

Constructing a Microbial Diversity Profile for Red Meat Abattoir Effluent

by
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Declaration of Independent Work

I, Nthuseng Phooko (student number #####), hereby declare that this research project submitted to the Central University of Technology, Free State, for the degree Master of Health Science in Environmental Health is my own independent work. This research project was conducted at the Central University of Technology, Free State under the supervision of Prof. O de Smidt and co-supervised by Prof. E Cason.

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Table of Contents

Declaration of Independent Work	ii
Acknowledgments	iii
Table of Contents	iv
List of Abbreviations	vii
List of Figures	viii
List of Tables	x
Abstract	xi
Chapter 1	1
Introduction	1
1.1 Background and Rationale	1
1.2 Problem Statement.....	2
1.3 Aims and Objectives.....	3
1.4 Chapter Layout.....	3
Chapter 2	6
Literature Review	6
2.1 Introduction.....	6
2.2 South African Water Legislation	8
2.3 Nature and Characteristics of Wastewater	10
2.4 Wastewater Treatment	11
2.4.1 Treatment processes utilised by municipal wastewater treatment plants	13
2.4.2 Problems experienced by WWTPs.....	14
2.5 Food Industry	15
2.6 The Red Meat Abattoir Industry.....	16
2.7 Abattoir Waste	18
2.8 Wastewater Generation Areas within a Red Meat Abattoir	19

2.8.1	Lairages	19
2.8.2	Stunning and bleeding area	19
2.8.3	Carcass dressing area	20
2.8.4	Pig scalding area	20
2.8.5	Rough offal	20
2.8.6	Miscellaneous wastewater sources	20
2.9	Handling of Effluent	20
2.9.1	Separation of blood	21
2.9.2	Screening of solids	21
2.10	Characterisation of Red Meat Abattoir Wastewater	21
2.10.1	Physico-chemical parameters	22
2.10.2	Microbial Diversity	29
2.10.3	Culture-dependent approach	30
2.10.4	Culture-independent approach	32
2.11	Conclusion	33
Chapter 3		35
Materials and Methods		35
3.1	Introduction	35
3.2	Study Area	35
3.3	Study Design	36
3.4	Sampling Site	37
3.5	On-site Sample Collection	38
3.6	Physicochemical Analyses	39
3.7	Biological Analyses	41
3.7.1	Culture-dependent analysis (enumeration)	41
3.7.2	Culture-independent analysis (genetic diversity)	44
3.7.3	Statistical analysis	47

Chapter 4	48
Results and Discussion.....	48
4.1 Introduction	48
4.2 Experimental Design	48
4.3 Sampling	48
4.4 Physicochemical Parameters	49
4.5 Biological Diversity	58
4.5.1 Culturable Microbiome	58
4.5.2 Functional Diversity.....	60
4.5.3 Genetic Diversity	67
Chapter 5.....	78
Correlations and Relationships	78
5.1 Introduction.....	78
5.2 Microbial Community and Related Environmental Parameters in Abattoir Effluent	78
5.3 Linking the Microbial Genetic Diversity profile and the functionality profile of the Effluent	80
5.4 Cluster Analysis to Compare the Microbial Diversity Profile	83
Chapter 6.....	87
Concluding Remarks.....	87
6.1 Introduction.....	87
6.2 Concluding Remarks	87
References	91
Appendix.....	115

List of Abbreviations

Abbreviation	Description	Abbreviation	Description
WWTP	Wastewater Treatment plant	HT	High Throughput
COD	Chemical Oxygen Demand	tCOD	Total Chemical Oxygen Demand
TS	Total Solids	sCOD	Supernatant Chemical Oxygen Demand
EC	Electric Conductivity	CAFSaB	Centre for Applied Food Sustainability and –Biotechnology
GDP	Gross Domestic Product	TKN	Total Kjeldahl Nitrogen
BOD	Biological Oxygen Demand	FOG	Fats, oils & Grease
TKNH	Total Kjeldahl Nitrogen	CLPP	Community-Level Physiological Profiling
DWS	Department of Water and Sanitation	TOC	Total Organic Compound
WSA	Water Services Authorities	NGS	Next Generation Sequencing
WSP	Water Service Provider	PCA	Principle Component Analysis
DO	Dissolved Oxygen	DWA	Department of Water Affairs
VOC	Volatile Organic Compound	RBC	Rose Bengal Chloramphenicol
SS	Suspended Solids	NA	Nutrient Agar
RMAA	Red Meat Abattoir Association	YPD	Yeast peptone Dextrose
TDS	Total Dissolved Solid	AWCD	Average well colour development
TVS	Total Volatile Solid	UV	Ultraviolet
STS	Settleable Solids	ITS	Internal Transcribed Spacer
PCR	Polymerase chain reaction	ASV	Amplicon Sequence Variants
PD	Phylogenetic Diversity	AHC	Agglomerative Hierarchal Clustery
D	Simpson index	H	Shannon index
E	Evenness	R	Relative Abundance

List of Figures

Figure	Title	Page
Figure 2.1	Schematic demonstration of different treatment processes in municipal wastewater treatment plants	12
Figure 2.2	Relationship between the different types of solids	25
Figure 2.3	The Nitrogen Cycle which is a biogeochemical process that explains how nitrogen is converted into the different chemical forms, consecutively passing from the atmosphere to the soil, organisms and back into the atmosphere.	26
Figure 2.4	The BioLog Ecoplate™ which contains 96 well plates of 31 carbon sources repeated in triplicate; the carbon sources are the most useful for community analysis. The other 3 wells contain water and are the control wells	31
Figure 2.5	The process of next-generation sequencing. A- Extracted gDNA from a sample, B- Preparation fragments of genomic DNA and addition of adaptors to generate a library, C- DNA fragments within the library are each sequence in parallel, D- Individual sequence reads are reassembled by aligning to a reference genome, E- The whole genome sequence is derived from the consensus of aligned reads.	33
Figure 3.1	Experimental Design of the study, depicting when sampling occurred and the different physicochemical, microbiological, and microbial diversity properties that were analysed.	37
Figure 3.2	Abattoir sampling site: Rotary screening machine that the effluent goes through before being discharged into the municipal sewer system. Solids in the effluent that are greater than the mesh are trapped and later recycled.	38
Figure 4.1	Specific livestock and the number slaughtered on each sampling day.	49
Figure 4.2	Boxplot showing the different solids within the effluent.	51
Figure 4.3	Boxplot showing tCOD and sCOD of the effluent.	53
Figure 4.4	Relative abundance of metabolic activities according to substrate AWCD of substrate categories consumed by microbial communities in the effluent.	54
Figure 4.5	A heat map showing different carbon groups with the specific carbon sources within that group, found on the Biolog Ecoplate™.	62
Figure 4.6	A rarefaction curve. To evaluate the sampling depth, rarefaction curves of the microbial communities based on 16S rRNA and ITS gene sequences are shown	63
Figure 4.7	Distribution of bacterial phyla and genera diversity expressed as relative abundance % of Amplicon Sequence Variants (ASV) in red meat abattoir effluent.	69
Figure 4.8	Distribution of Fungal phyla and genera diversity expressed as relative abundance % of Amplicon Sequence Variants (ASV) in red meat abattoir effluent.	71

Figure 5.1	PCA biplots showing the correlation between the various physicochemical parameters with identified and enumerated bacteria and fungi from abattoir effluent.	79
Figure 5.2	PCA biplots showing the correlation between the Carbon Substrate Groups with identified and enumerated bacteria and fungi from abattoir effluent.	82
Figure 5.3	Cluster Analysis AHC Dendrogram showing the order of relatedness among the diversity profile of the different Abattoir Effluent Samples.	84
Figure 5.4	The Dice Coefficient is determined by measuring the Euclidean distance between the Microbial Diversity in the Abattoir Effluent Samples.	84

List of Tables

Table	Title	Page
Table 2.1	The main South African legislation and institutions governing wastewater management.	9
Table 2.2	Quantifiable unit standards for Red Meat Abattoir slaughter units	17
Table 2.3	Mangaung Local Municipality by-laws relating to industrial effluent discharge.	28
Table 2.4	Advantages and disadvantages of microbial diversity approaches relevant to this study	30
Table 3.1	Physicochemical parameter analysis methods and ranges used for Spectrophotometer readings.	40
Table 3.2	Formulae for the calculation of indices	44
Table 4.1	The highest and lowest total viable microbial populations for abattoir effluent. The selective media used were conducive for different types of microbes; but for the purpose of this study, only the above-specified microbes were looked at.	59
Table 4.2	Richness and diversity indices of the physiological profile at the community level from BioLog Ecoplate™ analysis of the 10 red meat abattoir wastewater samples.	61
Table 4.3	DNA absorbance values for A_{260}/A_{280} , determining the DNA quality.	68
Table S1	Physicochemical parameters tested, including compliance to Mangaung Effluent Discharge by-laws	114

Abstract

Abattoirs are critical components of the global food supply chain as they provide essential resources for populations worldwide. However, the environmental footprint of these facilities, particularly through the generation of effluent, poses significant challenges to sustainable water management and environmental conservation. Abattoir effluent is distinguished by its high biological load, which refers to the concentration of organic materials including stomach content, blood, and large amounts of fats, oils, and grease (FOG). While the physicochemical properties of abattoir effluent have been extensively studied, little is known about the microbial diversity within the effluent. This knowledge gap represents a critical barrier to understanding the full environmental and health implications of abattoir effluent discharge, especially in regions where it is released into municipal sewer systems without adequate pre-treatment.

Understanding the microbial constituents of abattoir effluent can inform targeted interventions to remove harmful pathogens and reduce the organic load, thereby alleviating the burden on municipal wastewater treatment systems. Ensuring compliance with existing municipal by-laws and regulatory standards that govern effluent discharge is also important in alleviating this burden. This study aimed to construct a detailed profile of the microbial diversity present in red meat abattoir effluent by focusing on both genomic and metabolic capabilities as well as physicochemical compliance with local regulatory standards.

The sampling site was a high throughput red meat abattoir in Bloemfontein, Free State. An abattoir is considered to have a high throughput if it processes more than 20 units daily, with the unit count based on the type of animal slaughtered. Red meat abattoirs are facilities where various animals such as cattle, sheep, pigs, or horses are slaughtered. Daily sampling was performed for 10 consecutive days by collecting composite samples from the pipe through which effluent was discharged into the sewer system. Physical and chemical parameters were considered significant when characterising the abattoir effluent environment were analysed. Community Level Physiological Profiles (CLPP) were constructed using BioLog EcoPlate™ sole-carbon substrate utilisation analysis while culturable bacteria and fungi were quantified. Enumerations of total microbial load, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and yeast and mould were performed using rich (Nutrient) and/or selective agars (Harlequin®, Nutrient Agar and Rose Bengal

Chloramphenicol). The genetic diversity was determined through 16S- and ITS-targeted metagenomic sequencing on the Illumina MiSeq platform.

Physicochemical parameters indicated 37.5% non-compliance with the Mangaung effluent discharge limits. Non-compliance was attributed to high levels of solids, mostly volatile, high chemical oxygen demand (COD) due to the organic loads as well as high alkalinity levels. The environment had a neutral pH and low EC levels that were within the Mangaung effluent discharge limits while sufficient levels of dissolved oxygen (DO) were also detected. The temperature profile was consistent throughout ranging between 24°C-28°C. Nutrients such as phosphate, total organic carbon (TOC), sulphate, and chloride were also detected at levels that were within the discharge limits.

As expected, high microbial loads were noted, with bacteria being the more dominant microorganisms compared to fungi. Bacterial coliforms, *Pseudomonas aeruginosa* (8.04×10^5 CFU.mL⁻¹), *Enterobacter aerogenes* (1.18×10^5 CFU.mL⁻¹) and *E. coli* (2.78×10^4 CFU.mL⁻¹) were present. Low counts of yeast (5.32×10^3 CFU.mL⁻¹) and mould (7.10×10^1 CFU.mL⁻¹) were observed. CLPP displayed an abundant, diverse, and functionally active community. Simpson index (0.962) measurements displayed diversity, the evenness index (0.97) depicted the presence of an abundance of species, and the average well colour development symbolised a functionally active consortium. The CLPP showed consistency amongst all the sample days. The relative abundance of metabolic activities was related to polymer, carbohydrate, and amino acid category metabolism. Glycogen and L-asparagine were the most utilised substrates while 2-hydroxy benzoic acid and α -ketobutyric acid were not utilised.

The taxonomic bacterial and fungal biodiversity showed a broad similarity. Bacterial diversity showed consistent dominance of Bacteroidetes, Firmicutes, and Proteobacteria phyla. Genus richness varied in dominance distribution, with *Acinetobacter*, *Bacteroides*, and *Rikenellaceae_RC9_gut_group* being the most abundant genera with the dominance varying for each sample. Dominant fungal phyla were either Ascomycota or Neocallimastigomycota. Basidiomycota appeared in small, varied levels for each sample. The distinctive presence of Mucoromycota was observed on days when pigs were slaughtered, which deviated from the normative microbial composition encountered during the other sampling days, thus signifying an exception within the microbial community dynamics. The fungal community was dominated by a particular genus in each sample, namely *Neocallimastix*, *Caecomyces*, and *Ciliophora* respectively.

Both the culture-dependent and culture-independent analyses of the microbial content yielded noteworthy findings that indicated an exceptional degree of uniformity across all sample days. The calculated similarity rate ranging from 96-98% highlighted a distinct resemblance in microbial diversity among the samples. The effluent consistently exhibited a comparable microbial content except for sample day 6 phylum discrepancies, which accounted for the 2-4% difference in the similarity rate calculated. These findings strongly indicate a substantial overlap in microbiomes between the meat, stomach content, and hide of different animals, suggesting a commonality in the microbial species present in the effluent.

Given the limited information available on the microbial diversity of abattoir effluent, this research filled a significant gap in the environmental science and public health domain as it was able to contribute significant knowledge of the complex microbial ecosystems within abattoir effluent. The findings could pave the way for innovative treatment strategies using indigenous microbes and may thus contribute to more sustainable abattoir effluent discharge practices.

Chapter 1

Introduction

1.1 Background and Rationale

Wastewater generated from food production and agricultural activities is a significant source of environmental pollution worldwide, and such water poses a dire threat to water resources and ecosystems. As one of many related industries, red meat abattoirs have emerged as prominent contributors to water pollution while also presenting formidable challenges to waste management. Abattoir operations require substantial water usage to maintain stringent hygiene standards, resulting in the generation of copious volumes of wastewater characterised by high organic concentrations (Calverley & Walther, 2022; Ungureanu et al., 2020).

The scale of water consumption within abattoirs is staggering, with estimates suggesting that as much as 82% of the water utilised in these facilities is ultimately discharged as effluent. In South Africa, the demand for meat products has surged alongside population growth, leading to the considerable expansion in the number of abattoirs from 25 in 1988 to 420 in 2021 (Moreroa & Basitere, 2022; Water Research Commission [WRC], 2017). Despite South Africa's status as a comparatively minor global meat producer in comparison to countries like the United States, it grapples with severe strains on its water resources, and many of these challenges can be attributed to abattoir water pollution. This pollution exacerbates water scarcity challenges as abattoir effluent is often discharged directly into municipal sewer systems without adequate pre-treatment measures. Consequently, this practice places unnecessary strain on already overburdened sanitation systems and contributes to downstream contamination that jeopardises water sources and ecosystems (Iloms et al., 2020).

The prevalent lack of accountability and awareness regarding the environmental impacts of abattoir wastewater further compounds the issue, leading to a continuation of poor waste treatment and disposal practices. As a result, it has become imperative to address these challenges, which means

that there is a critical need for research to inform interventions aimed at improving wastewater management practices within the red meat abattoir sector (Mema, 2019).

1.2 Problem Statement

The environmental and operational challenges posed by abattoir effluent, particularly in circumstances such as in South Africa, are multifaceted as they not only directly impact ecosystems and public health, but also place a strain on wastewater treatment infrastructure. The literature broadly discusses the environmental impacts of abattoir effluent, treatment technologies, and microbial risks in an effort to alleviate the threats posed by this industry (Bustillo-Lecompte & Mehrvar, 2017; Shukri et al., 2017). For instance, abattoir effluent contains high levels of organic matter, nutrients, pathogens, and sometimes veterinary pharmaceuticals that, when discarded in effluent, significantly impact water bodies and ecosystems. This impact manifests as eutrophication and reduced levels of dissolved oxygen in water and causes harm to aquatic life, particularly when the effluent has not been adequately treated. Various treatment technologies that range from mechanical to biological processes have been explored in research that has aimed to reduce the pollution load. However, the effectiveness, cost, and practicality of these technologies vary, accentuating the need for adaptable and balanced wastewater management strategies (Mema, 2019.; Müller, 2017).

A notable gap in current research is the examination of how abattoir effluent affects municipal wastewater treatment works. Earlier research focused predominantly on the direct discharge of effluent into water bodies and the environmental and health implications of this practice, but the disruption caused to wastewater treatment works by high-strength effluent from abattoirs is a critical area that requires further investigation. Such effluent can compromise the operational efficiency of treatment plants due to the insufficient treatment of both the effluent itself and the municipal wastewater mixed with it. Inadequately treated wastewater that is released into the environment thus exacerbates environmental and health problems (Moreroa & Basitere, 2022; Phungela et al., 2022).

Furthermore, while significant attention has been paid to the physicochemical properties of abattoir effluent and its treatment, studies on microbial diversity have been less common. The microbial content of abattoir wastewater, which may even include pathogens, poses substantial health risks and this makes it essential to understand the threat through risk assessment and the development of effective treatment strategies (Carles et al., 2022; Sharma et al., 2023). The field also wrestles

with controversies and unresolved issues such as the inadequacy and lack of enforcement of regulatory standards, the need for the selection of sustainable and effective treatment methods, and the need to fully understand the microbial risks associated with abattoir effluent. Moreover, the potential for resource recovery from abattoir effluent presents a promising avenue for sustainable management, but it first requires practical solutions for widespread implementation (Eberhard, n.d.).

The concerns expressed about the impact of abattoir effluent on wastewater treatment operations in broad discussions on health-related threats highlight the complexity of managing such effluent and underline the need for comprehensive research that explores not only the direct environmental and health impacts of poorly treated abattoir wastewater, but also the operational challenges faced by wastewater treatment facilities. Addressing these gaps and challenges requires a thorough effort from researchers, industry stakeholders, and policymakers who need to work collaboratively to develop holistic and sustainable wastewater management solutions that will ensure environmental protection and public health safety now and in the future.

1.3 Aims and Objectives

The aim of this study was to assess and characterise the microbial diversity in red meat abattoir effluent. To achieve this aim, the study's objectives were to:

- I. Monitor effluent flow rates and determine suitable sampling intervals to obtain representative composite effluent samples;
- II. Analyse the physicochemical parameters of abattoir effluent to understand its composition and characteristics and to monitor compliance with local regulations;
- III. Employ conventional culturing to analyse the bacterial and fungal content of the effluent; and
- IV. Construct microbial diversity profiles using functionality and genomic diversity analyses to comprehensively characterise the microbial consortium present.

1.4 Chapter Layout

This thesis was structured in monograph (traditional) format and consists of six chapters.

Chapter 1: Background to and overview of the study

The problem statement, aim, and objectives of the study are presented in this chapter.

Chapter 2: Literature Review

The literature review chapter focuses on the intricate issues of water scarcity and pollution in South Africa. In its exploration of the prevailing challenges faced by water treatment works, the literature exposes the overwhelming implications of poor wastewater treatment practices as a key factor that worsens the water pollution crisis in this country. This chapter thus presents an overview of current wastewater treatment processes as well as water legislation and regulatory mechanisms. The literature discusses various attempts by regulators to enforce water compliance in industries, but argues that this has become a major problem. The chapter transitions to a perceptive analysis of abattoir wastewater treatment methodologies and sheds light on the current situation and the barriers delaying effective treatment processes. Furthermore, a breakdown of the sources contributing to red meat abattoir effluent generation is provided, while a comprehensive characterisation of the physicochemical, microbial, and genomic aspects of red meat abattoir effluent is also presented.

Chapter 3: Materials and Methods

The research methodology that was utilised to obtain abattoir waste water samples is discussed in detail. Composite effluent samples were taken for 10 consecutive days at a high throughput red meat abattoir in Bloemfontein in the Free State Province. A concise description of the methods used to assess the effluent's physicochemical properties is provided and the processes for constructing culture-dependant and culture-independent microbial components are discussed. The culture-dependent component comprised viable microbial counts and the determination of the CLPP functionality of the microbes, while the culture-independent component comprised 16S- and ITS-targeted metagenomic sequencing.

Chapter 4: Results and Discussion

The results that emerged from the analyses were obtained at both physicochemical and microbiological levels. Eight physicochemical parameters were analysed and monitored in terms of Mangaung Municipal by-laws and it was found that three of these were non-compliant. It was also found that pH, electric conductivity, fats, oils and grease, sulphates, and phosphates fell within acceptable limits, while total suspended solids, chemical oxygen demand, and alkalinity were above the limits. High viable bacterial counts were observed compared to lower yeast and mould counts. The functional metabolic activity displayed an abundant, diverse, and functionally active community. Genetic data showed both bacterial and fungal phyla to have common dominant species, but genus-level diversity differed.

Chapter 5: Correlations and Relationships

This chapter explores the correlations and relationships among the analysed data. The PCA findings revealed limited positive correlations between the microbial communities and physicochemical parameters. Culture-independent analysis determined that fungal diversity exhibited significant positive correlations with the most utilised BioLog EcoPlate™ carbon groups. The culture-dependent and culture-independent analyses of the microbial content revealed a high level of similarity across all samples, indicating a remarkable resemblance in microbial diversity among the samples.

Chapter 6: Concluding Remarks

This chapter concludes the dissertation by reiterating pertinent key findings and offering concluding remarks pertaining to the significance of the study.

Chapter 2

Literature Review

2.1 Introduction

Water is an essential natural resource for the sustainability of all forms of life. Apart from drinking it to survive, there are many other uses for water and it plays an important role in the economy, food production, health, and the environment. However, water quality has become a matter of great concern throughout the developing world (Halder & Islam, 2015).

Drinking water sources are under increasing threat of contamination, with extensive consequences for the health of people and animals and the economic and social development of communities and nations. Deteriorating water quality also threatens the achievability of the UN Sustainable Developmental Goals that target the rights of people who have to survive without access to safe water and sanitation infrastructure (Hailu, Ayenew & Mulu, 2015; Brown, 2016; Bustillo-Lecompte & Mehrvar, 2017; Seiyaboh & Izah, 2017; Ungureanu, Vladut & M, 2020). Freshwater scarcity and security have been recognised as global issues, and their consistent quantification is required to provide an integrated view of the water situation on our planet (World Economic Forum, 2016). The scarcity and inadequate quality of water in many areas around the world are mainly due to water pollution as well as increasing demands that persistently exceed available supplies (Abu-Zeid & Shiklomanov, 2004).

Water consumption is also increasing due to population and economic growth and this will continue at an accelerating rate if current attitudes and patterns of water utilisation and treatment remain unchanged. The rate of how much water is consumed varies depending on several factors, such as the nature of the development within a region and the availability of natural water sources (Akpore & Muchie, 2011; Ritchie & Roser, 2017). South Africa is both semi-arid and a water-scarce country due to its low average annual precipitation and the unevenness of surface and groundwater distribution. This situation results from its warm climate and geography, and thus water availability varies greatly throughout the country. For instance, the present water supply situation has created a false sense of water security within the privileged sectors of South African society (Ingrao et al., 2023). Marginalised

and poor communities have always experienced high levels of water insecurity. Currently, water scarcity is increasing in all parts of South Africa and the demand for water of potable quality is not being met in many areas. Although attention on water supply has primarily focused on water quantity, water use efficiency, and issues of water allocation, poor wastewater management has created serious water quality problems in many parts of the country, thus exacerbating the water crisis (Calverley & Walther, 2022; Itumeleng Phyllis Molobela, 2011; Martin Zhuwakinyu., 2012).

The quality and availability of water and sanitation services are of extreme importance if humans' living standards are to be improved and sustained. The most recent information has confirmed that efforts to extend access to these services are ongoing as several reform measures have been implemented by various stakeholders in the water sector to address challenges that have an impact on the reliability of water provision services and ultimately on the quality of water itself (Department of Water Affairs, 2012; Mavasa, 2023). However, these efforts are being constrained by knowledge and skills shortages within the sector. For instance, the latest Blue Drop Report by the Department of Water Affairs (DWA) revealed a decline in drinking water quality and an increase in non-revenue water from 2014 to early 2023, while the Green Drop Progress Assessment Report also indicated increasingly poor performances by municipal wastewater treatment agents (Department of Water Affairs, 2023a, 2023b). The Department of Water and Sanitation (DWS) has been endeavouring to provide some support to struggling municipalities and has also urged water services authorities (WSAs) with compromised water or wastewater systems to submit detailed corrective plans. However, the ongoing deterioration of water services that has been signalled by the Green Drop report has prompted the need for fundamental reform. To address this need, the DWS released the Water Services Amendment Bill for public comment. This Bill clarifies the roles of WSAs and water services providers (WSPs) and introduces a mandatory operating license system for WSPs. The latter are thus overseen by the DWS to ensure minimum competency and adherence to performance standards (Mavasa, 2023).

The situation the country currently faces puts water authorities under pressure to find new and innovative ways to achieve and sustain water of a desirable quality. In addition, the DWA also developed and introduced a wastewater discharge fee system that works on a 'polluter pays' principle (Department of Environmental Affairs, 2014). The aim of this initiative is to recover the costs associated with different wastewater treatment and water quality management programs and to provide incentives for large water users to treat their waste in-house rather than discharging it untreated into a water source. Wastewater charges will therefore be payable by polluters who exceed

pollution load standards, but unfortunately the implementation of this initiative had not yet commenced by the time this study was conducted.

The main culprits that contribute to wastewater and water quality problems are mines (acidity and increased metal content), industries (chemicals and toxins), agriculture (sediment and the destruction of soil nutrients), and urban development (the release of microbiological content and the destruction of nutrients) (Mitchell et al., 2014). Moreover, abattoirs are integral to the food processing industry and play a pivotal role in the food provision chain as they serve as primary facilities for the slaughter and processing of animals for human consumption. However, their role in compromising scarce water sources should not be underestimated considering that the supply of potable water in sufficient quantities and of required quality is important for the health of communities and the environment and for the economic growth of the country (Bhagwat & Vinod, 2019; García-Díez et al., 2023) .

The agricultural sector, which contributes 3% to the GDP, is one of the largest users of water as it utilises approximately 60% of South Africa's water resources. The lack of or poor quality of water is a major factor that impedes the growth of this sector as poor water quality harms agricultural exports and, in turn, diminishes foreign income. It is for all these reasons that the efficient management of the country's available water sources should be prioritised. To this end, South Africa has a well-structured legislative framework that governs water and sewage services (Department of Water Affairs, 2012) ; however, the question that is often posed is whether this framework is implemented effectively.

