Cape Peninsula University of Technology

Monitoring extracellular enzyme activities and microbial population numbers during composting of winery solid waste

By

Yandiswa Mtimkulu

Thesis submitted in fulfilment of the requirements for the degree

Master of Technology: Horticulture

in the Faculty of Applied Sciences

Supervisor: Dr F. Nchu

Co-supervisor: Mr A. Meyer

Bellville

September 2016

Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signed:

Date: 24 : September : 2016

DEDICATION

This work is dedicated to my husband Galili Patrick Mtimkulu who has always supported and believed in me, and without who's constant love, encouragement and editing assistance, I would not have finished my studies.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to the following people and institutions for making this research possible:

- Most importantly, my God for health and strength throughout my life and giving me courage and ability to finish my studies.
- My supervisors, Dr F. Nchu and Mr A. Meyer for their advice, patience, motivation, continuous support and the valuable contribution and constructive criticism they have provided throughout the study.
- Mr Reckson Mulidzi and Ms Philisiwe Shange for giving me the opportunity to take part in the project.
- The staff in the Department of Soil and Water Science, ARC Infruitec – Nietvoorbij for their support and advice, particularly the technical assistants for their extra efforts and contributions made towards the construction of the compost heaps.
- Mrs Deborah Erasmus and students in the Department of Horticultural Sciences, Cape Peninsula University of Technology for their support during the course of my study.
- The ARC for the facilities.
- Bemlab with chemical analyses of the composts samples.
- Winetech, ARC and CPUT for financial support.
- And last, but not least, I would also like to thank my family for the constant love and support they provided me through my entire life.

SCIENTIFIC CONTRIBUTIONS FROM THIS STUDY

Parts of this study have been presented at the following scientific meetings:

(i) Combined Congress of the South African Society of Crop Production, Soil Science Society of South Africa, South African Society for Horticultural Sciences and the Southern African Weed Science Society held on 20-23 January 2014, Grahamstown (Rhodes University).

Topic of the poster: Temporal Fluctuations in Extracellular Enzyme Activities, Microbial numbers, Moisture and Temperature During Composting of Winery Solid Waste.

(ii) 36TH Congress of the South African Society of Enology and Viticulture held on 12-14 November 2014 at Lord Charles Hotel, Somerset West, South Africa.

Topic of the poster: Extracellular enzyme activities and microbial counts during composting of winery solid waste as influenced by environmental factors.

TABLE OF CONTENTS

Chapter One

Introduction, background to research problem and literature review

LIST OF ACRONYMS

NEMA - National Environmental Act

IPW - Integrated Production of Wine

DE - Diatomaceous Earth

ARC - Agricultural Research Council

DWAF - Department of Water Affairs and Forestry

IWMI - International Water Management Institute

CPUT - Cape Peninsula University of Technology

ANOVA - Analysis of Variance

OM - Organic material

CFU/g^{-1} - colony forming units per gram

WOSA -Wines of South Africa

OIV - International Organisation of Vine and Wine

NWQMS - National Water Quality Management Strategy

LIST OF TABLES

LIST OF FIGURES

CLARIFICATION OF CONCEPTS.

Winery waste - Is a by-product eliminated or discarded during production of wine as no longer useful or required after the completion of the process.

Compost - An organic waste that is not phytotoxic, free of pathogens, and that can be used as a substrate and nutrient source for plant growth or as a conditioner to improve soil properties (Huang *et al*., 2006).

Compost maturity - Generally relates to the agricultural value of the compost in relation to its effect on the soil and plant responses to its application (Cabañas-Vargas *et al*., 2005).

Extracellular enzyme - an enzyme that is secreted by a cell and functions outside of that cell.

API ZYM ™ - a semi-quantitative method designed for a systematic and rapid study of 19 enzymatic reactions.

Microbial population - The sum of living microorganisms in a given volume or mass of winery compost.

Two types of spent wine filter materials used

Douglas green waste - is made up of diatomaceous earth (DE) filter powder, wine and spirit lees, more clay and appeared to have more cementing nature, yellowish brown in colour when fresh, but would turn to purple when exposed to air for a long time.

Koelenhof waste - is a product of drum filtration that contained perlite from Chemserve and wine lees waste, and it is dark brown in colour.

Final product - Product that has been developed from treatments after the end process of composting.

Commercial products - Products manufactured and sold in retail stores.

Integrated Production of Wine - is a voluntary environmental sustainability scheme established by the South African wine industry in 1998. Guidelines for sustainable Viti-viniculture: Production, processing and packaging of products.

Abstract

Waste management in winery and distillery industries faces numerous disposal challenges as large volumes of both liquid and solid waste by-products are generated yearly during cellar practices. Composting has been suggested a feasible option to beneficiate solid organic waste. This incentivized the quest for efficient composting protocols to be put in place. The objective of this study was to experiment with different composting strategies for spent winery solid waste. Compost materials consisting of chopped pruning grape stalks, skins, seed and spent wine filter material consisting of a mixture of organic and inorganic expend ingredients were mixed in compost heaps. The filter material component varied (in percentage) among five treatments: T1 (40%) lined, T2 (20%) lined, T3 (0%) lined, T4 (40%) grinded material, lined and T5 (40%) unlined.

Composting was allowed to proceed in open air over 12 months, from autumn to summer. Indicators such as temperature, moisture, enzyme activities, microbial counts, pH, and C/N ratio, were recorded. Generally, season ($df = 3, 16$, $P < 0.05$) had significant effects ($df = 1, 3$, $P < 0.05$) on heap temperature and moisture in all treatments. Similarly, microorganisms (actinobacteria and heterotrophs) varied significantly in all treatments in response to seasonal change ($df = 3, 16$; $P < 0.05$). Enzyme activities fluctuated in accordance with seasonal factors and compost maturity stages, with phosphatases, esterases, amino-peptidases, proteases and glycosyl-hydrolases being most prominent. Compared to treatments T2 and T3, compost treatments with higher percentage waste filter materials (T1, T4 and T5) had higher N (16100-21300 mg/kg), P (1500-2300 mg/kg), K (19800-28200 mg/kg), neutral pH, and lower C/N ratios (13:1-10:1), which were also comparable with commercially produced composts. Filter materials therefore, appears to be a vital ingredient for composting of winery solid waste.

Chapter One

1.1. Introduction

Recycling of organic waste is one of the successful waste treatment systems used worldwide. Wineries are increasingly using solid waste as part of the composting material. Composting of organic waste is based on the transformation of biodegradable organic material from various sources including winery waste into humic substances (Golueke, 1977). It is mainly a microbial process because microorganisms through different kinds of substrate-based hydrolytic enzymes, promote the degradation of organic materials (De Bertoldi *et al*., 1983). These enzyme activities vary in time as a consequence of a complex sequence of microorganisms, where populations of bacteria, actinobacteria and fungi change in time depending on the specific conditions during composting evolution (MacKinley *et al*., 1985).

Whilst it is well- recognized that composting mimics the natural biodegradation process in soil (De Bertoldi et al., 1983) and could yield stable end-product from biological oxidative transformation of organic wastes, there are challenges that are retarding the implementation of sustainable and efficient composting programmes. Firstly, there are gaps in the current knowledge of the composting process, especially with respect to microbial and enzyme activities. Enzyme activities vary in time as a consequence of a complex sequence of microorganisms, where populations of bacteria, actinobacteria and fungi change in time depending on the specific conditions during composting evolution (MacKinley *et al*., 1985). Therefore, detailed characterization of microbial communities along the process of composting may provide valuable information regarding the evolution of the process, the rate of biodegradation and the measure of compost maturity. Secondly, the current composting procedures are not very efficient; hence it is important to optimize current composting protocols in order to improve on the agronomic and environmental qualities of the end product. Many workers are investigating the potential benefits of incorporating used organic materials during composting of winery waste materials.

Hauck (1981) projected that the application of organic waste residues to improve soil productivity in developing countries could contribute more than 50 percent of

the increased food production that is currently needed worldwide. Organic amendment has become particularly important in the restoration of C stocks in dry and semiarid soils, since it maintains soil organic matter levels, supplies nutrients and enhances microbial proliferation (Ros *et al*., 2003 & Tejada *et al*., 2006).

A study by Doublet *et al*. (2011) suggested that the inclusion of bulking agents influenced the time to reach organic matter stability and the biochemical evolution of OM during composting. Hornick *et al*. (1979) also discussed the use of sewage sludge compost for soil improvement and reclamation of disturbed lands. Bustamante *et al*. (2010) assessed the effect of compost stability on C mineralisation dynamics by applying organic materials (grape stalk, grape marc, exhausted grape marc and vinasse, with sewage sludge or animal manure) from different stages of the composting process, and results obtained showed that the addition of exogenous stimulated microbial growth, enhanced soil respiration and increased water-extractable C contents in both soils, particularly in the days immediately following amendment. While many studies have looked at the enhancement effects of organic filter materials composting, very few studies have investigated the beneficial effects of including inorganic filter materials during composting of solid agricultural wastes. Preliminary results obtained by our colleagues, Mulidzi & Shange (Unpublished) suggested that the addition of filter material that consisted of inorganic and organic filter ingredients to solid winery wastes could improve composting of the latter, thus warranting further evaluations.

1. 1.1. Structure of the research

This thesis comprises five chapters, which are briefly described.

Chapter One: presents the conceptual framework of the research, provides scientific justification of the study, hypotheses, aim and specific objectives of the study.

Chapter Two: Materials and method.

The chapter contains information on the experimental site, treatments, formulation of the compost heaps, API, microbial counts, measurement of heap temperature and moisture.

Chapter Three: Results

Temperature, moisture, microbial numbers (actinobacteria and heterotrophs), extracellular enzyme activity in the compost heaps, pearson correlation coefficients between enzyme activities and (environmental factors, microbes and chemical factors). Comparison between chemical analysis in compost end product and commercial produced compost.

Chapter Four: Discussion

Relationship between temperature and extracellular enzyme activities, moisture, relationship between extracellular enzyme activities and microbes, relationship between extracellular enzyme activities and chemical properties, C/N ratio.

Chapter Five: Conclusion and recommendation of the study.

1.2. Background to the research problem

Winery and distillery industries generate an increasing amount of both liquid and solid waste by-products during cellar practices. These by-products show different management and disposal challenges. Integrated production guidelines have been successfully developed for liquid waste produced by wineries (IPW guidelines, 2008). There are no established procedures, however, for the utilization of winery solid waste in a sustainable manner. Suggested strategies for biodegradation of solid waste include composting, which is one of the most promising strategies (Bustamante *et al.,* 2008). Composted winery materials can serve as organic fertilizers in crop production (Arvanitoyannis *et al*., 2006). Composted winery materials have high agronomic value in the sense that they have high organic content, they are important sources of nitrogen and have antimicrobial properties (Bertran *et al.,* 2004; Carmona *et al.,* 2012). Composted winery materials could also serve as replacements for well-known substrates such as coconut fibre and peat (Carmona *et al*., 2012).

Although good plant growth can be maintained by using inorganic fertilizers alone, best results are often achieved with a combination of inorganic fertilizers and organic residues applied to the soil (Raath & Fourier, 2006). Since exclusive use of chemical fertilizers is no longer considered the best method to supply plants with nutrients due to their negative environmental impacts and high cost, composting

has become a good alternative (Sinha *et al*., 2010), not only because it provides nutritional and energy benefits, but also because it does so with minimal harm to the environment (Azadi & Ho, 2009). Inorganic fertilizers also improve the structural and physical components of soils, minimize the leaching of important minerals and rapid depletion of nutrients by plants, and improve water holding capacity of soils (Steiner *et al.*, 2007). Although a large proportion of wineries are disposing their waste by dumping in specialized municipal landfills, which are generally not cost-effective, many are realizing the need to incorporate winery waste in compost (Schoeman, 2012).

Composting material is transformed through a variety of biological processes, which involves the use of enzymes (Garcia *et al*., 1992, 1993; Vuorinen 1999, 2000). Enzymes in compost can be categorized as intracellular enzymes inside viable cells or extracellular enzymes outside viable cells (Vuorinen, 1999) Enzymes in soil microbiology include cellulases which degrade cellulose nitrogenase, which converts dinitrogen gas into biologically available ammonia, sulphatases which release protein and other related organic compounds and phosphatases, which remove the phosphate groups from organic compounds (Burns 1978; Tate 1995; Nannipieri & Fusi, 1996). Often, mesophilic bacteria are the first to appear during composting, partly because they prefer temperatures around 20-35 ºC, and in this temperature, it is easy for worms and insects to work in cycle to the benefit of the bacteria. The increase in temperature during the early stage of composting results in the thermophilic phase of composting (Ros *et al*., 2006), characterised by thermophiles which thrive between 40 ºC and 70 ºC. Thermophiles normally work at these high temperatures for 3-5 days continuing until all the organic material is reduced to its smallest components. Once the more easily degradable materials have decomposed, the temperature falls to that of the environment and the process is stabilized (Nogueira *et al*., 1999). As the organic matter becomes more stable, microbial activities and decomposition rates are decreased marking the end of the thermophilic phase (Ros *et al*., 2006). Reduction of temperature following decomposition favours growth of beneficial microbes (Bernal *et al.*, 2009).

Previous studies at Nietvoorbij experiment farm, Stellenbosch investigated the use of filter material especially spent wine filter material during composting and recycling of winery material (Mulidzi & Shange, 2011). Preliminary results

suggested that incorporation of filter material could improve composting of solid winery wastes, thus warranting further evaluations.

1.2.1. Statement of the research problem

Handling treatment of solid winery wastes is challenging in 'South Africa' as these wastes can be toxic to the environment. Most wineries have tried one or more methods of disposing waste such as dumping in specialized municipal landfills or disposing on sites at wineries. These methods may initiate various environmental hazards such as soil and water pollution, bad odours and spread of diseases (Takele, 2011). In order to overcome setbacks which are associated with the disposal of winery waste, composting is believed to be the most promising strategy for treatment of winery waste because it improves soil structure, increases nutrient content of the soil and eliminates toxic residues to the environment.

1.3. Literature review

1.3.1. Wine industry and waste production in South Africa

Globally, grapes (*Vitis vinifera*) are one of the most important fruit crops with more than 60 million metric tons produced annually (Alleweldt *et al*., 1991). The family (Vitaceae) is most commonly cultivated for wine production. Climatic conditions in certain parts of the Western Cape region of South Africa are favourable for cultivation of grapes (Goldblatt & Manning, 2002). Wine industry is an important contributing sector to the South African economy especially in the Western and Northern Cape, where most winelands and wine cellars are situated (Schoeman, 2012). Wine production has increased over the past decade and this growth has increased pressure on the natural resources such as water, soil and produce (Van Schoor, 2000). This increase has occurred at a time when national legislation and foreign markets are becoming increasingly strict in their demands that all factors which have the potential to affect the environment should be controlled. At the same time, local winemakers have had to take notice of tighter legislative controls on environmental impacts, and a rise in consumer demand for 'enviro-friendly' products vegetation (Van Schoor, 2001). Excellent technology is an important criterion for global competitiveness, and the wine industry in South Africa is

currently undergoing a renaissance to establish itself in the international market (Malandra *et al*., 2003).

The wine industry in SA is comprised of a group of closely related industrial operations engaged in the production and processing of grapes to a variety of alcoholic and non-alcoholic products (Robertson & Kirsten, 1993). The rapid growth in wine production in most wine making regions of the world during the last decade needs to be matched with greater emphasis on minimizing the impact of winery operations on the natural and human environment (Gajdos, 1998). The winery industry has increased in popularity all around the world and consequently an increase in its contribution to environmental pollution. South Africa and the Western Cape are confronted by waste management challenges driven by population growth and the need to redress environmentally unacceptable waste management practices. In seeking solutions, South Africa has engaged in debates raised at international forum, which has influenced domestic laws and waste management trends locally (Glazewski, 2000).

1.3.2. Description of winery waste

Wine production process generates large quantities of waste annually, including organic solid wastes (solids, skins, pips marc, etc.), inorganic solid wastes (diatomaceous earth, bentonite clay perlite) liquid waste (cleaning waste water, spent cleaning, solvents, cooling water) and gaseous pollutants (carbon dioxide, volatile organic compounds, ammonia, sulphur dioxide, etc. (Catherine, 2011). The by-products of cellar practices that most commonly have negative impacts on the environment are: wastewater generated during cleaning, process water, solid wastes such as skins, pips, stems and lees, filter materials and filter aids and sedimentation substances (Van Schoor, 2000; Van Schoor, 2001; Chapman *et al.*, 2001). Where winery wastes are applied to land there is an imperative requirement to avoid adverse effects of such practice on aquatic environments and soil/plant health. To manage winery wastes and their potential environmental impacts effectively and to make provision for emergency situations, it is important for cellar managers to know what the potential pollutants are, how they are generated and what management options are available to minimise their impacts (Van Schoor, 2000).

1.3.3. Winery liquid waste

The South African wine industry generates more than 1000 million litres of wastewater annually (Sheridan, 2004). Winery effluent requires treatment before discharge, but remediation is complicated by the fact that the composition and volume fluctuates on a seasonal basis, depending on cellar activities (Arienzo *et al*., 2009; Malandra *et al*., 2003; Mosse *et al*., 2010). Wastewater necessity to be disposed in accordance with government legislation and approved by the Department of Water Affairs and Forestry (DWAF) (National Water Act, 1998). Winery wastewater is mostly generated during the cleaning of winemaking equipment and facilities as wineries must be kept clean to avoid contamination and spoilage (Catherine, 2011). Winery wastewater composition varies daily and throughout the year, depending on activities within the winery. Overall, wastewater is high in salts, contains moderate nutrient loadings, and has a low pH (Sheridan, 2004). The organic composition of winery wastewater is dominated by simple dissolved compounds such as organic acids, sugars and alcohols. The effluent has a high requirement for oxygen for biological decay (Chapman *et al.*, 2001). Wastewater handling involves collection, possible treatment, then disposal and/or reuse. Winery waste water can cause soil sodicity, salinity, contamination with a wide range of chemicals, waterlogging and anaerobiosis, loss of soil structure and increased susceptibility to erosion, if not handled properly (Sheridan, 2004).

1.3.4. Winery solid waste

Winery solid waste consists of the stalks, pips and skins, which remain behind after the grapes have been crushed and pressed and require specific handling if stored on-site. Grape solids are sometimes referred to as ‘marc’ in international literature (Van Schoor, 2005). Grape marc (pomaces), is the left-over after wine grape processing and filter solid waste, could be recycled as a soil conditioner due to its organic and nutrient contents (Catherine, 2011). Grape pomace is a fibrous material that consists of approximately 8% seeds, 10% stems, 25% skins and 57% pulp and its composition varies depending on the wine variety produced (Van Schoor, 2005). Grape marc has moisture content of about 65%, and it represents as much as 20%

of the wet weight of the original fruit (Hang, 1988). Grape marc contains a large amount of N, P and K, however, the availability of these nutrients is generally low (Laurenson & Houlbrooke, 2012). Inbar *et al.* (1991) reported that the direct incorporation of grape marc into agricultural land, a common practise, can produce serious problems since degradation products prevent root growth due to high phenols and tannins. Grape marc decomposes slowly due to the large portion of seeds that are high in lignin (Fernández *et al*., 2008). However, depending on the characteristics of the raw material and the management of the process, composts may also contain substances harmful to the environment such as pathogens, bioaerosols, heavy metals and toxic organics (Deportes *et al*., 1995). Such negative effects are mainly associated with an initial net immobilisation of nitrogen after the application of winery and distillery wastes into soil (Bustamante *et al*., 2007). According to the National Environmental Act (NEMA) of 2008, where waste generation cannot be avoided, waste should be reduced, re-used or recycled, but not without continual assessment and monitoring. Integrated Production of Wine (IPW) guidelines state that solid waste should be handled in such a manner that there is minimal detriment to the environment and that composting of waste is a proper waste management measure. The final grape marc, both fermented and unfermented, contain a variety of chemical components including cellulose, tartaric acid, unfermentable sugars tannins, phenolic substances and alcohol (Crowe, 2005).

