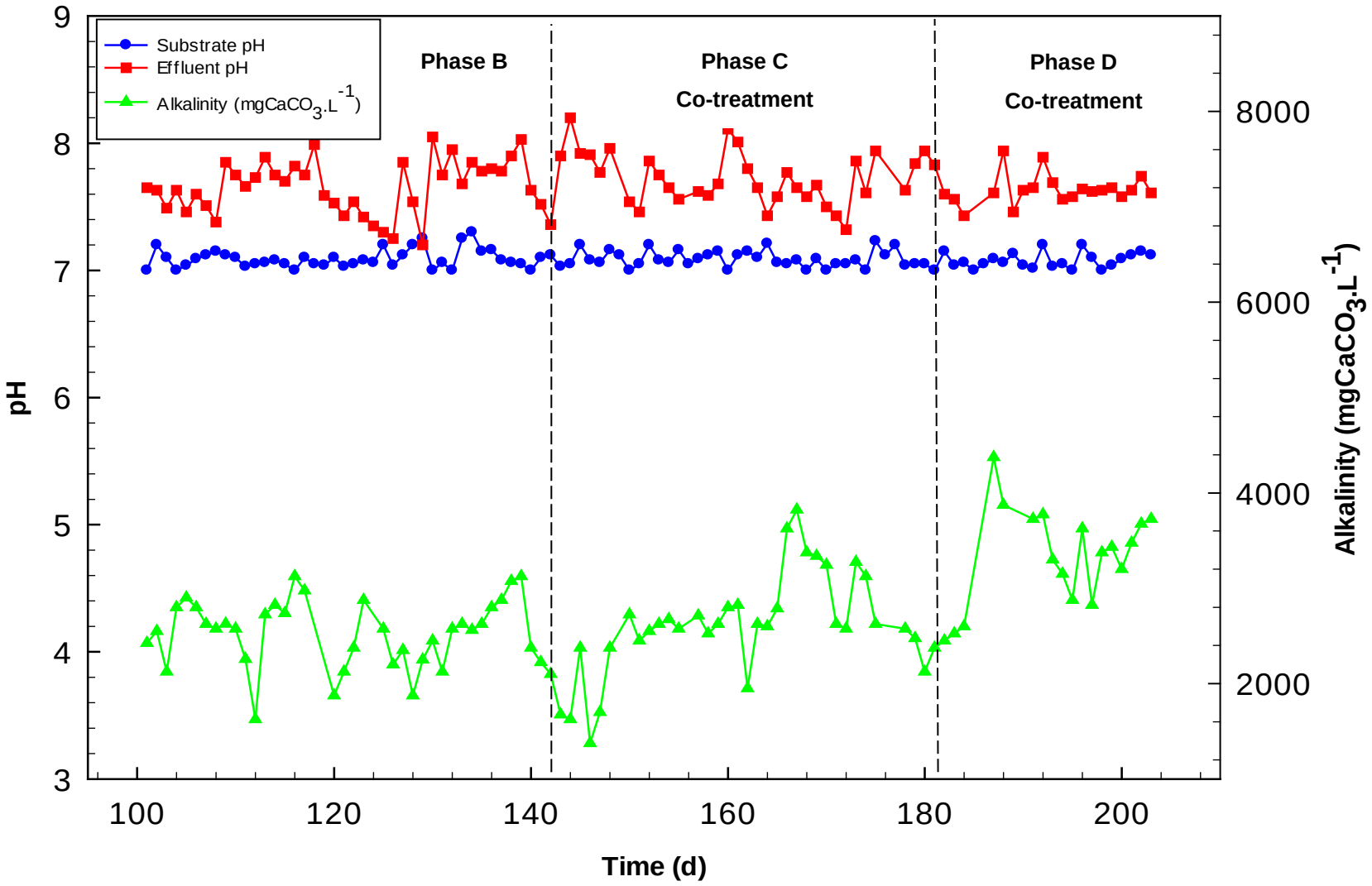

Figure 4.3 Substrate pH, effluent pH and alkalinity of the UASB reactor co-treating WWW and composting leachate.