2.2 South African Water Legislation

The South African water sector does not have a distinct or independent regulator as there are multiple stakeholders involved in the provision and management of water and sanitation services. Key institutions are responsible for water and sewage service provision, oversight, the management of water resources, and the availability of water of good quality for human consumption and other purposes. Moreover, several pieces of legislation also guide both fresh- and wastewater management, and non-compliance with these laws could result in a demand for the payment of severe fines or even imprisonment (Eberhard, n.d.). Table 2.1 presents a summary of the legal framework that guides wastewater management in South Africa.

Table 2.1: The South African legislative framework and institutions governing wastewater management.

Legislation	Institutions
Constitution of South Africa (Act 108 of 1996)	Department of Water and Forestry (DWAF) The custodian of water resources and overall sector leader, policy maker, and regulator.
National Environmental Management: Waste Bill of 2007	Catchment Management Agencies (CMA) Responsible for water resources planning at the catchment level.
The Water Act (Act 36 of 1998)	Water Service Authorities All metropolitan, many Districts, and authorised local municipalities.
Environmental Conservation Act (Act 73 of 1989)	Water Service providers Agencies that assume operational responsibility for providing water and/or sanitation services as a bulk or retail service.
Water Services Bylaws	Water Bodies Regional or bulk water service providers, sell water to or accept wastewater from other water service providers.
Green Drop Certification Programme	All metropolitan areas, many Districts, and authorised local municipalities.
National Environmental Management Act (Act 107 of 1998)	Department of Water Affairs
South African Water Quality Guidelines (TWQGR)	Department of Water Affairs and Forestry
The Health Act (Act 63 of 1977)	National Department of Health

Source: Adapted from the Department of Environmental Affairs and Development Planning, 2015

2.3 Nature and Characteristics of Wastewater

Wastewater generation is an inevitable consequence of human activities that cause modification to the original characteristics of water such as contaminating it and making it unfit for further use. Wastewater or effluent refers to any water which has been negatively affected in quality by anthropogenic activities. These waste products can be liquids or solids and can be composed of a range of negative biological, chemical, or radioactive properties (Naidoo & Olaniran, 2013).

Municipal wastewater is produced in homes and by businesses and industries and is referred to as domestic or industrial wastewater (or effluent) depending on its origin. Groundwater, stormwater, and surface waters also enter the wastewater collection system and add to the volume of wastewater. The latter types of wastewater characteristically contain pollutants; for instance, domestic wastewater may contain faecal and vegetable matter, grease, scum, detergents, rags, and sediment, while industrial wastewater commonly contains toxic chemicals and metals, concentrated organic waste, radioactive matter, large volumes of sediment, and high-temperature or acidic waste. Moreover, much of the ground, storm, and surface wastewater contains motor oil, gasoline, pesticides, and sediment (Mothetha, 2016).

Dumping of raw effluent into water courses often causes irreversible damage to the environment, while the discharge of untreated and inadequately treated effluent into water courses poses a risk to public health. The most significant environmental threat to public health due to untreated effluent is inadequate access to clean drinking water and sanitation facilities. Additionally, while direct discharge of effluent is a serious problem in South Africa, some wastewater treatment plants (WWTPs) are generally ill-equipped to remove large quantities of non-biodegradable waste and recalcitrant heavy metals from the water they treat, and the resultant contaminated water is eventually discharged into surface water sources (Mema, 2019). Most WWTPs also do not have sufficient capacity to treat the volumes and nutrient load of wastewater and sewage influents on site. Inflow loads that are beyond the capacity of WWTPs result in malfunctioning, poor operations, poor maintenance, and poor levels of wastewater and sewage purification. The result is that contaminated effluent is discharged into natural environments, and this negative consequence makes effective wastewater treatment a pivotal concern in various areas (Iloms, 2018; Iloms et al., 2020).

2.4 Wastewater Treatment

Effective wastewater treatment is a global imperative as municipal and industrial effluent should be appropriately treated before it is discharged into a water body or the environment. The conventional way of treating effluent is in a WWTP where a series of physical, chemical, and biological processes are used to reduce the concentration of contaminants before the effluent is discharged. The mandate of any WWTP is to minimise all risks to the environment and the population (Metcalf et al., 2003).

Effluent that is generated by domestic and industrial activities is a major cause of the pollution of natural water. Poor wastewater management could therefore lead to a point-source pollution problem and, to solve it, high treatment costs are incurred while a wide array of chemical pollutants and microbial contaminants are released into natural water sources (Iloms, 2018). Two fundamental reasons for treating wastewater are: (i) to prevent the pollution of water sources, and (ii) to protect public health by safeguarding water supplies against the spread of disease. The prevention of these threats is accomplished by utilising a process that separates and settles solids to create an acceptable quality of effluent and by removing substances that have high oxygen demand through metabolic reactions of microorganisms, thus removing excess microorganisms from the water system (Akpor & Muchie, 2011b; Sathya et al., 2022).

In a municipal wastewater system, the common water quality variables of concern are biological oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), biodegradable organic compounds, volatile organic compounds (VOC), toxic metals, suspended solids (SS), nitrates, ammonia nitrogen, phosphates, salinity, microbial pathogens, and the presence of a range of trace metals. High concentrations of these pollutants above the limits set by national and international regulatory bodies negatively impact receiving water bodies as they cause a major challenge in wastewater systems such as eutrophication and different health impacts on humans and animals (Verlicchi & Grillini, 2020). In the past, wastewater treatment plants' operations were limited to the removal of organic matter and suspended solids (Stare et al., 2007). Today, due to the efforts and results of research investigations, these plants also have to focus on the removal of substances such as nitrogen, phosphorus, odours, volatile organic compounds, metals, and toxic organics that commonly exacerbate the quality of the wastewater found in treatment plants (O'Dwyer et al., 2020; Zaborowska et al., 2021).

The main objective of the wastewater treatment process is the reduction, or preferably the removal, of organic content (i.e., BOD) and trace organics that are recalcitrant to biodegradation and that may be toxic or carcinogenic. Such materials include toxic metals and nutrients such as nitrogen and phosphorus. Removing them reduces the pollution levels of receiving surface waters or groundwater by minimising the prevalence of pathogenic microorganisms and parasites in the effluent (Akhtar et al., 2021; Bitton, 2010). According to a report by the Water Research Commission (Oliveira & Von Sperling, 2008), wastewater treatment technologies and practices commonly used in South Africa include screening, de-gritting, oxidation ponds, primary settling, biological filters, activated sludge treatment, settling, maturation ponds (natural UV treatment), wetland polishing treatment, disinfection, sludge thickening, sludge drying beds, and anaerobic digestion. These treatment processes are illustrated in Figure 2.1.

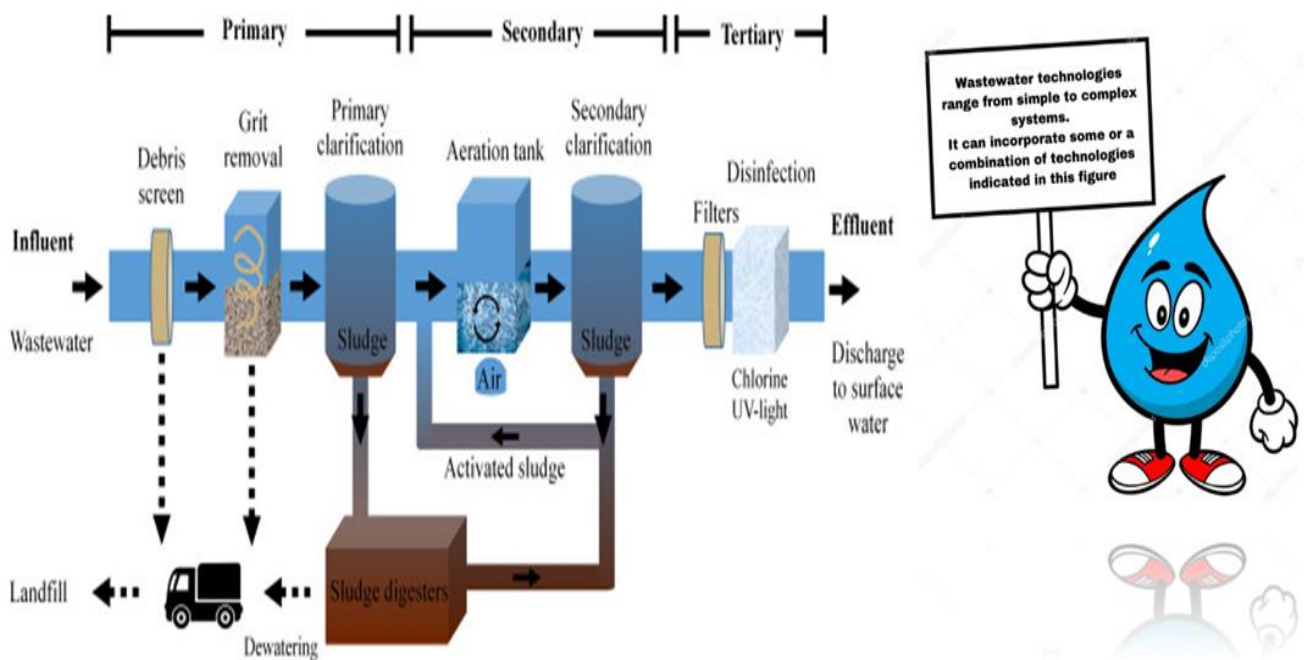


Figure 2.1: Schematic illustration of the treatment processes in municipal wastewater treatment plants

Source: Water Research Commission, 2016

2.4.1 Treatment processes utilised by municipal wastewater treatment plants

2.4.1.1 Preliminary treatment

Preliminary treatment is the first treatment process as the waste enters the WWTP. It removes large solid and grit materials that can hinder the flow through the treatment process. These materials are removed by screening and preventing them from getting caught in the mechanical equipment and causing abrasions or blockages in the pumps. These particles are commonly disposed of at an approved landfill. It is estimated that this stage removes about 60% of total suspended solids and only 10 - 20% of total nitrogen and total phosphorus (Department of Environmental Affairs, 2014).

2.4.1.2 Primary treatment

Primary treatment comprises a mixture of biological and physical processes such as screening and sedimentation. The velocity of the water is lowered or it is left to stand, causing the solids to sink to and settle at the bottom while floatable materials such as oils and grease rise to the top. The suspended solids are scraped off the bottom and the oil and grease (scum) are washed off with water jets. The scum and the solids are then collected and combined to form sludge and sent off for treatment or are disposed of. If not removed, these materials cause odour problems and attract vectors that could spread infections (Department of Environmental Affairs, 2014).

2.4.1.2 Secondary treatment

In the secondary treatment process, the dissolved organic material in the wastewater is used as food for microorganisms. As the microorganisms consume the organic matter, they convert it into more microorganisms that are then separated from the water by gravity settling. This can be accomplished in many different types of secondary treatment systems such as trickling filters, rotating biological contactors, activated sludge, and lagoons (Mothetha, 2016). According to Naidoo and Olaniran (2013), this stage removes 90% of organic nitrogen, organic phosphorus, and heavy metals associated with solids when they settle in the tank and form sludge.

2.4.1.3 Tertiary treatment

During tertiary treatment, physical and chemical processes are used to further remove BOD, nutrients, pathogens, parasites, and sometimes toxic substances (Bitton, 2010). The sludge is converted to biogas, which is a mixture of methane (CH_4) and carbon dioxide (CO_2), that is then used to generate electricity for the plant.

The liquid is treated by bacteria that break down the organic matter remaining in the solution. It is then sent to the oxidation ponds where heterotrophic bacteria continue the breakdown of the organics and solar UV light destroys the harmful bacteria (de Souza & Moorgas, 2016). Disinfection is the final step in this process before the wastewater is released into the environment. This can be done in several ways which include chlorination, ozonation, ultraviolet light exposure, and long detention times in lagoon cells. Chlorination is the most popular method of disinfection.

2.4.2 Problems experienced by WWTPs

The chemical and physical contamination of water sources that occurs both naturally and due to pollution is a very serious problem, but even more serious is the microbiological contamination of drinking water sources. As populations, pollution levels, and environmental degradation increase, so does the chemical and microbiological contamination of water streams. This is due to a variety of factors, including population pressure, urbanisation, and the inadequate construction, operation, and maintenance of wastewater treatment systems (Edokpayi, Odiyo & Durowoju, 2017). The increasing demand for water and large volumes of untreated effluent cause strenuous operations within WWTPs. This occurs concomitant with a decline in the state of municipal wastewater treatment infrastructure, which is a key contributing factor to multiple pollution problems experienced in most parts of the country as well as the health problems in poor communities. Increasing concerns have therefore been raised regarding the influence of untreated or poorly treated effluent from industries on the already overburdened sanitation systems across South Africa (Mema, 2019).

Water is an important requirement in industrial processes such as production, cooling, cleaning, and rinsing and it is an undeniable fact that industries generate a considerable proportion of the total volumes of wastewater that are released into the environment. Industrial discharge can contain a wide range of contaminants that originate from numerous sources. Some of the biggest generators of toxic industrial wastewater are mines, pulp mills, pharmaceutical industries, and food/agricultural producers (Muralikrishna & Manickam, 2017). In many undeveloped countries, wastewater from industries is not only discharged directly into water sources, but also seeps into the ground where it contaminates groundwater. Polluted water supplies therefore often go undetected as monitoring is expensive and, even if pollution is detected, remediation generally does not occur as the source of the pollution must be addressed and decontamination carried out at the same time, which can be extremely difficult and costly (Edokpayi, Odiyo & Durowoju, 2017).

In many countries, including South Africa, industries that are located within municipal jurisdictions discharge their effluent into municipal sewer systems and it becomes the taxpayer's and municipalities' responsibility to treat and dispose of that wastewater. In the absence of a 'user pays' system for pollution control, large volumes of contaminated industrial wastewater end up in municipal sewage treatment plants that are expensive to construct, operate, and maintain. To contain this threat, South Africa introduced municipal by-laws and Green Drop regulations that govern effluent discharge limits according to the operational capabilities of the receiving WWTP (Department of Water Affairs, 2011).

According to the Department of Water and Sanitation (2017), it is the responsibility of the industry to establish monitoring programs to assess the wastewater quality and quantity, to address any failures in the discharge system, and to report any threats to the local authority. This form of self-regulation aims to ensure that industries reduce pollution at source rather than opting for the more expensive end-of-pipe solution of government WWTPs (van der Merwe-Botha & Manus, 2011). Industrial effluent is usually pre-treated before being discharged into sewage systems to reduce pollution loads and to avoid disturbing the wastewater treatment process. Industrial effluent that is discharged into a sewage system may adversely affect the operation of a municipal WWTP. For instance, different flow rates, extreme concentrations of pollutants, and pollutants that are potentially resistant to biological treatment may disturb technological processes, increase operational costs, and deteriorate the quality of the WWTP effluent. If the WWTP cannot deal with such problems, it causes the discharge of inadequately treated effluent into receiving water bodies, and water bodies that are contaminated by abattoir effluent pose significant environmental and public health hazards (Bitton, 2010; Department of Water and Sanitation, 2023b; Mema, 2019).

2.5 Food Industry

The food and beverage industry is one of the most important sectors in terms of financial and social significance due to its continuous growth. Whenever food in any form is handled, processed, packaged, and stored, there will always be the accompanying generation of wastewater, and this is why the food industry is associated with high emissions of organic waste into water resources (Al-Rumaihi et al., 2020). Wastewater generated from food production and agricultural activities is a major cause of environmental pollution. Such waste is also amongst the most difficult and costly to manage because food processing effluent can contain large quantities of nutrients and organic carbon as well as nitrogenous organic, inorganic, suspended, and dissolved solids. Moreover, it is

also associated with high biochemical and chemical oxygen demands. The most serious environmental impacts are high levels of water consumption, wastewater discharge, and energy consumption. The effluent must be treated to levels that will not damage receiving waters when directly discharged into the environment or that will not disrupt WWTP processes due to excessive nutrients or oxygen demand when discharged into municipal sewers (Noukeu et al., 2016). Examples of food produced by this industry include meat, poultry, and dairy products, fruit and vegetables for canning and preserving, grain products, sugar, oils, beverages, and brewing, among others. The large variety of food and agricultural waste presents different challenges as special factors have to be considered with each waste type, such as technical performance issues and the seasonality of production which add to the complexities of the treatment choices and operations in these industries (Cotruvo, 2018).

Knowledge of the characteristics of wastewater is essential for the development of economically and technically viable management systems that follow current environmental policies and regulations. Management methods that may be adequate for certain industrial wastewater may be less feasible for food and agricultural wastewater unless these methods are modified to reflect the characteristics of the wastewater produced and the opportunities it may pose. Wastewater that results from agricultural activities and food processing varies in quantity and quality, with the streams from fruit and vegetable processing typically having low strength and high volumes and those from animal operations having high strength and low volumes. These differences in quantity and quality dictate the type and capacity of wastewater management systems that should be utilised (Climate Policy Watcher, 2020).

2.6 The Red Meat Abattoir Industry

The South African red meat industry is one of the main producers of red meat in Africa as it contributes to 21.4% of meat production in Africa and 1% globally. Red meat provides more than a third of the protein needs of the African population. The key determining factor of the quantities of meat produced in an area is the number of feedlots and abattoirs that are located there and not the size of the livestock herds in that area. Meat is then produced and distributed all over South Africa and its neighbouring countries (Swanepoel, 2014; Bustillo-Lecompte & Mehrvar, 2017).

An abattoir is a specialised facility that must be approved and registered by the regulatory authority that is mandated to inspect the animals and ensure the hygienic slaughtering, processing,

preservation, and storage of meat products before they are released to the public for human consumption. Abattoirs are obligated to produce edible and fit-to-consume meat for the public; however, large quantities of secondary organic and inorganic waste is generated in the process. Due to the nature of its operation, the abattoir industry contributes significantly to air pollution, loss of biodiversity, climate change, land degradation, and water shortage and pollution (Pinto et al., 2022; Red Meat Abattoir Association [RMAA], 2011; Tolera et al., 2022).

Abattoirs in South Africa are graded by the number of units that can be processed in a specific time frame. According to the Gauteng Department of Agriculture and Rural Development (GDARD) (2009), abattoirs use a quantifiable standard to determine their throughput of slaughter units. An abattoir is therefore graded according to its throughput capacity and its structural requirements. The unit standards for and grading of red meat abattoirs are presented in Table 2.2.

Table 2.2: Quantifiable unit standards for red meat abattoir slaughter units

Category	One slaughter unit =
Red Meat	1 cow, ox, or bull (including heifers) 2 calves younger than 6 months 1 horse 6 sheep or goats 4 small pigs 2 bacon pigs 1 sausage pig
Category	Slaughter units per day
Rural abattoir	0-2
Low throughput abattoir	3-20
High throughput abattoir	>20
Grade A	More than 100
Grade B	51-100
Grade C	16-50
Grade D	9-15
Grade E	1-8

2.7 Abattoir Waste

Abattoir waste is defined as “the collective by-products of an abattoir that are not fit for human consumption and are then discarded”. This waste results because of the nature of the slaughtering and operational activities that are employed at an abattoir. The main purpose of an abattoir is to recover the edible parts of slaughtered animals for human consumption (Red Meat Abattoir Association [RMAA], 2011). During the slaughtering process, substantial volumes of waste material are generated that can be divided into solid, liquid, and gaseous waste. Solid waste consists mainly of bones, undirected ingests, and hairs; liquid waste consists of blood, urine, water, dissolved solids, gut content, and cleaning detergents; and gaseous waste comprises of odour and gaseous emissions (Roberts, de Jager & Blight, 2009; Bandaw & Herago, 2017).

The red meat abattoir industry is one of the largest water-consuming industries world-wide. Globally, agriculture accounts for 92% of the global freshwater footprint while 29% of the water that is used in agriculture is directly or indirectly used for animal production. The wastewater pollution streams generated by this industry depend on the size of the abattoir, the units slaughtered, the type of meat that is produced (cattle, pigs, sheep, poultry), and the processes subsequent to slaughtering such as the cleaning of the facility (Charlebois & Summan, 2014).

The increasing demand for meat products has resulted in an increase in abattoir facilities and escalating volumes of wastewater. The concentration of organic matter in abattoir effluent is usually high while residues are moderately solubilised, leading to a polluting effect due to high levels of organics and pathogens present in the wastewater as well as detergents used for cleaning purposes (Singh, 2018). Water consumption and waste generation differ among the various categories of abattoirs, which means that the variation in water use among abattoirs results from operations within facilities, the scale of these operations, what it is that regulations require and how they are implemented, and other circumstances such as the cost and availability of water.

It is mandatory for the red meat abattoir industry to monitor the effluent that it generates and that is discharged into municipal sewer systems to ensure that it complies with municipal by-laws that govern discharge limits according to the operational capability of each receiving WWTP. Municipal by-laws must thus be adhered to by abattoirs and administrative bodies as a tool for pollution control (Water Research Commission, 2016). If this and other industries are not compliant given the limited resources and skills in most municipalities, legislative control alone cannot address the problem of contaminated wastewater, thus the development of a practical but scientific-based guide to enable

the effective management of polluted effluent has become vital. Developing this guide requires detailed understanding of the processes that generate such effluent, a complete characterisation and quantification of the effluent stream, and an understanding of the economic limitations faced by the industry that may hinder pollution control and prevention (Ntuli, 2012).

2.8 Wastewater Generation Areas within a Red Meat Abattoir

According to a Water Research Commission report on water and wastewater management in the red meat abattoir industry, the use of water in abattoirs varies greatly among the process areas in these facilities (Müller, 2017). The average water consumption of a red meat abattoir is approximately 900 L per slaughter unit, while the total volume of water required depends on the size of the abattoir. However, regardless of their different operational practices and processes, a considerable volume of effluent is generated by all abattoirs while approximately 82% of freshwater that is consumed at an abattoir is discharged as wastewater (effluent) that contains high organic loads. This effluent contains blood from the slaughtered animals, solids such as meat and pieces of bone, as well as grease/fat. Effluent is generated in different areas in red meat abattoirs that are briefly discussed below.

2.8.1 Lairages

Animals excrete urine and faecal matter while they are being kept in lairages before slaughter. Livestock is usually held overnight or for some time before being slaughtered to allow the animals to rest, and thus the volume of excreta is high. These lairages are supposed to be dry-cleaned by using a broom to sweep the holding surface before washing it down using a high-pressure hose, but the floors are often just washed down without sweeping, which means that a large volume of solids are washed into the wastewater (Hearle, 2016).

2.8.2 Stunning and bleeding area

In many abattoirs, instead of blood being separated and discarded or reused, it is washed down the sewer as part of the continuous cleaning process. Blood is one of the biggest wastewater pollutants in the red meat abattoir industry. Raw blood contains a COD of about 400 000 mg.L⁻¹, which is extremely high. This concentration significantly surpasses the regulatory threshold of 5000 mg.L⁻¹, as prescribed by a Mangaung effluent discharge by-law. Moreover, a constant flow of water is used in abattoirs to sterilise equipment such as evisceration pans and slaughter knives (Müller, 2017).

2.8.3 Carcass dressing area

During the dressing process, pieces of meat, fat and bones from carcasses are deposited on the floor. These are supposed to be dry-swept and removed but, in some cases, they end up being swept away by wet-broom operations along with the wastewater. Carcasses then receive a final wash which causes excess contamination elements such as bone chips, pieces of fat, meat, and blood to be washed down into the sewer (Cross & Singh, 2020).

2.8.4 Pig scalding area

There is a continuous flow of hot water for scalding the hard skin of pigs and sterilising equipment and this water is discarded as wastewater that contains hairs from pigs' skins (Müller, 2017).

2.8.5 Rough offal

In this area, paunches and intestines are slit open and receive an internal rinse to remove patch and gut content. Large quantities of water are used in this area and very high levels of contamination occur if the water is not treated effectively (Water Research Commission, 2016).

2.8.6 Miscellaneous wastewater sources

Large volumes of effluent are generated at the end of each day when intensive cleaning of the premises is done. Process areas, fittings, and floors are washed, rinsed, and disinfected using sanitisers, detergents, and/or enzymatic cleaning systems. For modern cleaning purposes, cleaning agents that are complex mixtures of alkaline, acid, or neutral chemical substances are used. Other sources of wastewater are laundry, boot washing, and knife sterilising basins (Hamawand et al., 2017).

2.9 Handling of Effluent

For most abattoirs in developing countries, the easiest method to dispose of their effluent is sending the effluent into existing pools, rivers, or lakes. However, this practice, which is not compliant with current international and South African legislation, leads to pollution problems in terrestrial and aquatic environments and may affect the health of humans and animals in surrounding areas. It therefore follows that such effluent needs to be disposed of sustainably. To ensure the safe disposal of liquid and solid waste, abattoirs are required, at the very least, to use the following methods before discharging effluent (Bustillo-Lecompte & Mehrvar, 2017):

2.9.1 Separation of blood

Blood from slaughtered animals contains nitrates that coagulate into a solid mass that will block open and closed drains. It is therefore recommended that the blood is collected and used in products that are suitable for human consumption, stock feed production, fertilisers, or the pet food market.

2.9.2 Screening of solids

Discarded and suspended solids and fats must be screened by inserting vertical sieves in drains that act as filters that catch the solids but let the water through. The solids can then be discarded separately or reused for other viable purposes. All South African abattoirs are obliged to follow a strategic approach towards water and waste management in accordance with the Department of Water Affairs' water conservation, waste minimisation, and progressive waste treatment philosophies. These practices should be employed in conjunction with good housekeeping and low-cost technologies for recovery and treatment of slaughtered animal waste. Toxopeus (2019) argues that water-use licenses and permits for on-site disposal should be mandatory to ensure that abattoirs will incrementally progress towards predetermined water quality and waste management objectives that include minimising waste generation at source, curbing the practice of washing solids into drains, and promoting research into cleaner technology and better recovery of high-value products from the waste stream.

2.10 Characterisation of Red Meat Abattoir Wastewater

According to Mittal (2004), Bustillo (2017), and Benit (2015), abattoir wastewater quality depends on water usage, the type of animals slaughtered, and the nature of the rendering or processing that are done on site (Seiyaboh & Izah, 2017; Ungureanu et al., 2020). Three major categories of pollutants are commonly detected in abattoir effluent:

- **Disease-causing agents:** These are viruses, parasites, and bacteria. Because of the richness of these microbes, the effluent becomes a source for the transmission of diseases such as cholera and typhoid.
- **Oxygen-demanding waste:** This waste consists of biodegradable matter such as plant residues and animal manure. This biological waste uses oxygen present in the effluent and results in oxygen depletion.

- **Water-soluble inorganic pollutants:** These are salts, acids, and metals and also include ammonium salts, nitrates, and phosphates that are important nutrients. These nutrients favour algae growth which causes eutrophication.

Abattoir effluent is composed of high concentrations of suspended solids and may include fat, grease, hair, feathers, flesh, manure, grit, undigested feed, blood, as well as about 80% fresh water. This organic matter provides a nutrient-rich source for microbial growth and reduces the dissolved oxygen contained in wastewater, resulting in foul odours and darkening of water. Blood and undigested feed constitute the highest pollution loads and account for high COD and BOD levels (Shukri, Kyambadde & Hawumba, 2017).