Where solid wastes are present, offensive odours may be generated and leakage may result in the contamination of soil and water resources, preventing vegetative performance (Chapman *et al*., 2001). There is another part of solid waste that is used in the current study i.e. spent filter materials, which mainly contain certain filtration agents such as diatomaceous earth (DE) and perlite (perlite is used for juice lees filtration and diatomaceous earth (DE) for wine filtration). According to Zingelwa (2012), DE is the most used filtering aid for bulk filtration in conjunction with cellulose in cellars while perlite powder is normally used for white wines in drum filters. These agents contain proteins, yeasts and tartrates which are bound to these agents making re-use in composting an advantageous option. Some wineries use both diatomaceous earth and perlite (wine filters). Both spent filter materials are routinely mixed and dumped in the same place or container (Catherine, 2011).

1.3.5. Perlite

Perlite is a manufactured product that originates from volcanic rocks that are mined and heated (Daniel *et al.*, 2014). Perlite is normally used in horticulture as an amendment with properties such as uniform particle size and a natural sharpness (Zmora-Nahum *et al*., 2007). When mixed in proper portions with other materials such as sandy soil can contribute to creating an exceptional plant growth medium which can improve the physical characteristics of the soil e.g. high ability to retain water and nutrients and improved aeration (Hodges, 2012).

1.3.6. Diatomaceous earth

Diatomaceous earth (DE) is a manufactured product that comes in the form of a white to off-white powder from natural mineral deposits that accumulated over centuries under huge lakes or oceans and it originates from the remains of microscopic one-celled floating plants which are called diatoms (Halliday, 2010). It is made up of silica (85%), aluminium (8%), iron (2%), sodium (5%), and many trace minerals such as titanium, it can also be used as a soil amendment and can be dusted into lawns and beds, or it can be mixed into potting soil or bed preparation (Athanassiou *et al.*, 2005).

1.4. Composting of solid winery waste

The wine industry and regional governing programmes are increasingly focusing on land application of winery wastes as the most cost effective and environmentally sound means of disposal (F. Smith, personal communication, November 8, 2015). This form of dumping does however raise unease over potential impacts on soil and crop health, and off-site environmental pollution associated with nutrient leaching and run-off (Laurenson & Houlbrooke, 2012). In actual fact, wineries are obliged to dispose of grape marc and waste water in a sustainable manner that does not contaminate drinking water sources or results in off-site pollution. There is a developing concern relating to land degradation, the inappropriate use of inorganic fertilizers, atmospheric pollution, soil health, and sanitation. These have favoured

worldwide interest in organic recycling practices such as composting (Misra *et al.*, 2003).

Composting is defined as the aerobic biological decomposition and stabilization of organic substrates, under conditions that allow the development of thermophilic temperatures as a result of biologically produced heat, to obtain a final product that is stable, free of pathogens and plant seeds, and can be beneficially applied to land (Golueke,1982; Haug,1993). Composting of organic material is a simple and efficient manner of transforming agro-industrial waste into the products suitable for use as soil conditioners (Ferrer *et al*., 2001). Composting of winery waste is also a substitute to the traditional disposal of residues, and also involves a commitment to reducing the production of waste products (Bertran *et al.*, 2004). Winery materials are composed of two components, i.e. liquid waste (effluent) and solid wastes (NWQMS, 1998). According to Levay (1995), winery solid wastes consist of the following:

-Stalks, seeds and skins (marc) produced during the crushing, draining and pressing stages.

-Sediments (lees) containing pulp, tartrates and yeasts from the fermentation stage.

During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a role (Garcia *et al*., 1992, 1993; Vuorinen 1999, 2000). According to Ipek *et al*. (2002) composting microorganisms consume oxygen for the bio-oxidation of the organic material resulting in the generation of heat, carbon dioxide and water vapor, which are released into the atmosphere. At the same time, the volume and mass of the organic raw material is reduced significantly converting it into a stable organic final product, which can be used as soil conditioner, as well as for land reclamation (Kiyasudeen *et al*., 2015). Carlos *et al.* (2004) states that composting of solid organic waste involves three levels of consumer organisms. First level which are the true decomposers composed of microflora, actinobacteria, bacteria and fungi. Protozoa and arthropods form second levels which feed on first level organisms, while the third level is composed of higher arthoropods such as ants and beetles, predators of the second level organisms (Carlos *et al.*, 2015). In this ecological network, biotransformations of organic material during composting are mainly due to enzyme activities of first level microorganisms leading to mineralization process (Carlos *et al.*, 2004). The evolution of environmental conditions temperature,

moisture and oxygen, is dependent on the decomposition rate of an organic material (Carlos *et al.,* 2015). Temperature, water content, C/N ratio, pH level, aeration rate, and the physical structure of organic materials are important factors influencing the rate and competence of composting (Khalid *et al.,* 2011).

The respiratory activity, carbon dioxide production and oxygen consumption rates, and microbial biomass, have been successfully employed to understand the composting process and to assess compost maturity (Iannotti *et al*., 1994; Insam *et al*., 1996; Tiquia *et al*., 1996; Epstein, 1997).

Often, mesophiles, which are in mesophilic phase are the first group of bacteria to appear during composting, partly because, they prefer temperatures around 20-35 ºC, and in this temperature, it is easy for worms and insects to work in cycle to the benefit of the bacteria. It has been reported that the increase in temperature during the early stage of composting may be the results of thermophilic phase of composting (Ros *et al*., 2006) in this phase thermophiles, which take over at around 40 ºC and continue until the temperature stabilizes at 70 ºC, burn through the organic material quickly. Normally they work at these high temperatures for 3-5 days and if the conditions in the compost pile are not changed, they should complete their work (Epstein, 1997). This phase will continue until all the organic material reduced to its smallest components. Once the more easily degradable materials have decomposed, compost temperature falls to that of the environment temperature and the process is stabilized (Nogueira *et al*., 1999). As the organic matter become more stable, the microbial activities and the organic material decomposition rate decreased with the temperature gradually decreasing to ambient levels, marking the end of the thermophilic phase (Inbar *et al*.,1993).

Therefore, composting is the process of controlled biological decomposition of biodegradable materials under managed conditions that are predominantly aerobic and that allow the development of thermophilic temperatures as a result of biologically produced heat, in order to achieve compost that is sanitary and stable (Litterick *et al.,* 2003). Microbes can convert organic wastes to humus in the soil (Levay, 1995). In nature composting has always occurred naturally. The addition of non–stabilized compost to the soil may cause several phytotoxicity effects and adversely affects the environment (Butler *et al.,* 2001*).* Extensive research has demonstrated that

many biodegradable organic wastes can be composted in an appropriate and cost-effective way (Ioannis *et al*., 2006). The essential components of compost making are organic material, water, air, and nitrogen (Raath & Fourie, 2006). High temperatures generated during composting may kill most parasites, rendering the material relatively safe to handle (Raath, 2002). Since exclusive use of chemical fertilizers is no longer considered the best method to supply plants with nutrients due to their negative environmental impacts and high cost, composting has become a good alternative, not only because it provides nutritional and energy benefits, it does so without any harm to the environment if carefully managed. It also improves the structural and physical components of soils. It minimizes the leaching of important minerals and rapid depletion of nutrients by plants, and improves water holding capacity of soils. On the basis of the established benefits associated with composting the development of efficient composting procedures is imperative.

Most wineries have tried some other methods of disposing their waste such as, dumping their waste in specialized municipal landfills or disposing on sites at wineries or reuse through incorporating it in compost (Manuel, 2004). Raut *et al.* (2008) states that shortening of composting period with considerable reduction in the C/N ratio is one of the options considered for making composting business lucrative. Wineries have to follow the guidelines or legislations set by the government or the municipalities on the handling of solid waste (Zingelwa, 2012). Site factors include property size, available land onsite for disposal proximity to nearby surface waters, natural surface drainage the depth of groundwater, and soil type (Zingelwa, 2007). Other factors include winery wastewater loads, waste constituent levels, seasonal load variation, future plans for expansion, economic considerations, adjacent land-uses, and proximity to residents. Storage of waste in enclosed containers could avoid bad odours and pest breading (flies). Disposal of wastes on sites or landfill should be monitored in order for the leachate not to seep and contaminate ground water and the surrounding soil (Nathanson, 2015). The Scheme for Integrated Production of Wine (IPW) requires all producers (farms) and cellars that subscribe to the scheme to complete an annual self-evaluation form and or cellar to evaluate compliance with the IPW guidelines. The purpose of the guidelines is to promote suitable production, and by implication the IPW evaluation forms for farms and cellars also serve as a "barometer'' to measure the relative impact of the farming and or winemaking practices on the environment (Allsopp &

van Schoor 2008). Drainage into water systems like rivers and dams would also be disastrous, sludge disposal into land should be analysed to ensure that applications and application rates do not have adverse effects on the environment. Filter residues such as bentonite and diatomaceous earth have been disposed unlawfully in the past and potential re-use and recycling has been ignored especially in the field of agriculture, as these can be very beneficial (Zingelwa, 2007). Composting done on site by wineries has some advantages; it reduces cost of transportation and risks of environmental contamination during transportation (e.g. Caborn dioxide emissions) and composted materials could be used to improve soil quality onsite (Catherine, 2011). Also, the composted material could serve as an additional source of income for wineries.

It is worthwhile to investigate the benefits of combining grape marc materials and filter materials during composing products (Catherine, 2011).

1.5. Relevance of composting: Application and usefulness of composting

Among different strategies suggested for use in biodegradation of winery solid waste, composting is one of the most promising strategies (Bustamante *et al.,* 2008). Extensive research has demonstrated that many biodegradable organic wastes can be composted in a convenient and economical way (Ioannis *et al*., 2006). Composting of organic material is a simple and efficient manner of transforming agro-industrial waste into the products suitable for use as soil conditioners (Ferrer *et al*., 2001).

These by- products (solid and liquid waste) when composted are valuable fertilizer resources with high contents of macro-and micro-nutrients, primarily nitrogen and potassium for crop growth (Gómez-Brandón *et al.,* 2011). Winery waste is characterised by the presence of natural antioxidants that are much safer than synthetic antioxidant (Ioannis *et al.,* 2006). Wine waste derived antioxidants have been recently used in the food industry. Moreover, wine waste can be potentially used as soil conditioner as adsorbent for heavy metals and for fertilizers (Ioannis *et al.,* 2006). Grape pomace represents a rich source of various high value products such as ethanol, grape seed oil hydrocolloids and dietary fibre. Previously, Solivia *et al.* (2002) compared the best compost obtained from winery wastes with those from other organic wastes and found that the chemical values of the compost obtained fell within the same range in most cases, with the exception of a high -

calcium value owing to the nature of the wine making process. Because of these beneficial properties, winery by-products are now being sold to the rapidly growing dietary supplements industry (Ioannis *et al*., 2006). This may be the most effective and economically viable waste management method in wineries. Exclusive additions of chemical fertilisers are not considered the best method to feed plants and keep plant pathogens under control. Growers now understand that some type of organic material need to be added to the soil whether as compost or another type of soil amendment (Arvanitoyannis *et al*., 2006). The use of compost in vineyards is of growing interest due to the general poverty of soils, characterised by low levels of humus and their exposure to erosion (De Bertoldi *et al*., 1986; Balanyá *et al*., 1994). Composting may also replace traditional farm manure in areas of intensive agriculture (Ayari *et al*., 2010).

1.6. Waste management in South Africa

South Africa particular in the Western Cape are confronted by waste management challenges driven by population growth and the need to redress poor legacies of environmentally unacceptable waste management practices (Catherine, 2011). In seeking solutions, South Africa has engaged in debate raised at international forums which has influenced domestic law and waste management trend locally (Glazewski, 2000). Local agreements have emerged and, combined with the international debate, South Africa waste management strategies have been guided by various instruments, including: the convention on the control of transboundary movements of hazardous wastes and their Disposal, 1989 (the Basel Convention). A draft of National Integrated Waste Management Bill was submitted to Parliament in 2006 for declaration and was approved in 2008. Presently, waste management is the responsibility of the Provincial District Municipalities in partnership with private companies.

1.7. Compost parameters

1.7.1. Temperature and composting

Temperature is one of the main control parameters of the composting process and constitutes a by-product of the microbial activity during organic material biodegradation (Litterick *et al.*, 2003). Steger *et al.* (2007) describe temperature as

the most influential parameter, although some other conditions also favour the presence of microorganisms with specific metabolic capabilities. The importance of temperature monitoring lies on the fact that it reflects the activity of microorganisms in the substrate and also represents an indicator of the proper evolution and occurrence of the composting process (Diaz & Savage, 2007). Microorganisms require a certain temperature range for optimal activity (Beales, 2006). High temperatures result in faster breakdown of organic materials that destroy weed seeds and kill pathogens (Cooperband, 2000). Other temperature benefit to compost include its effect on microbial growth within piles and activities and hence the rate at which the raw materials decompose (Bernal *et al.*, 2009). Common methods used for adjusting temperatures are aeration, turning and changing pile moisture contents and pile sizes (Chen *et al.*, 2011).

1.7.2. Aeration (effects of turning winery solid waste)

Aerobic organisms need to inhale air to survive. Oxygen is required to support the growth of beneficial organisms and to eliminate the risk of pathogens and other toxic compounds (Richard, 2012*).* Aeration is essential in high temperature aerobic composting for rapid odour-free decomposition (Misra *et al*., 2003). Liang *et al.* (2003) also state that aeration is correspondingly beneficial in reducing high initial moisture content in composting materials. Ndegwa & Thompson, (2001) also confirm that turning material is the most common method of aeration when composting is done in stacks. Mechanical turning or static pile systems with a forced air system have higher capital costs in large municipal or commercial operations (Tardy & Beck, 1996). Studies at the University of California indicated that turning at fairly frequent intervals during the first 10 to 15 days of composting achieved approximately the same degree of stabilization as making the same number of turns over a longer period (Diaz & Savage, 2007). The air requirement for biological activity depends on the availability of nutrients in the feedstock (e.g. a very high C/N ratio material would not support as large a biological population) (Haug, 1993). Turning schedule will permit rapid decomposition at thermophilic temperatures (Kalamdhad & Kazmi, 2009).

1.7.3. Oxygen flow

The oxygen that is required for the composting process is essential for the aerobic metabolism and respiration by microorganisms, and also for the bio-oxidation of the organic molecules present in the substrate (De Bertoldi *et al*., 1988). Oxygen consumption during composting is directly proportional to the microbial activity providing a direct relationship between oxygen consumption, temperature, moisture and aeration (EA, 2001). Therefore, aeration is a key factor for composting since proper aeration controls the temperature, removes excess moisture and provides oxygen for the biological processes (Bernal *et al.*, 2009). According to Miller (1992) the optimum oxygen concentration is between 15% and 20%. If there is insufficient oxygen, the process can become anaerobic involving a different set of micro-organisms and different biochemical reactions, which result in the production of methane gas and malodorous compounds, such as hydrogen sulfide gas and ammonia (Manyi-Loh *et al*., 2013). Aeration of the organic substrate is achieved through agitation, active aeration (air blowing) and natural diffusion of air (International Water Management Institute, [IWMI] 2003).

1.7.4. Moisture

Moisture supports the metabolic and biodegradation processes by the micro-organisms, since water is the medium for biochemical reactions, transportation of nutrients and allows the microorganisms to move about (Gajalakshmi & Abbasi, 2008). However, the optimal moisture level depends upon the composted material and more specifically on its porosity (Diaz & Savage, 2007). Organic mix with a low porosity requires higher moisture content than a substrate with a higher porosity level (Diaz & Savage, 2007). Moisture content which is lower or higher than the optimum range results in the inhibition of microbial activity due to early dehydration and the formation of anaerobic conditions respectively (De Bertoldi *et a*l., 1983; Gajalakshmi & Abbasi, 2008). When moisture content exceeds 70%, oxygen movement is inhibited and the process tends to become anaerobic (Tiquia, *et al*., 1996, 2002). On the other hand, if the moisture content is lower than required, 'microorganisms' growth and the subsequent decomposition rate of organic material are significantly reduced creating a final product that is physical, but not biologically stabilized (De Bertoldi *et al*., 1983; Diaz & Savage, 2007).

1.7.5. Microbes

1.7.5.1. Actinobacteria

Microorganisms in the compost pile cannot directly absorb the insoluble atoms of organic matter instead, they produce hydrolytic extracellular enzymes to depolymerize the larger compounds to smaller fragments that are water soluble (Chanyasak *et al.*, 1982; Godden *et al*., 1983). Actinobacteria are a group of prokaryotic organisms belonging to subdivision of the Gram-positive bacteria phylum (Zhang *et al*., 2003). The term aerobic actinobacteria is an informal designation for bacteria that belong to the order actinomycetales (Michael & June, 1994). Originally, microorganisms of this order were classified with the fungi because they possessed true aerial hyphae, which were considered to be a fungal characteristic (Chanyasak *et al.,* 1982). However, on the basis of their cell wall components, in particular, their cell envelope lipid and peptidoglycan compositions, these microorganisms are now recognized as true bacteria that are aerobic (Michael & June, 1994). Most actinobacteria are typically gram-positive, filamentous, partially acid-fast, branched bacteria that have many microbiologic characteristics in common with members of the genera Mycobacterium and Corynebactenium (Ventura *et al*., 2007).

Although aerobic actinobacteria are infrequently encountered in clinical practice, they are important potential causes of serious human and animal infections. When actinobacteria are characterised as a host cells to enzymes two striking characteristics are highlighted first, they exhibit a unique metabolic diversity and enzymatic capabilities (Michael & June, 1994). The compounds they produce as secondary metabolites are valuable for industrial and pharmaceutical purposes (Tokiwa *et al*., 2004), and the enzymes themselves are also valuable. Actinobacteria are the most widely distributed group of microorganisms in nature and are also well known as saprophytic soil inhabitants (Takizawa *et al.,* 1993). The actinobacteria give a pile a pleasing earthy smell, a result of special enzymes they excrete. Actinobacteria play a special role in creating humus. They work many feet below the surface of the soil (most bacteria stays in the top foot), creating humus under the roots of the plants. Actinobacteria and bacteria turn to have antagonistic relationship (Michael & June, 1994). Actinobacteria produce an antibiotic that kills off the bacteria through also a normal part of the decomposition process (Sullivan & Miller, 2001).

1.7.5.2. Heterotrophic

Heterotrophic microorganism are organisms that cannot produce their own food but instead obtain their food and energy by taking in organic substances, usually plant or animal matter that uses organic compounds as sources of energy and carbon (Soni, 2007). Heterotrophic bacteria include all bacteria that use organic nutrients for growth (Martin *et al.,* 2004) and cannot survive on inorganic matter. Heterotrophs can be further divided based on how they obtain energy if the heterotroph uses light for energy, then it is considered a photoheterotroph, while if the heterotroph uses chemical energy, it is considered a chemoheterotroph (Hogg, 2013). These bacteria are collectively present in all types of water, food, soil, vegetation and air (Martin *et al.,* 2004). Bacteria depend on many episodic events, such as rainfall and root growth or ingestion by various soil fauna, for passive movement to enable them to move about (David *et al*., 2004). All heterotrophs, of whatever size or volume, are involved in ingesting organic carbon and associated nutrients and assimilating them into carbohydrates, lipids, and proteins (David *et al*., 2004). However, there are specialized heterotrophic bacteria capable also of decomposing cellulose, lignin keratin, hydrocarbons and other substances (Soviet, 1979). Compounds such as lignin and cellulose are oxidised by microorganisms to produce metabolic energy, as they also carbon source for the biosynthesis of their own biomolecules (Carlos *et al*., 2004). Heterotrophic plate count bacteria represent those microbes isolated by a particular method, whose variables include media composition, time of incubation, temperature of incubation, and means of medium inoculation (Martin *et al.,* 2004).