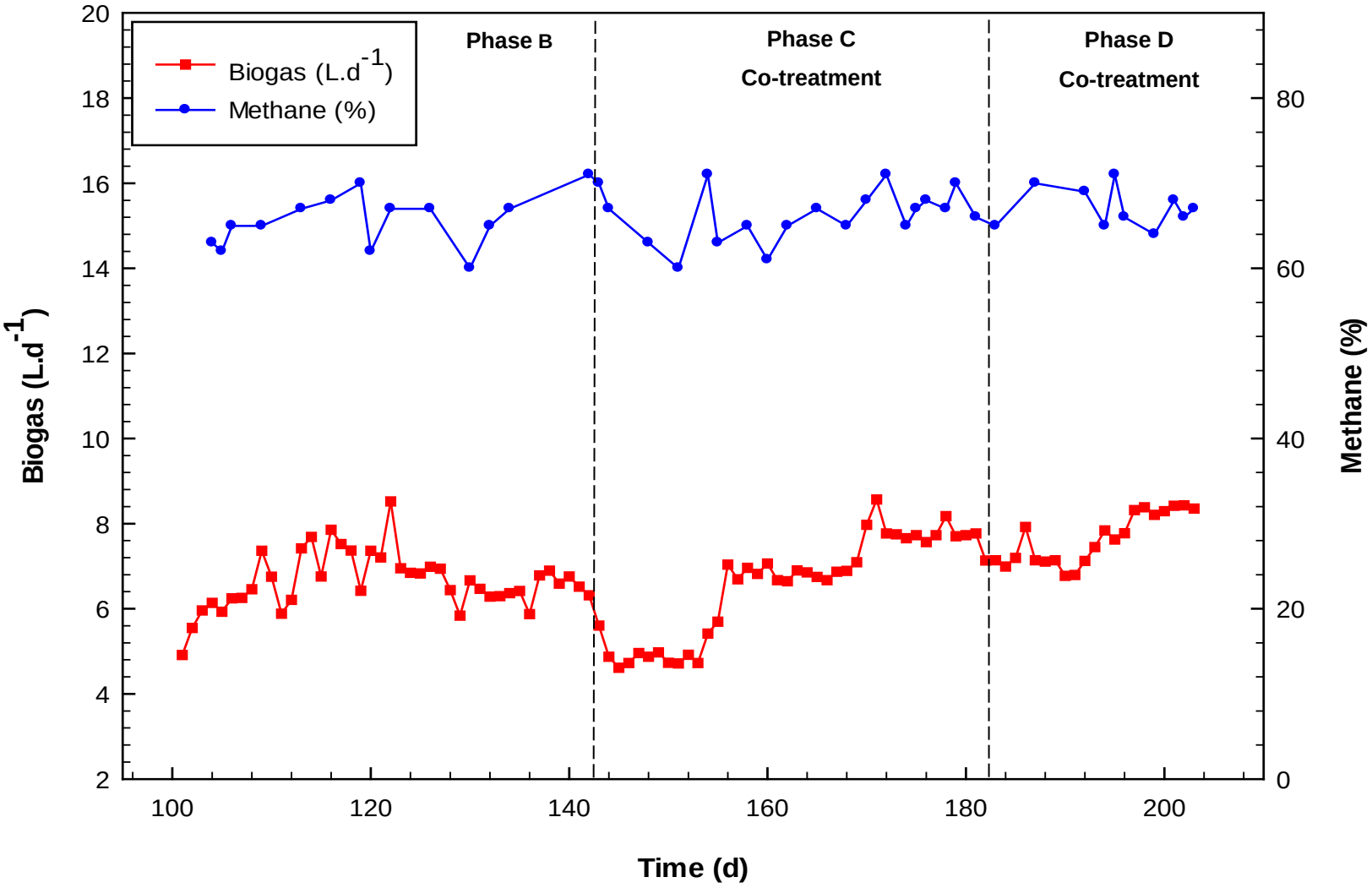

Figure 4.4 Biogas volume and methane percentage of the UASB reactor co-treating WWW and composting leachate.

a change in the type of methanogens (i.e. activity) (Gerardi, 2003; De Lemos Chernicharo, 2007). As the biogas and methane generation stayed fairly stable during Phase B (Fig. 4.4), as well as the COD reduction and effluent pH it was concluded that the reactor was in a stable phase and ready to start the co-treatment.