2.10.1 Physico-chemical parameters

Several physical and chemical parameters are considered when characterising wastewater. For instance, Wu and Mittal (2012) published a review article on the characterisation of effluent from abattoirs for land application. Numerous studies have determined parameters associated with abattoir effluent depending on the operating practices of the premises; however, certain parameters are consistent and appear to be more important than others in abattoir effluent (Benit & Roslin, 2015).

2.10.1.1 Temperature

It is a measure of the intensity of heat stored in a volume of water and is measured in °C. Water temperature is very important because it controls the rate of all chemical reactions and regulates various abiotic characteristics and activities in water (Benit & Roslin, 2015).

2.10.1.2 pH

The pH value is measured to determine the acidity or alkalinity of water and the number expresses the concentration of hydrogen ions indirectly. A value of 7 is neutral, less than 7 is acidic, and greater than 7 is base saturation or alkaline. The pH changes in water are governed by the amount of free CO₂, carbonates and bicarbonate and is an important parameter in evaluating the acid-base balance of water. The pH concentration range for the existence of biological life is quite narrow and is typically 6-9. Conditions that favour the production of hydrogen ions result in the lowering of pH and is referred to as the acidification process, while conditions favouring the neutralisation of hydrogen ions result in an increase in pH, which is referred to as the alkalinisation process (Talukder & Selvakumar, 2015).

2.10.1.3 Alkalinity

Alkalinity is understood as the ability of water to neutralise acid or absorb hydrogen ions . It comprises carbonate and bicarbonate and acts as a stabiliser for pH. The need to maintain a proper pH balance makes its evaluation a crucial part of wastewater treatment. Factors that contribute to alkalinity include the type of dissolved inorganic and organic compounds present, the quantity of suspended organic matter, whether the water is strongly or weakly buffered, the presence or absence of free hydroxyl alkalinity, and the quantity of bicarbonate in the water. The quantity of bicarbonate to dissolved CO₂ ratio is indirectly correlated to the number of dissolved solids (Marietta, 2014).

2.10.1.4 Electric conductivity (EC)

EC is a measure of the ability of water to conduct an electric current. This ability is the result of the presence of ions in the water such as chloride, bicarbonate, nitrate, and sodium, all of which have the ability to carry an electrical charge. Most organic compounds dissolved in wastewater do not dissociate into ions, therefore they do not affect the EC. The EC depends on dissolved solids in the effluent. High EC values indicate the presence of a high amount of dissolved inorganic substances in ionised form while fluctuations in EC in any location depend on the fluctuation in total dissolved solids (TDS) and salinity (Wu & Mittal, 2012).

2.10.1.5 Dissolved oxygen (DO)

The DO is a measure of the degree of pollution by organic matter, the destruction of organic substances, and the self-purification capacity of the water body. DO is associated with the corrosives of water and photosynthetic activity and is essential for many chemical reactions such as oxidation of metals and the decomposition of dead and decaying matter. The actual quantity of oxygen that can be present in a solution is governed by the solubility, temperature, and partial pressure of the atmosphere as well as the concentration of impurities, such as salinity and suspended solids, in the water (Muller, 2004; Benit & Roslin, 2015).

2.10.1.6 Biochemical oxygen demand (BOD) and chemical oxygen demand (COD)

Oxygen demand may be in the form of BOD or COD and can be defined as the amount of oxygen used by microorganisms as they degrade organic solids in the wastewater. BOD is the amount of dissolved oxygen required for the biochemical decomposition of organic compounds and the oxidation of certain inorganic materials. COD measures the oxygen equivalent of that portion of the organic matter in a sample that is susceptible to oxidation by a strong chemical oxidant (Mittal, 2004).

The COD and BOD values are measures of the relative oxygen-depletion effect of a waste contaminant. COD is similar in function to BOD, but BOD measures only biodegradable organic matter while COD measures the oxygen equivalent of the organic material that is oxidised chemically and biologically. The COD value is usually higher than that of BOD because some organic materials in water are resistant to microbial oxidation and hence not involved in BOD. The COD test can be completed in 2.5 hours compared to the 5-day period required for the BOD test (Mittal, 2004).

2.10.1.7 Total organic carbon (TOC)

TOC measures the organic compounds in various oxidation states and the amount of CO₂ produced during the oxidation of this organic material, which determines the degree of organic contamination. The larger the carbon or organic content, the more oxygen is consumed. A high organic content means an increase in the growth of microorganisms which contributes to the depletion of the oxygen supply (Talukder & Selvakumar, 2015).

2.10.1.8 Fats, oil, and grease (FOG)

These compounds create a thin film on the surface of water which impedes the exchange of air and water, thereby increasing oxygen demand in the water. Abattoir wastewater contains animal fats that are made up of long-chain fatty acids and glycerol. Fats can be difficult to hydrolyse and can diminish process performance by coating the granules of organic materials, attaching to the granules, and causing washout or inhibiting activity. Fats clog pipes, pumping systems, and screens and can reduce oxygen transfer, thereby seriously reducing the efficiency of an aerobic treatment system (Mittal, 2004a).

2.10.1.9 Solids

According to (Mittal, 2004), the detection of total solids is an indication of the amounts of solids in a liquid, but it does not describe what kinds of solids are present. Total solids can be broken down based on two distinctions: (i) if the solids are suspended or dissolved in liquid; or (ii) if they are organic or inorganic material. Figure 2.2 depicts the relationship between different types of solids. Solids are an important constituent to measure as effluent solids represent a pollutant load in addition to a potential sediment load in the effluent from a receiving plant.

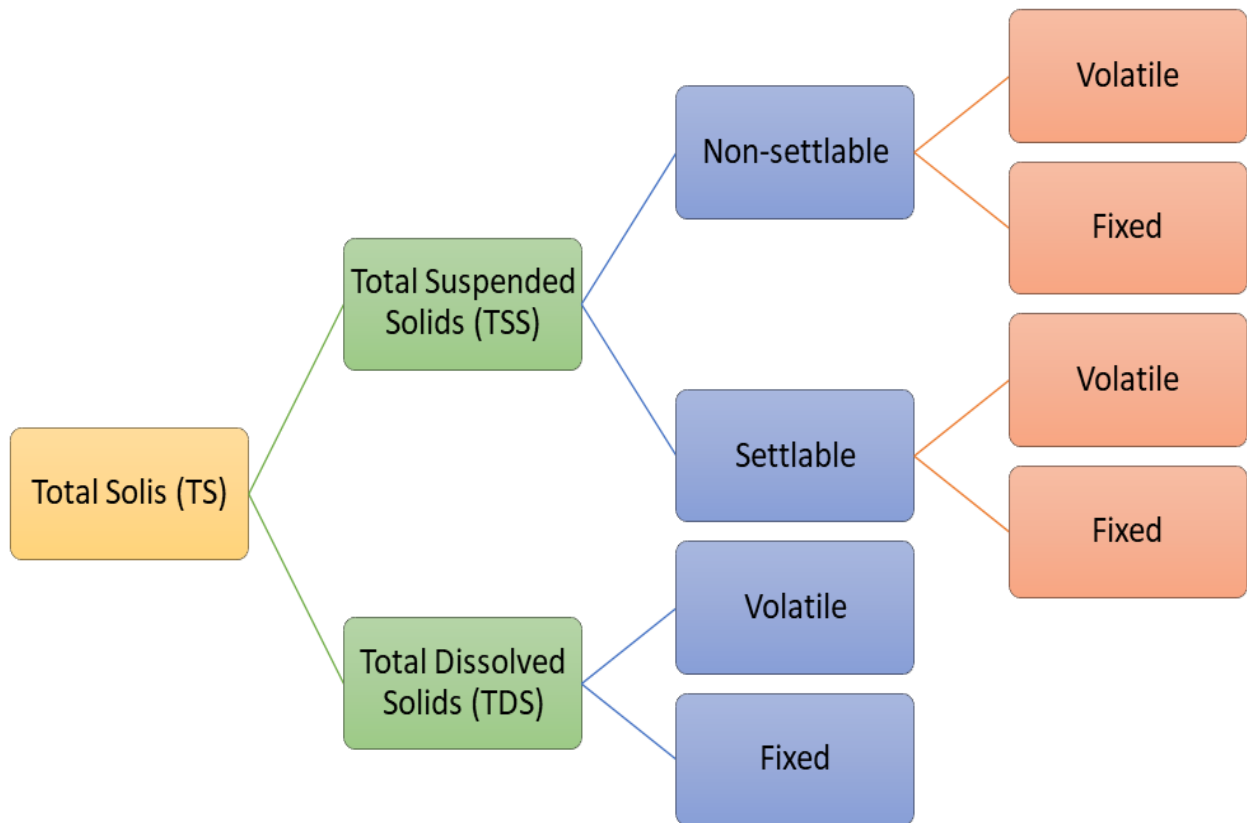


Figure 2.2: Relationship between the different types of solids in wastewater treatment

Suspended solids (SS) and settleable solids (STS): These are residual matter that is insoluble and is suspended in the wastewater and can also settle out of wastewater. STSs constitute that portion of suspended solids that will become sediment and settle out of suspension after a certain period, which is a process that generally occurs when the wastewater is stagnant. When effluent enters the treatment plant, all particulate solids are suspended due to water velocity. Their concentration in the water determines the design of the treatment process and the degree of treatment required and it indicates changes in influent water quality.

Total dissolved solids (TDS): This refers to the quantification of the number of inorganic salts and small matter that are dissolved in water. The concentration is directly proportional to the EC.

Total volatile solids (TVS): These are solids in water that are organic and that combust and volatilise at 550°C. The volatile content of suspended solids relates to the efficiency of pre-treatment techniques and indicates changes in the organic content of the incoming effluent. In a biological

treatment system, volatile solids are associated with biomass and can be used as a measure of the microorganism population. This correlation often needs careful and stringent determining.

2.10.1.10 Nitrogen

Nitrogen is required by organisms to produce proteins, grow, and reproduce and is recycled continuously by plants and animals. Most organisms cannot use nitrogen in its gaseous form (N_2), so they convert it into other forms known as organic nitrogen, ammonia, nitrate, and nitrite. Blood, meat particles, and manure from meat plants are significant sources of nitrogen due to their high protein content (Mittal, 2004).

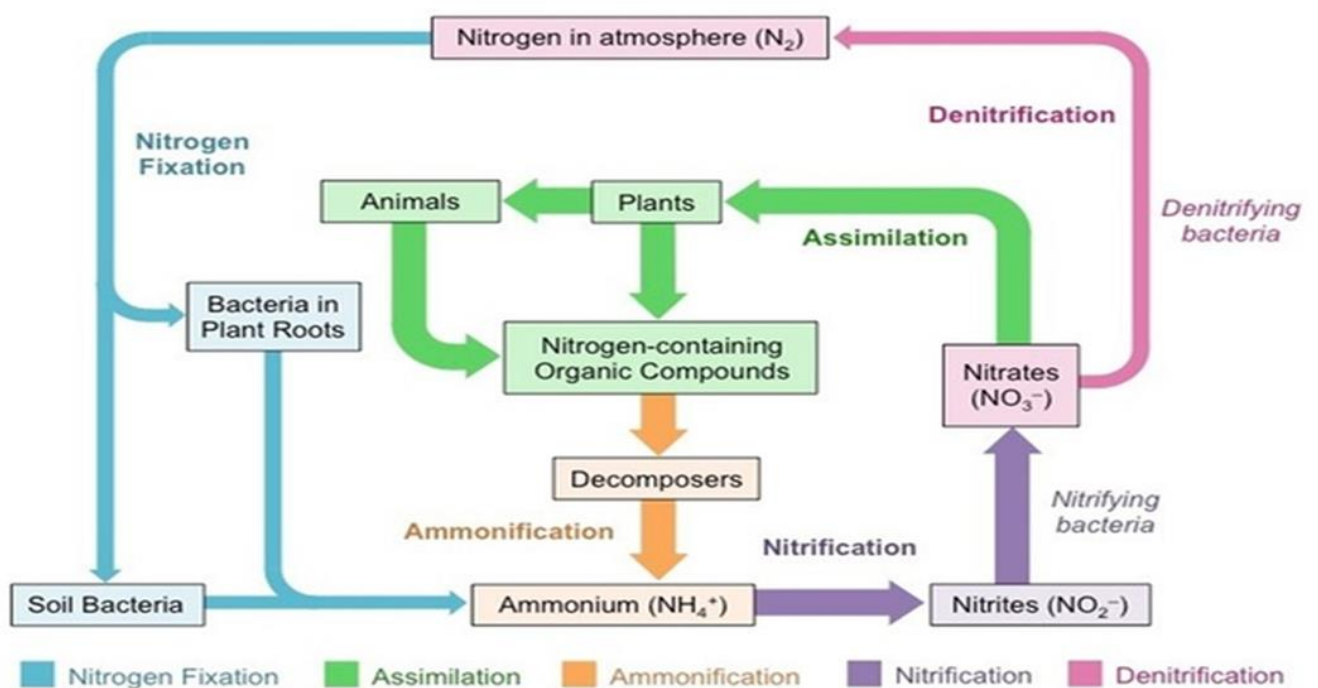


Figure 2.3: The Nitrogen Cycle. This is a biogeochemical process in which nitrogen is converted into different chemical forms that consecutively pass from the atmosphere to the soil, to organisms, and back into the atmosphere.

Ammonia (NH_3): Ammonia is a form of nitrogen. Nitrification, the oxidation of ammonia to nitrite and then to nitrate is a fundamental process in aquatic environments brought about by both autotrophic and heterotrophic microorganisms. The chemical reactions and toxicity effects of ammonia are closely correlated to pH. It is more toxic under alkaline than neutral conditions but has very low toxicity under acidic conditions. Ammonia can also form complexes with many of the transition metals such as copper (Hua Li et al., 2017).

Nitrate NO_3 : Nitrate is the highest oxidised form of nitrogen. It occurs naturally in source water due to decaying plants. Agricultural sources of nitrates include livestock waste matter and chemical fertilisers. The presence of nitrates in water samples is suggestive of some bacterial action and bacterial growth. Under oxidising conditions, nitrite is converted to nitrate, which is the most stable positive oxidation state of nitrogen and is quite common in the aquatic environment.

2.10.1.11 Phosphorus

Phosphorus occurs in wastewater almost solely as phosphates and is derived primarily from blood, bone, manure, detergents, and sanitising compounds. In natural conditions, phosphorus concentration in water is balanced, but if input is higher than what the population of living organisms can use up, this leads to eutrophication. Organic, inorganic, and condensed forms of phosphorus are present in wastewater and are of concern as pollutants because of their increased availability and slow hydrolysis (Dabkowski & White, 2016).

2.10.1.12 Sulphates

Sulphates are formed due to the decomposition of various sulphur-containing substances such as animal feed present in water. The sulphate ions (SO_4^{2-}) occur naturally in water because of leaching from gypsum and other common minerals. Elevated sulphate concentrations increase the erosion rate of metal fittings in distribution systems. It is also responsible for acute health effects in humans such as diarrhoea (Dabkowski & White, 2016).

2.10.1.13 Chloride (Cl^-)

Chloride is a naturally occurring major anion found in all natural waters. In wastewater, chloride sources could be soluble salt (NaCl and KCl) from blood that is discharged into effluent and NaCl used in detergents. The presence of chloride in effluent is a concern because high concentrations result in the saltiness of water which accelerates the corrosion rate of metals. These high concentrations can also be detrimental to chloride-sensitive plants.

Water authorities should be cognisant of the parameters discussed above as they are all essential in determining the toxicity of effluent discharged into municipal sewage systems. If these discharge levels are too high, a fee for excessive polluters can be charged (Iloms, 2018). By-laws issued by the Mangaung Municipality intend to regulate and manage the discharge of industrial effluent. These standards are depicted in Table 2.3 below:

Table 2.3: Mangaung Local Municipality by-laws governing the discharge of industrial effluent.

Parameters	Limit Value
pH	6-10
EC	500 mS.m ⁻¹
Caustic alkalinity (CaCO ₃)	1000 mg.L ⁻¹
Vegetable oils, fats, grease, or wax (FOG)	400 mg.L ⁻¹
Non-organic matter in suspension	100 mg.L ⁻¹
COD	5000 mg.L ⁻¹
Sulphate (expressed as SO ₃)	1500 mg.L ⁻¹
Suspended solids (SSs)	1000 mg.L ⁻¹
Phosphate and phosphate-containing compounds (expressed as P)	100 mg.L ⁻¹

Source: Adapted from Local Government Notice No 60 of 26 September 2008 (To, Discharge & Industrial, 2008).

Due to high pollution concentrations contained in abattoir effluent, it is important to minimise waste volumes, conserve all water sources, and limit housekeeping water usage. Most South African abattoirs have trouble implementing the required measures, therefore a degree of on-site pre-treatment is recommended. Characterisation of the effluent can then be used to conceptualise, control, and optimise a series of physical, chemical, and biological processes for treating the effluent (Akpore & Muchie, 2011).

Due to the physical and chemical composition and large amounts of residual waste from meat processing, it is necessary to treat all waste and effluent before disposal in the environment, a sewer system, or for reuse to comply with environmental legislation. Various physicochemical methods may be used in these treatment processes, but wastewater is often not treated comprehensively as the processes require high capital, specialised operations, and maintenance costs for the adjunction of non-reusable chemicals. Moreover, such processes remove only 25 - 40% of BOD, 50 - 70% of SSs, and 35% of total COD while they do not remove soluble COD (sCOD), N, and protein concentrations (Crini & Lichtfouse, 2019).

Biological treatment is an alternative as it removes organic compounds and pathogens from the effluent using microorganisms, and this process can thus remove more than 90% of pollutants from

wastewater (Bustillo-Lecompte & Mehrvar, 2017). Moreover, analysis of the microbial community can provide very important information for wastewater biological treatment (Samer, 2015). Studies of this nature are innovative and can help to maintain sustainability in the production processes of meat products, yet limited studies have been conducted on abattoir effluent, especially on effluent from red meat abattoirs (Mittal, 2004b; Moreroa & Basitere, 2022; Wu & Mittal, 2012).

2.10.2 Microbial Diversity

Microbial diversity refers to biological diversity that includes genes of the species, species number, and ecological diversity (Fakruddin, 2015). Major microorganisms commonly found in wastewater are viruses, bacteria, and fungi. These microorganisms are very important because they contribute to numerous waterborne disease outbreaks but can also be beneficial in wastewater treatment. Potential health risks are associated with waterborne pathogens found in abattoir effluent, and these constitute significant environmental and public health hazards. Pathogens present in animal carcasses or in animal waste may include *Escherichia coli* and other faecal coliforms (Osibanjo & Adie, 2007; Nafarnda et al., 2012).

It is iterated that not all microorganisms present in effluent are necessarily harmful. Several studies have shown that bioremediation using living organisms is a feasible solution for treating contaminants found in wastewater. Depending on the final use of the water (drinking, recreation, or irrigation), several technologies can be used to remove these compounds. Biological wastewater treatment systems' biotransformation, bioremediation processes, and bioenergy generation depend on the use of microorganisms. The unique abilities of microbes to degrade organic matter, remove nutrients, and transform toxic compounds into harmless products make them essential players in waste removal (Ayilara & Babalola, 2023; Ferrera & Sánchez, 2016; Sharma & Sharma, 2020).

An understanding of consortia functioning could be achieved by gaining knowledge of different co-existing microbial populations through the use of culture-dependent and culture-independent approaches. Microbial diversity is a growing field of study, and our increased knowledge of wastewater microbial diversity depends on the improvement of research approaches, such as those employed by the current study. In the past few years, culture-dependent and culture-independent technologies have been developed to assess microbial diversity. The conventional culture-dependent methods illuminate information about the active heterotrophic component and the functional role of the population. However, the evaluation of microbial diversity based on these

methods has been limited to cultivatable cells and has accounted for the presence of only 1% of microorganisms. However, culture-independent methods allow for a better estimation of the microbiome diversity and the functionality of a community (Paul et al., 2016).

Table 2.4: Advantages and disadvantages of the microbial diversity approach

Method	Advantages	Disadvantages
Plate counts	Fast Inexpensive	Unculturable microorganisms cannot be detected. It is biased towards fast-growing microorganisms. It is biased towards fungal species that produce large quantities of spores
Community-level physiological profiling (CLPP)	Fast Highly reproducible Relatively inexpensive Able to differentiate microbial communities	Only represents a culturable fraction of the community Favours fast-growing organisms Represents microorganisms capable of utilising available carbon sources Potential metabolic diversity Sensitive to inoculum density
Nucleic acid re-association and hybridisation	Total DNA extracted Not influenced by PCR biases and DNA or RNA can be studied in situ	Lack of sensitivity Sequences need to be in high copy number for detection Dependent on lysing and extraction efficiency

Source: Adopted from Fakruddin, 2015

2.10.3 Culture-dependent approach

2.10.3.1 Plate count

The most traditional method for assessing microbial diversity is selective and differential plating and viable counts. It is fast, inexpensive, and provides information about active and culturable heterotrophic microbial populations. The method selects microorganisms with fast growth rates and fungi that produce large numbers of spores. However, the culture method cannot reflect the total diversity of the microbial community (Wang et al., 2021; Zhang et al., 2021), but it does allow for the isolation, identification, and characterisation of individual microbes.

2.10.3.2 Community-level physiological profiling (CLPP)

Microorganisms play an important role in many biological processes within an ecosystem and especially in the decomposition of organic matter. It is important to assess the entire population of the ecosystem because it makes it possible to obtain the most likely reflection of the natural environmental conditions. The enzymatic activity of microorganism populations reflects their composition (Teng et al., 2019). Changes in the enzymatic activity could be an indicator of the changes occurring in the microorganism populations under a wide range of conditions. The characteristics of the microbe community are detected by inoculating Biolog EcoPlate™ with a mixed culture of microorganisms within an environmental sample. The plate consists of 96 well microplates in which every well is a coat lyophilise substrate; the microbial population then gives a characteristic response pattern called a metabolic fingerprint by producing a colour (Mulcahy et al., 2007). The colour produced from the reduction of tetrazolium violet is used as an indicator of respiration of sole carbon sources. Direct incubation of these environmental samples produces patterns of metabolic response called the community-level physiological profile (CLPP) (Gryta, Fraç & Oszust, 2014).

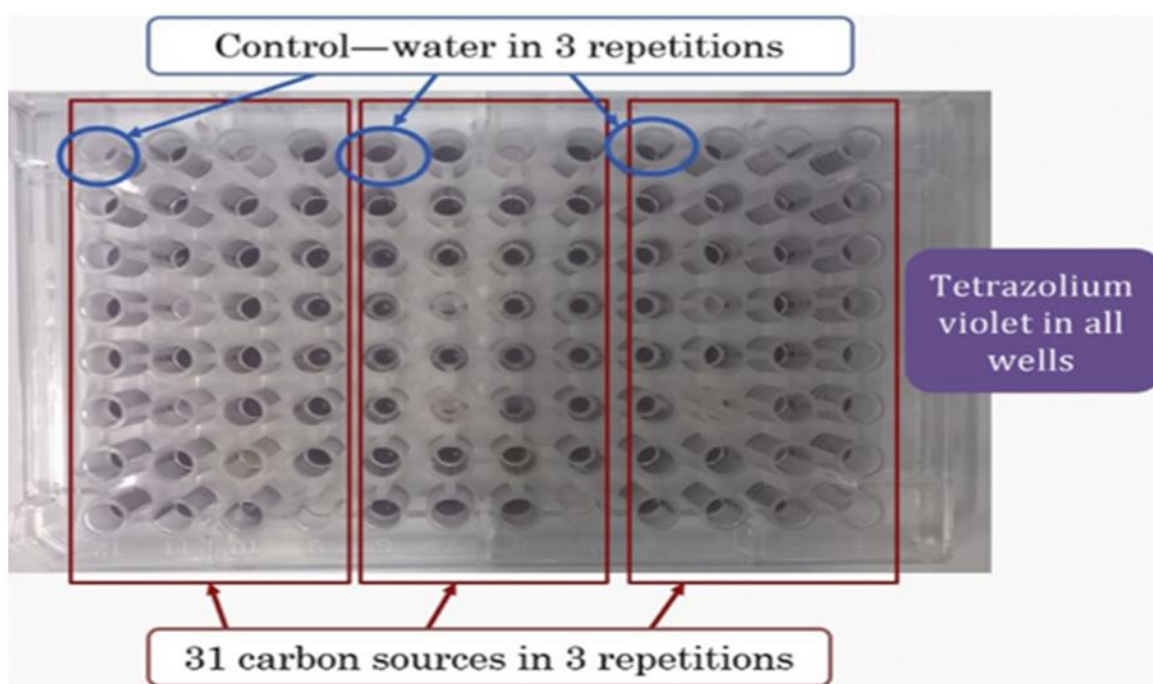


Figure 2.4: The Biolog EcoPlate™ which contains 96 well microplates of 31 carbon sources repeated in triplicate. The carbon sources are the most useful for community analysis. Three control wells contain water (Gałązka & Furtak, 2019).

Several studies have used CLLP as part of microbial community analysis for wastewater treatment. This method has also been used in soil quality analysis (Gałązka & Furtak, 2019), olive mill

wastewater sludge (Martínez-Gallardo et al., 2021), and winery wastewater (de Beer, Botes & Cloete, 2018).

2.10.4 Culture-independent approach

2.10.4.1 Next generation sequencing

Genomic analysis of composite environmental samples is an important tool that can be used to understand ecological biodiversity instead of focusing on laboratory cultivation or isolation of individual specimens. Advancements in conventional DNA-sequencing technologies as proposed by Sanger and colleagues (1977) have led to large-scale, broad-scope biosystematics projects with a wide range of applications such as the Barcode of Life initiative (Hajibabaei et al., 2007). DNA barcoding uses standardised species-specific genomic regions (DNA barcodes) to generate many DNA libraries for the primary purpose of the identification of unknown specimens. The 16S ribosomal RNA (16S) gene is commonly used in studies on bacteria, whereas the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA is used in studies on fungi (Nilsson et al., 2008). In different cases, several other marker genes have been used for biodiversity analyses at different phylogenetic depths or in certain taxonomic group identification investigations.

According to Sanger, Nicklen and Coulson (1977), the traditional DNA-sequencing method can only sequence specimens individually; therefore, it is inadequate for processing complex environmental samples, especially in large-scale studies. These samples often contain mixtures of DNA from hundreds or thousands of microbial species. Although conventional sequencing has provided the most efficient method for the development of large DNA barcode reference libraries, determining the number of individuals in an environmental sample is beyond its ability (Wilkinson et al., 2017). Recovering DNA sequences from the thousands of specimens present in an environmental bulk sample requires the ability to read DNA from multiple templates in parallel, which is something that next-generation sequencing technologies do effectively and at ever-lowering costs.

Next-generation sequencing (NGS) platforms have made it possible to recover DNA sequence data directly from environmental samples (Sogin et al., 2006). The taxa present in an environmental sample can be identified by comparing obtained sequences to a growing standard reference library of known organisms. To this end, the Illumina genome analyser was introduced in 2007. Due to its high capacity, it soon became a workhorse for whole-genome resequencing applications, including human and model organism genomic projects. There are multiple versions of Illumina sequencers

commercially available, but the most recent MiSeq2000 enables researchers to sequence an entire genome in 24 hours (Shokralla et al., 2012).