1.7.6. Enzymes

Enzymes are the main mediators of various degradation process (Goyal *et al*., 2005) and quantitative determination of enzyme actions can be used to assess the dynamics of the composting process. Enzymes released by the microorganisms during composting also play a key role in the biological and biochemical transformations of the matrix (Castaldi *et al.,* 2008). Microorganisms facilitate metabolic processes mainly through the synthesis of enzymes in reaction to availability of mineralizable substrates (Cardoso *et al.,* 2013). For example, *β*-glucosidase, phosphatase and urease facilitate cycling of C, P and N, respectively

(He *et al.*, 2013). Densities of microorganisms reflect concentrations in mineralizable substrates, therefore, variability in factors, notably that of pH, temperature and moisture that affect microbial populations, also affect the availability and activity of enzymes produced by those microorganisms, and hence, the rate of degradation of organic material and nutrient cycling in compost (Cardoso *et al.*, 2013). Bioactivities such as respiration, carbon dioxide production and oxygen consumption rates, and microbial biomass, have been successfully employed to understand the composting process and to assess compost maturity (Gómez-Brandón *et al*., 2008). Hence, characterizing and quantifying enzymatic activities and microbial populations as well as physical and chemical properties during composting can reflect the dynamics of the composting process in terms of the decomposition of organic material and nitrogen transformations, and may provide information about the maturity and quality of composted product (Tiquia *et al*., 2002). It is a challenge to accurately determine qualitatively and quantitatively the chemical and biological indicators of the real degree of the organic material evolution during composting, the need for efficient monitoring of biological, chemical and physical parameters during composting processes is vital. Such information could serve as baseline for monitoring composting evolution of winery solid waste.

Benitez *et al*. (1999) mentioned that microbial enzymes are responsible for the breakdown of several organic compounds which generally characterised by complex structure, leading to the solubilization of simple water-soluble compounds. Monitoring the presence and activity of specific intracellular and/or extracellular enzymes during composting may improve our understanding of the development of waste biodegradation processes (Benitez et *al*., 1999). Examples of important enzymes in soil microbiology include celluloses, which reduce the polymer cellulose into smaller components; nitrogenase, which converts dinitrogen gas into biological available ammonia; sulphatases, which release protein and certain organic compounds; and phosphatases, which remove phosphate groups from organic compounds (Burns, 1978; Tate, 1995; Nannipieri & Fusi, 1996). Enzymes in compost can be classified as intracellular enzymes, found inside living cells or in soil/compost due to cell lysis, and extracellular enzymes purposely released from viable cells to catalyse the degradation of extracellular polymeric materials (Carlos *et al*., 2004). According to Priest (1984) enzymes that catalyse the degradation of polymeric substances such as cellulose hemi-cellulose and lignin are extracellular

enzymes because the polymer is too large to be transported across the cellular membrane. Enzyme activities during composting have been studied in the past (Godden *et al*., 1983; Garcia *et al*., 1992, 1993; Vuorinen 1999, 2000). However, most of these studies have been restricted to monitoring the changes of total enzyme activities (intracellular and extracellular) during composting. This newly soluble organic matter formed is metabolised again by the microbial biomass as a source of carbon, energy and nutrients (Bernal *et al*., 2009).

1.7.7. Indicators of compost maturity

The composting procedure is a valuable method of generating a stabilized material that can be used as a source of nutrients and soil conditioner in fields (Castaldi *et al*., 2005). It is difficult to determine the degree of maturation of composting organic material by one indicator. This difficulty can be overcome by learning about the transformations which the organic material undergoes during the composting process and by using the techniques that can help to obtain a complete knowledge of the processes involved (Adani *et al*., 1999). Nevertheless, most studies on composting have focused on physico-chemical parameters to evaluate both process evolution and compost quality (Castaldi *et al*., 2005; Said-Pullicino *et al*., 2011; Albrecht *et al*., 2011; Aslam *et al*., (2008). Inbar *et al*. (1993), Castaldi *et al*. (2004) and Mondini *et al.* (2004) defined stability as a point at which the rate of oxygen consumption is shortened so that anaerobic or odorous conditions are not produced and maturity as condition where compost does not pose any adverse effects on plants, which implies a stable organic matter content is attained. Stability is also related to the level of biological activity of the compost and depends on the degree of degradation achieved during composting process while maturity is related to the lack of phytotoxicity on vegetation growing in the soil treated with compost (Hue, 1995).

1.7.8. Carbon: Nitrogen ratio

According to many studies (Pascual *et al*., 1997; Raath & Schutte, 2001), the C/N ratio decreased as the strength of the increased and is usually between 13:1 and 10:1. Consequently, the C/N ratio is sometimes used as an indicator of compost stability. In Bernal *et al*. (1996) study C/N ratio decreased from 24.0 to 12 and

further explained that C/N ratio is proved to be the most suitable parameter for assessing the maturity of compost.

1.7. 9. Enzyme profiles using API ZYM ™ kit

API ZYM ™ is a semi-quantitative method designed for a systematic and rapid study of 19 enzymatic reactions. The kit has been successfully used to study extracellular enzyme profiles during co-composting of poultry manure and yard trimmings (Tiquia *et al*., 2001). API ZYM ™ was used to detect the presence of 19 different enzymes and to indicate compost maturity. The findings by Tiquia *et al*. (2002) suggest that the API test was suitable not only in monitoring the quantitative and qualitative fluctuation of the available substrate during composting, but also to reveal differences in composts and compost maturity. Results from that study showed an overall increase in diversity and relative abundance of enzymes present.

1.8. Hypotheses of the study

- Incorporating spent wine filter materials in a winery waste mix during composting will produce compost of better quality as opposed to using zero filter material in mixes.
- An array of extracellular enzymes are produced during composting. Due to the continual change in temperature and progressive breakdown of complex compounds to simpler ones (by enzymatic activity), different enzyme profiles:

  (1) should reflect qualitative and quantitative fluctuation of the amount of substrate during composting.

  (2) should reflect different stages of composting and thus can be used as an index (marker) of compost maturity.

- Changes in microbial numbers (actinobacteria and heterotrophs) and heap temperature and moisture should also indicate the state of compost maturity if these parameters correlate with extracellular enzymes activity.

- The use of a cheap and convenient composting protocol would yield compost with comparable agronomic quality as commercially-produced winery waste compost.

1.9. Overall aim of the study

The main goal of the study was to monitor composting indicators such as extracellular enzyme activities, microbial population numbers, heap temperature and moisture during an open air composting process. To improve composting of solid winery waste with the view of developing efficient technology for open air composting of spent winery solid waste in the Western Cape region of South Africa.

1.10. Specific objectives of the study

(a) To assess the relationship between five different winery waste mixes (as main treatments) and heap temperature, heap moisture, extracellular microbial enzyme activities, numbers of functional microbial groups (heterotrophs and actinobacteria) and selected chemical properties.

(b) To determine shifts in extracellular microbial enzyme activity profiles, changes in the numbers of functional microbial groups (heterotrophs and actinobacteria), heap temperature and moisture fluctuations during composting of winery solid waste over 12 months (during autumn, winter, spring and summer), with respect to the varied compost treatments.

(c) To compare the agronomic quality of five different composted winery waste mixes assayed in this study with commercially-produced winery waste composts.

Chapter Two

Materials and Methods

2.1. Experimental site

The experiment was carried out on a designated research plot at Agricultural Research Council Infruitec-Nietvoorbij for Deciduous Fruit, Vines and Wine, situated just outside Stellenbosch (GPS Coordinates: Latitude: -33.9262 | Longitude: 18.897162). The research site has dark alluvium and clay soils, which are well-drained and on a hilly terrain (Figure 2.1). The area is endowed with a Mediterranean climate, which is characterized by summer that are dry and warm to hot, and winter that are cool, rainy and sometimes relatively windy. Spring and autumn are colder seasons. These climatic conditions have been proven to be excellent for viticulture.

Figure 2.1. Aerial photo of compost site (source: Google Maps). The red arrow points to the compost heaps.

2.2. Compost materials

Compost heaps were made from a combination of the following materials (Figure 2.2): chopped pruning canes, grape stalks, skins and seeds (standard ingredients) from Nietvoorbij farm, and spent wine filter material from the Douglass Green (Wellington), [GPS Coordinates :S 33° 52’ 27.5”, E 018° 58’ 34.4] and Koelenhof [GPS Coordinates: 33°50'04.92”S. 18°47'52.68”E] wineries. Nietvoorbij farm vineyards have existed for more than 20 years (K, Guillaume 2014, personal communication, 11 October) with pruned season in winter before bud break according to each cultivar’s own requirements. Plant materials were collected after the harvest from the fields and used immediately after chopping and grounded. Filter materials were raw, moist, and have some waste effluent (water flowing out of the waste) in them, and wine aroma. Koelenhof waste was dark brown in colour according to the wine maker, it was a product of drum filtration that contained perlite from Chemserve PTY and wine lees. Douglas green’s waste was made up of diatomaceous earth (DE) filter powder, wine and spirit lees, was of a small volume, more clay and appeared to have more of a cementing nature than that of the Koelenhof winery. Douglas green waste was yellowish brown in colour when it was fresh, but would turn to purple when exposed to air for a long time. Filter materials were not mixed together but were layered on compost heaps individually, after they have been weighed in bags according to its treatment requirements. Stalks were firm in texture when chopped; for treatment 4 (T4), stalks were fine in texture after grinding.

Figure 2.2. The compost trial and arrangement of heaps at Nietvoorbij experiment farm.

2.3. Treatments

Five treatments were applied (Table 2.1), each replicated five times: T1 (40% spent wine filter materials + 60% standard ingredients (consisting of chopped pruning canes, grape stalks, berry skins and seeds,) lined with black plastic underneath each compost heap), T2 (20% spent wine filter materials + 80% standard ingredients (consisting of chopped pruning canes, grape stalks, berry skins and seeds), lined with black plastic underneath each compost heap), T3 (100% standard ingredients (consisting of chopped pruning canes, grape stalks, berry skins and seeds) + 0% spent wine filter material, lined with black plastic underneath each compost heap), T4 (40% spent wine filter materials + 60% standard ingredients grinded (consisting of grounded pruning canes, grape stalks, berry skins and seeds), lined with black plastic underneath each compost heap), and T5 (40% spent wine filter materials + 60% standard ingredients (consisting of chopped pruning canes, grape stalks, berry skins and seeds) unlined and compost heaps were laid on hardened soil surface. Prunings for T1, T2, T3 and T5, were chopped using hand pruning cutters, whereas prunings for T4 were grinded using a wood chipper (Bearcat 12.7cm [5 inches] PTO chipper, L x W x H 49 X 50 X 187, weight 720) of particle size approximately 7.6 cm (3 inches).

All treatments were prepared in one week, one treatment a day (with its five replications). Total volume per heap was 1 m^3. Using T1 as an example, volume per treatment can be explained as follows: Total heap volume was 40% of spent wine filter material and 60% of pruned canes and grape stalks, skins and seeds. Therefore, volume for spent wine filter material was 0.4 x 1 m^3 = 0.4 m^3 and for standard ingredients, 0.6 x 1 m^3 = 0.6 m^3.

100% of spent wine filter material was made up of two types of materials (Koelenhof's 90% and Douglass green 10% spent wine filter material). Total waste volumes for spent wine filter material were; Koelenhofs: 90% (0.9 m^3 x 0.4 m^3 = 0.36 m^3) and Douglass green 10% (0.1 m^3 x 0.4 m^3 = 0.04 m^3). Standard ingredients were made up of 80% Pruning canes (0.8 m^3 x 0.6 m^3 = 0.48 m^3), 10% grape stalks (0.1 m^3 x 0.6 m^3 = 0.06 m^3) and 10% of skins and seeds (0.1 m^3 x 0.6 m^3 = 0.06 m^3).

The study was conducted over a year from autumn (March, April and May 2013), winter (June, July and August 2013), spring (September, October and November 2013) to summer (December 2013, January and February 2014). Water was supplied to the compost heaps through an irrigation system. Irrigation pipes were linked to the tap next to the dam (water source) for irrigation. Heaps were watered twice a week for one hour, except during the rainy season (winter), which no watering was required. Mixing of the heaps was done twice a week in the beginning of the trial till the end of May. After May 2013, heaps were turned once a week using a spade to promote aeration.

Table 2.1. Treatments (T1-T5) applied to the experiment farm at Nietvoorbij during composting of spent winery waste under open- air conditions from autumn to summer.

Treatment	Description
T1	40% spent wine filter materials + 60% standard ingredients *, lined with black plastic underneath
T2	20% spent wine filter materials + 80% standard ingredients *, lined with black plastic underneath.
T3	0% spent wine filter material + 100% standard ingredients*, lined with black plastic underneath.
T4	40% spent wine filter materials + 60% standard ingredients *, grinded, lined with black plastic underneath.
T5	40% spent wine filter materials + 60% standard ingredients *, unlined.

Chopped pruning canes, grape stalks, berry skins and seeds

2.4. Experimental layout

Treatment combinations were randomly allocated to five blocks (Figure 2.3), each treatment consisting of 5 experimental units (25 heaps). The total number of experimental heaps was 25. Trenches were dug between rows for run-off, the space between heaps was 2 cm. The site where the trial was located was steep (20° angle), surrounded by vineyards and adjacent to a dam (Figure 2.2).

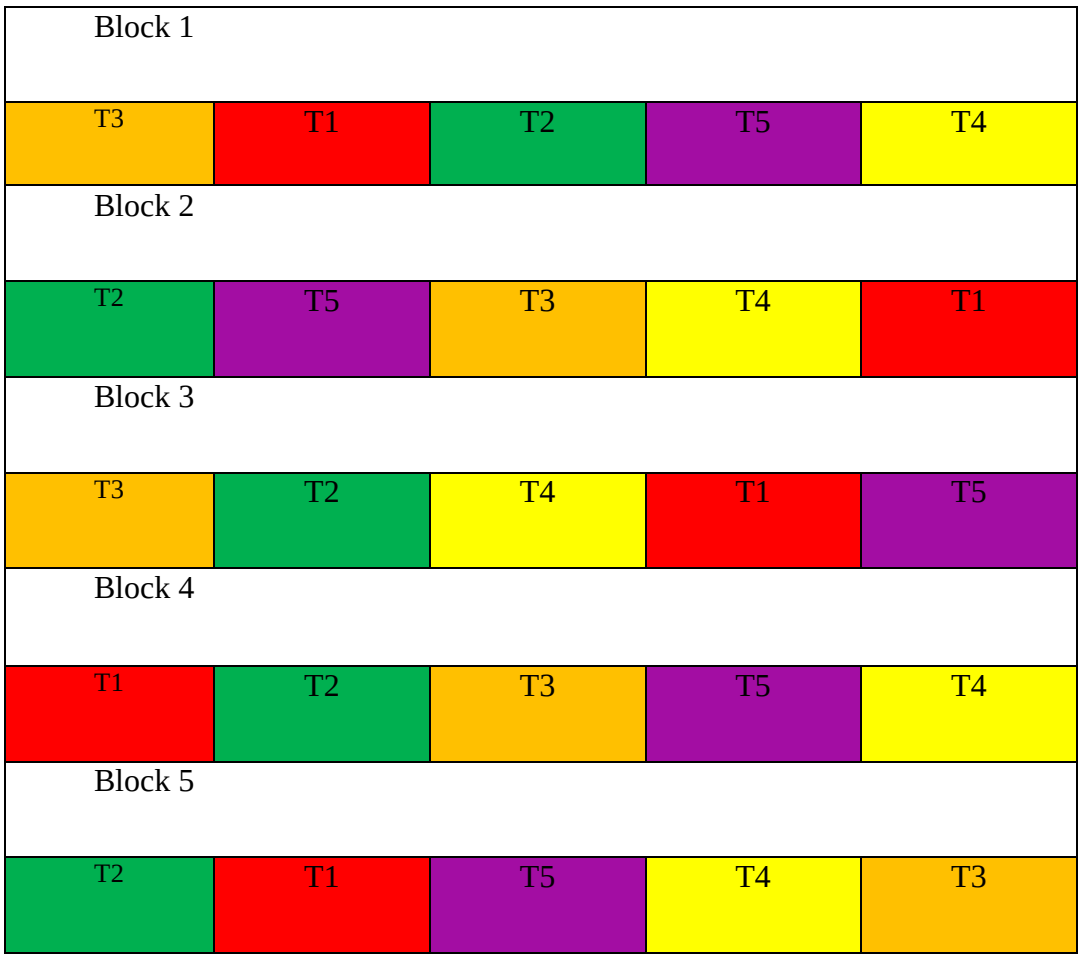


Figure 2.3. Experimental layout of the heaps at Nietvoorbij farm during composting of spent winery waste under open- air conditions.

2.5. Compost heaps layering

Soil surface was first cleaned and levelled before the layering of the heaps. Plastic was placed underneath heaps in the various treatments T1, T2, T3 and T4 with the exception of T5. The compost heaps were built following the same protocol and comprised a layer of pruning stalks followed by a layer of spent wine filter material (Figure 2.4), and according to the design of the experiment (Bertran *et al*., 2004). All treatments were uncovered and exposed to environmental conditions.

Figure 2.4. A diagram showing compost layers in a heap (repeated 4 times across the height of each heap).

In order to determine how much winery material to add onto each heap, the density of each ingredient were determined through weighing each material in a container of a known volume. Densities were found to be 1.2439kg/ m^3 = Koelenhof's spent wine filter material, 0.7552kg/ m^3 = Douglass green spent wine filter material, 0.1085 kg/ m^3 = Berry stalks, 0.8500 kg/ m^3 = skins and seeds and 0.1579 kg/ m^3 =

Prunings. Total weight occupied by each material in each heap as well as the amount in weight of each material required per heap is shown in Table 2.2.

Table 2.2. Amount of waste materials making up each compost treatment during composting of spent winery waste under open- air conditions.

Waste Material	Pruning Canes.	Koelenhof waste.	Douglass green waste.	Grape stalks.	Berry skins and seeds.
Treatments	(Kg)	(Kg)	(Kg)	(Kg)	(Kg)
T1	38	224	15	3.3	26
T2	56	112	7.5	4	34
T3	63	0	0	5.3	43
T4	38	224	15	3.3	26
T5	38	224	15	3.3	26

2.6. Assessing effects of filter material treatments in composting process

The following parameters were recorded overtime; enzyme activities, heap temperature and moisture and microbial counts. The techniques employed to capture these data are described fully below.

2.6. 1. API ZYM^{TM} assay Method

Sampling was done once a month from the two horizontal sides of the heap taken in the centre 50 cm from the base of the pile, and then subsamples collected from the compost heaps were mixed homogenously to generate one composite sample. Enzyme extracts were prepared by mixing 2 g of compost sample from each heap, with 50 ml sterile water. The solution was shaken 10 min for consistency using a

shaker (SK-L180-Pro Digital Linear Shaker from, Merck [Pty] Ltd, South Africa) and allowed to settle for 10 min, and the supernatant fluid was used for enzyme analysis. Thereafter, an aliquot (65 µl) of the supernatant was dispensed into each of the 20 microcupules on the API ZYM™ strips. API ZYMTM strips (BioMerieux, Marcy l' Etoile, France) consist of 20 microcupules containing dehydrated chromogenic substrates of 19 different enzymes and a control (a microcupule that does not contain any enzyme substrate). The enzyme substrates in the system are shown in (Table 2.3).

Table. 2.3. Enzymes present in API kit, Substrate, pH and expected results from the test.