Phase C (Day 143 – 180)

After the amount of leachate to be co-treated was calculated, the COD of the substrate was increased gradually from *ca.* 4 300 – 6 700 $mg.L^{-1}$ (day 146 - 180) corresponding to an OLR of 4.3 – 6.7$kgCOD.m^{-3}d^{-1}$ (Fig. 4.2). An overall COD reduction > 90% was maintained during Phase C (Fig. 4.2). Although the effluent pH was fairly stable between pH 7.5 and pH 8.0, the value is higher than the recommended value for optimum anaerobic digestion.

Due to the fact that the COD reduction stayed constant although the COD concentration of the substrate increased (Fig. 4.2), it was expected to also see an increase in biogas production. This, however, was not the case as an initial decrease in both the biogas production (to *ca.* 5.0 $L.d^{-1}$) and methane content (60 - 63%) was seen after the co-treatment started (Fig. 4.4). This could probably be attributed to the introduction of a new waste stream that could have influenced the activity of the microbial consortium responsible for generating biogas or due to CO_2 being converted to bicarbonates (Gerardi, 2003) and thus alkalinity. Although it was expected that the introduction of a new waste stream would result in an increase in the VFA's, the effluent pH and COD reduction remained fairly stable (Fig. 4.2, Fig. 4.3). This can possibly be ascribed to the alkalinity, formed by the additional CO_2 production that was utilised to buffer the increased amount of VFA's and, therefore not resulting in changes in the total alkalinity. As higher leachate volumes in the substrate were maintained the consortium of methanogens started to adapt and degrade the VFA's, resulting in an increase in biogas content as seen from day 153 (*ca.* 6.8 $L.d^{-1}$), after which it remained stable until day 169. Another increase in biogas volume was seen around day 170, to *ca.* 7.6 $L.d^{-1}$, after which it remained stable at this level until the end of Phase C. From day 162 the methane content remained within the recommended range of 65 – 70% (Jönsson *et al*., 2003, Rasi *et al*., 2007) until the end of Phase C (Fig. 4.4).

Phase C showed that the maximum volume of leachate to be co-treated could be attained in 40 days of co-treatment starting, while COD reductions in excess of 90% were achieved, pH of the effluent remained above 7.2 and alkalinity varied between 1 370 – 3 800 $CaCO_3$ $mg.L^{-1}$. Having achieved stable COD reductions at the desired co-treatment substrate COD of *ca.* 6 700 $mg.L^{-1}$, it was decided to increase the volume of co-treated leachate to *ca.* double its volume, simulating a "shock loading" scenario.

Phase D (Day 181 – 205)

During Phase D the COD and OLR was thus rapidly increased to *ca.* 8 500 $mg.L^{-1}$ and 8.5 $kgCOD.m^{-3}d^{-1}$ (Fig. 4.2). A COD reduction > 90% was maintained during this phase with an

effluent COD lower than 1 000 mg.L^{-1} (Fig. 4.2). An initial drop below a pH of 7.5 was seen by day 181 (Fig. 4.3), after which it stabilised to a pH between 7.5 and 8.0 after day 192. The drop in pH can probably be ascribed to the rapid increase in COD concentration at the start of Phase D (day 181) (Fig. 4.2). The alkalinity during this phase was found to vary between 2 400 – 4 500 $CaCO_3$ mg.L^{-1} (Fig. 4.3). These values are higher than recommended by Gerardi (2003) (1 500 – 3 000 $CaCO_3$ mg.L^{-1}) and Anderson *et al*. (2003) (2 000 – 3 000 $CaCO_3$ mg.L^{-1}) (Fig. 4.3) for optimal anaerobic digestion. A similar trend in terms of alkalinity content and biogas volumes as found during Phase C were seen during Phase D as the results obtained, showed that biogas volumes initially decreased and then increased, whereas alkalinity increased and then stabilised.