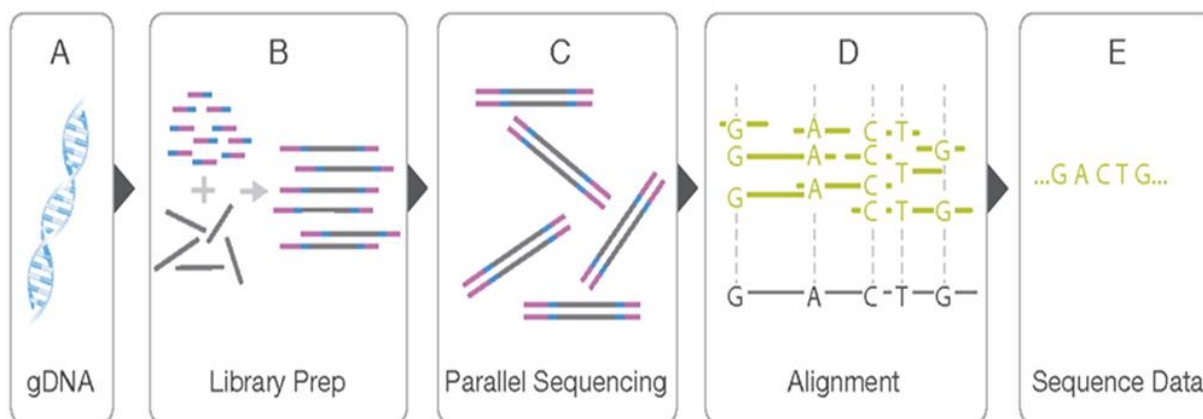


Figure 2.5: The process of next-generation sequencing. A: Extracted gDNA from a sample, B: Preparation fragments of genomic DNA and addition of adaptors to generate a library, C: DNA fragments within the library are each sequence in parallel, D: Individual sequence reads are reassembled by aligning to a reference genome, E: The whole genome sequence is derived from the consensus of aligned reads.

The literature highlights the importance of integrating metagenomic profiles, physicochemical parameters, and metabolic profiling (Fang et al., 2018). By combining these approaches, researchers can obtain a comprehensive understanding of the microbial community structure, function, and interactions within an effluent environment. Metagenomic profiling provides information on the taxonomic composition and functional potential assessed through BioLog Ecoplate™ data, while physicochemical parameters offer insights into environmental conditions. Integrating these approaches provides a holistic view of the microbial consortium in effluent samples.

2.11 Conclusion

Untreated effluent from red meat abattoirs poses severe problems in South Africa, and addressing this issue requires a multifaceted strategy. While a legislative framework exists for the treatment and handling of red meat abattoir effluent, the lack of enforcement necessitates a proactive approach to mitigate the environmental and financial impacts this threat poses. Pre-treatment is a good place to start to alleviate the burden on WWTPs. Among the pre-treatment methods, biological treatment stands out as a promising and potentially cost-effective solution.

However, the non-continuous flow and intricate composition of red meat abattoir effluent present formidable challenges to the application of conventional biological treatment methods. To overcome these challenges, a nuanced understanding of the effluent beyond regulatory compliance is imperative. It is particularly important to understand the intricacies of the composition of such effluent, with particular emphasis on physicochemical parameters, nutrient concentrations, and the microbial community.

Customising the application of indigenous microbial communities can address the variable nature of effluent; however, before implementing such a strategy, the characterisation of the indigenous microbial community's capabilities is essential in understanding the microbial diversity and the metabolic pathways of the native microbiota. The comprehensive analysis of red meat abattoir effluent will ensure legislative compliance and will contribute to the broad goal of environmental conservation.

Chapter 3

Materials and Methods

3.1 Introduction

This study was carried out to establish a microbial diversity fingerprint for red meat abattoir effluent. The wastewater was assessed by measuring the physical, chemical, and microbial quality of the effluent. Microbiological diversity was also analysed using culture-independent methods because these approaches give more abundant and accurate information on microbial diversity than culture-dependent methods (Liu & Haynes, 2011).

3.2 Study Area

The red meat industry is the second largest commodity in the food-production field following the poultry industry. It employs over 2 125 000 people involved in its value chain (Department of Agriculture, 2021a). Even though the industry plays a major role in the livelihood of people and in providing animal protein to the nation, it faces numerous challenges that prevent the industry from producing more meat and animal products. The country thus imports large quantities of various meat products from other countries (Department of Agriculture and Rural Development, 2009; Department of Agriculture, 2021b; South African Pork Producers Organisation, 2022).

Eastern Cape, Northern Cape, Free State and Western Cape provinces predominantly farming sheep and cattle while goats are the least produced livestock in all provinces. South Africa slaughters on average 12 000, 10 000, and 27 000 cattle, sheep, and pigs respectively every week for red meat (Red Meat Producers Organisation (RPO), 2020). According to RPO statistics, a total of 603 835 cattle, 526 859 sheep, and 1 354 924 pigs were slaughtered in 2019. The Free State is geographically situated in the centre of South Africa and is the third largest province with an area of approximately 129 825 km². Bloemfontein is the capital city of the province. The Mangaung Metropolitan Municipality where the city of Bloemfontein is located has the largest share of the province's population (Food and Drug Administration, 2014). The area is dominated by agricultural activities that

contribute significantly to the province's economy. Agricultural produce includes, among others, maize, wheat, potatoes, sunflower, red meat, vegetables, cherries, and dairy products. Industrial development, agricultural activities, and population growth are the reasons for the increase in water demand in the province. While high-quality water is essential for most industrial processes, many of these processes generate large effluents of contaminated wastewater, whose safe disposal into receiving water bodies is of great environmental and health concern (Botai et al., 2016).

There are approximately 64 high throughput (HT) and 45 rural and low throughput red meat abattoirs in the Free State. The capacity of slaughter units slaughtered per annum at the HT abattoirs ranges from 12 316 up to 90 000 per year, with an average of 40 865 units slaughtered per year. The LT abattoirs average between 520 and 5 200 slaughter units per annum (Swanepoel, 2014). The sampling location for this study was an abattoir located in the industrial area of Bloemfontein City. It is a high throughput red meat abattoir (Grade A) that slaughters and processes $\pm 1\,900$ beef, $\pm 2\,600$ lamb, and ± 500 pork sources per month, depending on availability and demand. The abattoir uses an average of 400 000 litres of water per day that is derived from a combination of municipal and borehole water. Approximately 82% of that fresh water is discharged as wastewater into the municipal sewer system with no pre-treatment other than a solids screen in the pipe system.

3.3 Study Design

This study was conducted in two sampling phases, namely a pilot study and a 10-day sampling event. A site visit to the abattoir was conducted to survey the area, and due to the complexity of the study, a pilot study was necessary to determine effluent flow rates, consistency, and adequate sampling intervals as well as a suitable sampling protocol to obtain a composite sample.

The consecutive 10-day sampling event occurred during spring (October) when 5 litres of wastewater were sampled each day. The effluent quality was analysed to determine 12 physical, 8 chemical, and 5 microbiological parameters. The biologically active microbial community was analysed using BioLog EcoPlates™ and the rest of the microbial community was analysed using Next Generation Sequencing (Figure 3.1).

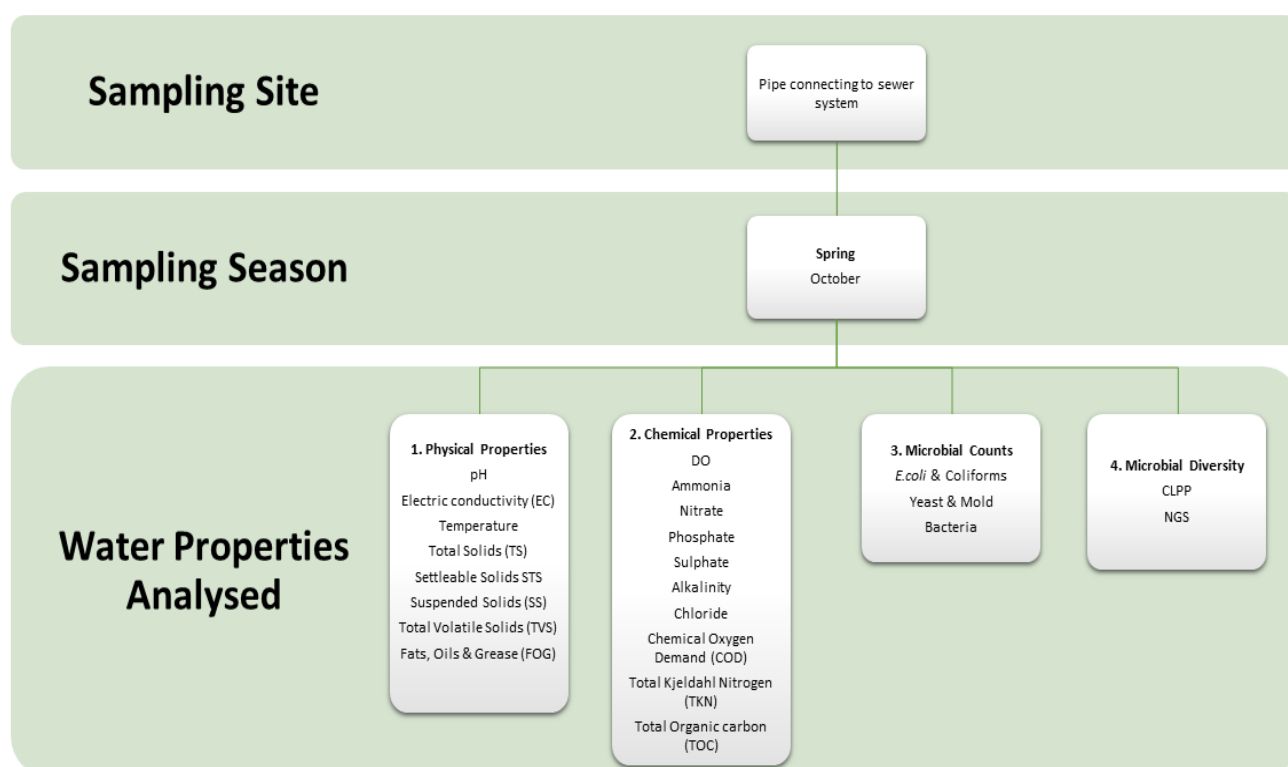


Figure 3.1: Experimental design of the study. The diagram depicts when sampling occurred and the different physicochemical, microbiological, and microbial diversity properties analysed.

3.4 Sampling Site

The abattoir discharges its wastewater into the municipal sewer system. The sampling site of choice was the pipe that connects to the sewer, as depicted in Figure 3.2. This was deemed the best location as the effluent moves through this area with enough velocity to prevent the settling out and accumulation of solids, and thus a true reflection of the quality of the effluent being discharged was obtained. The effluent passes over a rotary screening mechanism which is the most often used and easiest process of primary effluent treatment (Flugaur, 2003; Naidoo, 1999). It was currently the only form of treatment used at this abattoir to remove large solids and grit material. These particles are then dried and recycled to be used as agricultural manure.

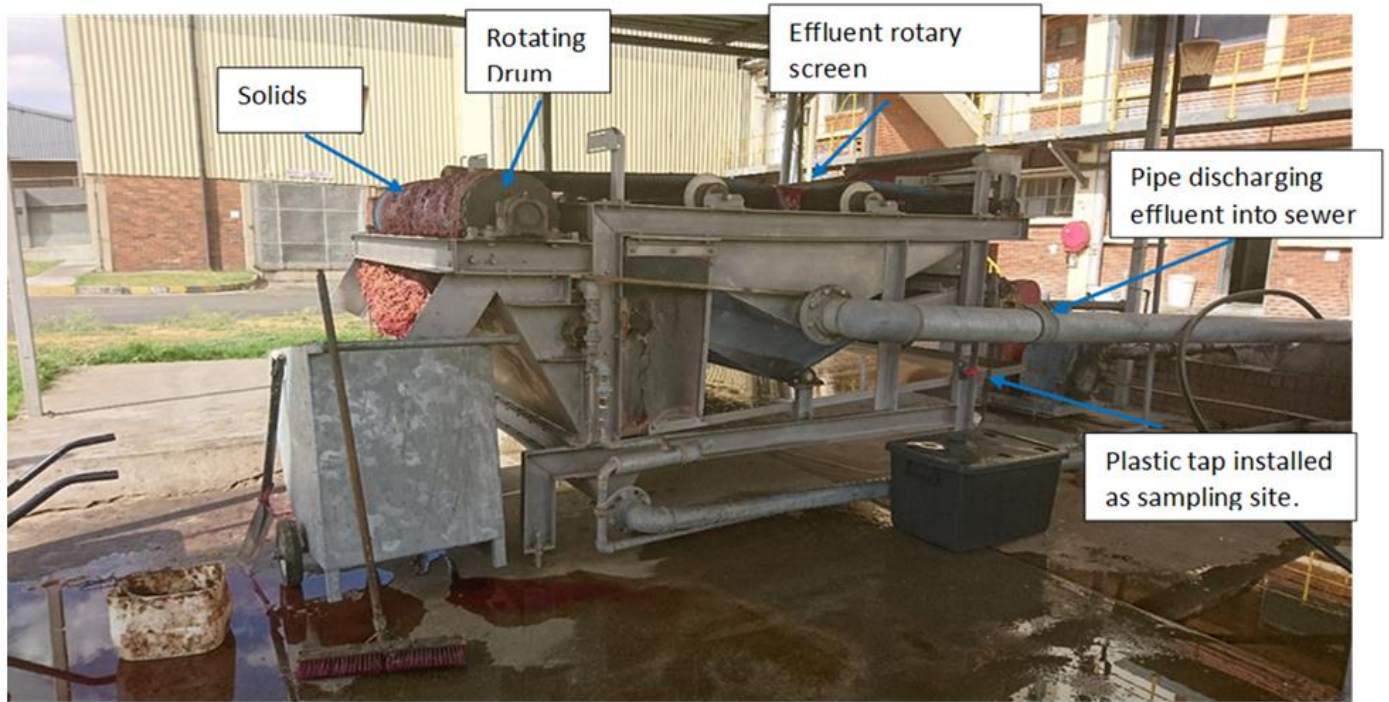


Figure 3.2: Abattoir sampling site. The image shows the rotary screening machine that the effluent passes through before being discharged into the municipal sewer system. Solids in the effluent that are greater than the mesh are trapped and later recycled.

Rotary screening comprises of rotating cylindrical rollers that receive wastewater on one end. The effluent passes over a mesh that traps solid particles greater than the mesh size (such screens commonly use mesh that is 25-150 mm in diameter) and it is then ultimately deposited in the sewer. A continuous water stream from external spray nozzles is usually present. These nozzles spray water over the drum to prevent any clogging (Flugaur, 2003; Thajudeen, 2017).

3.5 On-site Sample Collection

Daily composite samples were collected by drawing a 10-second flow sample every 10 minutes throughout the day while the abattoir was fully functional. The samples were collected in a 110 L sterile collection vessel. A composite sample was deemed statistically more reliable than a grab sample because effluent volume, composition, and pollutant concentrations varied during the day based on process flow in the abattoir. Thus, the composite samples that were collected represented an average concentration of the effluence that was collected (Kinzelman et al., 2006; Nickel & Fuchs, 2021).

Once the collection vessel was filled up, a sterile metal rod was used to stir the effluent for proper mixing of any settled solids. A sterile 1 L glass beaker was used to sample 2.5 L of the effluent and it was stored in a sterile 5 L plastic vessel and placed on ice in a cooler box. Depending on the production activities for the day, this process was repeated for a second time, unless production stopped early and only one collection vessel could be filled. On days when two collection vessels were filled, the 2.5 L of samples were combined before analysis.

3.6 Physicochemical Analyses

Five physicochemical properties were analysed on site: pH, temperature, electric conductivity, total dissolved solids, and dissolved oxygen. A battery-operated HQ40d multi-meter with the HACH LDO probe was used to determine DO and temperature and a Sension+ MM150 portable meter with pH+EC sensor probe was used to determine pH, EC, TDS, and temperature. The HACH direct measurement methods were 8157 and 8160 respectively. Calibration of the probes was done in the laboratory before sampling commenced and by following the manufacturer's instructions (HACH, 2016, 2017). On site, the appropriate probe was rinsed with distilled water before measurement and then dipped into the effluent storage container and measurement was recorded. The rest of the physicochemical properties were analysed in the laboratory at the Centre for Applied Food Sustainability and Biotechnology (CAFSaB) at the relevant tertiary institution.

Settleable solids, ammonia, nitrate, phosphate, alkalinity sulphate, chloride, total chemical oxygen demand, and supernatant chemical oxygen demand were analysed using a spectrophotometer (Esterhuizen, 2014). A DR 6000 UV VIS spectrophotometer was used for all the HACH methods and the Orbeco Hellige SP600 spectrophotometer was used for the Lovibond methods. The DRB 200 reactor (HACH) was used to digest samples for COD analysis. Each sample was diluted 10x. This was done to reduce the analyte that would be tested and to be within range for selected analysis methods. Table 3.1 indicates which method was used for which parameter.

Table 3.1: Physicochemical parameter analysis methods and ranges used for spectrophotometer readings

Parameter	Method	Method number	Range (mg. L ⁻¹)	Type	Supplier
Ammonia	Salicylate	8115	0.01-0.50	Powder Pillow	HACH
Nitrate	Chromotropic Acid	10020	0.2-30	Test 'n tube vials	HACH
Phosphate	Ascorbic Acid	8048	0.02-2.50	Powder Pillow	HACH
COD	Reactor Digestion	8000	20-1500	Test 'n tube vials	HACH
Suspended solids	Photometric	8006	5-750	N/A	HACH
Sulphate	Sulfa Ver	8051	2-70	Powder Pillow	HACH
Alkalinity	Alkalinity HR	31	5-500	Tablet	Lovibond
Chloride	Silver Nitrate	93	5-250	Tablet	Lovibond
TKN	Nessler	8075	1-150	Reagent solution	HACH
TOC	Direct	10129	0.3-20	Test 'n tube vials	HACH

The following general procedure was followed when using the spectrophotometer (HACH, 2021):

- Start a program for the specific parameter being analysed.
- Prepare a blank with either distilled water or the sample and shake the sample cell or test tube vial.
- The cell or vial was inserted into the cell holder and zero was pressed.
- A reagent powder pillow or tablet was added into the vial, shaken, and left for reaction time.
- A vial was inserted into the cell holder and 'read' was pressed.
- The value was the reading of that parameter within the effluent.

Settleable solids were analysed using direct measurement, namely HACH method 8165. In this method, 1 L of the sample was poured into an Imhoff cone and left to stand for 1 hour to let the sample become stable. Once the time had lapsed, the graduated scale on the Imhoff cone at the top of the solids layer was reads in mL.L⁻¹. The value was then multiplied by 1 000 to convert mL.L⁻¹ to mg.L⁻¹.

Total solids and total volatile solids were measured using the gravimetric method using HACH method 8271 and 8276 respectively. An aluminium dish was placed in a drying oven at 500°C for 1 hour. The dish was removed from the oven and left to cool in a desiccator. An analytical balance was used to weigh the dish (Value C), and then 50 ml of the sample was mixed and poured into the dish.

The sample was placed in the preheated oven and evaporated at 103°C for 4 hours. The dish was removed from the oven and left to cool in a desiccator. Once cool, it was weighed on an analytical balance (Value A). The dish was then put in a pre-heated muffle furnace at 500°C for 30 minutes, after which it was placed in a desiccator and left to cool. It was then weighed on an analytical balance (Value B). The loss of weight was the total volatile solids and the weighed residue was the total solids.

The results were calculated as follows:

$$\text{Volatile solids (mg.L}^{-1}\text{)} = (A-B) \times 1000 \quad (1)$$

A- Weight of sample + weight of aluminium dish (from oven)

B- Weight of sample + weight of aluminium dish (from muffle furnace)

$$\text{Total solids (mg.L}^{-1}\text{)} = (A-C) \times 1000 \quad (2)$$

B- Weight of sample + aluminium dish (from the oven)

C- Weight of empty aluminium dish

FOG analysis, total Kjeldahl nitrogen (TKN), and TOC were conducted at A L Abbott and Associates laboratory based in Cape Town. This laboratory is SANAS-accredited for the specific methods that were performed. Samples were poured into 100 mL glass bottles and stored at -20°C. The FOG analysis samples were preserved with 200 µl sulphuric acid and the samples were transported to Cape Town using an overnight service with a refrigeration facility, cold chain preserved.

3.7 Biological Analyses

3.7.1 Culture-dependent analysis (enumeration)

Three different types of selective media were used to study the viable microbial community according to the intended groups of microorganisms. The culture media were prepared by mixing specified commercial components according to the manufacturer's instructions. The media were sterilised in an autoclave at 121°C for 20 minutes, then poured into 90 mm Petri dishes and left to dry overnight. Selective media isolates specific organisms and provides a growing environment conducive to some types of organisms and not others (Chiemchaisri et al., 2023; Jałowiecki et al., 2016; Tang & Lau, 2022).

3.7.1.1 Rose Bengal Chloramphenicol Agar (RBC)

RBC is recommended for the selective isolation and enumeration of fungi from environmental and food services. It contains chloramphenicol which inhibits most bacterial growth. Expected organisms are *Candida albicans* (pink, smooth, raised colonies) and *Aspergillus brasiliensis* (white and filamentous, black specks on colonies and mould) (Hardy Diagnostics, 2019).

3.7.1.2 Nutrient agar (NA)

This agar is a rich, general-purpose medium for the cultivation of less fastidious microorganisms and it can be enriched with blood or other biological fluids. Expected microorganisms were *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella enteritidis*, *Salmonella Typhimurium*, and *Yersinia enterocolitica*, etc.

3.7.1.3 Harlequin® *E. coli*/Coliform agar

This agar is a selective medium for the simultaneous enumeration of *Escherichia coli* and coliforms in food and other environmental samples. The dual chromogenic substrate media make the different colony types easy to distinguish and allows rapid counting of both *E. coli* and coliforms on a single plate. *E. coli* (dark blue to violet), *Enterobacter aerogenes* (rose pink), and *Pseudomonas aeruginosa* (beige) were envisaged.

The isolation of microorganisms from the effluent was achieved using a serial dilution; thus 1 mL of the sample was added to 9 mL saline, and so forth. Samples were diluted to 10^{-3} . Aliquots of 100 μ L of each dilution were plated on RBC, NA, and Harlequin® agar using the easy Spiral® pro automatic plater and incubated at 25°C for 72 hours and 30°C for 24 hours, respectively. Enumeration was performed using the Scan® 1200 automatic HD colony counter to determine the number of viable colonies in the samples.

All yeast and mould isolates were cryopreserved in Yeast extract Peptone-Dextrose (YPD) medium (2% yeast extract, 4% peptone, 4% dextrose) that also contained 15% glycerol at -80°C. Yeast extract is a water-soluble compound generated when yeast is forced to self-digest, while peptone is a mixture of peptides and amino acids prepared by digesting animal protein with proteases and dextrose, or glucose, which is the favoured carbon source of yeast (Sherman, 2002). Bacterial isolates were cryopreserved in Microbank™ vials at -80°C. These vials contained 25 sterile coloured beads and a cryopreservative. These beads allow microorganisms to readily adhere to the bead

surface and can be kept for long periods at -80°C. The beads offer the least possibility of disturbance to the stored organism yet permit ready and rapid access (Pro-Lab diagnostic, 2012).

3.7.1.4 Community level physiological profiling (CLPP)

The metabolic potential of the effluent was evaluated using BioLog EcoPlate™ (Biolog Inc, n.d.). Measuring the community metabolic fingerprint over time ascertains characteristics of that community of microbes. CLPP is an effective way to distinguish spatial and temporal changes in microbial communities (Furtak et al., 2019). BioLog EcoPlates™ contain 96 wells containing 31 different carbon sources plus a blank well, in three replications. Formulation of purple occurs when the microbes can utilise the carbon sources and begin to respire, reducing a tetrazolium dye included in the carbon sources. In addition, the substrates are subdivided into six groups: amino acids (n=6), amines (n=2), carbohydrates (n=10), carboxylic acids (n=9), phenolic acids (n=4), and polymers (n=4) (de Beer et al., 2018).

Experimental setup and spectrophotometry

Each BioLog EcoPlate™ was inoculated with 100 µL of 10⁻³ dilution of the sample and saline into the control well using a 12-channel micropipette. The plates were then incubated at 25°C for a total of 72 hours. Absorbance at 590 nm was measured using the SpectraMax® M2 microplate reader after 24, 48, and 72 incubation hours. Optical density (OD₅₉₀) values obtained at 72 hours represented the optimal range of optical density readings and were used for assessing the microbial functional diversity and statistical analyses (Teng et al., 2019).

The community of microorganisms exhibited a characteristic reaction pattern reflecting the metabolic properties of the community, which was the CLLP. The CLPP was assessed using functional diversity indices which were average well colour development (AWCD) (Equation 1), Simpson diversity (D) (Equation 2), Shannon evenness (E) (Equation 4), and richness (S) (Equation 6) (Grzadziel et al., 2018). These characteristics in each reading interval for all samples were calculated using the formulae presented in Table 3.2.

Table 3.2: Formulae for the calculation of indices for average well colour development (AWCD), Simpson diversity (D), Shannon diversity (H'), Shannon evenness (E), and richness (S)

Formula #	Index	Formula	Definition	Reference
3	AWCD	$AWCD = \sum ((C-R))/n$	C—reading of the well OD ₅₉₀ R—reading of the control well OD ₅₉₀ n—the number of substrates on an EcoPlate™ (31)	Garland & Mills, 1991
4	Simpson diversity	$D = 1/\sum P_i^2$	P _i —proportional colour development of the well over the total colour development of all wells of a plate	Grzadziel et al., 2018
5	Shannon evenness	$E = H'/\ln S$	S—the number of wells with colour development (substrate utilisation richness)	Zak et al., 1994
	Shannon diversity	$H' = -\sum P_i \ln P_i$	-	Hill et al., 2003
6	Richness	S = number of utilised substrates with OD ₅₉₀ ≥ 0.25	-	Jalowiecki et al., 2016

EcoPlate™ data processing and analysis

The OD₅₉₀ values of each plate were corrected by subtracting the water blank (well A1) absorbance value at t=0 from each well, which eliminated interference from the density of the inoculum with the results. The colour score in each well was expressed as AWCD (Equation 1). The normalised absorbance was used as a colour score for substrate utilisation in the form of a heat map. Wells with OD₅₉₀ of less than 0.5 were considered as having no substrate utilisation. Wells with OD₅₉₀ between 0.5 and 2 were considered positive substrate utilisation and OD₅₉₀ and above 2 were considered to have strong substrate utilisation.