Enzymes assayed for	Substrate	pH	Results	
			Positive	Negative
1. Control	-	-	Colourless or pale yellow	Colourless or pale yellow
2. Alkaline phosphatase	2naphthyl-phosphate	8.5	Violet	Colourless or pale yellow
3. Acid phosphatase	2 naphthyl-phosphate	5.4	Violet	Colourless or pale yellow
4. Phosphohydrolase	Naphthyl AS-BI-phosphate	8.5	Blue	Colourless or pale yellow
5. Lipase	2- naphthyl-myristate	7.5	Violet	Colourless or pale yellow
6. Lipase- esterase	2- naphthyl-caprylate	7.5	Violet	Colourless or pale yellow
7. Esterase	2- naphthyl-butyrate	6.5	Violet	Colourless or pale yellow
8.Leucine-amino peptidase	L-leucyl-2-naphthylamide	7.5	Orange	Colourless or pale yellow
9.Valineamino-peptidase	L-valyl-2-naphthylamide	7.5	Orange	Colourless or pale yellow
10.Cystineamino-peptidase	L-cystyl-2-naphthylamine	7.5	Orange	Colourless or pale yellow
11. Chymotrypsin	N-glutaryl-phenylalanine-2-naphthylamine	7.5	Orange	Colourless or pale yellow
12. Trypsin	N-benzol-DL-arginine-2-naphthylamide	8.5	Orange	Colourless or pale yellow
13. α -galactosidase	6-Br-2-naphthyl-α-D-galactopyranoside	5.4	Violet	Colourless or pale yellow
14. *β* - glucosidase	6-bromo-2-naphthol-α-D-galactopyranoside	5.4	Violet	Colourless or pale yellow
15.*N*-acetyl-*β*-glucosaminidase	1naphthyl-N-acetyl-βD-glucosaminide	5.4	Brown	Colourless or pale yellow
16. α-glucosidase	2naphthyl-2-D- glucopyranoside	5.4	Violet	Colourless or pale yellow
17. *β*-galactosidase	2naphthyl-βD- galactopyranoside	5.4	Violet	Colourless or pale yellow
18. *β* -glucuronidase	Naphthyl-AS-BI-Bd-glucuronide	5.4	Blue	Colourless or pale yellow
19. α-mannosidase	6-bromo-2-naphthyl-2-D-mannopyranoside	5.4	Violet	Colourless or pale yellow
20. α – fucosidase	2-naphthyl-αL-fucopyranoside	5.4	Violet	Colourless or pale yellow

The API ZYM™ strips were subsequently covered with foil and incubated in an oven (Merck brand) at 37 ºC for 4 h. After incubation, 30 µl of each reagent (ZYM A and ZYM B) were added to all the microcupules.

Following a waiting period of 5 min for colour development, a numerical value of 1-5 was assigned to each microcupule according to the colour chart provided by the manufacturer. For the purposes of this study, the results reported as having low intensity reaction was assigned a value of 1, of moderate intensity a value of 2-3, and of high intensity a value of 4 (Figure 2.5 A-F). Raw data were recorded onto the recording sheets, provided by the supplier, and subsequently computed in Excel in preparation for statistical analyses.

Figure 2.5. API procedure: (A) Sample taken from the field (B) 2 g compost sample weighed and dissolve into 50 ml sterile H_20 (C) An aliquot (65 µl) of the extract supernatant dispensed into each of the 20 microcupules (D) API ZYM™ strips before incubation (E) Typical colour reaction after 4 hours incubation of API ZYM™ strips at 37 °C (F) Reagent (ZYM A and ZYMB).

2.6.2. Microbial population

Plate count agar bought from (Sigma-Aldrich [Pty] Ltd, Johannesburg) and actinomycete isolation agar (Sigma-Aldrich [Pty] Ltd) were used to isolate heterotrophic bacteria and actinobacteria, respectively, using the standard spread plate count method. The growth media were prepared in accordance with the manufacturer's instructions.

The microbial enumerations, performed on air-dried samples, were diluted 10-fold in sterile water by suspending 1 g of the compost material into 9 ml of autoclaved distilled water in a test tube (Figure 2.6 A & B). Samples were mixed until dissolved using a vortex mixer. Aliquots (0.1 ml) at dilutions ranging from 10^{-1} and 10^{-6} $CFUg^{-1}$ were plated in triplicate (Figure 2.6 C & D). The inoculated plates were incubated in an inverted position and incubated at 26 ºC for 120 h for total heterotrophs (Prescott *et al*., 2008) and at 30 ºC for 72 h for actinobacteria. Colonies were counted using an åCOLyte colony counter (Figure. 2.6 E & F). Results were reported as colony-forming units per gram ($CFUg^{-1}$) of compost dry weight.

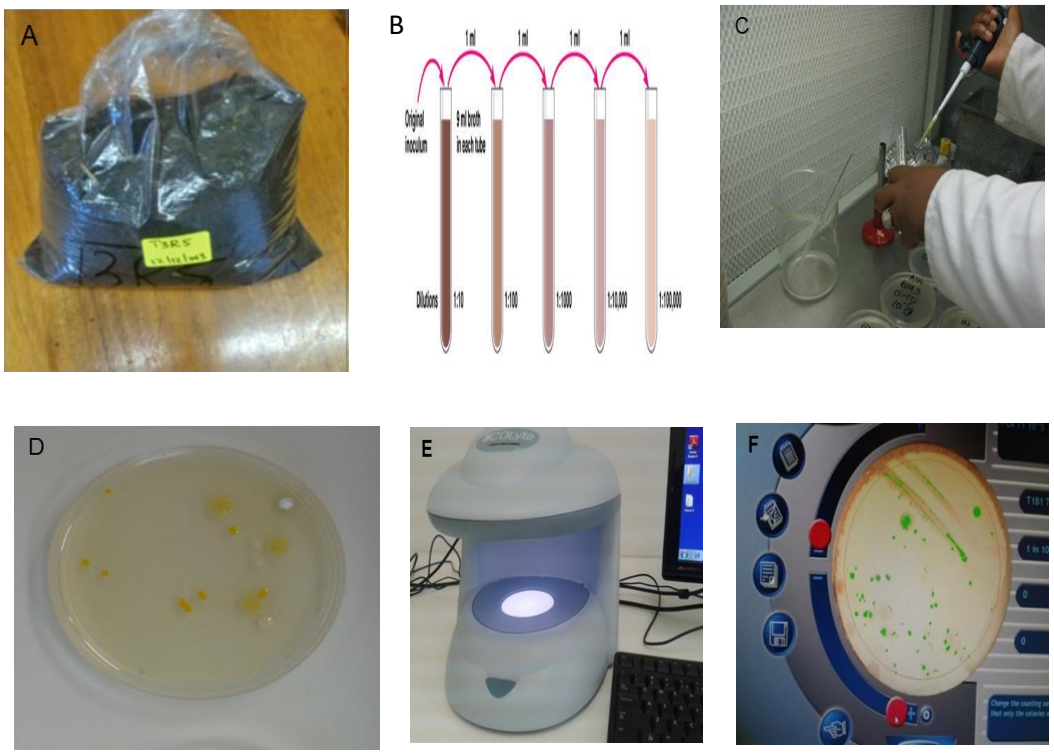


Figure 2.6. Microbial plating procedure; A) Compost sample (1g) (B) Dilution series (C) Plating (D) Petri dish after incubation (E) ÅCOLyte colony counter device (F) Colonies counted in the colony counter device.

2.6.3. Determination of heap temperature and moisture content

After preparation of the heaps, moisture and temperature were measured twice a week, Monday's and Friday's, and twice each day in the mornings and afternoons. These were pooled to obtain a seasonal data to obtain means e.g. (autumn = March, April and May) until the 12-month period of the trial (February 2014). Moisture content was measured using Mudder 3-in-1 Soil Moisture Meter with Plant Light & pH Test Gauge Function (Product Dimensions: 27.5 x 3.5 x 4.5 cm; Boxed-product Weight: 91 g). Reference scale is 1-10, (1-3 [dry], 4-7 [Moist] and 8-10 [Wet]). Temperature readings were measured using a 1 m long thermometer probe (Major tech [supplier] Milnerton in Cape Town). Both measurements were taken in the centre of the heap avoiding the side walls as these could be influenced by wind and direct sunlight (Mulidzi & Shange, 2011). Drop in heap temperatures was taken as an indication that turning was required.

2.6.4. Commercial products

The Commercial compost materials were used as positive control. The commercial composts were sourced from Agrimark PTY (Stellenbosch in Cape Town), Stodels and Game stores (Somerset West Cape Town). For comparison purposes, data of each of the physicochemical parameters obtained from the commercially-produced composts were pooled and the means were compared to those of the end-products of treatments. Based on the information provided by the manufacturer. The commercial compost types used are described below;

2.6.4.1 Reliance compost

Reliance compost is made from plant based organic material containing microorganisms to refresh soils for healthier plants and people. Reliance uses the controlled microbial composting process which involves the aerobic composting of an organic material using natural synergy of bacteria fungi and yeasts. Organic matter is blended in a specific carbon to nitrogen ratio turned daily with a composts tuner releasing carbon dioxide, adding oxygen and water, and controlling the

temperature. This process destroys unhealthy pathogens, weeds seeds and odours, and promotes multiplication of the beneficial organism.

2.6.4.2 Double grow compost

This is made up of organic material and create ideal environment for development of soil micro-organisms, helps retain moisture, improve aeration and drainage of the soil and is void of weeds as it is steam sterilized.

2.6.4.3 Culterra compost

It is produced from dead or decaying plant materials, which are free of weeds and other harmful soil pathogens. This product decomposes slowly, benefitting the soil condition and texture over an extended period. It contains no inorganic fertilizer and is manufactured is South Africa.

Meteorological data, wind speed, temperature, and rain, were obtained from the nearest weather station located at ARC-Nietvoorbij Campus South Africa, approximately 5 m from the trial site. Meteorological data were recorded daily for the duration of the experiment and are presented below (Table 2.4).

Table. 2.4. Meteorological data taken from the nearest weather station on a monthly basis (Averages).

Month	Temperature °C	Rainfall mm	Wind speed ms
March	21 -37	0.3 - 15	1 - 5
April	19-38	0.3 - 21	1 - 3
May	16-31	0.3 - 17	1 - 2
June	10-27	0.3 - 34	0.4 - 2
July	14-29	1 - 18	0.4 - 4
August	12-29	1 - 41	1 - 2
September	12-25	1 - 27	1 - 2
October	18-32	0.3 - 27	1 - 1.6
November	16 – 34	1 - 8	1 - 3
December	25-35	0.3 - 3	1 - 5
January	25-39	2 - 14	1 - 4
February	26-42	1 - 3	1 - 4

2.6.5. Chemical analysis

Total P, K, Ca, Mg and micro-nutrient analyses in plant tissue were determined as described in Campbell & Plank (1998) and Miller (1998). The cations and micro nutrient (B, Fe, Zn, Cu, and Mn) content of the extract was measured with a Varian ICP-OES optical emission spectrometer against suitable standards. Total *N* was determined directly using total combustion on a Leco N-Analyser". The pH readings of composts were determined in accordance with procedures by The Non-affiliated Soil Analyses Work Committee (1990). Compost was dried over-night at 105 ºC. Electrical conductivity of compost was determined from a water extract made from a suspension of 50 g dried compost in 100 ml deionised water after centrifugation. The EC of the clear solution was then determined with a CRISON GLP 32 Conduct meter. A solubility test for dry materials was determined by weighing exactly 10 g material into 100 ml of water. It was shaken for 5 min at 25 °C. Thereafter, it was filtered through a Whatman No. 2 filter paper, which was dried to determine the weight of the insoluble fraction. This weight was expressed as percentage of the original 10 g material.

2.6.6. Color and odour

Other physical parameters such as color and odour were observed at the end of the experiment respectively. Compost heap odour were classified by sense of olfaction and color by Munsell book of color (Baltimore, 1976).

2.6.7. Data analysis

One-Way ANOVA was used to compare treatment groups. Statistical significance was maintained at ($P < 0.05$). To compare the differences between specific treatments, the post hoc Tukey-b test was used ($P < 0.05$). Repeated measure Anova was used to analyse effects of various treatments on composting overtime. Statistics were analysed using the following; STATISTICA software (StatSoft, 2014) and PAST, free statistical software (Hammer *et al*., 2001).

Chapter Three

Results

3.1. The effect of treatment on compost heap temperature

Heap temperature was significantly (P <0.05) higher in T1, T4 and T5 in the initial stage of composting (autumn) compared to T2 and T3 (Table 3.1). There were no differences among treatments with 40% spent wine filter material T1, T4 and T5 in autumn. Generally, heap temperature in all treatments dropped below 10 °C in winter, although treatments containing spent wine filter materials (T1, T2, T4 and T5) appeared to retain heat better than T3 (df = 1.4, F = 18.21, P < 0.05). However, in spring and summer, heap temperatures increased in all treatments, ranging from 20.5 to 21.9 ^{o}C in summer; these did not vary significantly among treatments as was the case in winter. The treatment with no spent wine filter material (T3) had the lowest heap temperature in summer (1 year after commencement of experiment). Generally, significant variation in compost heap temperature across season were confirmed by repeated measure ANOVA (df=3,16, P< 0.05).

Table. 3.1. Heap temperature in all treatments (T1-T5) applied to the experiment farm at Nietvoorbij during composting of spent winery waste under open- air conditions from autumn to summer. Values are mean % ± SE.

Season	T1 (40 %)	T2 (20 %)	T3 (0 %)	T4 (40 %)	T5 (40%)
Autumn	23.8±0.26 a	20.6±0.36 b	15.2±0.09 c	24.5±0.52 a	24.7±0.59 a
Winter	9.22±0.08 ab	9.5±0.06 b	8.66±0.07 c	9.01±0.03 a	9.31±0.09 ab
Spring	14.6±0.05 a	14.6±0.18 a	14.7±0.18 a	14.4±0.06a	14.5±0.03 a
Summer	21.8±0.19 a	21.6±0.10 a	20.5±0.05 b	21.8±0.10 a	21.9±0.07 a

Means followed by the same letters in the same row indicate no significant difference ($P > 0.05$), following comparison using the post hoc Tukey HSD test.

3.2. Heap Moisture

Moisture content in T3, ranging from 4 to 7 was significantly lower compared to treatments with spent wine filter material in all seasons (autumn, winter, spring and summer) (Table 3. 2), perhaps demonstrating an inadequacy to retain moisture. The highest value of moisture content during all seasons was recorded in winter especially in treatments with spent wine filter material; these were between 9 and 10 suggesting that filter material had more water holding capacity. During summer, moisture readings were significantly lower in all the treatments as compared to the past seasons ranging from 5 -7. These seasonal variations in moisture over time were confirmed by repeated measure ANOVA (df =3, 16, $P < 0.05$).

Table. 3.2. Heap moisture in all treatments (T1-T5) applied to the experiment farm at Nietvoorbij during composting of spent winery waste under open- air conditions from autumn to summer. Values are mean % ± SE.

Season	T1 (40 %)	T2 (20 %)	T3 (0 %)	T4 (40 %)	T5 (40%)
Autumn	8±0.11a	7±0.01 a	4 ±0.01b	8.1±0.01 a	8.1±0.01 a
Winter	10±0.1a	9±0.1 a	7±0.1 b	10±0.1 a	10±0.1 a
Spring	8±0.1a	8±0.1 a	6±0.1b	8±0.1 a	8±0.1 a
Summer	7±0.1 a	6±0.1 b	5±0.1 c	7±0.1a	7±0.1a

Values presented are means % ± SE (standard error). Means followed by the same letters in each row indicate no significant difference ($P > 0.05$), following comparison using Tukey HSD test.

3.3. Characterisation of chemical and physical properties (comparison with Commercial product)

3.3.1. pH

In general, there were some similarities between the commercial and final product of the compost. Data from the current study shows that pH values for treatments with filter wine waste material (T1, T2, T4 and T5) were alkaline, ranging from (7.8 - 8.5), closely resembling the commercial product (7.6) (Table 3.3).

3.3.2. Color and odour

Composting heaps with different treatments showed variation in colour, the munsell book of colors was used (Baltimore, 1976), the order is as follows: hue, value and chroma. The colour of the compost made according to the method for T1, T2, T3 and T4 was 5 YR 3/3 (dark brown in colour), and T5 was 5YR 4/4 (brown in colour). Generally, compost heaps with dark brown in colour, were similar to the commercial products. Heaps produced more pleasant odour especially at the end of composting period.

3.3.3. Resistance

Resistance in T2 (92.5 ohm) was comparable to the commercial product (100 ohm) (Table 3.3). T3 exhibited significantly higher ($df = 1, 4$, $P < 0.05$) resistance values (307.5 ohm) compared to the treatments containing spent wine filter material, which coincides with low moisture content in the corresponding heaps. It is worthwhile to note that all the standard ingredient materials used had high resistance (prunings = 260 ohm, grape skins + pips = 90 ohm and Grape marc = 70 ohm). Resistance ranged from 72.5 to 85 ohm in treatments with spent winery waste and was significantly lower ($P < 0.05$) compared to the commercial products (100 ohm).

3.3.4. Macronutrients

Treatments were significantly different in N levels from commercial product (Table 3.3). The highest value of nitrogen concentrations was recorded in T1 and T4. Nitrogen levels for T2 T3 and T5 were comparable with the commercial product

(15700 mg/kg). There were no significant differences ($P > 0.05$) in phosphorus levels amongst the final products obtained in the treatments and the commercial product. The highest K content in the final product was associated with T1 and T4; the lowest was measured in the commercial products. Relatively higher values of Ca (22000 mg/kg) were observed in commercial products compared to other treatments. Magnesium content was significantly higher in T3 paralleled to other treatments and commercial product. Sodium levels were statistically higher ($P < 0.05$) in all the treatments with spent wine filter material with the exception of T3, which had the lowest value of Na. The commercial product had lower Na levels than the rest of the other filter material treatments.

3.3.5. Micro nutrients

The commercial product (Table 3.3) showed significantly ($P < 0.05$) enriched levels of Mn, Zn and Fe compared to end-products of the treatments (T1-T5). There were no differences among treatments T1-T5 in Mn levels, including in T3, which had no spent wine filter material. Fe concentration in treatments with spent wine filter material was generally lower ($P < 0.05$) than the commercial product, with the exception of T1. There was no difference ($P > 0.05$) in Zn levels between any of the treatments, albeit high values were recorded in T3 (44.4 mg/kg). Ash particles in T3 were noticeably lower compared to all other treatments with spent wine filter material and the commercial product.

Table 3.3. Chemical composition of the end product of composting of winery solid waste and commercial compost product.

Parameters	Commercial	Treatment 1 *	Treatment 2*	Treatment 3*	Treatment 4 *	Treatment 5*
pH	7.6±0.10 b	8.5±0.10 a	7.8±0.52 a	6.4±0.04 b	7.8±0.21 a	8.2±0.23 a
Resistance ohm	100±4.08 b	72.5±2.5 c	92.5±2.5 b	307.5±2.5 a	80±0 c	85±5 bc
Moisture %	44.5±3.71 b	64.2±0.82 a	60.01±2.19 a	58.6±2.08 a	63.27±2.8 a	58.8±3.54 a
Density kg/m3	555.7±18.1 c	795.9±23.2 ab	716.2±14.02 b	598.9±26.7 c	790.6±31.5 ab	829.9±24.69 a
N mg/kg	15700±0.07 b	21300±0.18 a	17100±0.05 b	15300±0.17 b	17900±0.02 a	16100±0.03 b
P mg/kg	2000±0.07 a	2300±0.01 a	1300±0.01 a	1100±0.004 b	1600±0.01 a	1500±0.01 a
K mg/kg	6800±0.19 c	28200±0.14 a	19800±0.12 b	8100±0.03 c	21300±0.16 a	20300±0.17 b
Ca mg/kg	22000±0.43 a	6100±0.02 b	6800±0.03 b	10300±0.03 b	5400±0.07 b	4400±0.06 b
Mg mg/kg	1700±0.03 ab	1200±0.002 bc	1400±0.004 bc	2400±0.01 a	1100±0.01 c	900±0.01 c
Na mg/kg	1511±386 c	3357±80.4 a	2270.4±156.1b	683.7±32.2 d	2624±268.3 a	2331.7±297.2b
Mn mg/kg	230.5±40.9 a	49.6±0.92 b	52.5±1.75 b	57.47±2.98 b	49.03±2.63 b	44.95±2.23 b
Fe mg/kg	35816±5210 a	24400±6522 ab	17538±1390 b	16232±695.5 b	18114±19.98 b	19697±1397 b
Cu mg/kg	16.2±2.14 b	26.05±1.24 a	26.5±1.37 a	22.45±1.33 ab	25.18±1.59 ab	21.88±0.60 ab
Zn mg/kg	77.9±17.0 a	26.57±0.34 b	34.57±1.55 b	44.40±1.38 b	28.08±2.8 b	23.51±1.90 b
B mg/kg	25.4±1.60 e	53.01±2.44 a	42.46±2.28 c	32.34±0.99 d	42.39±2.90 b	39.87±3.13 c
C mg/kg	168±2.32 ab	1283±1.33 bc	1278±1.17 bc	2157±1.30 a	1169±1.70 bc	942±1.17 c
Ash %	65.1±5.89 a	63.73±1.47 a	60.89±1.24 a	46.07±0.89 b	65±5.19 a	71.6±2.97 a
C/N ratio	10:1 ± 1.81 b	10:1± 0.35 b	13:1±1.56 ab	20:1±2.30 a	10:1±0.72 b	15:1 ± 1.34 ab

Values presented are means % ± SE (standard error). Values followed by the same letter in a row do not show significance at $P > 0.05$ following comparison using Tukey Test.*(T1 = 40% spent wine filter materials + 60% standard ingredients filter material lined, T2 = 20% spent wine filter materials + 80% standard ingredients, T3= 0% filter material + 100% standard ingredients lined, T4= 40% spent wine filter materials + 60% standard ingredients grinded filter material lined and T5 = 40% spent wine filter materials + 60% standard ingredients filter material unlined.