Although the substrate COD concentration doubled to 8 500 mg.L^{-1} during Phase D the biogas volumes remained mostly in a stable range (*ca.* 7.0 – 8.0 L.d^{-1}) (Fig. 4.4). A possible explanation for not achieving a higher biogas production during Phase D is probably due to population changes within the consortium as a result of the rapid addition of a higher COD waste stream. Methanogen populations often have doubling times of several days (Zinder, 1993) and Phase D (24 days) could have possibly been too short for the methanogens to adapt and produce more methane. This would explain the fact that biogas volumes did not increase substantially, although alkalinity and COD reduction remained stable. The decrease in the initial alkalinity content as seen on day 192 was probably due to the system utilising it to buffer the increased amount of VFA's generated as increased volumes of leachate were added to the substrate. As the alkalinity decreased, the CO_2 formed during the degradation of the VFA's probably resulted in the slight increase of biogas volume as seen from day 190, which stabilised to *ca*. 8.1 L.d^{-1} until the end of Phase D (Fig. 4.4). Another possibility for the overall increase in alkalinity during Phase D could be due to the addition of increased amounts of leachate as obtained from the anaerobic composting of grape skins during Chapter 3 (Experimental Study 7, day 1) of this study, which had a high alkalinity.

In conclusion, during Phase D it was found that the reactor continued to operate optimally even after the amount of leachate to be co-treated was doubled with a final COD reduction > 90%, effluent pH = 7.61, an alkalinity of 3 281 $CaCO_3$ mg.L^{-1} and a methane content of 67%. As the COD reduction remained constant (> 90%), the biogas volumes produced where lower than expected and the alkalinity content was within the recommended range, the excess carbon was probably utilised to form biomass (sludge bed height increased from *ca.* 32.5 - 64 cm) or unutilised carbon sources such as lignin and cellulose.

Effluent quality

The final COD concentration (< 1 000 mg.L^{-1}) and pH (7.61) of the reactor effluent was within the regulatory limit for wastewater if land irrigation (< 50 m^3) is the intended end use (Republic of South Africa, 2004) but advanced treatments are often necessary to reduce or remove nitrogen, phosphorous and suspended solids in order to meet specific regulations (Srinivasan, 2008).

Therefore, further analyses of the reactor effluent would be necessary to identify if it complies with all regulations regarding disposal by means of land irrigation.

The *E. coli* loads of the reactor effluent were found to be 3 870 × 10^2 MPN.100 mL^{-1}. This value is higher than the recommended value for faecal coliforms (*E. coli*) (< 100 000 per 100 mL) if land disposal is the intended end use for volumes of up to 50 $m^3.d^{-1}$ (Republic of South Africa, 2004).

Conclusion

The generation of wastewater is a characteristic part of wine production, and thus it is necessary to minimise the volumes produced. In order to prevent ecological pollution, this wastewater (WWW) must be treated efficiently. This study showed that the co-digestion of winery wastewater with leachate from the anaerobic composting of grape skins was treated successfully, in an UASB reactor at OLR of 8.5 $kgCOD.m^{-3}d^{-1}$ with biogas volumes and methane content (%) of *ca.* 7.0 – 8.0 $L.d^{-1}$, and 65 - 71%, respectively. The final COD concentration of the reactor effluent was within the regulatory limit (< 5 000 $mg.L^{-1}$), but the faecal coliform (*E. coli*) levels were too high, which makes the reactor effluent unsuitable for land application (irrigation) (Republic of South Africa, 2004).

The feasibility of co-treating liquid waste (WWW) from wine production and liquid waste (leachate) from the AnC of grape skins in a lab-scale UASB reactor was shown. It is necessary that in order to provide a more economically viable solution for wineries, especially during peak harvest season, the up-scaling of this process needs to be investigated. It is also recommended that longer trial periods must be evaluated to determine the full potential of biogas generation during the co-treatment of composting leachate and WWW so that the consortium can acclimatise better to the additional waste stream that could possibly favour higher methane production.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