3.7.2 Culture-independent analysis (genetic diversity)

3.7.2.1 DNA extraction

DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components (Gupta, 2019). A

15 mL falcon tube of each sample was centrifuged at 7000 x *g* for 5 min. The supernatants were discarded and the pellets were stored at -80°C as an intact natural community. After the sampling period, all the frozen pellets were processed as one batch. Pellets were thawed on ice for 15 min. and then weighed on an analytical balance, ensuring that there was more than 80 mg of the pellet. Genomic DNA was extracted using the NucleoSpin DNA stool extraction kit according to the manufacturer's instructions (Macherey-Nagel, 2023), with minor modifications. The kit contained a lysis buffer which, in combination with 5 min. heating, led to a chemical disruption of the membranes before mechanical lysis using ceramic beads.

The undissolved material and beads were subsequently removed by short centrifugation. Proteins and PCR inhibitors in the sample were precipitated by adding a lysis buffer and short incubation at refrigerated temperatures, followed by an additional centrifugation step to remove all impurities. The supernatant was cleared by passing it through an inhibitor removal column that completely removed substances that could interfere with enzymatic reactions. Residual contaminants and other PCR inhibitors were removed by a washing procedure using binding and wash buffers. After drying, the DNA was eluted with elution buffer.

The nucleic acid concentration and purity were confirmed on the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). A purified gDNA sample of 1 µl was pipetted onto the pedestal and measured at a wavelength of 260 nm (A_{260}). The elution buffer used for the DNA extraction (Tris/HCL) was used as a blank. The remaining purified gDNA was stored at -20°C until required. These composite samples were then sent to the Agricultural Research Council Biotechnology Platform (ARC-BTP) (Pretoria, South Africa) for Next Generation Sequencing (NGS) using an Illumina MiSeq system.

3.7.2.2 Targeted metagenomic sequencing

Analysis of the 16S ribosomal DNA gene was employed to identify the bacterial community, and the internal transcribed spacer (ITS) served to identify the composition of the fungal community. Region of interest-specific primers with Illumina adapter overhang nucleotide sequences were used for library construction. For the amplification of the 16S rDNA V3/V4 region the 16S forward primer: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and 16S reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTA

ATCC-3' were used (Dos Santos et al., 2021). For the amplification of the ITS2 region, the ITS3 forward primer: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCATCGATGAAGAA CGCAGC-3' and ITS4 reverse primer: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTC CTCCGCTTATTGATATGC-3' were used (Yang et al., 2018). Both the bacterial- and fungal-targeted PCR products were approximately 550 bp in length.

Impurities introduced during the initial amplification of the genes were removed by using AMPure XP beads (Beckman Coulter Genomics, USA). Negatively charged nucleic material bound to the magnetic beads, enabling easy separation from impurities within the supernatant. An index PCR was performed to introduce dual indices, which uniquely marked and distinguished samples from one another using Illumina sequencing adapters. The indexed PCR samples were subjected to a second clean-up with AMPure XP beads.

Library quantification, normalisation, pooling, and denaturing were done before being subjected to 2 x 300 cycle sequencing on the Illumina MiSeq sequencing platform using the MiSeq v3 reagent kit (Illumina). The MiSeq platform utilised a sequencing-by-synthesis approach, where DNA fragments were amplified, attached to a solid surface, and then sequenced in a cyclic process. Relatively short read lengths were generated that typically ranged from 150 - 600 base pairs depending on the specific sequencing chemistry and kit used (Illumina, 2021).

Bioinformatic processing of sequence read data was performed using the QIIME2 program. Demultiplexed paired-end sequences were trimmed, denoised, and chimeric sequences were removed. DADA2 was used for quality control processing and grouped the sequences into amplicon sequence variants (ASV) based on 99% sequence similarity (Cason et al., 2020). The sequence reads were first filtered with an expected error threshold of 2, combined with the trimming of 10 nucleotides from the start and end of each read. Filtered reads were then de-replicated by combining identical reads into unique sequences and constructing consensus quality profiles for each combined lot of sequences. The consensus quality profiles then informed the de-noising algorithm which inferred error rates from samples and removed identified sequencing errors from the samples. After building the ASV table and removing chimeras, taxonomy was assigned using the SILVA 132 database for the bacterial data (Quast et al., 2013) and UNITE for the fungal data (Abarenkov et al., 2010).

Output files from QIIME2 were imported into R v3.6.11 (R-Core Team, 2013) and the phyloseq package (McMurdie & Holmes, 2013). ASVs were rarefied. Rarefy is used to normalise microbiome counts from libraries of different sizes by resampling the ASV table to ensure that all samples have the same library size. Normalisation refers to the process of eliminating artefactual biases within a dataset by transforming the data, which intends to enable an accurate comparison of statistics from varying measurements (Weiss et al., 2017). The richness and phylogenetic diversity (PD) were calculated using the package picante in R (Kembel et al., 2010).

3.7.3 Statistical analysis

The statistical analyses and graphical representation of the research data were conducted using Microsoft Excel. To begin the analysis, the raw research data were inputted into Excel, organising the information in a structured manner. Excel's built-in functions such as mean, median, standard deviation, and correlation were employed to compute descriptive statistics and explore relationships between variables. These calculations provided valuable insights into the central tendencies, variability, and associations within the dataset.

Furthermore, Excel's charting capabilities were utilised to generate visual representations of the research data. Box plots and bar graph heatmaps were constructed based on the nature of the variables and the intended communication of the results. Pearson's correlations were made and analysed by principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) using the XLSTAT Premium 2022.3 x 64 version (Maguvu et al., 2020).

In addition to the statistical analyses and graphing, Excel's spreadsheet functionalities facilitated data management and organisation. The software allowed for data manipulation, sorting, filtering, and the creation of calculated columns while it also ensured flexibility and control during data exploration and preparation.

Chapter 4

Results and Discussion

4.1 Introduction

A holistic approach was adopted to monitor the physicochemical properties of the abattoir environment and determine the microbial diversity and functionality of the red meat abattoir effluent that was analysed. This approach elicited information on the environmental characteristics, active heterotrophic components, functional roles, and identities within the microbial population that were observed (Ma et al., 2015; Paul et al., 2016; Safford et al., 2022).

4.2 Experimental Design

The pilot study showed where the main sampling failures could potentially occur, and the researcher was able to determine where research protocols might not be followed and whether proposed methods or instruments would be inappropriate or too complicated. By adopting this approach, potential practical problems that could occur were identified. A consecutive 10-day sampling period was subsequently conducted and pH, temperature, EC, TDS, and DO were analysed on site due to sensitivity to change during storage and transportation. Portable pH probes (which could also measure EC, temperature, and TDS) and DO probes were used. The rest of the physicochemical parameters were analysed in a well-equipped laboratory.

4.3 Sampling

Sampling was conducted for 10 consecutive days and could only occur on days when the abattoir was operational, thus excluding weekends and cleaning days when a thorough cleaning and disinfection of the premises occurred. Each of the sampling days produced enough effluent to fill the collection vessel for analysis (i.e., a total of 5 L). A diverse array of animals was slaughtered at the abattoir each day, and this contributed to the dynamic nature of the abattoir's operations. The selected abattoir's role is to serve as a central facility for processing multiple species of livestock such as cattle, sheep, goats, and other red meat-producing animals. The daily intake of different

animals creates a challenging and complex environment and demands versatile processing techniques to cater for the specific requirements of slaughtering each species in a humane and hygienic manner. Figure 4.1 illustrates the different livestock slaughtered on each sampling day.

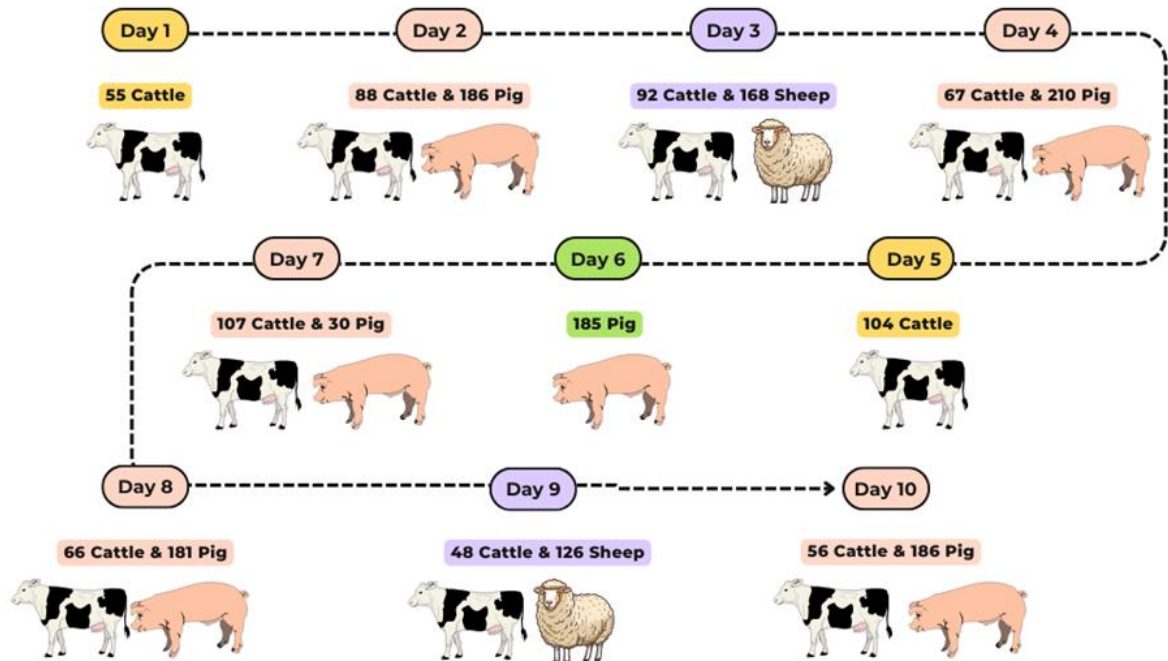


Figure 4.1: Specific livestock and their numbers slaughtered on each sampling day

The slaughtering of diverse animal species at one abattoir introduces a wide range of unique microbial communities associated with skin, fur, and internal organs, and this variety contributes to a complex and diverse microbial ecosystem within such an abattoir. Moreover, the influx of metabolic activities from different animals enriches microbial diversity and enhances functional versatility (Madoroba et al., 2021). Moreover, physicochemical parameters play a crucial role in shaping the overall microbial diversity at an abattoir and influence the stability and functionality of this environment. The coexistence of microbial communities from various animal sources further provides opportunities for intricate interactions, and these impact the complex ecosystem dynamics.

4.4 Physicochemical Parameters

The design, control, and operation of a wastewater treatment system mostly depend on the nature of the wastewater, and it is therefore essential to characterise the main components of wastewater,

especially its organic content for efficient treatment. Parameters such as COD, TSS, and nitrogen levels are carefully monitored in South Africa, and the primary goal for regulatory bodies is to minimise the values of these factors (Modin et al., 2016; Moreroa & Basitere, 2022).

By analysing the chemical and physical parameters of the wastewater, the characterisation of the sampled red meat abattoir effluent and the identification and quantification of different compounds found within the wastewater were facilitated. The results of the physicochemical qualities of the wastewater samples are presented in Table S1 (Supplementary Table 1). In South Africa, industrial effluent discharge is regulated by the Department of Water Affairs (1998). Most abattoirs are located within municipal boundaries and their effluent is discharged and treated at a municipal sewage treatment plant. Municipalities impose minimum discharge standards but, unfortunately, there are no specific standards or guidelines in existence that regulate all the physical and chemical parameter limits for the discharge of industrial effluent (GDARD, 2009).

According to Mangaung Municipal by-laws, eight physicochemical parameters should be monitored, and these were the ones analysed in this study, namely: pH, electric conductivity, fats oils and grease, total suspended solids, chemical oxygen demand, alkalinity, sulphates, and phosphates. The samples of three of the parameters (37.5%) showed non-compliance with the set limits (Supplementary Table 1). Wastewater discharge non-compliance is very costly for any municipality, and such non-compliant industries affect the bottom-line profitability through increased charges, fines, and high monitoring costs. The inflow of non-compliant effluent also leads to poor levels of sewage purification and such sewage that is then discharged into water courses poses various threats to humans, animals, and the environment (Iloms et al., 2020; Mema, 2019).

The overall total solids that were detected ranged between 1327 - 7050 mg.L⁻¹, with varying values on the different sampling days. No pattern was identified between the comparison of solids and the species slaughtered. A total of 85% of the TS belonged to TVSS which obtained an average of 3278 mg.L⁻¹ (Figure 4.2). TVSS are organic and thus volatilise during combustion. Organic solids, whether dissolved or suspended, have a greater impact on wastewater treatability, potential toxic by-product formation, and membrane fouling than inorganic solids (Hamilton & Zhang, n.d.; Hande Gursoy-Haksevenler & Arslan-Alaton, 2020). The prevalence of high TVSS in abattoir effluent is a widespread and anticipated characteristic as it is inherent to the nature of slaughterhouse activities (Gutu et al., 2021). The diverse range of animal tissues processed, coupled with variations in processing

techniques, contributes to the ubiquity and variability of TVS levels in the effluent. This common occurrence necessitates a thorough understanding of the specific challenges posed by elevated TVSs in abattoir wastewater (Michael et al., 2020).

High levels of TVS pose challenges to biological treatment processes within wastewater treatment plants. The organic load can strain microbial communities responsible for decomposition, potentially resulting in reduced treatment efficiency and prolonged treatment times. This may also contribute to undesirable odours and thus require additional operators to implement effective odour control strategies.

The study found that TDSs (825 mg.L^{-1}) averaged higher values than TSSs (739 mg.L^{-1}); however, only TSSs are regulated due to the possibility of sewer pipe blockages and reducing the effluent flow rate (Otsuka et al., 2020). Samples 3, 5 and 7 were above the limit of 1000 mg.L^{-1} in the Mangaung by-laws. Cattle, sheep, and pigs were slaughtered on these sampling days in varying numbers. In Figure 4.2 below the box plot shows how largely distributed the values of the TSSs (Range $176\text{--}1430 \text{ mg.L}^{-1}$) were compared to those of the TDSs (range $628\text{--}1217 \text{ mg.L}^{-1}$).

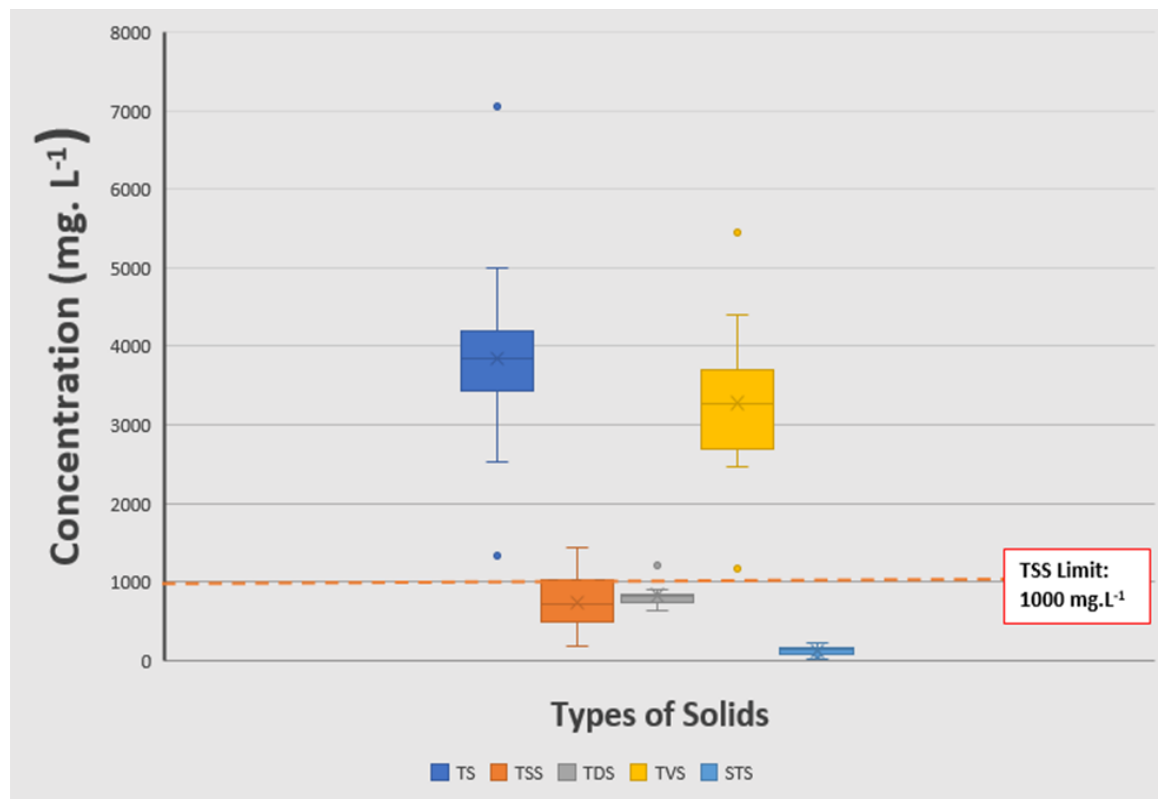


Figure 4.2: Boxplot showing the different solids within the effluent. The values within the box (X) are the average of 10 samples. The dots above the box represent the outliers

It was found that 30% of the abattoir effluent samples had TSS concentrations higher than the acceptable limit, indicating that the screening process that is implemented as a form of pre-treatment may not be consistently effective in removing solids to meet the specified standards. The higher-than-acceptable concentrations suggest that the screening system may need adjustment, improvement, or more frequent maintenance to enhance its performance. Possible considerations and actions could include screen maintenance and improved screen design and size. However, if the process consistently falls short of meeting the acceptable limits, considering the incorporation of additional pre-treatment steps such as sedimentation or additional filtration to complement the screening process may help address variations in the concentration of solids and improve overall treatment efficiency.

FOG is another regulated parameter of concern in abattoir wastewater. This arises from the fat being trimmed in the slaughtering process, and this fat often solidifies at suitable temperatures and thus forms part of suspended solids (Mittal, 2006; Moreroa & Basitere, 2022). As mentioned previously, TSSs can cause operational damage such as clogging pipes and causing bad odours. They may also create a breeding site for flies, insects, and pathogens. FOG values were <1 on all the sampling days, indicating that the screening process was efficient in removing suspended solids as FOG was not present.

The overall COD ranged between 1220 - 9160 mg.L⁻¹. Figure 4.3 shows that the levels of five samples were above the limit set by the Mangaung by-laws of 5000 mg.L⁻¹ (samples 1, 3, 4, 5, and 7). COD is the amount of strong oxidant required to break down organic matter. Total tCOD is made up of a particulate and soluble fraction. It has been reported that large abattoirs in South Africa may generate wastewater with COD values of around 1217 and 5025 mg.L⁻¹ (GDARD, 2009; Müller, 2017). The main pollution source in abattoir effluent is the high quantity of blood which contains high levels of COD and organic matter that can significantly influence microbial activity.

Microbes, particularly bacteria, thrive on the organic compounds present in high COD wastewater as they are sources of carbon and energy. However, excessively high COD levels can present challenges, such as oxygen depletion in aerobic conditions and potential toxicity due to breakdown products. This overwhelms microbial communities, reduces treatment efficiency, and leads to incomplete degradation of organic pollutants. Adequate oxygen supply is also crucial in aerobic treatment processes to avoid anaerobic conditions and the production of undesirable by-products.

High COD levels can also impact sludge production and negatively influence the characteristics and management of the sludge generated during treatment (Silva & Rosa, 2021). Pluciennik-Koropczuk and Myszograj (2019) argue that distinguishing between total COD and soluble COD is essential. Total COD encompasses both soluble and insoluble organic compounds, offering a comprehensive measure of the overall organic load. Soluble COD, on the other hand, specifically represents the fraction of organic matter readily metabolised by microorganisms during treatment (Pluciennik-Koropczuk & Myszograj, 2019; Zhang et al., 2021). In the current study, soluble COD (sCOD) was analysed by centrifuging each sample and analysing the solubility without the interference of settleable particulates.

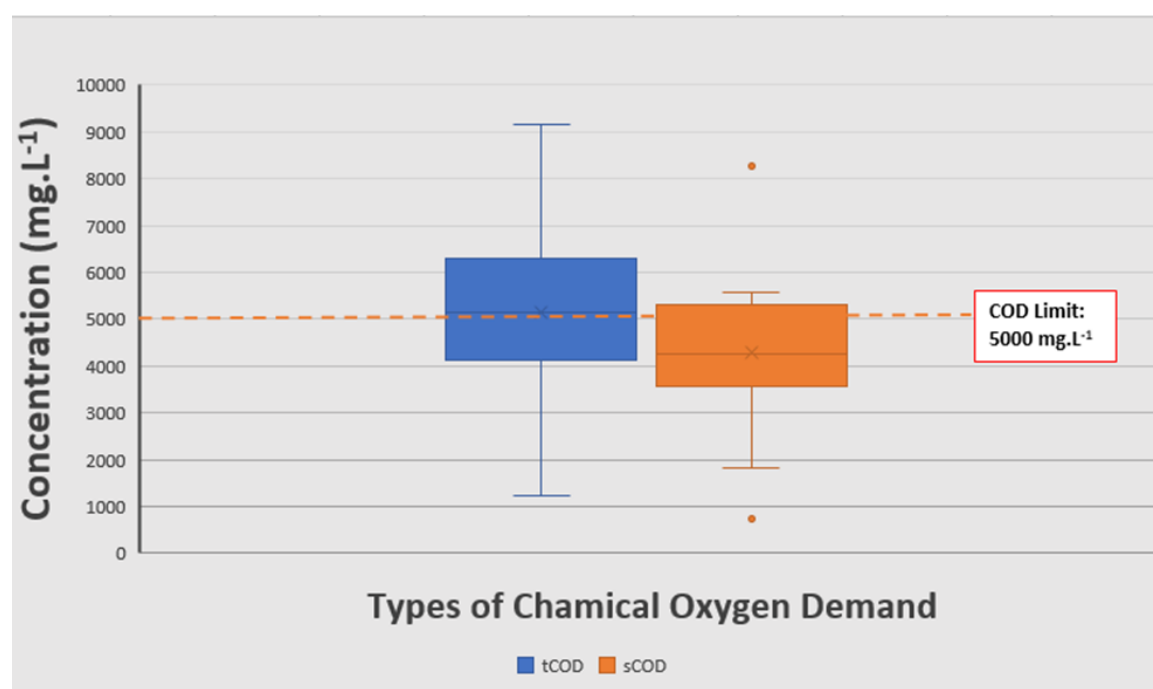


Figure 4.3: Boxplot showing tCOD and sCOD of the effluent. The values within the box (X) are the average of 10 samples. The dots above the box represent the outliers. Mangaung Municipality by-laws threshold limit is noted with a dashed red line and text on the right.

Figure 4.3 depicts a slight shift in the tCOD and sCOD values. Due to the removal of the particulates in the samples, the COD was reduced. Two outlying values are evident for sCOD, as sample 3 was slightly reduced from 9160 mg.L⁻¹ to 8270 mg.L⁻¹ but still above being an outlier. Sample 6 was considerably lower and further reduced from 1220 mg.L⁻¹ to 740 mg.L⁻¹, which was well below the other values. According to Kink (2016) and Turano et al. (2002), screening and filtration are effective methods to determine COD load reduction, which has a significant impact on effluent. It is thus proposed that settling the effluent as a form of pre-treatment at this abattoir could aid in the removal

of soluble COD. During settling, solid particles, including some organic matter, settle to the bottom of a tank, resulting in a clarified effluent. While settling primarily targets suspended solids, it can also contribute to the removal of some dissolved organic compounds, thereby reducing the soluble COD content.

DO values ranged from 0.9 - 3.32 mg.L⁻¹ for 90% of the samples, which indicated that the condition of the effluent was aerobic, except sample 3 of 0.48 mg.L⁻¹ that was anaerobic. Dissolved oxygen is a critical factor for aerobic microorganisms involved in the breakdown of organic matter within effluent. Adequate DO levels sustain aerobic conditions and support the growth and activity of these microorganisms, while insufficient levels can lead to anaerobic conditions that potentially compromise treatment efficiency and cause the production of undesirable by-products (Skouteris et al., 2020). WWTPs therefore regularly monitor DO levels because they are instrumental in optimising treatment processes. The information that is elicited is then used to make necessary adjustments to aeration systems or other components to ensure favourable conditions for aerobic microbial activity. Inadequate DO levels not only impact microbial health, but can also lead to the generation of foul-smelling compounds, and this necessitates vigilant monitoring to prevent odour issues (Daskiran et al., 2019; Meng et al., 2019). Figure 4.4 shows the results of the remaining physicochemical parameters that were measured.

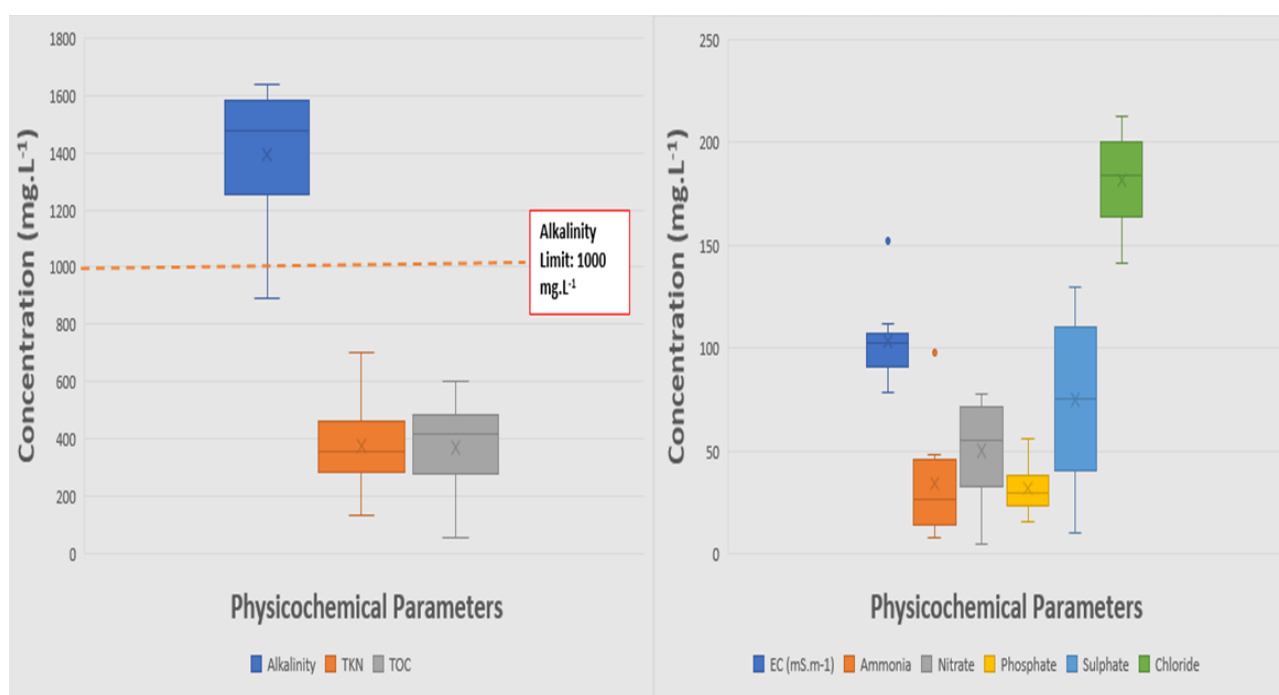


Figure 4.4: Boxplot showing the results of physico-chemical parameters of the red meat abattoir effluent. Mangaung Municipality by-laws threshold limits are indicated with a dashed red line and text on the right for alkalinity. The X within each box is the average of the 10 samples and the line in the interior of the box is the median. The top whisker above each box represents the highest value and the bottom whisker below each box represents the lowest value. The plots outside are the outlier values.