3.3.6. Carbon to Nitrogen ratio

At the final stage of composting there was an inverse relationship between spent wine filter material content in heaps and C/N ratio (Figure 3.1). T1, T4 and commercial products all had similar values of C/N ratio (10:1) compared to T3 (20:1) and T5 (15:1) (Table 3.3). There was a significant difference (df = 1, 5; F = 6.4673; P < 0.05) between the treatments and commercial products when means were separated using Tukey HSD test. The highest C/N ratio was recorded in T3 (20:1). T3 had better carbon concentration (2157 mg/kg) than the rest of the treatments.

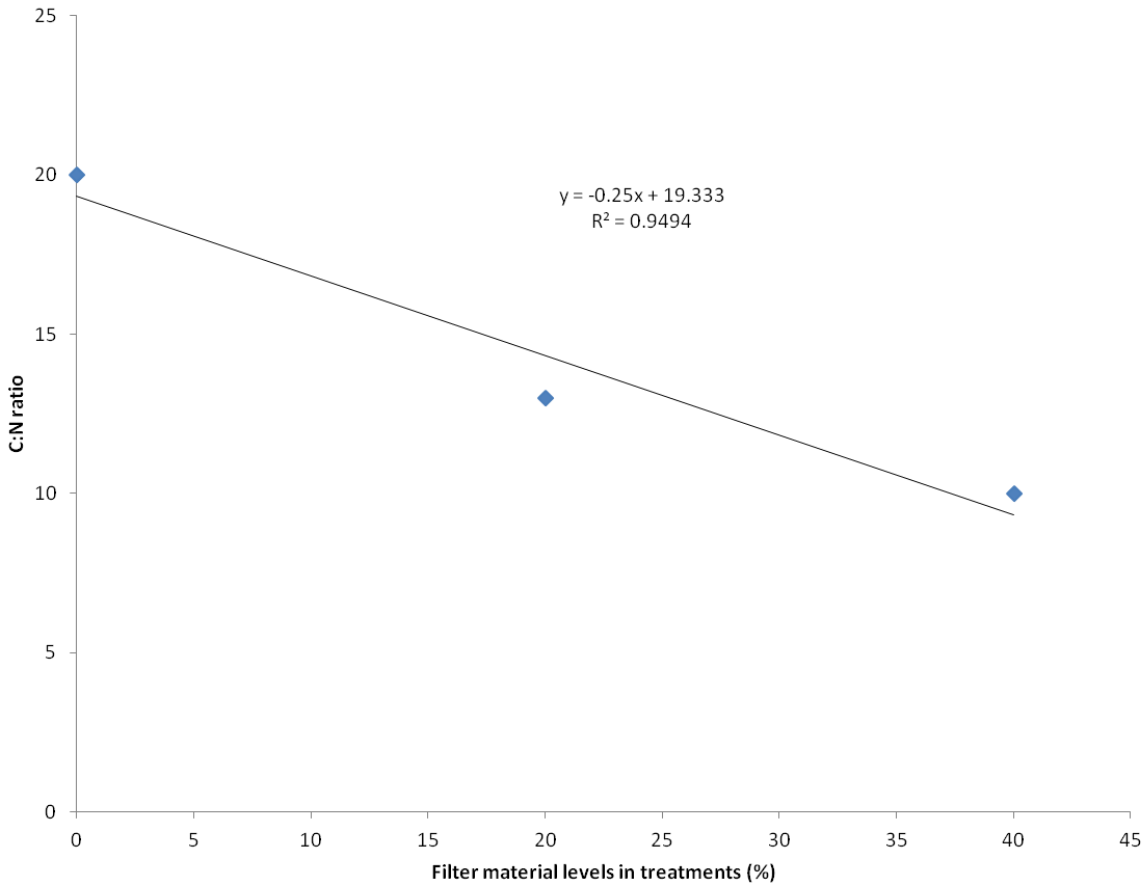


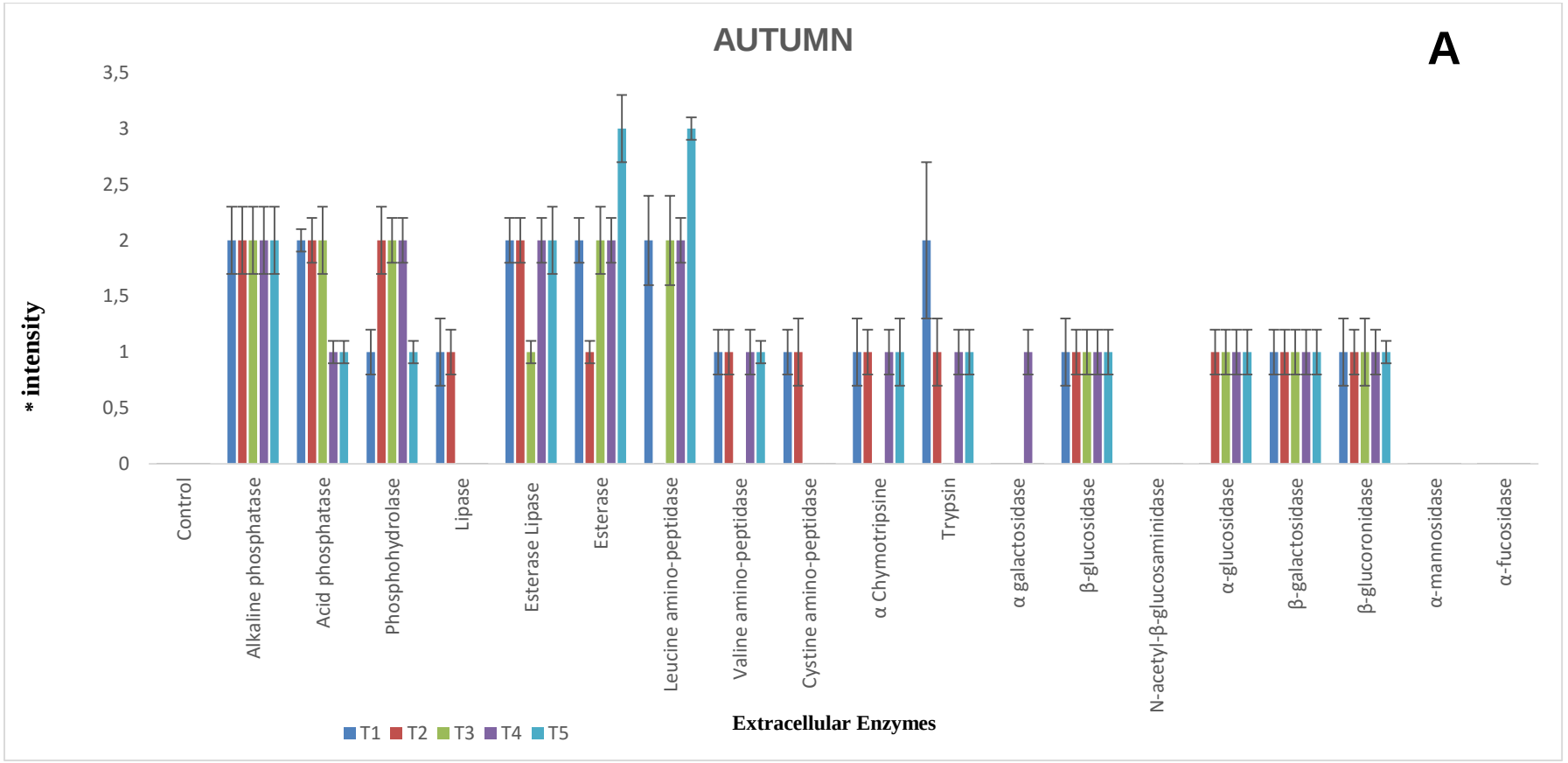
Figure 3.1. Relationship between content of spent wine filter material in compost heaps and C/N ratio.

3.4. Extracellular enzyme profiles at different stages of composting

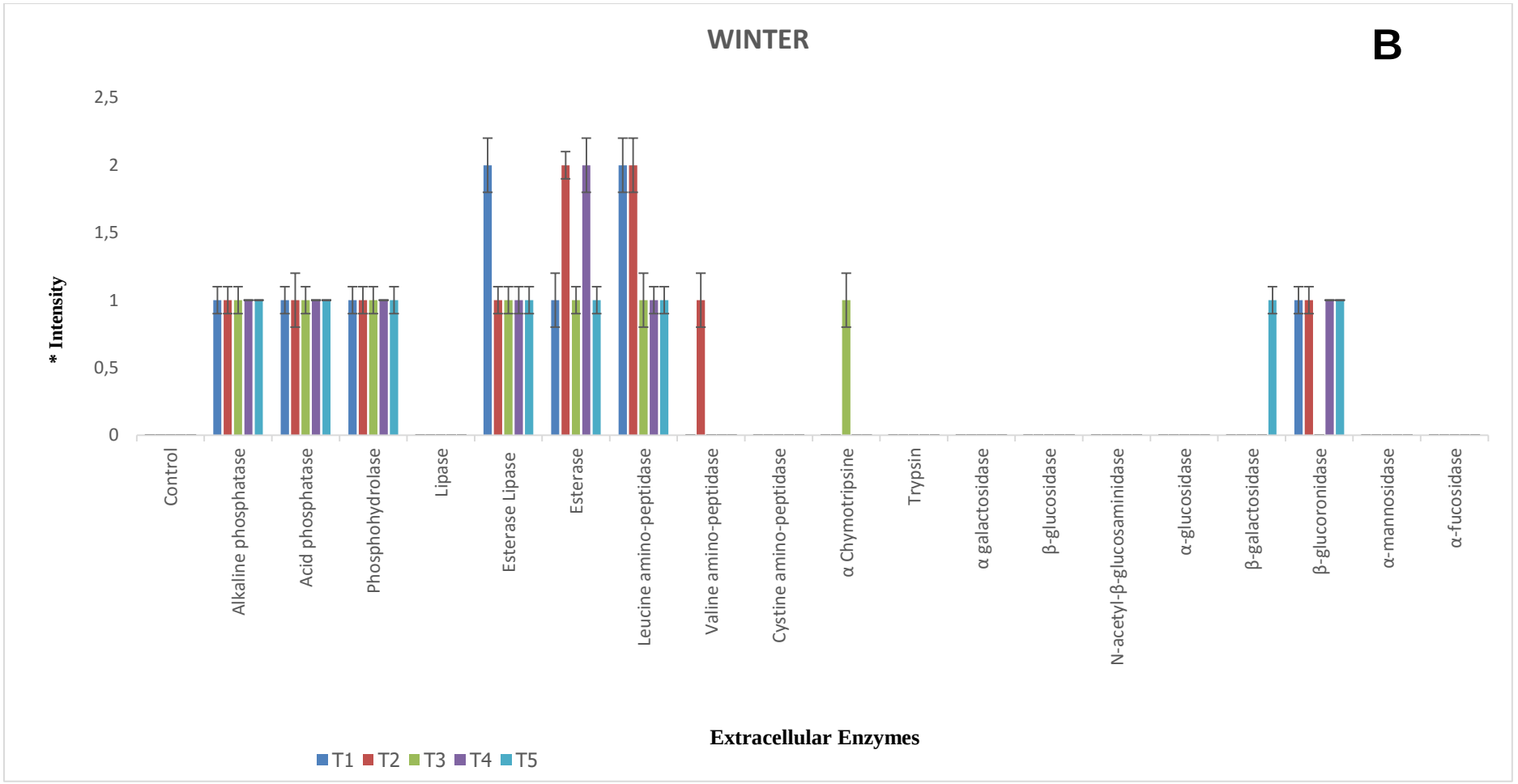
At each sampling period, 19 extracellular enzymes were identified (Figure 3.2). These enzymes include three phosphatases (alkaline phosphatase, acid phosphotase and phosphohydrolase), three esterases (lipase, esterase-lipase and esterace), three amino-peptidases (leucine amino-peptidase, valine amino- peptidase and cystine amino-peptidase), two proteases (chymotrypsin and trypsin), and eight glycosyl-hydrolases (*β*-galactosidase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, *ά*–glucosidase, *ά*-galactosidase, *β*-glucuronidase, ά- mannosidase and *ά*–fucoside) and one control (a microcupule containing no enzyme substrate).

Alkaline phosphatase activity had lowest intensity (1) in the winter reaching maximum (4) intensity by the end of the trial (summer) in all the treatments. Acid phosphatase activity started moderately (2) in T1, T2 and T3 and showed low intensity (1) in T4 and T5 in autumn, then gradually dropped in winter in all the treatments. Activities subsequently improved to moderate intensity (2-3) during the last two seasons (spring and summer). Overall, phosphohydrolase activity stayed consistent at 2 and only dropped in winter to 1. The low intensity in lipase activity that was observed during autumn in T1 and T2 disappeared in winter in all the treatments before showing moderate intensity in T4 and T5 by the end of composting process.

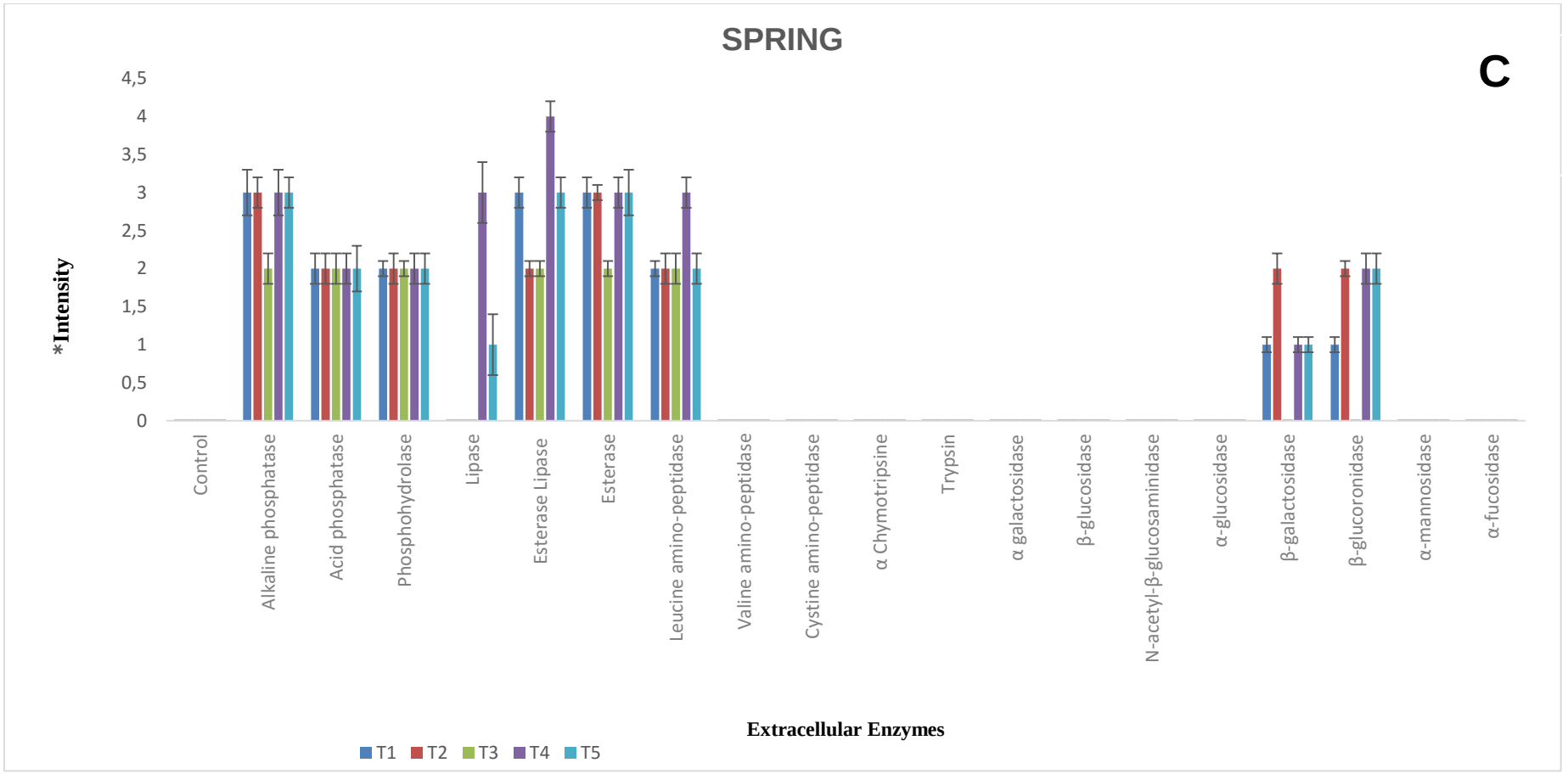
The activities of esterase lipase and esterase increased from moderate (2-3) to high intensity (4) as composting progressed. Leucine amino-peptidase activity was moderate in T1, T3, T4 and T5 during the entire period of composting, however the activity was not spotted in T2 in autumn. Valine amino- peptidase, cystine amino-peptidase, chymotrypsin, trypsin, ά–glucosidase, ά-galactosidase and *β*-glucosidase only showed low level of intensity (1) in the initial stage of composting, thereafter declined throughout the entire period of composting in all treatments. N-acetyl- *β*-glucosaminidase, ά- mannosidase and *ά*–fucoside activities were not spotted from the start till the end of composting. Overall, *β*-galactosidase and *β*-glucuronidase showed level of low to moderate intensity throughout the process. Seasonal variation significantly influence enzyme activities in all treatments as confirmed by repeated measure ANOVA ($df = 3, 76$, $P < 0.05$).