South Africa is a developing country that relies heavily on agricultural activities as a main source of overall economic growth (Anon., 2009). The production of wine is such an activity with an estimated 915.5 million litres produced in 2013 (SAWIS, 2014). The South African wine industry surpassed its export record, with approximate volumes of 527.7 million litres, a 26% increase from 2012 to 2013 (Anon., 2014). Increases in wine production not only places immense pressure on the usage of natural resources such as water, soil and vegetation but also on the wine industry itself, to comply with legal environmental requirements whilst upholding a competitive place in the international market (Oliveira & Duarte, 2010). More than 95% of South African wineries dispose of winery effluent by means of irrigation (Van Schoor, 2005) which could have severe environmental and health risks if disposed of in an uncontrolled and untreated manner. Solid grape waste (grape pomace), directly disposed onto land is a common practice, which results in ecological problems. Liquid and solid waste generated by winemaking needs to be treated in an appropriate manner to create sustainable water and waste utilisation practices within the wine industry. Anaerobic digestion (AD) is a proven biotechnology for the treatment of liquid, solid and semi-solid carbon-based wastes (Marín *et al*., 1999). AD offers advantages over other (aerobic) biotechnologies, mostly in terms of energy and environmental aspects (Moletta, 2005). A major drawback of AD is the uneven nutritional content in the waste substrate (Khalid *et al*., 2011) that can only be overcome by applying a co-treatment to supply the missing nutrients and optimise the substrate composition (Mata-Alvarez *et al*., 2000; Pagés-Díaz *et al*., 2014).

The objective of this study was to investigate the operational feasibility of the co-treatment of leachate produced during the anaerobic composting of grape skins in an upflow anaerobic sludge blanket (UASB) reactor, while treating winery wastewater. The first aim was to investigate the efficiency of the anaerobic composting of grape skins. This was accomplished by the set-up and optimisation of the composting system, evaluating the quantity and the quality of the final compost and investigating a possible treatment option for the composting leachate. The data obtained showed that the control of the inoculum composition, inoculum size, the pH as well as moisture content was of significant importance to produce a quality end-product. Since it was essential to control the pH, the most suitable method was investigated so as to increase and control the pH of the digesters. This was accomplished by adding pH adapted UASB effluent every 24 h, whilst removing the leachate produced during the digestion process to avoid acidification of the system. When using UASB effluent with a low alkalinity (obtained during the start-up phase), the composting system pH failed to reach a pH > 6.5 even over 21 days. It was therefore of importance that the inherent alkalinity of the reactor effluent that was used as moisturising liquid was high (3 500 $mg.L^{-1}$). This also played a significant role in buffering the

volatile fatty acids (VFA) formed during the initial stages of composting. Preliminary experimental studies also showed that grape skins cannot be composted alone due to their high carbon content and low pH. Thus, green waste (as source of nitrogen) and calcium oxide (CaO) were added and allowed to soak overnight at 37°C. The pH after the CaO soaking showed that is was necessary to only add the inoculum after the CaO soaking. The reason for this is that the lime solution probably was an unfavourable environment for the organisms. During preliminary experimental studies, it was also found that the waste needed to be shredded to speed up the composting process and to reach a final product by 21 days. The inoculum size and composition were found to be important during the digestion of the solid waste as they are directly responsible for the degradation. A 10% ($m.m^{-1}$) anaerobic compost inoculum was found to give the best compost, especially in terms of a pH stable and odour free compost. Using the lowest possible AC inoculum, results in more grape waste to be treated over time allowing the process to be more feasible. This study showed that AC as an inoculum also led to a more rapid attainment of pH > 6.5, which could possibly lead to a more pH stable end product as microbes responsible for the degradation of the waste, have previously adapted to the anaerobic conditions. Over the 21 day study period, colour changes were observed for both the grape skins (red-purple to brownish-green) and the composting leachate (dark green to yellow). By the end of the 21 day study the grape skins also changed into a uniform soil-like texture, with little recognisable grape skin pieces. A general trend was seen for all experimental studies, as the leachate pH increased and leachate volume decreased throughout the 21 day study period. The leachate produced by the AnC of grape skins could be utilised as a liquid fertiliser or as a co-substrate during anaerobic digestion of winery wastewater. The generation of leachate was not only of significant importance in monitoring the digesting content, but also gave an indication of the activity within the digesters, as it appeared that digesters generating higher volumes of leachate produced composts with a higher end pH and a larger mass reduction.