Total Kjeldahl nitrogen (TKN) indicates a combination of organic nitrogen and ammonia nitrogen concentrations, the highest values for TKN were observed on sampling day 3 (701 mg.L⁻¹), day 7 (491 mg.L⁻¹), and day 5 (456 mg.L⁻¹). The observation of elevated TKN levels on specific sampling days highlights a substantial nitrogen load on those days. To elucidate the composition further, a separate analysis of ammonia is suggested. This can be achieved by subtracting its value from TKN to determine the concentration of organic nitrogen. This analytical approach will enhance understanding of the nitrogen concentrations present in the effluent and aid in the evaluation of potential treatment processes.

The average concentrations of ammonia and nitrate of the sampling days were 34 and 50.1 mg.L⁻¹ respectively. These concentrations highlight the importance of these key nitrogen forms in the nitrogen cycle. Explorations of ammonia in biological treatment systems have reflected the dynamic nature of nitrogen transformations. The current study found that, when TKN exceeded ammonia concentration (Figure 4.4), the intricate interplay between ammonia addition from organic nitrogen breakdown and removal through nitrification complicated the determination of nitrification efficiency. This imbalance holds implications for the nitrogen cycle as it suggests that it affects ammonification and nitrification processes. Overall, scientific insights derived from analysing TKN and ammonia concentrations offer a valuable perspective on the nitrogen dynamics within red meat abattoir effluent. From the biological treatment system perspective, this information can contribute to better informed and more effective wastewater management strategies (Applequist, 2012; Wastewater Blog, 2022).

The profile of EC observed throughout the samples varied from 78.6 - 152.15 mS.m⁻¹. The highest value was observed for sample 3 when cattle and sheep were slaughtered, and the lowest value was observed for sample 6 when pigs were slaughtered. EC values below the recommended limit of 500 mS.m⁻¹ (Figure 4.4) were observed for all the samples (i.e., 100% compliance). The observed EC values suggest the existence of dissolved inorganic substances in ionized form within the effluent, and that this contributed to the TDS and salinity levels. The presence of these dissolved inorganic

substances also suggests the potential existence of chemicals that may have inhibitory effects on microbial growth. The mean TDS concentration was 825.25 mg.L^{-1} , which demonstrated a direct proportionality with the EC values as it followed a consistent pattern. Specifically, the highest TDS concentration in sample 3 (1217 mg.L^{-1}) corresponded with the peak EC value, while the lowest TDS concentration in sample 6 (628 mg.L^{-1}) aligned with the lowest EC value. This correlation highlights the interdependence between EC and TDS concentrations in the effluent, emphasising the significance of EC measurements as an indicator of the chemical composition and salinity of the wastewater, as proposed by Wu and Mittal (2012).

The permissible value of alkalinity as recommended by Mangaung Municipal by-laws is 1000 mg.L^{-1} for CaCO_3 . However, the alkalinity concentration levels of the wastewater samples collected in the study site ranged from $890 - 1640 \text{ mg.L}^{-1}$, and thus a 90% non-compliance with the by-laws was observed (Figure 4.4). Sample 6 (890 mg.L^{-1}) was the only sample below the acceptable limit of 1000 mg.L^{-1} . Talukder and Selvakumar (2015) state that alkalinity and pH are important factors in determining the amenability of wastewater to biological treatment as these variables affect the rate of microbial growth and the function of metabolic enzymes.

The phosphate levels observed in the samples ranged between $15.5 - 56.2 \text{ mg.L}^{-1}$ and were thus within the recommended guideline of 100 mg.L^{-1} . Phosphate is generally considered an undesirable factor in aquatic environments as it leads to various ecological challenges in water systems. High levels of phosphate and nitrates increase oxygen demand and low levels favour the growth of filamentous bacteria (particularly at activated sludge treatment plants), which results in bad settling and thickening properties of the sludge. A decline in the ability of sludge to thicken can serve as a practical indicator of phosphate deficiency. Phosphate plays a crucial role in wastewater treatment processes and biological treatment systems, particularly in terms of the formation of microbial biomass. When phosphate is limited, microorganisms may struggle to form an effective biomass, leading to a decrease in the ability of the sludge to thicken (Kroiss et al., 2011).

The analysis of sulphate and chloride in the effluent was crucial due to their predominant presence in cleaning agents and detergents commonly used in abattoirs for hygienic purposes. It was found that sulphate concentrations were within the Mangaung Municipal by-law level of 1500 mg.L^{-1} as they ranged between $10 - 130 \text{ mg.L}^{-1}$, while it was found that chloride concentrations ranged between $141 - 213 \text{ mg.L}^{-1}$ over the sampling period. Mittal (2004a) argues that compounds such as sodium

lauryl sulphate, sodium laureth sulphate, and other sulphate-containing surfactants and residues containing sulphates are known to disrupt the cell membrane integrity of microorganisms by interacting with lipids and proteins, leading to cell lysis and ultimately cell death. This inhibitory effect can reduce the overall microbial population in wastewater, including beneficial microbes responsible for biodegradation processes. For instance, enzymes responsible for the breakdown of fats, oils, and grease (e.g., lipases) may be particularly sensitive to inhibition by these surfactants. This selective inhibition can compromise the efficiency of wastewater treatment systems and lead to the accumulation of organic pollutants (Chandra et al., 2020).

Another group of chemicals comprises chlorides that are commonly present in cleaning and disinfection agents. For example, sodium hypochlorite (bleach) is one such common ingredient. These chlorine-based compounds can lead to the presence of chlorides in red meat effluent. High concentrations of chloride ions contribute to the saltiness of water, thus increasing the aggressiveness of the aqueous environment towards metals and exacerbating the corrosion process. Additionally, chloride ions can enhance the conductivity of water, thus facilitating faster electrochemical reactions involved in corrosion which can compromise the structural integrity and longevity of equipment and pipes used for effluent discharge (Chen et al., 2021; Xu et al., 2022). Abattoirs therefore need to consider the types and quantities of cleaning agents they use and they should implement proper cleaning practices. They should also explore the viability of environmental-friendly cleaning products without compromising cleanliness and sterilisation.

In terms of pH, the levels in the samples ranged from 7.19 (sample 5) - 7.70 (sample 8) (see Supplementary Table 4.1). The mean pH values recorded for all the samples over the 10-day period were within the guideline limit for discharged effluents into the municipal sewer system. The overall pH of the wastewater that was tested was neutral (pH 7.48) and ideal for the existence of biological life. Bacteria and other organisms that play an active role in wastewater treatment are most effective at a neutral to slightly alkaline pH of 7 to 8 (Edokpayi et al., 2017; Z. Li et al., 2020).

A wastewater temperature of above 35°C is associated with negative effects on biological activity during the treatment process as it may reduce waste treatment efficiency (Mahgoub et al., 2016). The overall temperature of the current samples ranged between 24°C - 28°C on all sampling days. The temperature profile was quite consistent throughout and only a slight difference was observed among the different sampling days. The highest value was for sample 6 (28.2°C), followed by

samples 7 (26.53°C) and 5 (26.25°C), while sample 8 (24.85°C) had the lowest temperature. On sample day 6, only one species of animal was slaughtered, namely 185 pigs. The increased temperature on this day was not unexpected as an additional step of scalding is required during pig slaughtering. In this step, pigs are submerged in a scalding tank for around 6-10 minutes depending on the water temperature that should ideally range between 58°C - 62°C to loosen hair follicles for dehairing (Carroll et al., 2015; Kongsted et al., 2020).

4.5 Biological Diversity

4.5.1 Culturable Microbiome

The microbiological enumeration revealed the presence of both bacterial and fungal populations in all the samples on all the survey days. The bacterial species that were isolated included coliforms, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, faecal coliform *E. coli*, and non-fastidious bacteria. The fungal species that were isolated were yeast and mould. Table 4.1 below presents the total viable counts in the abattoir effluent. The microbial community was dominated by bacteria, with levels in the range of $1.08 - 5.26 \times 10^6$ CFU.mL⁻¹. The average value of the yeast count for the abattoir effluent was 5.32×10^3 , while that of the mould was 7.10×10^1 CFU.mL⁻¹. These results are consistent with those of other studies that found a predominance of bacteria versus fungi in abattoir effluent as well as considerable levels of *E. coli* and coliforms (Ijah et al., 2022; Jabari et al., 2016a).

The water samples harboured bacteria of varying counts on the different sampling days. It was observed that the counts were highest on sampling day 2 and lowest on sampling day 5. Sampling day 2 was when cattle and pigs were slaughtered and sampling day 5 was cattle only. This was not a consistent pattern because other sampling days when cattle and pigs were slaughtered, such as sampling days 7, 8 and 10, did not display such high microbial counts (Moreroa & Basitere, 2022).

Amongst the coliform bacteria, *Enterobacter aerogenes* had the highest average of 1.18×10^5 CFU.mL⁻¹, followed by *Pseudomonas aeruginosa* with an average of 8.04×10^4 CFU.mL⁻¹ and *E. coli* with an average of 2.78×10^4 CFU.mL⁻¹. *Enterobacter aerogenes* and *E. coli* are normal inhabitants of the intestinal tract of animals, including livestock processed in abattoirs. Contamination of abattoir effluent can occur through faecal material from slaughtered animals (Wang et al., 2022). *Pseudomonas aeruginosa* is a ubiquitous bacterium commonly found in soil, water, and various environments. *P. aeruginosa* can enter abattoir effluent through contaminated water sources used in

processing operations. Additionally, it may be present in the surrounding environment and introduced into the wastewater system through runoff water or other environmental pathways (Adesoji et al., 2016).

Table 4.1: Highest and lowest CFU.mL⁻¹ values of total viable microbial population in the abattoir effluent

Sample	Bacteria	<i>E. coli</i>	<i>Enterobacter aerogenes</i>	<i>Pseudomonas aeruginosa</i>	Yeast	Mould
1	4,24E+06	1,40E+04	1,17E+05	6,10E+04	7,75E+03	3,00E+00
2	5,26E+06	1,50E+04	2,01E+05	6,60E+04	6,82E+03	4,00E+00
3	1,32E+06	1,30E+04	1,48E+05	1,18E+05	5,63E+03	2,30E+01
4	3,02E+06	9,60E+04	9,20E+04	1,08E+05	6,15E+03	1,00E+00
5	1,08E+06	3,00E+04	1,60E+05	8,30E+04	4,30E+03	1,30E+01
6	1,69E+06	2,80E+04	2,08E+05	5,60E+04	1,66E+03	7,00E+00
7	1,52E+06	5,00E+03	5,60E+04	9,20E+04	4,81E+03	5,00E+00
8	1,47E+06	3,50E+04	7,20E+04	4,40E+04	5,38E+03	2,00E+00
9	2,18E+06	2,00E+03	2,70E+04	1,04E+05	7,71E+03	1,10E+01
10	2,75E+06	4,00E+04	1,02E+05	7,20E+04	2,98E+03	2,00E+00
Average	2,45E+06	2,78E+04	1,18E+05	8,04E+04	5,32E+03	7,10E+00

Note: The selected media were conducive for different types of microbes but, for the purpose of this study, only the levels of the specified microbes were determined.

Yeast counts were higher on sampling days 1, 2, and 9 than on the other days. On these former days cattle were slaughtered. Mould counts were highest on sampling days 3, 5, and 9, which were days when cattle and sheep were slaughtered. Fungi form a group of microscopic non-photosynthetic plants that include yeasts and moulds. Yeast is widely used as a feed supplement for livestock while moulds are filamentous fungi that bear a resemblance to higher order plants in structure with branched, fractal-like growths. Fungi tend to compete disadvantageously with bacteria for nutrients, so their numbers are low in the presence of bacteria. Fungi such as moulds are nuisances in many biological wastewater processes because of their filamentous nature which interferes with floc settling in flocculation and sedimentation basins (Climate Policy Watcher, 2023; Zhang et al., 2018).

4.5.2 Functional Diversity

When living as an organised consortium, bacteria exhibit enhanced efficiency in degradation processes. This efficiency is attributed to the employment of various communication strategies among the microbial community during the degradation of environmental substances. Their efficiency is due to the presence of multiple microorganisms within the consortium, each possessing distinct metabolic capabilities. These diverse metabolic pathways enable the bacteria to collaborate effectively within the consortium and facilitate the degradation of a broad range of materials found in the environment (Jamal et al., 2018; Koh & Khor, 2023).

The functional analysis of microbial communities allows the clarification of the interactions between the organisms and the environment. In this study, analyses were performed using Biolog Ecoplates™, which is a culture-dependent approach that reveals the most active populations within the microbial community and thus illuminates the community's physiological patterns (Feigl et al., 2017). The Biolog Ecoplates™ contain 31 substrates that can potentially be used as nutrients by microorganisms within a community. The substrates in the wells can be subdivided into five groups: carbohydrates ($n = 10$), carboxylic and acetic acids ($n = 9$), amines and amides ($n = 2$), amino acids ($n = 6$), and polymers ($n = 4$) (Martínez-Gallardo et al., 2021). Table 4.2 shows the average well colour development (AWCD), the number of substrates metabolised ('functional richness'), and other functional biodiversity indices of the microbial community of the samples measured after an incubation period of 72 hours. The number of substrates utilised by the effluent community averaged 30 out of the 31 carbon sources. A similar trend was also found in the carbon substrate categories for the intensity of metabolic activity, as shown by the AWCD in Figure 4.5 below. Metabolic activity was detected in all five the substrate categories.

The functional biodiversity that is expressed as Shannon index (H') was moderately high in all samples, with values ranging between 3.29 (sample 6) - 3.38 (sample 2). The Simpson index (D), which was used to quantify the diversity of the microbial community, was 0.962 (± 0.0012), thus displaying a very diverse community. While the evenness values (E -values) indicated a very similar metabolic profile for the samples ranging from 0.95 (sample 6) - 0.98 (sample 2), this compared the similarity of population sizes between each of the species present in the community. The E -values depicted the presence of a large abundance of species. These results revealed that the functional biodiversity of the effluent was quite consistent, which was irrespective of the number of animals and species of animals slaughtered on the day.

Table 4.2: Richness and diversity indices of the physiological profile at community level using BioLog Ecoplate™ analysis (10 red meat abattoir effluent samples)

Sample	H'	D	E	R
1	3,344026	0,961978	0,973803	30
2	3,375262	0,960817	0,982899	30
3	3,353774	0,963470	0,976641	30
4	3,353146	0,963668	0,976458	30
5	3,356902	0,963596	0,977552	30
6	3,292848	0,959138	0,958899	29
7	3,353449	0,960529	0,976547	30
8	3,326756	0,962031	0,968774	29
9	3,313916	0,961113	0,965035	29
10	3,349023	0,963062	0,975258	30

The relative abundance of the metabolic activities grouped according to the chemical family of the substrates is shown in Figure 4.5. The dominant functional activities were those related to the metabolism of polymers (24%) and carbohydrates (20.6%), followed by those of amino acids (20.3%), carboxylic acids, and amines and amides that were less represented with relative average abundances of 17% and 16.9%, respectively.

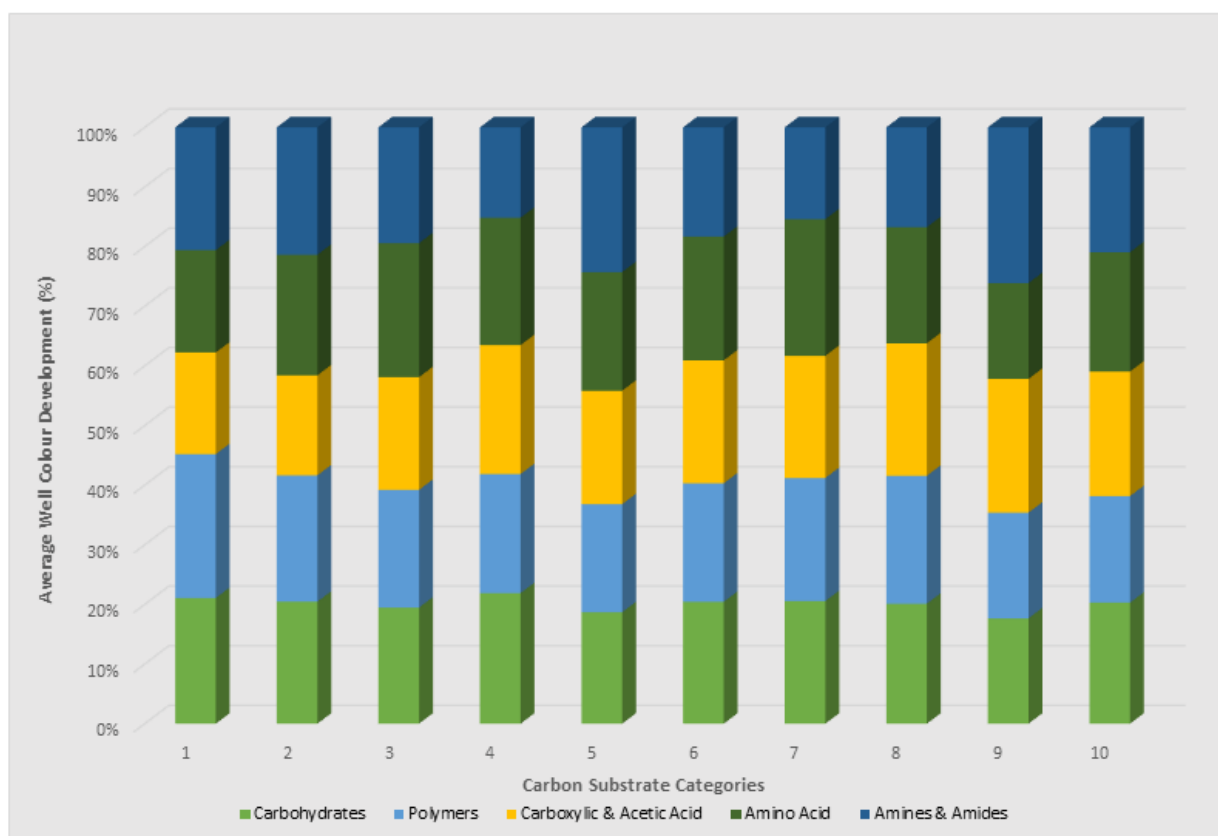


Figure 4.5: Relative abundance of metabolic activities according to average substrate well colour development (AWCD) of substrate categories consumed by microbial communities in the effluent

These results show that the microbial community found in the effluent was prone to biodegradation of polymers which was mainly associated with the activity of fungal and Actinobacteria populations. These are known to be the main microbial groups involved in the utilisation of polymers because of the production of extracellular enzymes (Khanna Kapur et al., 2018). Sample 2 appeared to be the extreme case as 29% of the overall metabolic activity was related to the polymer-utilising activity.

Carbohydrates are intermediates of organic matter degradation and they were the second highest utilised carbon source amongst the samples. They are precursors for the synthesis of many biochemically active compounds and are important in terms of interactions between organisms of different trophic levels, thus having great significance for plant nutrition and phytoremediation (Lojkova et al., 2020). Samples 4, 5, and 7 showed the highest utilisation of carbohydrates. These

samples had no notable similarity except for the fact that cattle were slaughtered, which was also evident in samples 1, 2, 8 and 10.

All the samples presented shared a common substrate utilisation pattern where L-arginine (average optical density [OD] value of 2,48, glycogen (average OD value of 2,47), and D-cellobiose (average OD value of 2.31) was the most utilised substrate by all the samples belonging to polymers, carbohydrates, and amino acid groups respectively. The substrate that was not utilised, namely 2-hydroxy benzoic acid (average OD value 0,15) and the substrate that had the second lowest utilisation, namely α -Ketobutyric acid (average OD value 0.68), both belonged to the carboxylic and ketonic acids (Figure 4.6).

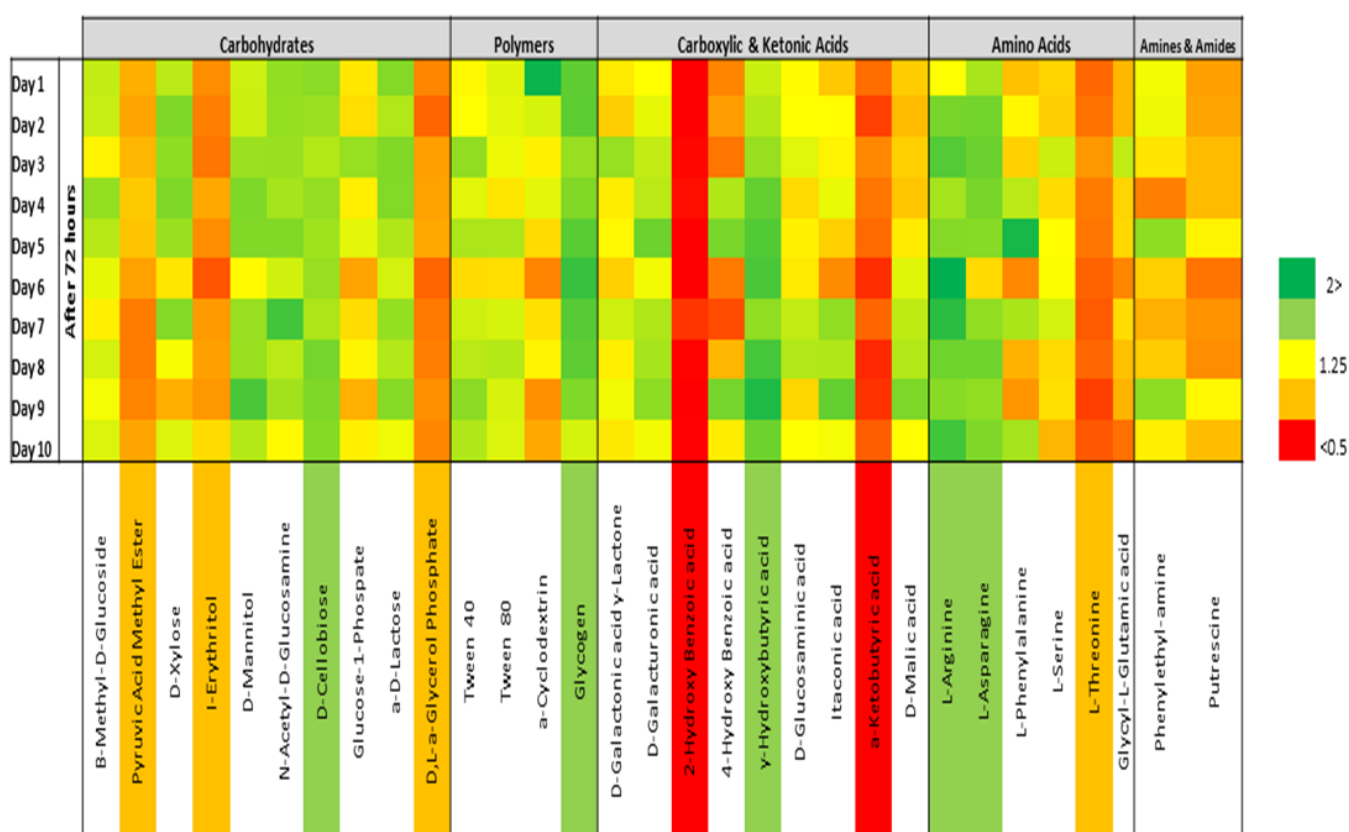


Figure 4.6: A heat map showing different carbon groups with the specific carbon sources within that group detected by Biolog Ecoplate™. The colours indicate a change in optical density (OD₅₉₀) calculated over 72 hours for abattoir effluent samples 1-10 taken over 10 days. Red reflects poor utilisation (OD change <0.5) and green (OD change >2) reflects the more active community with high utilisation.

In abattoir effluent, microorganisms play a crucial role in the degradation and transformation of various organic compounds, including amino acids such as L-arginine. The microbial community present in abattoir environments consists of bacteria and fungi that have developed metabolic capabilities to utilise amino acids as a source of carbon and nitrogen (Idrees et al., 2020; Ries et al., 2018). Certain bacteria possess enzymes called deaminases that can remove the amino group from amino acids like L-arginine, converting them into organic acids or other metabolites (Cooper, 2000). This deamination process allows bacteria to utilise the carbon skeleton of L-arginine as an energy source for their growth and metabolic processes. The released amino group is often incorporated into the microbial biomass or further metabolised (Motta et al., 2016; Saini et al., 2023). Similarly, some fungi can utilise amino acids such as L-arginine for their growth and metabolism. These fungi possess specific enzymes that break down amino acids and convert them into simpler compounds, which can then be used as a carbon and nitrogen source for their cellular processes (Ene et al., 2014).

The metabolic pathways involved in the utilisation of L-arginine in abattoir wastewater can vary depending on the specific microorganisms present. The pathways are largely dictated by the enzymatic capabilities of the microbial consortium, as briefly discussed below (Racké & Warnken, 2010):

- Arginine deamination: Bacteria possessing the enzyme arginine deaminase can deaminate L-arginine, removing the amino group and converting it into ornithine. This metabolic pathway allows the bacteria to utilise the carbon skeleton of L-arginine as an energy source while incorporating the amino group into their cellular biomass or further metabolising it.
- Arginine decarboxylation: Some bacteria possess the enzyme arginine decarboxylase, which enables the decarboxylation of L-arginine, resulting in the production of agmatine. Agmatine can serve as a precursor for the biosynthesis of various metabolites or be further catabolised by the microorganism.
- Arginine hydrolysis: Many bacteria and fungi produce enzymes called arginases that hydrolyse L-arginine, breaking it down into ornithine and urea. Ornithine can be further metabolised or it may serve as a precursor for the biosynthesis of other compounds, while urea can be utilised by microorganisms capable of urea hydrolysis.
- Arginine utilisation as a nitrogen source: In addition to carbon metabolism, microorganisms can utilise L-arginine as a nitrogen source. They may employ various transporters and

enzymes to internalise and metabolise L-arginine for the synthesis of amino acids, nucleotides, and other nitrogen-containing compounds required for their growth.

Glycogen is a storage form of glucose, and certain bacteria and fungi in abattoir environments can utilise this polymer as a carbon and energy source (Zeitz et al., 2019). Utilisation of glycogen by microorganisms involves the following metabolic pathways (McGinnis & Tying, 1996; B. T. Wang et al., 2020):

- Glycogen degradation: Microorganisms possessing the enzyme glycogen phosphorylase can break down glycogen into glucose-1-phosphate units. Glycogen phosphorylase cleaves the glucose units from the glycogen chain, releasing glucose-1-phosphate, which can then be further metabolised.
- Glycolysis: Glucose-1-phosphate, derived from glycogen breakdown, can enter the glycolytic pathway. In glycolysis, glucose-1-phosphate is converted to glucose-6-phosphate and subsequently metabolised to produce energy in the form of ATP. This pathway involves a series of enzymatic reactions and ultimately results in the production of pyruvate.
- TCA (tricarboxylic acid) cycle: Pyruvate generated from glycolysis can enter the TCA cycle. In the TCA cycle, pyruvate is further metabolised to generate additional ATP and reduce equivalents, and it is a precursor molecule for various biosynthetic pathways.