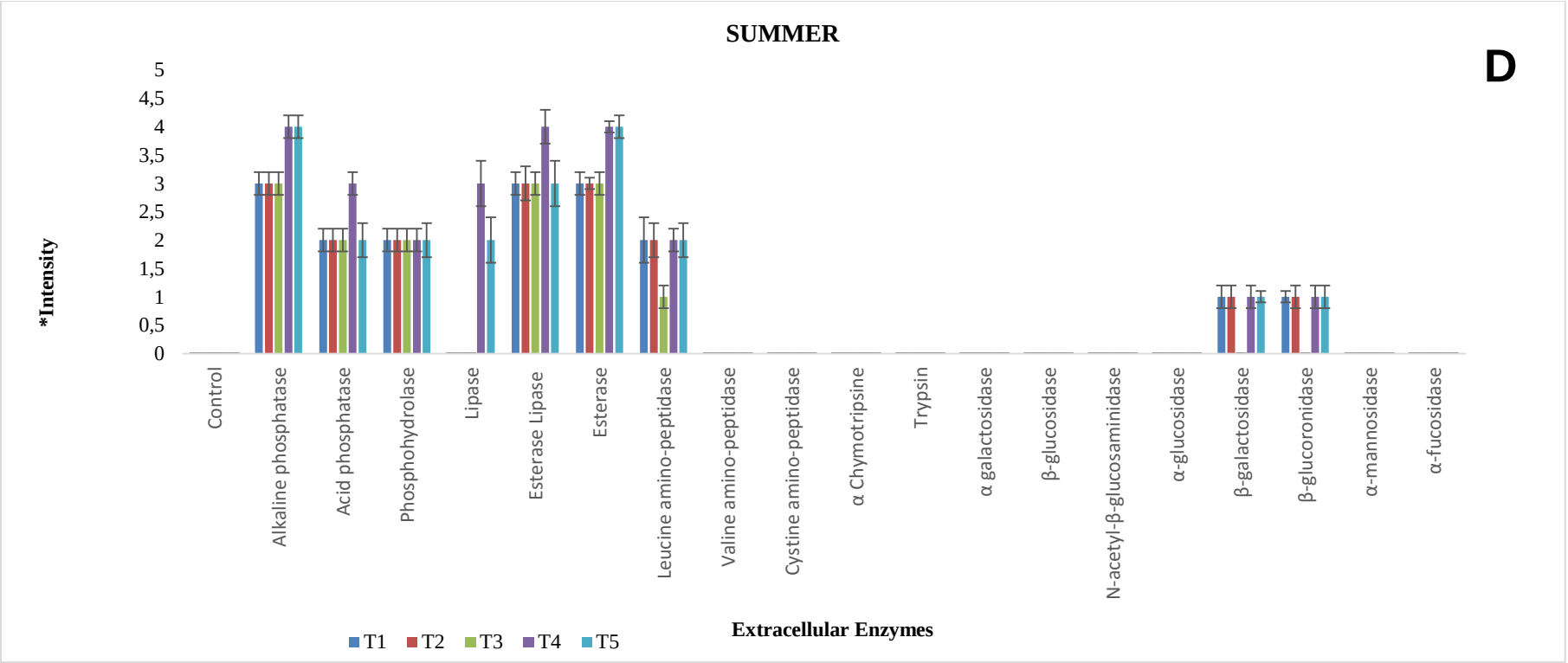
***Intensity of reaction - low intensity [value of 1], moderate intensity [values of 2-3] and high intensity [value of 4]**



***Intensity of reaction - low intensity [value of 1], moderate intensity [values of 2-3] and high intensity [value of 4]**



***Intensity of reaction - low intensity [value of 1], moderate intensity [values of 2-3] and high intensity [value of 4]**



***Intensity of reaction - low intensity [value of 1], moderate intensity [values of 2-3] and high intensity [value of 4]**

Figure 3.2. Enzyme activities associated with different compost treatments during (A) Autumn, (B) Winter, (C) Spring and (D) Summer.

3.5. Microbial count

The results of microbial counts (Table 3.4) showed that the total aerobic actinobacteria counts of compost samples in all the treatments were high and significantly different (df =1, 4, F = 11.303, P < 0.05) among treatments at the beginning of the composting process (autumn). Generally, during winter, there was a drop in numbers ($7x10^7 – 8x10^7$ CFU/g^{-1}) in all treatments. Marked increase in the count was recorded in all treatments ranging from $8x10^7$ to $1x10^8$ CFU/g^{-1} when the temperatures started to pick up again in the proceeding spring and summer seasons. Overall, seasonal changes had a significant effect P < 0.05 on both aerobic heterotrophs and actinobacteria counts during composting of spent winery waste.

Table 3.4. Effect of treatments (T1-T5) on seasonal aerobic actinobacteria counts (CFU/g^{-1}) during composting of spent winery waste under open- air conditions from autumn to summer.

Treatment	Autumn	Winter	Spring	Summer
T1 (40% Fm*)	$9x10^7$ab	$8x10^7$b	$1x10^8$a	$8x10^7$b
T2 (20% Fm*)	$1x10^8$a	$7x10^7$b	$1x10^8$a	$1x10^8$ab
T3 (0% Fm*)	$1x10^8$a	$7x10^7$b	$1x10^8$a	$1x10^8$ab
T4 (40% Fm*)	$7x10^8$b	$8x10^7$b	$1x10^8$a	$1x10^8$ab
T5 (40% Fm*)	$7x10^8$b	$1x10^7$a	$9x10^7$a	$1x10^8$ab

Values followed by same letter in the same column are not significantly different (P > 0.05) following comparison using Tukey test. Fm (Filter material).

Aerobic heterotroph counts (Table 3.5) during autumn were higher compared to all the treatments ($1x10^8$ CFU/g^{-1}) and there was no significant difference among the treatments (df = 1.4, F =1.918, P > 0.05). Numbers for aerobic heterotroph increased to a maximum of ($1x10^8$ CFU/ g^{-1}) during summer.

Table 3.5. Effect of treatments (T1-T5) on seasonal aerobic heterotroph counts (CFU/g^{-1}). during composting of spent winery waste under open- air conditions from autumn to summer.

Treatment	Autumn	Winter	Spring	Summer
T1 (40% Fm*)	$1x10^8$a	$1x10^8$a	$3x10^8$bc	$1x10^8$a
T2 (20% Fm*)	$1x10^8$a	$1x10^8$b	$5x10^8$a	$1x10^8$ab
T3 (0% Fm*)	$1x10^8$a	$1x10^8$c	$2x10^8$c	$1x10^8$ab
T4 (40% Fm*)	$1x10^8$a	$9x10^7$c	$4x10^8$ab	$1x10^8$ab
T5 (40% Fm*)	$1x10^8$a	$8x10^7$d	$3x10^8$bc	$1x10^8$ab

Values followed by same letter in the same column are not significantly different (P > 0.05) following comparison using Tukey test. Fm (Filter material).

Chapter Four

Discussion

4.1. Temperature, heap size, degree of grinding, season and extracellular enzyme activities

This study has shown that composting of spent winery waste material went through physical, chemical and biological changes. Temperature is a key parameter affecting composting, as it indicates the state of decomposition and guarantees sanitation of the final product (Remigio *et al.*, 2013). The highest recorded temperature in this study was 24.7 °C in autumn throughout the process of composting compared to other studies. For instance, Bustamante *et al.* (2008) observed a maximum temperature of 70 °C in their study that focused on the evolution of the pathogen content during co-composting of winery and distillery wastes. Bertran *et al*. (2004) also observed a maximum value of 74 °C during composting of winery waste containing sludges and grape stalks. However, the experimental conditions in the studies by Sánchez-Monedero *et al*. (2001), Remigio *et al*. (2013) and Bertran *et al*. (2004) differed from the present study. For example, the study by Bertran *et al*. (2004) included the use of sludge, which could have exerted different effects on the composing process. Furthermore, (Huang, 2006; Moretti *et al*., 2015) studies has indicated that composting was completed after 90 to 120 days, the process of composting in the present study appeared to have taken appreciably longer (12 months) to be completed. Whether the extra length of time for composting to be completed in the present study could have compensated for the overall lower heap temperatures that were observed, is arguable. Whether these results further suggest that high temperatures may not necessarily be a prerequisite for optimum composting is also debatable. Corroborated work by Remigio *et al.* (2013) also recorded low temperatures (17-30 °C) during composting of lignocellulosic winery waste. Possible reasons for the low temperatures of composting in the present study could have been due to one or more of the following factors: (a) exposed compost heaps to the environment (not protected from the rain, wind and sun during different seasons of the year), (b) an insufficient isolation of the wastes during composting; (c) a low volume of material (d) a lack of available nitrogen for microorganisms (Remigio *et al.*, 2013).

The extent to which heap volume contributed to the observed results in the present study could also have been important. The relatively small sized heaps (1 m^3) could have resulted in heat loss to the atmosphere through evaporation, convection and conduction more rapidly than e.g. large sized heaps (between 2.5 m diameter x 1.5 m height and 3 m diameter x 3 m height) used in the study by

Bertran *et al. (*2004). In the latter study the recorded heap temperature ranged between 25 °C and 74 °C. Despite the generally low temperatures recorded in current study, subtle differences in heap temperatures among the different treatments were noted. Treatments with high filter material content (T1, T2, T4 and T5) tended to have higher temperature compared to those with lower filter materials (T3). Heaps in T3 had low temperature values from autumn to spring. According to Mustin (1987) & Charnay (2005), the temperature measurement is an indirect approach of the biodegrading intensity.

The inclusion of filter materials such as perlite could have improved aeration (Hodges, 2012). In present study, heaps were turned twice weekly throughout the process of composting, which is relatively more frequent compared to other composting experiments on winery and distillery wastes, as by Bustamante *et al*. (2009). In the latter study they used heaps of 1800 kg each, which were turned only three times during the entire period of 130 days whereas, in the study by Inbar *et al*. (1993), 30 m^3 sized heaps were turned after 0, 7, 13, 33, 57, 86, 160 and 378 days. Main changes occurred at the later stages of composting, one of the highlighted findings from Inbar *et al*. (1993), was the decline in the C/N ratio value from 35-40:1 or higher to a final level of 18–20:1, strongly implying a substantial degree of stabilization.

Generally, most enzymes were active at the start of the composting process before becoming undetectable at a later stage. Some of these reappeared towards the end of the composting process (Figure 3.2). The decline in enzyme activities were also observed by Hankin *et al*. (1975) during the thermophilic stage of composting. For example, enzyme activities that appear to be prominent in the early stage of the composting (autumn) in the present study were: Alkaline phosphatase, acid phosphatase, phosphohydrolase, esterase-lipase, esterase, leucine amino-peptidase, β-galactosidase and β-glucosidase. The phosphatases and esterases appeared to be the most abundant at any given time during the composting period. They also seemed to increase with compost maturity (highest in end product). These results are in agreement with the results reported by Tiquia *et al*. (2002) and Tiquia *et al*. (2004). Other enzymes groups such as the proteases appeared to be detectable at the start, but were not detectable for most of the latter part of the process. Some of the enzymes in the glycosyl-hydrolases group such as α-mannosidase and α-fucosidase were not detected. These also agree with the findings reported by Tiquia *et al*. (2002) and Tiquia *et al*. (2004). These results support the assertion that change in extracellular enzyme activities is a good indicator of the composting evolution and could assist in determining compost maturity. Changes

in enzyme profiles could also potentially reflect the qualitative and quantitative fluctuation of the amount of substrate during composting since some enzymes are substrate-inducible. For example, the synthesis of phosphatases is induced by phosphohydrolate compounds and the presence of phosphatases is considered to be an indicator of microbial presence (Shemekite *et al*., 2014). Tiquia *et al*. (2001) also established a link between an increase in phosphatase enzyme activities and higher organic material and larger quantities of nutrients, which encouraged growth of total aerobic bacteria and successive phosphatase and peptidase production. Both alkaline and phosphatases activities are important enzymes in organic P mineralization and plant nutrition (Spier, 1978). There was an effect of season distinguished during the study on the enzyme activities, as temperatures dropped in winter also the intensity in enzyme activities dropped to low (1) intensity and some disappeared completely till the last stage of composting. As temperature rises in spring and summer activities subsequently improved from moderate (2-3) to high intensity (4) (Figure 3.2).

T4, particularly in the final stage of composting, showed moderate intensity (3) to high intensity (4) in acid phosphatase, lipase and esterase-lipase compared to other treatments (Figure 3.2 D), which could perhaps be attributed to the fact that the material was ground and nutrients were more freely accessible to micro-organisms for microbial breakdown. Results from a study by Betran *et al*. (2004) suggested that best composting results are obtained when grape stalk are ground because of increased surface area for microbial outbreak. (Amato *et al*., 1985; Goluke, 1982; George, 1989).

4.2. Moisture and extracellular enzyme activities

Moisture is a key environmental factor that affects many aspects of the composting process. Our data shows that the compost moisture (Table 3.2) was higher in all the treatments with spent wine filter material during autumn, winter and spring, but dropped towards the end of the composting process to 7. The lowest was associated with treatment with zero filter material. It is reasonable to suggest that the optimum range of moisture required for composting was 5 – 7 which is moderately moist according to the reference scale used in this study. This is in agreement with those of Willson (1989). It is worth-mentioning that the perlite content could have contributed to high retention of water (Hodges, 2012). These results highlight the potential of spent wine filter material as a composting enhancer. It is worth noting that chymotrypsin and *β*-galactosidase (enzymes involved in the hydrolysis of lactose) (Tiquia *et al*, 2001) had significant positive correlation with heap

moisture (Table 4.1), which corroborates the work of Burin & Pilar (2002) stating that *β*-galactosidase activity is affected by reduced moisture. The effect of season on enzyme activities and how it relates to moisture was evident in this study, as highest moisture illustrated in winter (Table 3.2) but generally lowest enzyme activities displayed (Figure 3.2 B).

Table 4.1. Pearson correlation coefficients between enzyme activities and environmental factors during composting of solid winery waste in open air conditions (n=25).

Enzyme	Environmental factors	
	HT*	HM*
Phosphatases		
Alkaline phosphatase	0.773***	-0.373*
Acid phosphatase	0.421*	-0.434**
Phosphohydrolase	0.629***	-0.453**
Esterases		
Lipase	0.487**	-0.197
Esterase-lipase	0.500**	-0.485**
Esterase	0.625***	-0.036
Amino-peptidases		
Leucine-amino peptidase	0.557**	0.001
Valine amino-peptidase	0	0.211
Cystine amino-peptidase	-0.081	0.143
Proteases		
Chymotrypsin	0.751***	-0.360*
Trypsin	0.697***	-0.294
Glycosyl-hydrolases		
α-galactosidase	0.520**	-0.03
β- glucosidase	0.487**	-0.118
N-acetyl- α-glucosaminidase	0.19	0.141
α-glucosidase	0.43*	-0.118
β-galactosidase	0.806***	-0.404*
β-glucuronidase	0.506**	-0.274
α-mannosidase	0.482**	-0.202
α – fucosidase	0.485**	-0.072

a ***, **, and * indicate correlations significant at 0.001, 0.01, and 0.05 probability levels, respectively. HT (Heap temperature) HM (Heap moisture). Correlation analyses were based on 35 observations.

4.3. Relationship between extracellular enzyme activities and microbes

Enzyme activities and microbial biomass are closely related because transformations of the important organic elements occur through microorganisms (Frankenberger & Dick, 1983). However, the results indicated a lack of positive correlations between population numbers of total aerobic heterotrophs and actinobacteria with enzyme activity (Table 4.2). This resonates with the statement by Alexander (1977) that it is not always possible to evaluate the ecological significance of a microorganism simply by knowing its number, but that it is more important to obtain information on its activity. This was reiterated by Naseby and Lynch (1997), who considered enzymatic determinations more useful than microbial measures, since they can be made with higher precision. Based on the results obtained in this study, the recorded enzyme activities may function as a good index of qualitative and quantitative assessment of composting, since some of these enzymes are substrate-inducible enzymes. These results demonstrate the applicability of the API ZYM^{TM} test.

Table 4.2. Pearson correlation coefficients between enzyme activities and microbes during composting of solid winery waste in open air conditions (n=25).

Enzyme	Microbes	
	Hete*	Act*
Phosphatases		
Alkaline phosphatase	-0.142	0.25
Acid phosphatase	0.178	0.225
Phosphohydrolase	-0.041	0.28
Esterases		
Lipase	-0.07	0.226
Esterase-lipase	0.247	0.239
Esterase	-0.034	0.107
Amino-peptidases		
Leucine-amino peptidase	-0.27	0.062
Valine amino-peptidase	-0.424*	0.062
Cystine amino-peptidase	-0.402*	-0.05
Proteases		
Chymotrypsin	-0.483*	0.437*
Trypsin	-0.400*	0.144
Glycosyl-hydrolases		
α-galactosidase	-0.186	0.248
β- glucosidase	-0.233	0.232
N-acetyl- *α*-glucosaminidase	-0.362*	0.056
α-glucosidase	-0.218	0.244
β-galactosidase	-0.252	0.328
β-glucuronidase	0.157	0.223
α-mannosidase	-0.187	0.27
α – fucosidase	-0.197	0.19

a ***, **, and * indicate correlations significant at 0.001, 0.01, and 0.05 probability levels, respectively. Act (Actinobacteria) Hete (Heterotrophs). Correlation analyses were based on 35 observations.

4.4. Extracellular enzyme activities and chemical properties

The decomposition of organic acids and microbial formation depend on the oxygen level and temperature (Vergnoux *et al.,* 2009). The turning of the compost increases oxygen levels that activate microbes, resulting in faster decomposition of the material. The pH values of treatments with spent wine filter material (T1, T2, T4 and T5) were significant higher than T3 (Table 3.3). The pH increment in treatments with spent wine filters material could be the result of high oxygen concentrations of organic acids in the compost and a faster decomposition of the acids, leading to the faster rise in pH (Beck-friis *et al.,* 2001). Previous work showed pH values ranging from 7.4 to 8.8 to be optimum for the microflora and were relevant to aerobic conditions (Michel & Reddy, 1998; Eklind & Kirchmann, 2000; Sánchez-Monedero *et al*., 2001). The pH of the compost has a marked effect on the microbial population and it increases because of protein decomposition, which liberates ammonium (Bertran *et al*., 2004). Ammonia assimilation by micro-organisms is one of the important functions for ammonia retention in the composting process (Sasaki *et al.,* 2005). Although it was not measured in the present study, it is interesting to know that phytotoxicity decreased as pH increased to values close to neutrality (Remigio *et al.*, 2013). The pH values in the reports by De Bertoldi *et al*. (1983) and Miller (1992) were closer to those observed in the present study (pH ranging between 7.8 – 8.5). This pH range is within the optimum range for composting thereby, suggesting that the compost in the present study was subjected to good oxidation. However, pH was only correlated with the esterase and leucine-amino peptidase activities (Table 4.3). In the treatment with zero filter materials (T3) pH levels were acidic (6.4). Paradelo *et al*. (2013) indicates that in all cases, the acidic pH could present a problem if composting is carried out at an industrial level.

Treatment with zero percent filter material (T3) also had high resistance and low moisture content when compared to other treatments (Table 3.3). Spry's (2009) stated that material with a low resistivity behave as good conductor and one with a high resistivity as bad conductor. In this regard, compost produced from the T3 will be rendered unsuitable. The dryer the compost the more is the resistivity of the soil due to the absence of soluble salts, whereas wet moist compost is associated with low resistivity (Chauvin, 2002), which was also in the present study.

The importance of grinding in T4 was also evident in the conservation of organic nitrogen in the final product of the compost. This condition was attested in the work of Bertran *et al.* (2004). T4

with ground pruning stalks had higher value of nitrogen compared to other treatments. Bertran, (2004) showed that ground stalks gave better results as opposed to where stalks were not ground as they integrated more easily with sludge and attains higher temperatures. Golueke (1982) findings also indicated that high temperatures were recorded in ground material and provided the best hygienization. Materials that are ground have greater surface areas, which make them more susceptible to microbial invasion. Enzyme activities that showed strong relationship with nitrogen in the current study were: alkaline (0.377) and acid phosphatase (-0.405), as opposed to the weak correlation with: esterase, luecine-amino peptidase, valine amino peptidase, N-acetyl-a-glucosaminidase and a-fucosidase (Table 4.3). That high phosphatase activity was observed at the end of the composting, in agreement with Ros *et al*. (2006), is suggestive of its known agronomic value. According to Raut *et al. (*2008) phosphatase hydrolyses compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which are assimilated by plants. P is important for overall plant health and P content in compost heaps was positively ($P < 0.05$) correlated with phophohydrolase (-0.36), valine- amino peptidase (0.503) and cysteine amino-peptidase (0.492). Other enzymes activities correlated positively with some of the heavy metal properties (Fe, Zn, Mn, Ca and C) (Table 4.3).

Table. 4.3. Pearson correlation coefficients between enzyme activities and chemical factors during composting of solid winery waste in open air condition.