The UASB reactor used during the co-treatment of winery wastewater and composting leachate had a double advantage: (i) the reactor effluent was used to wet the system, therefore eliminating the need to utilise clean tap water; and (ii) to increase and control the pH of the digesting units to better facilitate the composting process when effluent was re-added to the system. An additional benefit of using UASB reactor effluent as a moisturising liquid is the continuous addition of fresh and active micro-organisms to the digesting units.

With all the optimum operational parameters in place: 6 g CaO; 50% ($m.m^{-1}$) water; 20% ($m.m^{-1}$) green waste; white and red grape skins in an equal ratio (50:50) (150 g each); and 15% ($m.m^{-1}$) cow manure inoculum, a good, stable compost was produced. In order to create a method to produce a stable compost in only 21 days the optimum factors as found during the lab-scale experimental studies were up-scaled (1:10) (total mass of 5 550 g). Physico-chemical analysis of both the lab-scale and up-scale products showed favourable results that complied with guidelines

(pH, ash, N%, K%, P%, Ca%, Mg%, Na%, Cu%, Fe%, Zn%, B%, EC) obtained from a local South African composting company.

The second aim of the study was to investigate the combined anaerobic digestion of winery wastewater and leachate from the composting process. This was achieved by using a lab-scale UASB reactor (2.3 L). The final COD reduction (> 90%), effluent pH (7.61), alkalinity (3 281 $CaCO_3$ mg.L^{-1}) and methane content (67%) were obtained even when the volume of co-treated leachate was doubled to simulate a “shock loading” scenario as may be expected during peak harvest season for large wineries. By introducing this additional waste stream (leachate) to the UASB reactor, the volatile fatty acids formed during the initial composting stages were removed and carried over into the stable UASB reactor to be converted into biogas. This prevented instability and composting failure in the anaerobic digester while treating an additional waste product without any pre-treatment in an lab-scale UASB reactor. Microbial analyses (*E. coli*) from the compost were within standard guidelines (< 1 000 MPN.g^{-1}). In contrast the *E. coli* levels for the leachate and the subsequent UASB effluent, were too high (3 870 × 10^2 MPN.100 mL^{-1}), indicating that it is unsuitable for land disposal (irrigation) for volumes of up to 50 $m^3.d^{-1}$ (Republic of South Africa, 2004). It was concluded from the data obtained during this study, that when an UASB reactor and anaerobic composting digesters are operated together, more than one waste stream can be treated successfully that could possibly contribute to a more sustainable wine industry.

Concluding remarks

This study provides a possible solution for wineries to simultaneously treat liquid waste from wine production as well as the leachate obtained during anaerobic composting of grape skins.

Although this study showed that the AnC of grape skins on lab-scale is a possible solution in terms of solid waste recycling, when the AnC of grape skins on an industrial scale is considered various parameters (CaO addition, shredding waste, leachate removal, temperature control) would need to be investigated in order to create an economically viable process. Due to the fact that biogas values obtained during the anaerobic composting of grape skins were found to be low or even absent for both the lab-scale and up-scale digesters the co-digestion of grape skins with a carbohydrate rich waste such as green kitchen waste should be investigated. This could possibly provide nutrients to facilitate the generation of increased biogas and methane.

To better characterise the stability and maturity of the end-product, the germination index (based on the germination seed) and SOLVITA® (commercial maturity test based on CO_2 and NH_3) test could be evaluated to compare these results with general recommended physico-chemical characteristics. With increased environmental awareness, stricter legislation for waste disposal is increasing and new solutions need to be considered to develop a sustainable wine industry. This study showed the feasibility of co-treating winery wastewater and composting leachate in a lab-scale UASB reactor. It is recommended that longer trial periods should be

evaluated to investigate the full potential of biogas generated during the co-treatment process so that the microbial consortium can adapt to the additional waste stream, possibly allowing more methane gas to be produced. It is also recommended to further analyse the reactor effluent to identify possible post-treatment options to reduce the microbial counts of reactor effluent after co-treatment. Future research should include a complete study on industrial scale to determine the actual feasibility of the process.

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