Periyannan (2019) explains D-cellobiose as a disaccharide composed of two glucose units linked by a β -1,4-glycosidic bond, and it is derived from the hydrolysis of cellulose. Ruminants like cattle and sheep are known for their ability to digest cellulose, which is a complex carbohydrate found in plants. This is facilitated by the microbial consortium in their stomachs, which includes cellulolytic microorganisms capable of breaking down cellulose into simpler sugars. Pigs, on the other hand, are monogastric animals and traditionally not considered as efficient cellulose digesters as ruminants, yet the microbial communities can demonstrate adaptability and metabolic versatility even in environments where cellulose digestion is not the primary function (Jha et al., 2019; Seyedalmoosavi et al., 2022).

As anticipated, the detected microorganisms exhibited the ability to utilise D-cellobiose as a carbon source that occurred through the following metabolic pathways as described by Li et al. (2019):

- Extracellular hydrolysis: D-cellobiose can be hydrolysed by extracellular enzymes called β -glucosidases. These enzymes cleave the β -1,4-glycosidic bond between the two glucose units, releasing individual glucose molecules.
- Glucose metabolism: Once D-cellobiose is hydrolysed into glucose, microorganisms can metabolise glucose through various metabolic pathways. Glucose can enter glycolysis, where it is further metabolised to produce ATP and reduce equivalents, and these are precursor molecules for cellular processes.
- Phosphorylation and transport: Some microorganisms possess specific transport systems and enzymes that phosphorylate glucose. The phosphorylation of glucose allows the glucose molecule to enter metabolic pathways efficiently.

In the current study, the microbial consortium in the abattoir effluent did not utilise 2-hydroxybenzoic acid (salicylic acid) and α -ketobutyric acid due to several possible reasons. For instance, microorganisms have specific metabolic capabilities and not all microorganisms possess the necessary enzymes and pathways to utilise these organic compounds. The absence of these specific enzymes or metabolic pathways could thus have limited their utilisation. Additionally, microorganisms often have preferred carbon sources that they can metabolise more efficiently. If other readily available carbon sources were present in the abattoir effluent, microorganisms could have prioritised utilising those sources over 2-hydroxybenzoic acid and α -ketobutyric acid, as proposed by Preston-Mafham et al. (2002).

The literature proposes that the toxicity and inhibitory effects of these compounds can impact microbial utilisation. Therefore, if 2-hydroxybenzoic acid and α -ketobutyric acid are present in high concentrations, they may exhibit toxicity or inhibitory effects on microorganisms, affecting their ability to effectively utilise these compounds (Koh & Khor, 2023). However, it is essential to note that the EcoPlate™ assay, designed for qualitative assessments and relative comparisons, measures the ability of microbial communities to oxidise carbon substrates, and this does not facilitate the precise quantification of the compound concentrations (Biolog EcoPlate™, 2023). The current study thus highlights a potential limitation regarding the investigation of 2-hydroxybenzoic acid and α -ketobutyric acid within the scope of microbial interactions in red meat abattoir effluent. Specifically, while these compounds have been identified as not useful to the microbial community, their precise in-situ concentrations remain unexplored. This lack of detailed concentration data represents a gap in our

understanding, as it inhibits our ability to fully assess the potential of not utilising these compounds that could be caused by toxicity or inhibitory effects of these compounds on the microbial community.

Furthermore, the microbial community in abattoir effluent does not inherently consist of microorganisms with specialised metabolic pathways for these compounds. The absence or low abundance of microorganisms with the necessary metabolic capabilities can contribute to their limited utilisation. The utilisation of 2-hydroxybenzoic acid (also known as salicylic acid) and α -ketobutyric acid by microorganisms involves specific metabolic pathways that allow them to catabolise these compounds for energy and carbon. Degradation of salicylic acid typically proceeds via the salicylate degradation pathway, while the degradation of α -ketobutyric acid occurs through the ketobutyrate degradation pathway (Qin et al., 2023; Silva et al., 2007; Vila-Santa et al., 2021).

The absence of the salicylate and ketobutyrate degradation pathways as an inherent feature of abattoir effluent can be attributed to the typical composition of organic compounds found in such effluent and the metabolic capabilities of the microbial communities associated with it. Furthermore, the environmental conditions prevalent in abattoir wastewater may not favour the growth and activity of microorganisms capable of metabolising these specific compounds, as they may require specific environmental conditions and microbial consortia for efficient degradation that are not typically found in abattoir wastewater environments.

4.5.3 Genetic Diversity

4.5.3.1 Quality control and data hygiene

The quality of DNA can differ depending on the source or extraction method used. For this reason, a standardised and cost-effective workflow for the qualification of DNA preparations is essential to guarantee reproducible results. The qualification process consists of the quantification of double-strand DNA and the assessment of its suitability for downstream applications, such as high-throughput next-generation sequencing (NGS) (Brosnan & Lacobuzio-Donahue, 2012). While NGS sequencers yield robust results, the workflow process is lengthy, expensive, and labour-intensive and requires appropriate management of the input material. Moreover, non-standardised DNA qualification can negatively impact sequencing performances, resulting in a waste of samples, low confidence results, and higher costs (Montserrat García-Alegría et al., 2020). In the current study, once the DNA had been extracted, the concentration and purity were confirmed on the NanoDrop

2000 spectrophotometer (Thermo Scientific, USA). The NanoDrop results are presented in Table 4.3 below.

Table 4.3: DNA absorbance values for A_{260}/A_{280} , determining the DNA quality

Sample ID	Nucleic acid (ng. μL^{-1})	A_{260}/A_{280} ratio
gDNA_1	83.00	1.835
gDNA_2	102.70	1.840
gDNA_3	103.15	1.840
gDNA_4	115.75	1.825
gDNA_5	123.60	1.830
gDNA_6	148.90	1.845
gDNA_7	104.65	1.835
gDNA_8	102.35	1.835
gDNA_9	90.85	1.835
gDNA_10	94.00	1.830

Absorbance readings were performed at 260nm (A_{260}) where DNA absorbs light more strongly. The A_{260} value should be within the instrument's linear range of 0.1 - 1.0 as it is considered a reliable and reproducible value. Conversely, RNA also has a great absorbance at 260nm, and the aromatic amino acids present in protein absorb well at 280nm. Thus, both contaminants, if present in the DNA solution, will contribute to the total measurement at 260nm. These guidelines were considered in the study to avoid DNA over-quantification. Expected A_{260}/A_{280} values for good quality DNA are commonly within a range between 1.7 - 2.0. The DNA in the current study appeared to be of good quality (Table 4.3) as concentrations were above 50 ng. μL^{-1} as required by ARC-BTP, while the A_{260}/A_{280} ratios were 1.835 ± 0.005 .

The next generation sequencing data for the samples were rarefied. This step ensured that variations observed between different samples were statistically relevant and not the result of differing library sizes. The rarefaction curves (Figure 4.7) suggest that the sequencing depth was adequate to capture most of the prokaryotic diversity in each sample.

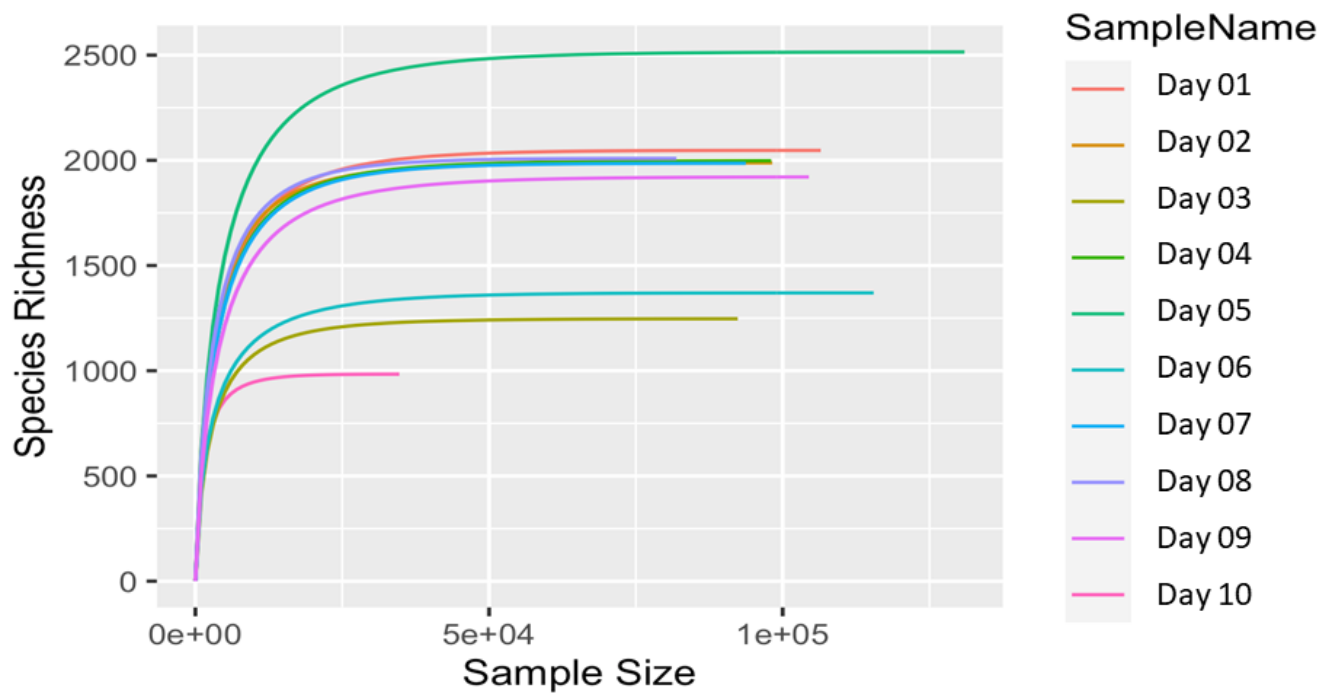


Figure 4.7: A rarefaction curve. To evaluate the sampling depth, rarefaction curves of the microbial communities based on 16S rRNA and ITS gene sequences are shown. Sample names refer to sampling days.

The flattening of the rarefaction curve means that the rate of increase in the number of observed species slowed down as more individuals were sampled. In other words, the curve reaches an asymptote, which suggests that most of the species present in the community were observed, and that adding more individuals to the sample was unlikely to reveal new species.

4.5.3.2 Bacterial diversity and abundance

Taxonomic classification of the clustered amplicon sequence variants (ASVs) revealed the presence of 26 bacterial phyla. Bacterial diversity showed consistent dominance of 3 phyla, namely Bacteroidetes ($39.40\% \pm 0.05$), Firmicutes ($33.23\% \pm 0.02$), and Proteobacteria ($20.1\% \pm 0.05$). These three phyla were present in varying degrees in all 10 samples, with Bacteroidetes being the dominant phylum (Figure 4.8A).

Bacteroidetes and Firmicutes represent major members of microbiota that are often found in the gastrointestinal tract of animals where they exist as degraders of carbohydrates, carbohydrate-based substances, and proteins through fermentation. They are often found in high concentrations, indicating their role in the decomposition of animal waste (Flint et al., 2012; Kirby et al., 2019). Bacteroidetes are Gram-negative bacteria while Firmicutes, on the other hand, are Gram-positive bacteria that are known for their ability to break down complex organic compounds and organic matter. The presence of Bacteroidetes and Firmicutes in abattoir effluent was significant because they play a crucial role in the degradation of organic matter that can be harmful to the environment if not properly treated (Cholewińska et al., 2021; Nishijima et al., 2016).

In wastewater treatment, organic matter is removed through a biological treatment process that relies on microorganisms to break down the organic material. Bacteroidetes and Firmicutes are two of the key groups of microorganisms that are involved in this process (Cholewińska et al., 2021). Bacteroidetes are particularly important in the initial stages of organic matter degradation where they break down complex organic compounds into smaller, more easily digestible molecules. These smaller molecules can then be further broken down by other microorganisms, including Firmicutes. Firmicutes are particularly important in the later stages of organic matter degradation where they break down the remaining organic material into simpler molecules such as carbon dioxide and water (Begmatov et al., 2022; Niestępski et al., 2020).

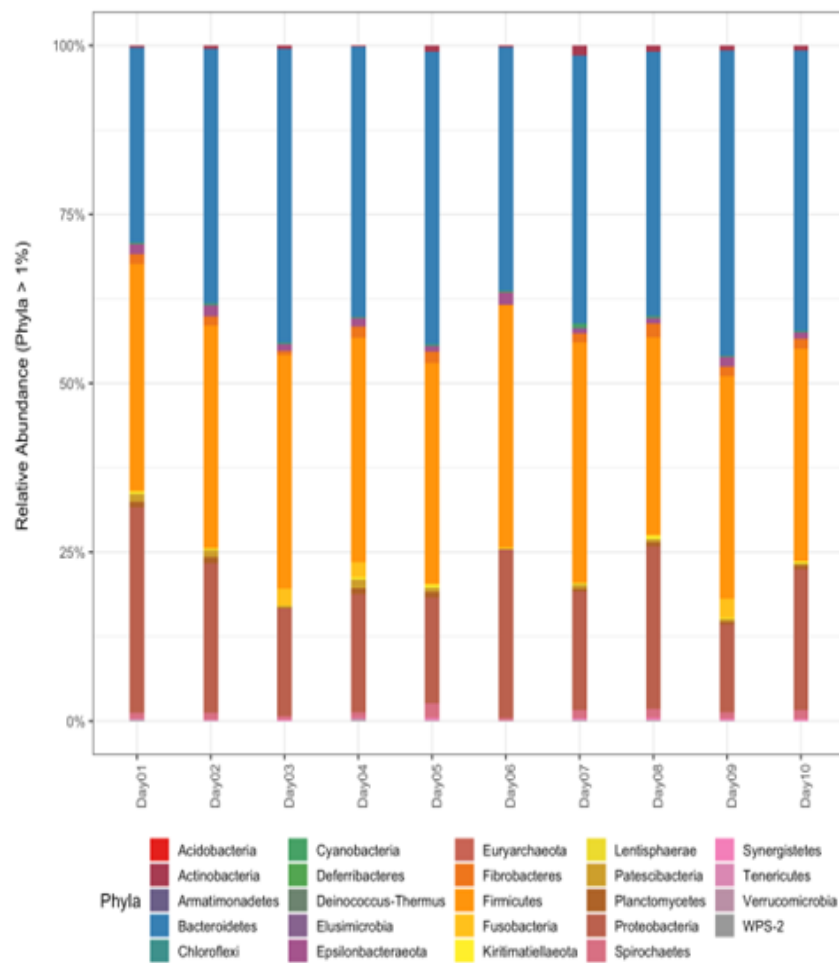
Proteobacteria are the most diverse phylum of Gram-negative bacteria commonly found in the faecal microbiota of animals. They include several well-known opportunistic pathogens such as *Escherichia coli*, *Salmonella*, and *Campylobacter*. Proteobacteria are a diverse group of bacteria that can survive and thrive in a wide range of physical and chemical conditions. Some of the key factors that can affect the growth and survival of Proteobacteria include temperature, pH, salinity, oxygen levels, and nutrient availability (Dias et al., 2019; Diyanoro & Wardhana, 2019). Proteobacteria play an important role in the hydrolysis and acetogenesis steps of anaerobic digestion and include delta, gamma, and beta varieties. Deltaproteobacteria contain many syntrophic anaerobic bacteria that participate in sulphate reduction. Among the Gammaproteobacteria, many denitrifying bacteria or bacteria accumulate phosphates. The Betaproteobacteria are involved in nitrification and are potentially also involved in denitrification (Begmatov et al., 2022; Jabari et al., 2016).

A

Bacterial Composition

Central University of
Technology, Free State

Phyla



Genera

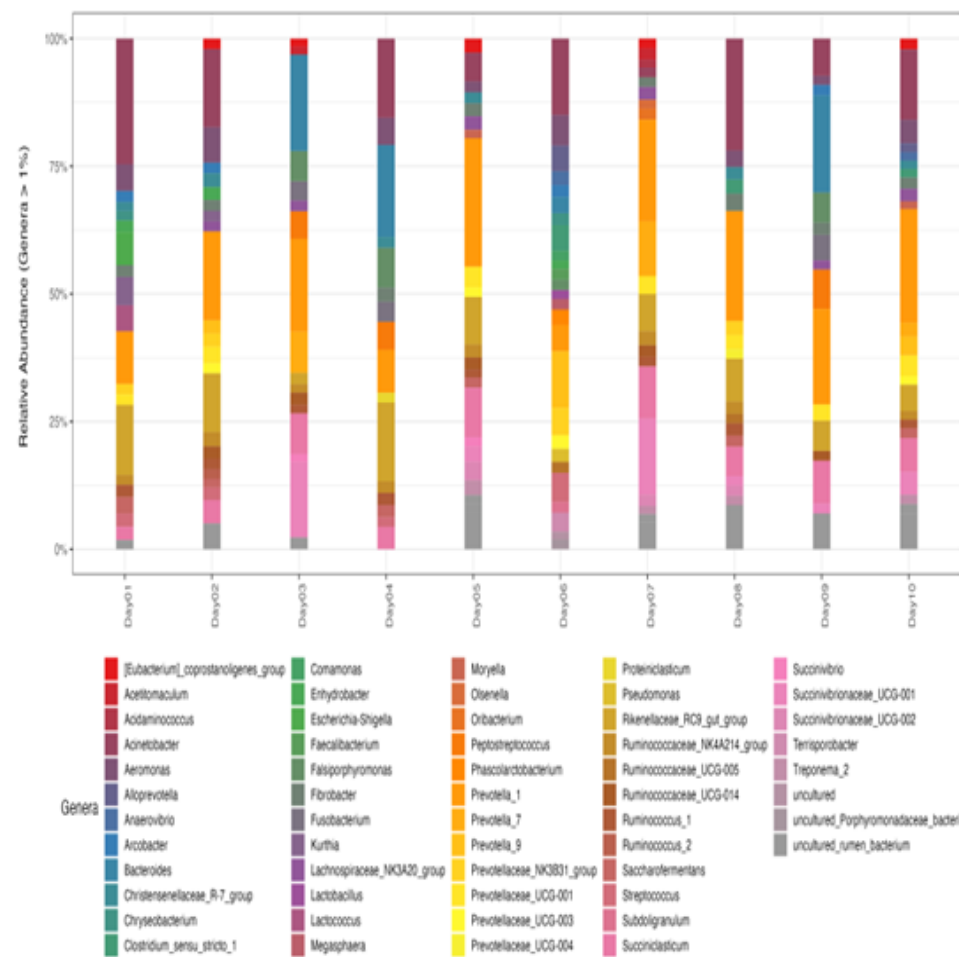


Figure 4.8: Distribution of bacterial phyla and genera diversity expressed as relative abundance % of amplicon sequence variants (ASV) in red meat abattoir effluent. (A) The most consistent dominant phyla are Bacteroidetes (blue), Firmicutes (orange), and Proteobacteria (purple). (B) The genus has no present pattern or consistency in distribution.

Figure 4.8B displays the most abundant bacterial genera detected, which revealed that the genus richness varied in dominance distribution. Certain bacterial genera were more abundant than others in the samples, and the degree of dominance varied across the different samples. A total of 683 bacterial genera were detected. Specifically, the most abundant genera observed were *Acinetobacter*, which belongs to the Proteobacteria phylum, and *Bacteroides* and *Rikenellaceae_RC9_gut_group*, which both belong to the Bacteroidetes phylum. The fact that these four genera were consistently observed across the different samples affirms that they play important roles in the microbial community of abattoir effluent. The variability in their dominance across the different samples reflected changes in the overall microbial community structure or the environmental conditions of the effluent.

Abattoir effluent contains a complex mixture of organic and inorganic materials that can provide a rich source of nutrients for bacterial growth. For *Acinetobacter*, *Bacteroides*, and *Rikenellaceae_RC9_gut_group*, the conditions that are most conducive to their growth and survival include the presence of organic matter, low dissolved oxygen, and a neutral to slightly alkaline pH. The high organic content of abattoir effluent provides these bacteria with a rich source of carbon and energy for growth, while the low oxygen levels and anaerobic conditions in WWTPs provide an ideal environment for these anaerobic bacteria to thrive. In addition, these bacteria are well adapted to the gut microbiota of animals and may be introduced into abattoir effluent through animal waste (He et al., 2021; Qiu et al., 2022), which is a major component of abattoir effluent. Overall, the conditions in abattoir effluent are conducive to the growth and survival of *Acinetobacter*, *Bacteroides*, and *Rikenellaceae_RC9_gut_group*, which all play important roles in the degradation of organic matter and the removal of nutrients from water.

Forcina et al. (2022) explored the gut microbiome in livestock, and they support the notion of a microbiome present in abattoir effluent. In the current study it was found that, in the cattle microbiome, the predominant phyla identified were Bacteroidetes and Firmicutes, which collectively constituted over 90% of the gastrointestinal tract bacterial community. The dominant genera observed not only in cattle but in adult ruminants overall were *Butyrivibrio*, *Prevotella*, and *Ruminococcus*. Similarly, the sheep gastro intestinal tract (GIT) microbiome exhibited substantial similarity in composition with that of cattle and other ruminants, with Bacteroidetes and Firmicutes comprising over 80 - 90% of the gut microbial community. The phyla Actinobacteria, Proteobacteria, Spirochaetes, and Verrucomicrobia were also identified, although in lower abundance. The most

prevalent genera in sheep were *Prevotella*, *Acinetobacter*, *Campylobacter*, *Bacteroides*, *Desulfovibrio*, *Oscillospira*, *Ruminococcus*, *Treponema*, *Fibrobacter*, and *Succinivibrio*.

In contrast, owing to the structural differences between ruminants and monogastric animals such as pigs, the gut microbiome of the latter was characterised by the dominance of the phyla Bacteroidetes and Firmicutes, with Proteobacteria being the subsequent abundant phylum. *Bacteroidia*, *Clostridia*, *Alloprevotella*, *Bacteroides*, *Escherichia*, *Lactobacillus*, and *Prevotella* were the most prevalent genera detected in the GIT of adult pigs, along with *Clostridium*, *Desulfovibrio*, *Enterococcus*, *Fusobacterium*, and *Streptococcus*. These findings provide valuable insights into the microbial composition of abattoir-sourced animals and shed light on the intricate dynamics of microbiomes found in abattoir effluent.

4.5.3.3 Fungal diversity and abundance

The fungal taxonomic ASVs classification revealed 11 types of phyla in total. Dominant fungal phyla were either Neocallimastigomycota (52.61% $\pm$ 0.30) or Ascomycota (36.18% $\pm$ 0.26), in contrast to the common phyla distribution of bacteria amongst the different sampling days. Basidiomycota appeared in small, varied levels for each sample, except on day 6 when only pigs were slaughtered. The presence of Mucoromycota was detected in this sample, which is associated with pigs as it was also detected in a study conducted by Li et al. (2022) who characterised the diversity and composition of fungi in the GIT of pigs using high-throughput sequencing. Mucoromycota was found to be one of the most prominent fungal phyla. The remaining abundance consisted of an unidentified phylum. Figure 4.9A illustrates the fungal phyla present in the abattoir effluent under study.

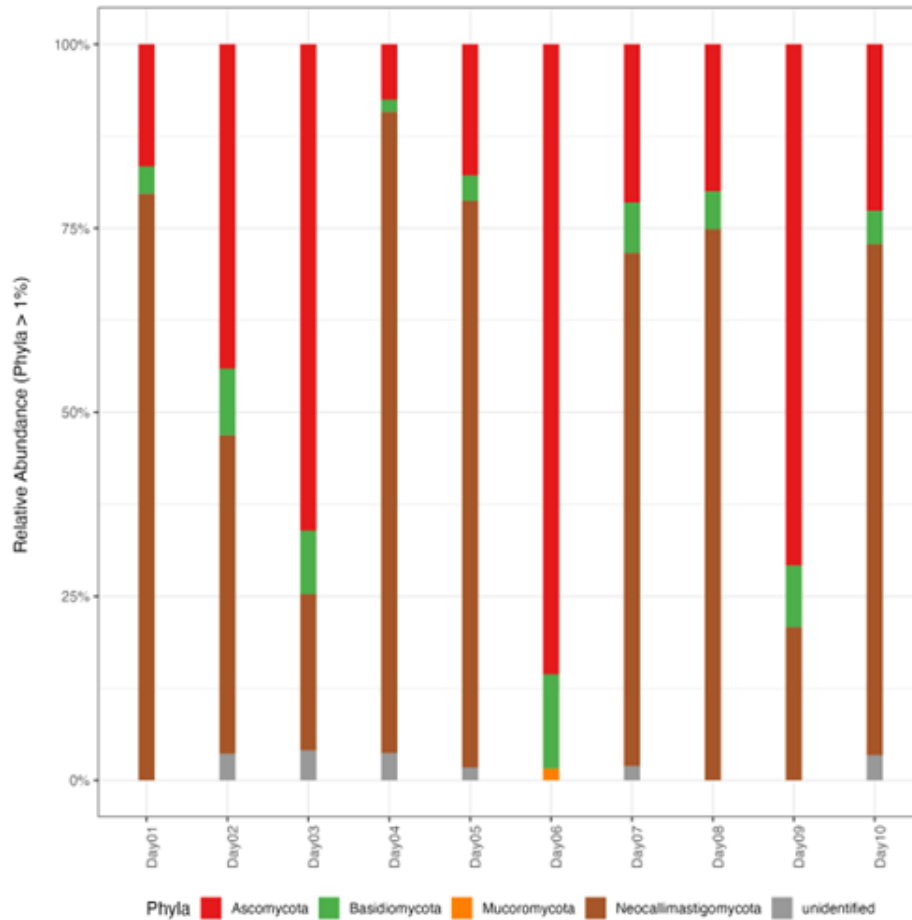
Neocallimastigomycota and Ascomycota fungi play distinct roles in different ecological contexts, including carbon and nitrogen cycling, and their significance in the treatment of abattoir effluent has been demonstrated. Ascomycota fungi are a diverse group of fungi that encompass a wide range of species that include yeasts, moulds, and truffles (Kirk et al., 2008). They are ubiquitous in various ecosystems and play crucial roles in carbon and nitrogen cycling. Ascomycota fungi are involved in the decomposition of organic matter as they break down complex compounds such as lignin and cellulose into simpler forms (Rytioja et al., 2014). This process releases carbon dioxide back into the atmosphere and enables the recycling of nutrients, including nitrogen, back into the ecosystem (Glass et al., 2013). As a result, Ascomycota fungi contribute to nutrient availability and overall ecosystem functioning.