Enzyme	pH	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	C
Phosphatases												
Alkaline phosphatase	0.146	0.377*	0.087	0.234	-0.177	-0.145	-0.027	-0.493**	0.009	-0.477**	-0.326	0.177
Acid phosphatase	-0.24	-0.405*	-0.568***	-0.428*	-0.103	-0.009	0.062	-0.003	0.649***	0.333	0.016	-0.403*
Phosphohydrolase	-0.134	-0.124	-0.363*	-0.208	-0.139	-0.09	0.158	-0.122	0.493	0.131	-0.144	-0.267
Esterases												
Lipase	-0.024	-0.035	-0.226	-0.091	-0.281	-0.209	0.113	-0.278	0.274	0.006	-0.26	-0.209
Esterase-lipase	-0.217	-0.325	-0.536***	-0.365*	-0.088	0.003	0.044	-0.052	0.601***	0.316	-0.035	-0.335
Esterase	0.424*	0.671***	0.496**	0.654	-0.261	-0.316	0.186	-0.535	-0.349	-0.713	-0.463	0.192
Amino-peptidases												
Leucine-amino peptidase	0.356*	0.691***	0.491	0.609***	-0.132	-0.19	0.106	-0.405*	-0.412*	-0.674***	-0.337*	0.291
Valine-amino peptidase	0.176	0.556***	0.503**	0.420*	0.059	-0.024	-0.089	-0.164	-0.652***	-0.476**	-0.108	0.526**
Cystine-amino peptidase	0.134	0.475**	0.492**	0.374*	0.121	0.047	-0.123	-0.119	-0.633***	-0.412*	-0.039	0.556***
Proteases												
Chymotrypsin	-0.043	0.143	-0.145	0.017	-0.158	-0.116	0.03	-0.255	0.165	-0.105	-0.223	-0.014
Trypsin	-0.043	0.143	-0.145	0.017	-0.158	-0.116	0.03	-0.255	0.165	-0.105	-0.223	-0.014
Glycosyl-hydrolases												
α-galactosidase	0.217	0.456**	0.228	0.307	-0.242	-0.221	-0.012	-0.422**	-0.122	-0.471**	-0.369*	0.141
β- glucosidase	0.197	0.491**	0.303	0.37	-0.141	-0.146	-0.081	-0.412*	-0.3	-0.562***	-0.325	0.318
N-acetyl- α-glucosaminidase	0.289	0.560***	0.522**	0.467**	-0.019	-0.155	-0.002	-0.335	-0.52**	-0.531**	-0.274	0.389*
α-glucosidase	0.084	0.436	0.184	0.23	-0.034	0.019	-0.132	-0.257	-0.146	-0.456**	-0.169	0.324
β-galactosidase	0.042	0.249	-0.057	0.079	-0.174	-0.127	0.07	-0.381*	0.112	-0.15	-0.313	0.049
β-glucuronidase	0.011	-0.187	-0.331	-0.162	-0.404*	-0.308	0.272	-0.332	0.607***	0.231	-0.337*	-0.446**
α-mannosidase	0.089	0.439**	0.19	0.246	-0.049	-0.051	-0.132	-0.356*	-0.22	-0.556***	-0.232	0.351*
α – fucosidase	0.211	0.544***	0.289	0.339*	-0.175	-0.208	0.017	-0.454**	-0.314	-0.605***	-0.367*	0.281

a ***, **, and * indicate correlations significant at 0.001, 0.01, and 0.05 probability levels, respectively. Correlation analyses were based on 35 observat

4.5. C/N ratio

Generally, a strong inverse relationship (R^2=-0.9494) was observed between spent wine filter material and C/N ratio (Figure 3.1) implying that inclusion of more filter material in compost heaps will favour the production of compost end product with enhanced C:N ratio. Commercial products, as well as T1 and T4, had similar C/N ratio of 10:1 (Table 3.3). According to Richard & Trautmann (1996), as carbon gets converted to CO_2 (presuming minimal nitrogen losses) the C/N ratio drops during the composting process, with the ratio of finished compost typically close to 10/1. T1 (10:1), T2 (13:1) and T4 (10:1) with spent wine filter material content yielded improved C/N ratio compared to T3 (20:1) with no spent wine filter material. The lower the C/N ratio the quicker the N would become available. Raath & Schutte (2001) stated that optimum C/N ratio for ripe compost should be between 13:1 and 10:1. Therefore, on the basis of our results, compost heaps in treatments T1 and T4 were matured and finished at the end of the trial period.

Chapter Five

Conclusion and Recommendations

Incorporating spent wine filter material in heaps during composting of winery waste under open air conditions is recommended. The treatment with 40% spent wine filter material that was grinded and lined with black plastic underneath gave best results in terms of compost quality. However, under open field conditions environmental factors can influence the composting process. These findings represent useful scientific information for farmers, compost producers, entrepreneurs, researchers and municipalities and can help improve the quality of compost and composting as a waste management strategy.

Preferably, composting should be done between spring and summers when the temperatures are high, and the decomposition rate of material is faster. Rain shelters should be considered that would allow composting to continue unhindered by rain. Although rather preliminary in nature, the results of this study suggest the potential use of the API ZYM^{TM} test as a tool for monitoring the course of the actual composting process, and by inference, as an indicator of compost maturity. Since some of these enzymes are substrate-inducible, they could potentially reflect the qualitative and quantitative fluctuation of the amount of substrate during composting.

To obtain more generally-acceptable data for composting of spent winery solid waste, more methodological work is needed. For example, a greater variety of different sources of compost materials (Chopped pruning canes, grape stalks, berry skins and seeds) and different ratios of composting mixtures and sizes, must be examined in order to determine the full potential of this assay as a means of testing compost maturity. The API ZYM^{TM} test may also potentially lends itself to wider application in other composting processes.

REFERENCES

Adani, F., Genevinia, P. L., Gasperib, F., Tambonea, F. 1999. Composting and humification. *Compost Science & Utilization* 7, 24 - 33.

Alexander, M. 1977. Effects of acidity on microorganisms and microbial processes in soil. *Springer US* 4, 363-374. Book Title: Effects of Acid Precipitation on Terrestrial Ecosystems .

Alleweldt, G., Spiegel-Roy, P., Reisch, B. 1991. GRAPES *(VITIS)*. *Acta Horticulturae*. 290, 291-330.

Allsopp, E., van Schoor, L. 2008. IPW Farm and Cellar Audits for 2005 and 2006 vintages Results and trends. Available from: http://www.wynboer.co.za/recentarticles/200712ipw.php3. Accessed: 21 /08/ 2014.

Albrecht, R., Joffre, R., Gros, R., Le Petit, J., Terrom, G., Périssol, C. 2011. Efficiency of near-infrared reflectance spectroscopy to asses and predict the stage of transformation of in the composting process. *Bioresourc*e *Technology* 99, 448 - 455.

Amato, M., Ladd, J. N., Van Veen, J. A. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C (U)] glucose and [^{15}N] $(NH_4)_2So_4$ under different moisture regimes. *Soil Biology and Biochemistry* 17(6), 747 – 756.

Arvanitoyannis, S., Ladas, D., Mavromatis, A. 2006. Review: Wine waste treatment methodology. *International Journal of Food Science & Technology* 41, 1117 - 1151.

Arienzo, M., Christen, E.W., Quayle, W., Kumar, A. 2009. A review of the fate of potassium in the soil-plant system after land application of wastewaters. *Journal of Hazardous Materials* 164, 415 - 422.

Aslam, D. N., Horwath, W., VanderGheynst, J. S. 2008. Comparison of several maturity indicators for estimating phytotoxicity in compost-amended soil. *Waste Management* 28, 2070 - 2076.

Athanassiou, C. G., Vayias, B. J., Dimizas, C., B. Kavallieratos, N. G., Papagregoriou, A. S., Buchelos, C. T. 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. *Journal of Stored Products Research* 41(1), 47–55.

Ayari, F., Hamdi, H., Jedidi, N., Gharbi, N., Kossai, R. 2010. Heavy metal distribution in soil and plant in municipal solid waste compost amended plots. *International Journal of Environmental Science and Technology* 7(3), 465 - 472.

Azadi, H., Ho, P. 2009. Genetically modified and organic crops in developing countries: A review of options for food security. *Biotechnology Advances* 28, 160 - 168.

Balanyà Martí, T., Saña Vilaseca, J., Gonzàlez Tardiu, M.L., Lapeña Kruter, Y De. M. 1994. Utilization composite Solid Waste urban vineyard in the Penedes.Viticulture / *Enology Professional* 31, 20 - 25.

Baltimore, M.D. 1976. Munsell Book of Color.

Beales, N. 2006. Adaptation of Microorganisms to Cold Temperatures, Weak Acid Preservatives, Low pH, and Osmotic Stress: A Review. *Comprehensive Reviews in Food Science and Food Safety Volume* 3(1), 1 - 20.

Beck-Friis, B., Smars, S., Jonsson, H., Kirchmann, H. 2001. SE-structures and environment: gaseous emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. *Journal of Agricultural Engineering Research* 78(4), 423 - 430.

Benitez, E., Nogales, R., Elvira, C., Masciandaro, G., Ceccanti, B. 1999. Enzyme activities as indicator of the stabilization of sewage sludge composting with Eiseniafoetida. *Bioresource Technology* 67(3), 297 - 303.

Bernal, M. P., Alburquerque, J. A., Moral, R. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. *Bioresource Technology* 100 (22), 5444 - 5453.

Bernal, M. P., Paredes C., Sánchez-Monedero M. A., Cegarra J. 1998. Maturity and stability parameters of composts prepared with a wide range of organic wastes. *Bioresource Technology* 63(1), 91- 99.

Bernal, M. P., Navarro, A. F., Roig, A., Cegarra, J., Garcia, D. 1996. Carbon and nitrogen transformation during composting of sweet sorghum bagasse. *Biology and fertility soils* 22,141 – 148.

Bertran, E., Sort, X., Soliva, M., Trillas, I. 2004. Composting winery sludges and grape stalks. *Bioresource Technology* 95 (2), 203 - 208.

Bewick, M. W. M. 1980. Wastes from the fermentation industries: their use as feedstuffs and fertilizers. In: Bewick MWM (ed) Handbook of Organic Waste Conversion, Van Nostrand Reinhold Company, New York, p.165 -186.

Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., Zoppini, A. 2013. Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology and Biochemistry* 58, 216 - 234.

Burns, R.G. 1978. *Soil Enzymes*. *Folia Microbiological* 24, 380-380. New York: Academic Press.

Burin, L., Pilar, B.M. 2002. Enzyme and Microbial Technology. *Elsevier* 30, 367 - 373.

Bustamante, M. A., Moral, R., Paredes, C., Marhuenda-Egea F., Bernal, M. P. 2009. Utilisation of manure composts by high-value crops: Safety and environmental challenges. *Bioresource Technology* 100, 5454 - 5460.

Bustamante, M. A., Moral, R., Paredes, C., Vargas-Garcia, M.C., Suarenz-Estella, F., Morenob, J. 2008. Evolution of the pathogen content during co-composting of winery and distillery wastes. *Bioresource Technology* 99(15), 7299 - 7306.

Bustamante, M. A., Pérez-Murcia, M. D., Paredes, C., Moral, R., Pérez-Espinosa, A., Moreno-Caselles, J. 2007. Short-term carbon and nitrogen mineralisation in

soil amended with winery and distillery organic wastes. *Bioresource Technology* 98, 3269 - 3277.

Butler, T. A., Sikora, L. J., Steinhilber, P. M., Douglas, L.W. 2001. Compost age and sample storage effects on maturity indicates of biosolids compost. *Journal of Environmental Quality* 30, 2141 - 2148.

Cabañas-Vargas, D.D., Sánchez-Monedero, M.A., Urpilainen, S.T., Kamilaki, A., Stentiford, E.I. 2005. Assessing the stability and maturity of compost at large-scale plants. *Ingeniería* 9 (2), 25-30.

Campbell, C. R., Plank, C.O. 1998. Preparation of plant tissue for laboratory analysis. In Y.P Kalra (ed) *Handbook of Reference Methods for Plant Analysis*, 37- 49.

Catherine, D. 2011.Waste Management in the South African Wine Industry. Dissertation submitted in partial requirement for the diploma of Cape Wine Master.

Carmona, E., Moreno, M. T., Avilés, M., Ordovás, J. 2012. Use of grape marc compost as substrate for vegetable seedlings. *Scientia Horticulturae* 137, 69 - 74.

Carry, C. W., Stahl, J. F., Hansen, B. E., Friess, P. L. 1990. Sludge management and disposal practices of the county sanitation districts of Los Angeles (USA). *Water Science and Technology* 22, 23 - 32.

Carlos, A. S., Susan, E. T., Eric, A. D., Sara, V., Janssens, I. 2015. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature. *Advances in Modeling Earth Systems* 7(1), 335 - 356.

Carlos, P., Alex, M., Antonia, P. 2004. Development of a solid phase kinetic assay for determination of enzyme activities during composting. *Process Biochemistry* 39, 971 - 975.

Cardoso, E. J., Vasconcellos, R. L., Bini, D., Miyauchi, M. Y., Santos, C. A. 2013. Soil health: looking for suitable indicators. What should be considered to assess the effects of use and management on soil health? *Scientia Agricola* 70 (4), 274 - 289.

Castaldi, P., Alberti, G., Merella, R., Melis, P. 2005. Study of the organic matter evolution during municipal solid waste composting aimed at identifying suitable parameters for the evaluation of compost maturity. *Waste Management* 25(2), 209 - 213.

Castaldi, P., Garau, G., Melis, P. 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water-soluble fractions. *Waste Management* 28, 534 - 540.

Castaldi, P. Garau, G., Melis P. 2004. Influence of compost from sea weeds on heavy metal dynamics in the soil–plant system. *Fresenius Environment Bulletin,* Vol. 13, pp. 1322-1328, ISSN 1018-4619.

Chanyasak, V., Hirai, M., Kubota, H. 1982. Changes of chemical components and nitrogen transformation in water extracts during composting of garbage. *Journal of Fermentation Technology* 60, 439 - 446.

Chapman, J., Baker, P., Wills, S. 2001. *Winery Wastewater Handbook*. Winetitles, Adelaide, South Africa, page 127.

Charnay, F. 2005. Composting of urban wastes in developing countries: Elaboration of a methodology for a production of compost. Doctorate thesis, University of Limoge.

Chauvin, A. 2002. AEMC® Instruments 200. Foxbororough Blvd. MA 02035 USA.

Chen, L., Marti, H., Moore, A., Falen, C. 2011. The composting process. Environmental, Microbiological and Utilization Aspects. Renaissance Publications, University of Idoho, page 1- 5.

Cooperband, L. R. 2000. Composting: art and science of organic waste conversion to a valuable soil resource. *Laboratory Medicine* 31(6), 283 - 289.

Crowe, A. 2005.The Pomace Predicament -Winemaker Magazine. Issue: 12/Aug/Sept 2005.

Daniel, M. L., McConnell, N. R., Ernest, E. 2014. Perlite toxicology and epidemiology-a review. *Inhalation toxicology* 26(5) 259-270.

David, C. C., Crossley, D. A., Paul, F., H. 2004. Fundamentals of Soil Ecology (Second Edition). Publisher Academic Press, page 386.

De Bertoldi, M., Rutili, A., Citterio, B., Civilini, M.1988. Composting Management: A New process control through O2 Feedback. *Waste Management & Research* 6 (3), 239 - 259.

De Bertoldi, M., Ferranti, M. P., L, Hermite, P., Zucconi, F. 1986. In: Compost: Production, Quality and Use. *Elsevier Applied Science*, London, page 852.

De Bertoldi, M., Vallini, G., Pera, A. 1983. The Biology of Composting: A Review. *Waste Management and Research* 1,157 - 176.

Deportes, I., Benoid-Guyod, J., Zmirou, D. 1995. Hazards to man and environment posed by the use of urban waste compost: a review. *The Science of the Total Environment* 172, 197 - 222.

Delbert, D., Hemphill, Jr., Ticknor, R. L., Flower D. J. 1984. Growth Response of Annual Transplants and Physical and Chemical Properties of Growing Media as Influenced by Composted Sewage Sludge Amended with Organic and Inorganic Materials. *Journal of environmental horticulture* 2(4), 112 - 116.

Diaz, L. F., Savage, G. M. 2007. Bioremediation. In: L. F. Diaz, M. De Bertoldi, B. W. and S. E. (eds.), *Compost Science and Technology*, 159 -176.

Disposal Act, 1989 (ACT 73 of 1989). *Gazette No. 11927, Notice No. 1188.* Commencement date: 9 June 1989.

Doublet, J., Francou, C., Poitrenaud, M., Houot, S. 2011. Influence of bulking agents on organic matter evolution during sewage sludge composting; consequences on compost organic matter organic matter stability and N availability. *Bioresource Technology* 102, 1298–1307.

EA (Environment Agency), (2001). Technical Guidance on Composting Operations (Draft), Environment Agency UK, Bristol.

Eklind, Y., Kirchman, H. 2000. Composting and storage of organic household waste with different litter amendments. II: nitrogen turnover and losses. *Bioresource Technology* 74(2), 125 -133.

Engeli, H., Edelmann, W., Fuchs, J. Rottermann, K. 1993. Survival of plant pathogens and weed seeds during anaerobic digestion, *Water Science and Technology* 27(2) 69 - 76.

Epstein, E.1997. The Science of Composting. Lancaster, PA: Techonomic Publishing Company, page 487.

Fernández, F. J., Sánchez-Arias, V., Villaseñor, J., Rodríguez, L. 2008. Evaluation of carbon degradation during co-composting of exhausted grape marc with different biowastes. *Chemosphere* 73 (5), 670 - 677.

Ferrer, J., Páez, G., Mármol, Z., Ramones, E., Chandler, C., Marín, M. Ferrer, A. 2001. Agronomic use of biotechnologically processed grape wastes. *Bioresource Technology* 76(1), 39 - 44.

Food and Agriculture Organization, 2008. The encyclopaedia of earth. Water profile of South Africa. Available from: www.eoearth.org/article/Water_profile_of_South_Africa.

Frankenberger, W. T., Dick, W. A.1983. Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal* 47(5), 945 - 951.

Gajdos, R. 1998. Bio-conservation of organic waste by the year 2010 to recycle elements and save energy. *Resource, Conservation and Recycling* 23, 67 - 87.

Gajalakshmi, S., Abbasi, S. A. 2008. Solid Waste Management by Composting: State of the Art. *Critical Reviews in Environmental Science and Technology* 38 (5), 311- 400.

Garcia, C., Hernandez, T., Costa, F. 1993. Hydrolases in the organic matter fractions of sewage sludge: changes with composting. *Bioresource Technology* 45 (1), 47 - 52.

Garcia, C., Hernandez, T., Costa, F., Ceccati , B., Ciardi, C. 1992. Changes in ATP content, enzyme activity and inorganic nitrogen species during composting of organic wastes. *Canadian Journal of Soil Science* 72(3), 243 - 253.

George, B. W. 1989. Combining raw materials for composting. The *BioCycle Guide to Composting Municipal Wastes*, 82 - 85.

Godden, B., Penninckx, M., Pierard, A., Lannoye, R. 1983. Evolution of enzyme activities and microbial populations during composting of cattle manure. *European Journal of Applied Microbiology and Biotechnology* 17(5), 306 - 310.

Goldblatt, P., Manning, J.C., 2002. Plant Diversity of the Cape Region of Southern Africa. *Annals of the Missouri Botanical Garden* 89 (2), 281 - 302.

Golueke, C. G. 1982. When is compost "safe"? A review of criteria for assessing the destruction of pathogens in composting. *Biocycle* 28, 28 - 38.

Golueke, C. G. 1977. Biological Reclamation of Solid Wastes. Rodale Press, Emmaus, PA, U.S.A.

Gómez-Brandón, M., Lazcanob, C., Loresc, M., Domínguez, J. 2011. Short – term stabilization of grape marc through earthworms. *Journal of Hazardous Materials* 187(1-3), 291 - 295.

Gómez-Brandón, M., Lazcano, C., Domínguez, J. 2008. The evaluation of stability and maturity during the composting of cattle manure. *Chemosphere* 70, 436 – 444.

Goyal, S., Dhull, S. K., Kapoor, K. K. 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresource Technology* 96 (14), 1584 -1591.

Glazewski, J. 2000. Environmental law in South Africa. *Durban: Butterworths*, page 903.

Hauck, F.W. 1981.The importance of organic recycling in the agricultural programmes of developing countries, in: organic Recycling in Asia. *Food and Agricultural Organization* 1, 17- 28.

Haug, R.T. 1993. The Practical Handbook of Compost Engineering. Lewis Publishers, Boca Raton, page 717.

Halliday, S. 2010. Home food grade diatomaceous earth and its health advantages, page 124-125.

Hang, Y. D. 1988. Recovery of food ingredients from grape pomace. *Process* 23, page 24.

Hammer, O., Harper, D. A. T., Ryan, P. D. 2001. PAST. Available from: http://palaeo-electronica.org/2001-1past/issue1-01.htm.

Hankin, L., Raymond, P. P., Sandra, L, A. 1975. Microorganisms from composting leaves: Ability to produce extracellular degradative enzymes. *Microbial Ecology* 2(4), 296 - 308.

He, H. Y., Qin, Y. L., Chen, G. G., Li, N., Liang, Z. 2013. *β*-Glucosidase in *Aspergillus oryzae* HML366 and enzymatic characterization. *Applied Biochemistry and Biotechnology* 169, 870 - 884.

Hodges, S. C. 2012. Soil Fertility Basics - Chapter 12 Nutrient Management & Realistic Yields. Soil Science Extension North Carolina State University.

Hogg, D., Lister, D., Barth, J., Faviono, E., Amlinger, F. 2009. Frameworks for use of compost in agriculture in Europe, Eunomia Research and Consulting.

Hogg, S. 2013. *Essential Microbiology* (2nd ed.). Wiley-Blackwell, page 86.

Hornick, S. B., Patterson, J. C., Chaney, R.L. 1979. An evaluation of urban soil, vegetation, and soil amendments. Paper in press: 2nd Conference on Scientific Research in National Parks. San Francisco, Ca.

Hue, N. V., Liu, J. 1995. Predicting compost stability. *Compost science & utilization 3* (2), 8 - 15.

Huang, G. T., Wu, Q. T., Wong, J. W. C., Nagar, B.B. 2006. Transformation of organic matter during co-composting of pig manure with sawdust. *Bioresoure Technology* 97, 1834 - 1842.

Iannotti, D. A., Grebus, M. E., Toth, B. L., Madden, L.V., Hoitink, H. A. J. 1994. Oxygen respirometry to assess stability and maturity of composted municipal solid-waste. *Journal of Environmental Quality* 23, 1177 - 1183.

Ioannis, S.A., Demetrios, L., Athansios, M. 2006. Potential uses and applications of treated wine waste. *International Journal of Food Science & Technology* 41(5),475-487.

Inbar, Y., Hadar, Y., Chen, Y. 1993. Recycling of Cattle Manure: The Composting process and Characterization of Maturity. *Journal of Environmental Quality* 22(4), 857- 863.

Inbar, Y., Chen, Y., Hadar, Y. 1991. Carbon -13 CPMAS NMR and FTIR spectroscopic analysis of organic matter transformations during composting of solid waste from Wineries. *Soil Science* 152(4), 272 - 282.

Insam, H., Amor, K., Renner, M., Crepaz, C. 1996. Changes in functional abilities of the microbial community during composting of manure. *Microbial Ecology* 31, 77 - 87.

IPW guidelines, 2008. Date access: 12/08/2014. Available from: http:www.ipw.co.za.

International Organisation of Vine and Wine - OIV. World wine production has increased significantly in 2013 while consumption is stabilising. Accessed 28/10/2013. Available from: www.oiv.int/oiv/info/enpoint2013.

International Water Management Institution. 2003. Co-composting of faecal sludge and municipal organic waste - A Literature and State-of-Knowledge Review. Available from:http://www.eawag.ch/forschung/sandec/publikationen/ewm/dl/cocomp_reviewdf.

Ipek, U., Obek, E., Akca, L., Arslan, E. I., Hasar, H., Dogru, M. Baykara, O. 2002. Determination of degradation of radioactivity and its kinetics in aerobic composting. *Bioresource Technology* 84, 283 - 286.

Ishii, K., Fukui, M. Takii, S. 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. *Applied Microbiology* 8, 768 - 777.

Juan, A. L., Joaquín, M., Vargas-García, M. C., Suárez-Estrella, F., López, M. J., Jurado, M. 2014. Exploiting composting biodiversity: Study of the persistent and biotechnologically relevant microorganisms from lignocellulose-based composting. *Bioresource Technology* 162, 283–293.

Kalamdhad, A. S., Kazmi, A. A. 2009. Effects of turning frequency on compost stability and some chemical characteristics in a rotary drum composter. *Chemosphere* 74(10), 1327 -1334.

Kiyasudeen, S. K., Ibrahim, M. H., Quaik, S., Ismail, A., S. 2015. Vermicomposting: an earthworm mediated waste treatment technique. *Applied Environmental Science and Engineering for A Sustainable future*,167-199.

Khalid, A., Arshad, M., Anjum, M., Mahmood, T., Dawson, L. 2011. The anaerobic digestion of solid organic waste. *Waste Management* 31(8), 1737 - 1744.

Lasaridi, K.E., Stentiford, M. A. 1998. Simple respirometric technique for assessing compost stability. *Water Research* 32(12), 3717- 3723.

Laurenson, S., Houlbrooke, D. J. 2012. Determination of soil dispersion in response to changes in soil salinity. AgResearch Client Report for Marlborough District Council, Invermay, New Zealand.

Lazarus, D. M. L. 1997. A model for an environmentally directed teaching approach. Submitted in accordance with the requirements for the degree of Doctor of education. University of South Africa, Pretoria.

Levay, G. 1995. Effluent management for wineries and distilleries. The Australian Regulatory Framework and Code of Practice. In Wark Research Institute, University of South Australia, Adelaide.

Liang, C., Das, K.C., McClendon, R. W. 2003.The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource Technology* 86 (2), 131 - 137.

Litterick, A., Harrier, L., Wallace, P., Watson, C. A., Wood, M. 2003. Effects of composting manures and other organic wastes on soil processes and pest and disease interactions. Scottish Agricultural College, *Organic eprints.*

Lynd, L. R., Weimer, P. J., van Zyl, W. H., Pretorius, I. S. 2002. Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology* 66 (3), 506 - 577.

Manuel, A., C. 2004. Performance of an Active Biological Contactor (ABC): Treating Winery Wastewater. Ph.D. University, Toronto.

Manyi-Loh, E. C., Mamphweli, S. E., Meyer, E. D., Okoh, G. M., Simon, M. 2013. Microbial Anaerobic Digestion (Bio-Digesters) as an Approach to the Decontamination of Animal Wastes in Pollution Control and the Generation of Renewable Energy. *International Journal of Environmental Research and Public* 10(9), 4390 - 4417.

Macauley, B. J., Stone, B., Liyama, K., Harper, E. R., Miller, F. C.1993. Compost research runs 'hot' and 'cold' at La Trobe University. *Compost Science and Utilization* 1, 6 -12.

Malandra, L., Wolfaardt, G., Zietsman, A., Vijoen-Bloom, M. 2003. Microbiology of a biological contactor for winery wastewater treatment. *Water Research* 37, 4125 - 4123.

Martin, J. A., Stephen, C. E., Donald, J. R. 2004. Heterotrophic plate count bacteria – what is their significance in drinking water. *International Journal of Food Microbiology* 92, 265- 274.

Michel, F. C., Reddy, C. A. 1998. Effect of oxygenation level on yard trimmings composting rate, odor production, and compost quality in bench – scales reactors. *Compost Science & Utilization* 6, 6 -14.

Michael, M. M., June, M. B. 1994. The medically important aerobic actinonomycetes: Epidemiology and Microbiology. *Clinical Microbiology Reviews* 7(3), 357 - 417.

Miller, F. C.1992. Composting as a process based on the control of ecologically selective factors. In: F. Blaine-Metting (ed.), *Soil Microbial Ecology*: Applications in Agriculture Environment Management, Marcel Dekker Inc., New York.

Misra, R.V., Roy, R. N., Hiraoka, H. 2003. On-farm composting methods. *Food and agriculture organisation of the United Nations*. Rome, page 51.

Mondini, C., Fornasier, F., Sinicco, T. 2004. Enzymatic activity as a parameter for the characterization of the composting process. *Soil Biology Biochemistry* 36, 1587 -1594.

Mosse, K. P. M., Patti, A. F., Christen, E.W., Cavagnaro, T. R. 2010. Winery wastewater inhibits seed germination and vegetative growth of common crop species. *Hazardous Material* 180, 63 -70.

Moretti, L., Bertoncini I. E., Hamilton, C. A. 2015. Composting sewage sludge with green waste from tree pruning. *Soil and plant nutrition* 72(5), 432 - 439.

Mulidzi, R. Shange, P. 2011. Composting using spent wine filter material from wineries. (Preliminary study) unpublished manuscript.

Mustin, M. 1987. Le compost – management of organic material. Editions François Dubusc – Paris, page 954.

Mckinley, V.L., Vestal, J.R.1985. Effects of different temperature regimes on microbial activity and biomass in composting municipal sewage sludge. *Journal of Microbiology* 31, 919-25.

Nannipieri, P., Fusi, P. 1996. Humus enzyme and enzyme activity. In *Humic Substances in Terrestrial Ecosystems* ed. Piccolo New York: *Elsevier*, page 293 - 329.

Naseby, D.C., Lynch, J.M. 1997. Rhizophere soil enzymes as indicators of perturbations caused by enzyme substrate addition and inoculation of a genetically modified strain of *Pseudomonas fluorescens* on wheat seed. *Soil Biology and Biochemistry* 29, 1353-1362.

Nathanson, J. A. 2015. Hazardous-waste management. 2015. *Encyclopedia Britannica Online*. http://www.britannica.com/technology/hazardous-waste-management.

Nogueira, W. A., Nogueira, F.N., Devens, D.C. 1999. Temperature and pH control in composting of coffee and agricultural wastes. *Water Science and Technology* 40 (1), 113 - 119.

Ndegwa, P. M., Thompson, S. A. 2001. Integrating composting and vermicomposting in the treatment and bioconversion of biosolids. *Bioresource Technology* 76 (2), 107 - 112.

NWQMS (National Water Quality Management Systems). (1998). Effluent management guidelines for Australian wineries and distilleries. Available from: http://www.environment.gov.au/water/publications/quality/pubs/australian-wineries-distilleries-paper20.pdf. Date accessed 07/08/2014.

Organic Materials Management. Available from: http://www.calrecycle.ca.gov/Organics/. Last updated: February 4, 2015.

Paradelo, R., Moldes, A.B., Barral, M.T. 2013. Evolution of organic matter during the mesophilic composting of lignocellulosic winery wastes. *Journal of Environmental Management* 116, 18 - 26.

Pascual, P. A., Ayuso, M., Garcia, C., Hernández 1997. Characterizaion of Urban Wastes according to fertility and phytotoxicity parameters. *Waste Management* 15,103 - 112.

Prescott, M.L., Harley, J.P., Klein, D.A. 2008. Microbiology, 7th edition, McGraw-Hill Companies, New York, page 215-340.

Priest, P. 1984. Extracellular Enzymes. Washington D.C.*: American Society for Microbiology*, page 136.

Raath, P. J., & Fourie, J. C. 2006. Guidelines for making compost on a wine grape enterprise. Date accessed: 12/08/2014. Available from: http://www.wynboer.co.za/recentarticles/200601compost.php3.

Raath, P. 2002. The importance of compost in organic crop production. ARC Infruitec nietvoorbij, Private bag x 5026, Stellenbosch, 7599, page1 - 8.

Raath, P., Schutte, C. 2001. Guidelines for monitoring soil fertility, plant nutrient status and compost quality in organic wine grape production systems. *WineLand*, page 90 - 94.

Raut, M. P., Prince - William S. P. M., Bhattacharyya, J. K., Chakrabarti, T., Devotta, S. 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid

waste—a compost maturity analysis perspective. *Bioresource Technology* 99(14), 6512 –6519.

Remigio, P., Ana, B.M., María, T.B. 2013. Evolution of organic material during the mesophilic composting of lignocellulosic winery waste. *Journal of Environmental Management* 1161, 8 - 26.

Richard, M. 2012. Turning compost by temperature Posted on Monday, December 17th, 2012 at 7:10 pm. Posted by admin .

Richard, T., Trautmann, N. 1996. Cornell Waste Management Institute Ithaca, NY 14853 607-255-1187 cwmi@cornell.edu.

Ros, M., García, C., Hernandez, T. 2006. A full-scale study of treatment of pig slurry by composting kinetic changes in chemical and microbial properties. *Waste Management* 6, 1108 - 1118.

Ros, M., Hernández, M.T., García, C. 2003. Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology & Biochemistry* 35, 463 - 469.

Robertson, S., Kirsten. F. 1993. Water and wastewater management in the Wine industry. WRC Report No. 416/1/99. Water Research Commission, Pretoria.

Said-Pullicino, D., M Erriquens, F., Gigliotti, G. 2011. Changes in the chemical characteristics of water-extractable organic material during composting and their influence on compost stability and maturity. *Bioresource Technology* 98(9), 1822 - 1831.

Sánchez-Monedero, M. A., Roig, A., Paredes, C., Bemal, M.P. 2001. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of composting mixtures. *Bioresource Technology* 78, 301- 8.

Sasaki, H., Yano, H., Sasaki, T., Nakai, Y. 2005. A survey of ammonia-assimilating micro-organisms in cattle manure composting. Applied Microbiology 99(6), 1356–1363.

Sinha, R., K. Agarwal, S., Chauhan, K., Valani, D. 2010. The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers from agriculture. *Agricultural Sciences* 2**,** 76-94.

Schoeman, C. 2012. Grape and wine quality of V. vinifera L. cv. Cabernet Sauvignon/99R in response to irrigation using winery wastewater. Thesis degree of Master of Science in Agriculture, Stellenbosch University.

Shikwambana, S., Kutu, F. R., Madiba, O. F. 2014. Quantification of microbial population dynamics and enzyme activity in soil amended with composts of varying degree of maturity.

Shemekite, F., Gómez-Brandón, M., Franke-Whittle, I. H., Praehauser, B., Insam, H., Assefa, F. 2014. Coffee husk composting: An investigation of the process using molecular and non-molecular tools. *Waste management*, 34(3): 642 - 652.

Sheridan, C. 2004. Empirical and economic modelling of winery and effluent parameters. *Wine Biotechnology*. Date updated December/2004. Available from:

www.wineland.co.za/.../empirical-and economic modelling of winery and effluent parameters.

Solvia, M., Felipó, M. T. 2002. Organic wastes as a resource for Mediterranean soils. Workshop ‘‘Biological Treatment of Biodegradable Wastes –Technical Aspects’’. DG Environment and the JRC, Brussels, p.19.

Soni, S. K. 2007. Microbes: A Source of Energy for 21st Century. New India Publishing.

Spier, T.W., Ross, D. F. 1978. Soil phosphatases and sulfatases. In: Burn, R.G. (ed) Soil Enzymes. Academic Press, New York, p.198 - 235.

Steiner, W.G., Johannes Lehmann,T.,Vasconcelos de Macêdo,J. L. Winfried. E. H., Wolfgang Zech, B. 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil 291(1),* 275-290.

Steger, K., Jarvis, A., Vasara, T., Romantschuk, M., Sundh, I. 2007. Effects of differing temperature management on development of Actinobacteria populations during composting. *Research in Microbiology* 158, 617 - 624.

Soviet Encyclopedia, 3rd Edition (1970-1979). © 2010 The Gale Group, Inc. All rights reserved.Availablefrom:
href="http://encyclopedia2.thefreedictionary.com/Heterotrophic+Bacteria">Heterotro
phic Bacteria.

Sullivan, D. M. Miller, R. O. 2001. Compost Quality Attributes, Measurements and Variability. Chapter 4 in: P. Stofella and B. Kahn (eds.). Compost Utilizaton in Horticultural Systems.

StatSoft, Inc. (2014), STATISTICA (data analysis software system), version 12, Available from: www.statsoft.com.

Spry's, T. 2009. Electrical Engineering Pages. Soil Resistivity Testing. Available from: myweb.tiscali.co.uk/gospry/gospry2009.htm.

Takizawa, M., Colwell, R.R., Hill, R.T. 1993. Isolation and diversity of actinomycetes in the Chesapeake Bay. *Applied Environmental Microbiology*. 59 (4), 997-1002.

Takele, T. 2011. Lecture Notes for Environmental and Occupational Health Students. *Solid Waste Management* University of Gondar.

Tardy, R. J., Beck, R. W. 1996. Composting Technology in the United States: Research and Practice. Springer, Netherlands, page 939 - 947.

Tate, R. L. 1995. Soil enzymes as indicators of ecosystem status. *In Soil Microbiology*, John Wiley, Chichester, page 123 -146.

Tejada, M., Hernández, M. T., García, C. 2006. Application of two organic amendments on soil restoration: effects on the soil biological properties. *Journal of Environmental Quality* 35, 1010 -1017.

Tiquia, S. M., Wan, J. H. C. Tam, N.F.Y. 2002. Microbial population dynamics and evolution of enzyme activities during composting. *Compost Science and Utilization* (in press).

Tiquia, S. M., Wan, J. H. C., Tam, N.F.Y. 2001. Extracellular enzyme profiles during co-composting of poultry manure and yard trimmings. *Process Biochemistry* 36, 813 - 820.

Tiquia, S.M., Tam, N. F. Y., Hodgkiss, I.J. 1996. Microbial activities during composting of spent pig-manure sawdust litter at deferent moisture contents. *Bioresource Technology* 55, 201-206.

Tokiwa, Y., Buenaventurada, P. C. 2004. Degradation of microbial polyesters. *Biotechnology* 26, 1181 - 1189.

The non-affiliated soil analysis work committee. 1990. Handbook of standard soil testing methods for advisory purposes. *Soil Science Society of south Africa*, Pretoria.

Van der Ham, I. 2015. The effect of inorganic fertilizer application on compost and crop litter decomposition dynamics in sandy soil. Master of Science in Agriculture at University of Stellenbosch, page 1- 94.

Van Schoor, L, H. 2005. Guidelines for the management of waste water and solid waste at existing wineries. *Winetech*, South Africa. Available from:

http//www.winetech.co.za/index.php.

Van Schoor, L. H., 2001. Proposed criteria in the system for integrated production of wine for managing wastewater, solid waste, noise and air pollution. *Wineland*, Available from:

http://www.wineland.co.za/technical/proposed-ipw-criteria-for-managing- wastewater-solid-waste-noise-and-air-pollution.

Van Schoor, L. H. 2000. Management options to minimise negative environmental Impacts at wine cellars. *Wineland,* July. Available from:

http://www.wineland.co.za/technical/management-options-to-minimise-negative- environmental-impacts-at-wine-cellars.

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., van Sinderen, D. 2007. Genomics of *Actinobacteria*: Tracing the Evolutionary History of an Ancient Phylum. *Microbiology and Molecular Biology* 71 (3), 495 - 548.

Vergnoux, A., Guiliano, M., Le Dréau, Y., Kister, J., Dupuy, N., Doumenq, P. 2009. Monitoring of the evolution of an industrial compost prediction of some compost properties by NIR spectroscopy. *Science of the total environment* 407, 2390 - 2403.

Vuorinen, A. H. 2000. Effect of bulking agent on acid and alkaline phosphomonoesterase and *β*-glucosidase activities during manure composting. *Bioresource Technology* 75,113 - 138.

Vuorinen, A. H.1999. Phosphatases in horse and chicken manure composts. *Compost Science and Utilization* 7(2), 47 - 54.

DWAF (Water Act), 1998 (Act No. 36 of 1998). Government Gazette No. 20526. Department of Water Affairs and Forestry, Pretoria.

Wiles, C. C. 1978. Composting of refuse. In *Composting of Municipal Residues and Sludges*. Information Transfer Inc. and Hazardous Material Control Research Institute, Rockville, MD, U.S.A. page 20.

Willson, G. B. 1989. Combining raw materials for composting. *BioCycle* 30, 82 - 89.

Zingelwa, N. S. 2012. Management of filing and filter waste in wineries. Wynboer Technical Yearbook 2012.*WineLand Publications*, page 115 - 117.

Zingelwa, N. S. 2007. The environmental impacts and benefits of winery solid waste, filter material and fining agents. Available from: http://www.sawislibrary.co.za/dbtextimages/FinalReport144.pdf. Accessed: 11 /11/ 2014.

Zhang, H., Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y., Nakamura, K. 2003. Nakamura, K. 2003. *Gemmatimonas aurantiaca* gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum *Gemmatimonadetes* phyl. *International Journal of Systematic and Evolutionary (53)*, 1155 - 1163.

Zmora-nahum, S., Hadar, Y., Chen.Y. 2007. Physico-chemical properties of commercial composts varying in their source materials and country of origin. *Soil biology and biochemistry* 39(6),1263-1276.