A

Central University of
Technology, Free State

Fungal Composition

Phyla



Genera

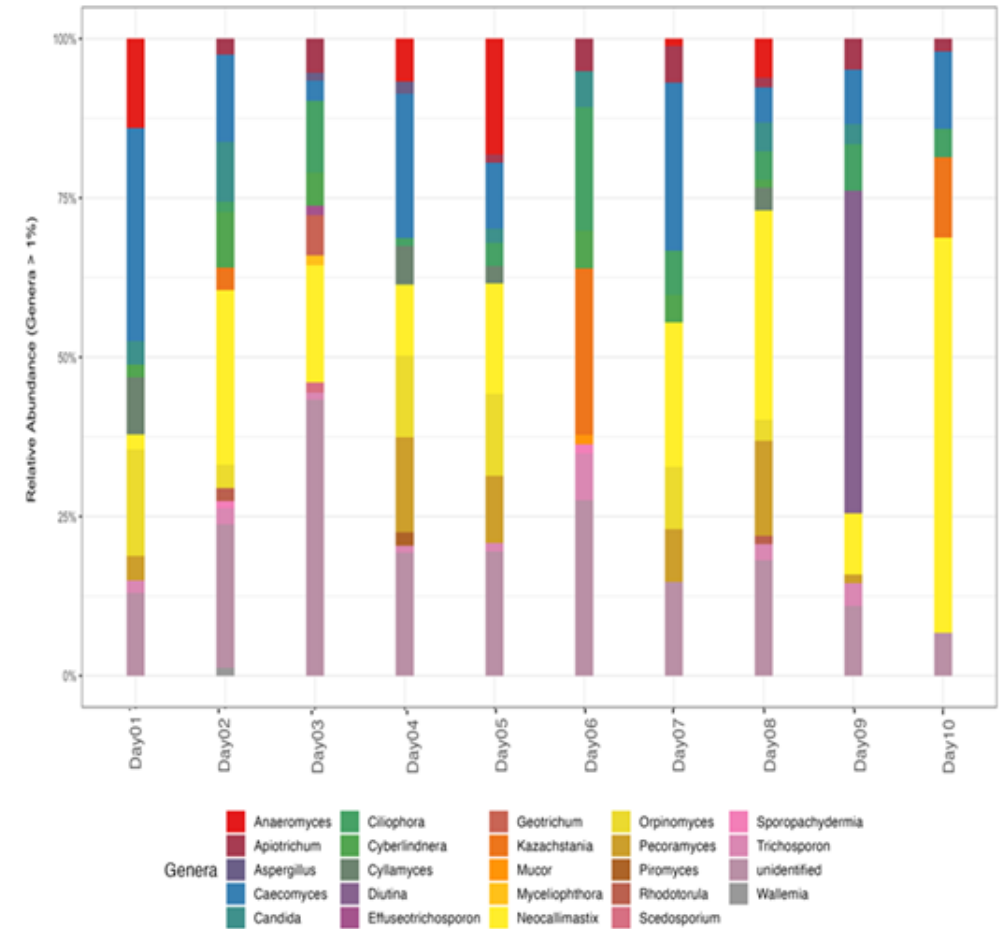


Figure 4.9: Distribution of fungal phyla and genera diversity expressed as relative abundance % of amplicon sequence variants (ASV) in red meat abattoir effluent. Uncultured or ambiguous sequences are indicated as unidentified. (A) The most consistent dominant phyla are either Ascomycota (red), Neocallimastigomycota (brown), or Basidiomycota (green). (B) The genus has no present pattern or consistency in distribution, but it is not so rich in diversity.

Neocallimastigomycota fungi belong to a specialised group of fungi known as gut fermenters or anaerobic fungi. They are primarily found in the rumen, which is the first chamber of the stomach in herbivorous animals (Joblin, 2010). Neocallimastigomycota fungi play a critical role in the digestion of plant material in the rumen. They produce specialised enzymes that break down complex carbohydrates, such as cellulose and hemicellulose, into simpler sugars that can be utilised by the host animal for energy production (Hess et al., 2020; Youssef et al., 2013).

Mucoromycota fungi can be found in the gastrointestinal tract of pigs and can subsequently be excreted in their faeces (Guedes, 2008; Mølbak et al., 2008). During the slaughter and processing of pigs, the faecal matter and other animal waste can mix with abattoir wastewater, leading to the introduction of Mucoromycota fungi into the effluent (Gutu et al., 2021). These fungi can then propagate and proliferate in the nutrient-rich and organic matter-laden environment of the effluent. Mucoromycota fungi possess the ability to degrade organic compounds and assist in the breakdown of complex organic matter present in the effluent (Shen et al., 2021). They contribute to the overall microbial diversity and enzymatic potential involved in the degradation processes.

A notable pattern emerged during the slaughter of the animals. On days when both pigs and cattle were slaughtered simultaneously, the microbial analyses revealed the consistent presence of Neocallimastigomycota and the absence of Mucoromycota. Intriguingly, on a distinct occasion when only pigs were slaughtered, a shift in the fungal community dynamics was observed when the presence of Mucoromycota and the absence of Neocallimastigomycota were detected. It is noteworthy that the absence of Mucoromycota on other days when pigs were slaughtered could have been due to cattle microbiota suppressing Mucoromycota. This could have resulted in Mucoromycota being overshadowed by other phyla present in higher abundances. This observation presents an interesting avenue for further exploration and warrants in-depth microbial ecological studies to elucidate potential interphylum relationships and their implications within the abattoir setting.

In contrast to the bacterial diversity, the fungal diversity differed notably in terms of genus richness (Figure 4.8B). The presence of 24 different genera was recorded, and they presented a much less varied genus dominance than the bacteria. However, similar to the bacterial community, the fungal community was also dominated by a particular genus in each sample. These included *Neocallimastix* (21.13%) and *Caecomyces* (13.39%) that belong to the Neocallimastigomycota phylum, *Ciliophora* (6.31%), and an unidentified (14.82%) genus that both belong to the Ascomycota phylum. The unidentified genera constituted 14.82%, which represented a large proportion of the fungal

community without taxonomic annotations. This finding suggests possible misconceptions about the true fungal diversity of such a community and highlights the need for more research on classification systems based on environmental samples (Hibbett et al., 2011).

Neocallimastix, *Caecomyces*, and *Ciliophora* are important microbial groups found in the rumen of herbivorous animals, particularly in the context of their role in fibre digestion and fermentation. These fungi specialise in breaking down complex plant cell wall components, such as cellulose and hemicellulose, which are abundant in the diet of herbivores (Kazemi et al., 2022; Saye et al., 2021). They possess a unique repertoire of cellulolytic enzymes that aid in the degradation of these complex polysaccharides into simpler forms that can be utilised by the host animal (Gordon & Phillips, 1998). These fungi have not been known to have any direct significance in conventional wastewater treatment as they are usually found in small proportions compared to other fungi.

The presence of the observed dominant fungal genus was markedly different from what had been observed in other studies. Literature based on studies investigating the microbial composition of abattoir effluent has identified different fungal genera present in wastewater. The presence of fungal genera such as *Aspergillus*, *Penicillium*, *Candida*, and *Mucor* has been reported to be among the most dominant (Rabah et al., 2010; Cardoso et al., 2012; Ediene et al., 2017). However, in the current study, those were identified as some of the minor genera of the population. In the case of rumen-associated microorganisms such as *Neocallimastix*, *Caecomyces*, and *Ciliophora*, their abundance is not usually associated with a large presence in effluent. The observed presence of these microorganisms could thus suggest disposal of the digestive tract contents of the slaughtered animals, such as rumen contents or faecal matter, into the drainage system. Paruch et al. (2017) and Paruch and Paruch (2021) support this conclusion, arguing that this occurs due to improper handling or inadequate separation of the digestive tract during the slaughtering process.

In South African abattoirs, the disposal of animal rumen content, along with other waste generated during the slaughtering process, is typically managed in compliance with local regulations and environmental guidelines. Schedule 3 of the NEMW Act (No.53) of 2008 requires abattoirs to ensure that all waste generated from the preparation and processing of meat should be separated and disposed of according to different disposal requirements. Rumen content and blood should be separated from water and rendered into useful products or ultimately disposed of at an authorised waste disposal facility (NEMW Amendment Act, 2014; NEMWA, 2009). The microbial diversity data

clearly suggest that the abattoir participating in this study lacked proper separation processes for rumen content at the time of the study.

Abattoirs in South Africa are mandated to adhere to all regulatory requirements and to implement appropriate waste management practices. They need to obtain all the necessary permits and licenses to ensure compliance with the law. Regulatory authorities such as the Department of Agriculture, Forestry, and Fisheries as well as local environmental agencies should play an active role in monitoring and enforcing these regulations to ensure that abattoirs comply with waste disposal guidelines and standards. The extent of compliance with waste management regulations in South African abattoirs can vary, and there have been instances where authorities have faced challenges in ensuring full adherence to these laws. However, this issue is not unique to South Africa and has been observed in various countries and industries worldwide.

To conclude, the effluent from the red meat abattoir under study could be comprehensively described using each of the characterisation techniques employed in the investigation. A deeper knowledge of microbial diversity was made possible by integrating the data produced by the complementing methodologies that were employed.

Chapter 5

Correlations and Relationships

5.1 Introduction

In the realm of environmental research, understanding the intricate interplay between physicochemical parameters and microbial communities is crucial, particularly in settings as complex as abattoirs and the effluent they produce. Multifaceted investigations may therefore delve into correlations and relationships between physicochemical analyses and functional diversity assessments using carbon substrate utilisation and genetic diversity (Hajibabaei et al., 2007; Safford et al., 2022). Abattoir effluent, which is a significant source of wastewater with diverse organic and inorganic constituents, presents an ideal ecosystem to study how physicochemical conditions may influence microbial diversity and function (Ma et al., 2020).

Each method used in this study was effective in describing the red meat abattoir effluent under study. The approaches yielded complementary data, which allowed conclusive deductions about the effluent environment, microbial communities present in the effluent, and their functionality. Understanding the relationships and associations using this holistic approach revealed several benefits associated with both environmental and operational perspectives.

5.2 Microbial Community and Related Environmental Parameters in Abattoir Effluent

A principal component analysis (PCA) biplot was used to illustrate the possible ecological relationships between the microbial community and physicochemical variables within the effluent (Figure 5.1). When analysed, PCA Axis 1 explained 42.27% of the variance, while Axis 2 explained 20.24% of the variance in the bacterial and fungal environmental parameter relationships. In essence, the length of the arrow in the plot was proportional to the rate of change, so a longer arrow indicated a larger change in variables.

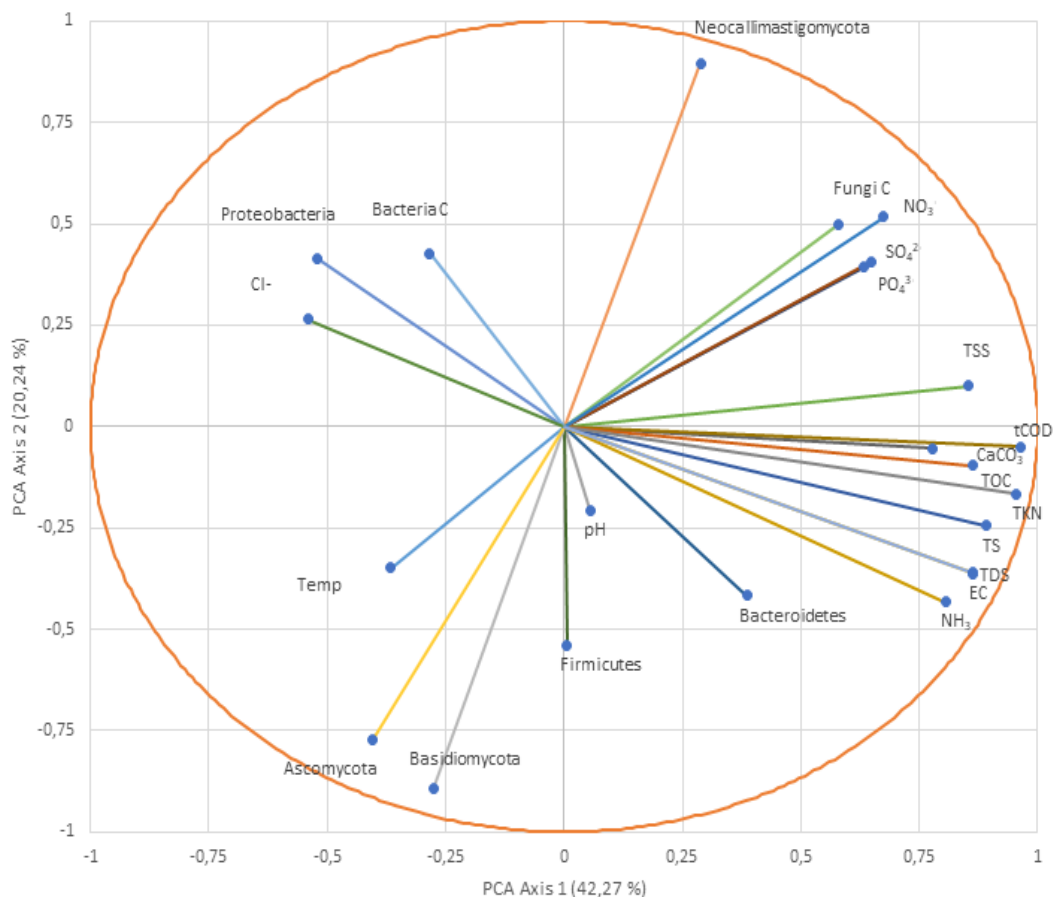


Figure 5.1: PCA biplots showing the correlation between the various physicochemical parameters with identified and enumerated bacteria and fungi present in abattoir effluent. Fungi C and Bacteria C represent culturable bacterial and fungal counts.

The PCA plot above indicates that NO₃⁻, SO₄²⁻, PO₄³⁻, and TSS strongly correlated with culturable fungi in the effluent. Fungi play an essential role in nutrient cycling and they are known for their ability to decompose organic matter, including complex compounds like lignocellulose. Nitrate, sulphate, and phosphate are essential nutrients that fungi require for their growth and metabolic processes. These nutrients can influence fungal community composition and activity (Murugesan, 2017; Osunmakinde et al., 2019).

The analyses also indicated that Neocallimastigomycota did not have a specific positive correlation with any parameter. Ascomycota and Basidiomycota both exhibited a positive correlation with temperature, implying that their growth and activity generally increased with higher temperatures. Ascomycota includes a diverse group of fungi, such as *Aspergillus*, *Candida*, moulds, yeasts, and many plant pathogens. These fungi are known to be active over a wide range of temperatures, but

they tend to thrive in moderate to warm temperatures. The positive relationship with temperature that was detected can be explained by metabolic activity (Naranjo-Ortiz & Gabaldón, 2019), sporulation (Hyde et al., 2019), decomposition, and nutrient cycling (Challacombe et al., 2019).

Bacteroidetes and Firmicutes were found to have a weak positive correlation with EC, while Proteobacteria and culturable bacteria had a weak positive correlation with chloride. Both EC and chloride are associated with salinity, which is an important predictor of bacterial community compositions and phylum distribution in wastewater. Salt concentration may also inhibit cell activity, performance, and efficiency (Gao et al., 2021), which could explain the weak correlation.

The findings revealed limited positive associations or identifiable correlations between microbial communities and measured physicochemical parameters. The bacterial populations exhibited no significant correlation with the measured physicochemical parameters, suggesting that other factors beyond those assessed in this study might have driven their distribution and abundance within the abattoir effluent. Conversely, the fungal communities exhibited some correlation with a few of the studied parameters; however, these associations were limited. This outcome highlights the complexity of environmental systems and the multifaceted interactions that govern microbial diversity and microbial community dynamics in abattoir wastewater.

5.3 Linking the Microbial Genetic Diversity profile and the functionality profile of the Effluent

The Biolog EcoPlate™ methodology was used to assess the metabolic capabilities of the diverse microorganisms present in each effluent sample. These microorganisms included bacteria, fungi, and other microbial groups that worked together to break down and utilise various compounds, as proposed by Barik (2020) and Kurten and Barkoh (2014). Barros et al. (2023) argue that, if an environment provides favourable conditions for life, it can support a diverse array of microorganisms with significant genetic variability. Conversely, in harsh or specialised environments, the microbial community tends to be less diverse and thus comprises a smaller number of microorganisms.

In this context, the Simpson index (D) is a widely used measure to quantify the diversity of a microbial community, and the higher the value of the Simpson index, the more diverse is the community being accommodated (Adams et al., 2015). In this study, the Simpson index revealed that the microbial community in the abattoir effluent was indeed highly diverse. The identification of a total of 683

bacterial and 24 fungal genera provided strong evidence of richness and a large variety of microorganisms present in the effluent, and the presence of such a diverse array of bacterial and fungal genera highlighted the complexity and ecological significance of the microbial community in the effluent environment under study. Moreover, evenness values (EVs) indicated a striking similarity in the metabolic profiles of the samples, reflecting comparable population sizes among the various species present in the microbial community (Balasaheb Gunwantrao et al., 2017). The high EVs further underscored the abundance of species, revealing a rich and diverse microbial community within the effluent. Interestingly, the functional biodiversity remained consistent regardless of the number of animals slaughtered on a given day and overall. These findings highlighted the remarkable stability and resilience of the microbial community in the effluent, regardless of the daily variations in the abattoir's activities. Knowledge of this diverse and balanced microbial composition in the effluent has implications for potential new adaptations and applications in wastewater treatment and environmental management.

PCA biplot was used to illustrate the possible relationships between the microbial community and carbon substrate when utilisation patterns were measured (Figure 5.2). PCA Axis 1 explained 37.50% of the variance and Axis 2 explained 28.85% of the variance. Culturable fungi in the abattoir effluent exhibited significant positive correlations with carbohydrates ($r = -0.707$) and polymers ($r = 0.861$). Moreover, the fungal phylum Neocallimastigomycota demonstrated strong correlations with the same substrate groups, achieving correlation coefficients of $r = 0.767$ and $r = 0.51$ respectively. The carbon substrate groups were identified as the most utilised according to the AWCD data presented in Chapter 4 (polymers 24% and carbohydrates 20.6%). The high correlations suggest a potential ecological specialisation niche for these fungi as they are allowed to thrive and dominate in environments rich in these specific carbon sources.

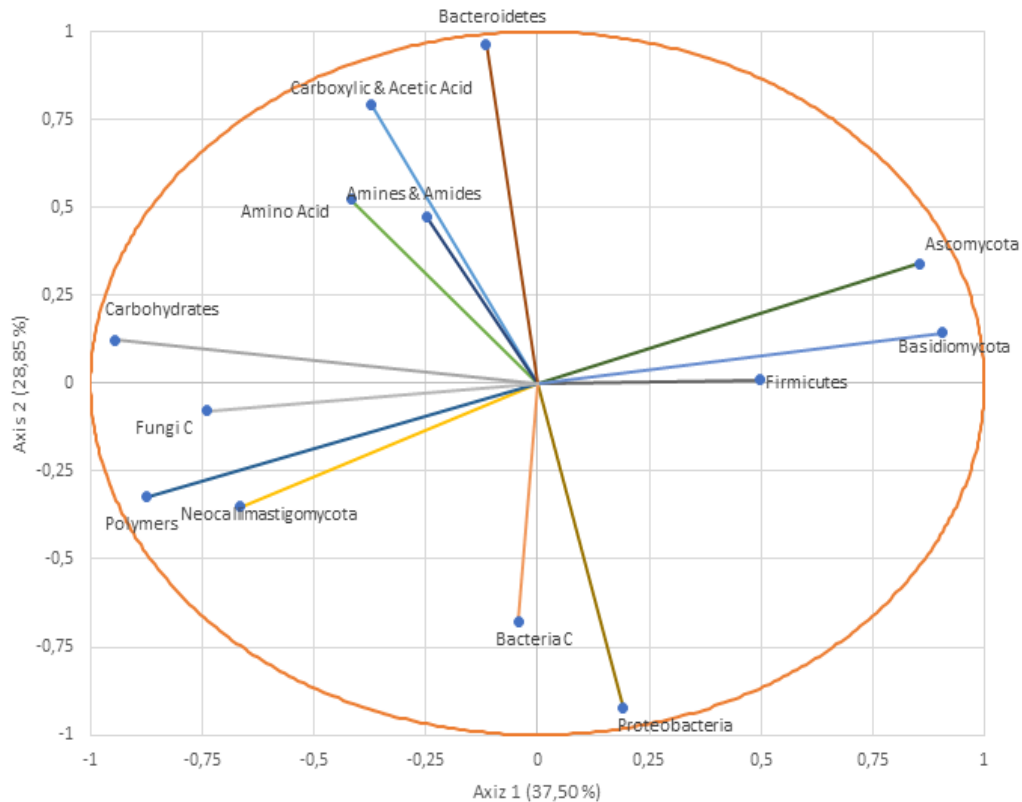


Figure 5.2: PCA biplots showing the correlation between the carbon substrate groups with identified and enumerated bacteria and fungi from abattoir effluent. Fungi C and Bacteria C indicate culturable bacterial and fungal counts.

These strong correlations could be attributed to the metabolic capabilities and adaptations of the fungal groups present in the abattoir effluent. Fungi possess diverse enzymatic machinery that allows them to efficiently degrade complex organic compounds like carbohydrates and polymers and to convert them into usable carbon sources for growth and metabolism (Viegas et al., 2016). As the effluent contained a substantial amount of organic matter from waste and processing residues, these fungi could have evolved to take advantage of these abundant carbon sources. Additionally, the high correlation of *Neocallimastigomycota* suggests that this phylum evolved specialised mechanisms to efficiently utilise these specific substrates, possibly because of their association with the digestive systems of herbivores, as proposed by Balasaheb Gunwantrao et al. (2017) and Molelekoa et al. (2021).

Furthermore, environmental factors such as pH, temperature, and oxygen levels could have influenced the activity and distribution of specific fungal groups in the effluent (Meng et al., 2019;

Rajhans et al., 2021). Fungi with a preference for carbohydrates and polymers could have been favoured under advantageous physicochemical conditions in the effluent, leading to their higher abundance and strong correlations.

Bacteroidetes showed a positive correlation with amino acids and amines and amides, and these were the least utilised substrates according to the AWCD (amino acids 20.3%; amines and amides 16.9%). The low utilisation of amino acids and amines and amides by the overall microbial community could have resulted from competition for these resources. Other bacterial groups might have outcompeted one another for these substrates, leading to lower utilisation rates. However, Bacteroidetes generally have a competitive advantage or evolve specialised adaptations and these abilities could have allowed them to thrive in the presence of these substrates, leading to a positive correlation (Naylor et al., 2022; Regueira et al., 2021). An important point to remember is that the approach that was adopted used DNA, whether that DNA was derived from a live or dead cell. Therefore, the presence of a certain group of microorganisms did not necessarily mean that that group played an active role within the community. Despite these limitations, the microbial diversity that was observed in the current study suggests that fungi played a crucial role in the microbial community's physiological profile in the effluent that was analysed.

5.4 Cluster Analysis to Compare the Microbial Diversity Profile

Agglomerative hierarchical clustering (AHC) analysis was used to estimate the order of relatedness among the different samples (Figure 5.3). Cluster analysis was thus conducted based on the taxonomically classified microbial ASVs and culturable microbial counts. These segregated the diversity profiles into three main cluster groups categorised as Cluster 1, Cluster 2, and Cluster 3, which allowed differentiation between the groups.

Cluster 1 contained samples 4, 1, and 2 as these samples seemed to harbour similar microbial compositions. In sample 1, only cattle had been slaughtered and in samples 2 and 4, both cattle and pigs had been slaughtered. Cluster 2 combined most of the samples apart from sample 6; thus sample 6 appeared to be under cluster 3 on its own. Samples 9 and 3 that belonged to cluster 2 were grouped together as cattle and sheep had been slaughtered on those days. The high similarity of microbial content in the sheep rumen and faeces was similar to that of cattle. This may be attributed to the fact that these species are ruminants and have a similar digestive system. Moreover, the bacterial and fungal genera found in sheep rumen and faeces were comparable with those found in

cattle rumen and faeces, which is a finding that is supported by Cholewińska et al. (2020), Regueira et al. (2021), and Szeligowska et al. (2021).

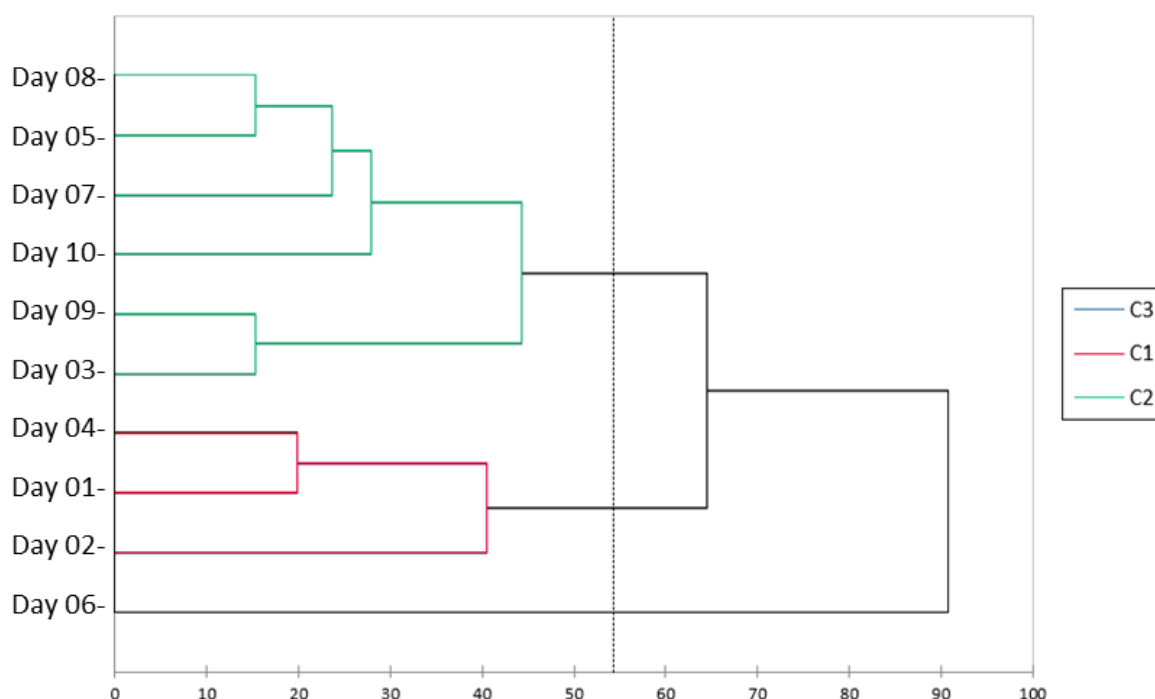


Figure 5.3: Cluster analysis (AHC) dendrogram showing the order of relatedness among the diversity profile of the different abattoir effluent samples. The diversity profiles are segregated into three main clusters labelled as C1, C2, and C3.

Sample Name	Sample 1									
Sample 1		Sample 2								
Sample 2	97,89		Sample 3							
Sample 3	97,33	97,62		Sample 4						
Sample 4	98,35	97,72	97,29		Sample 5					
Sample 5	98,04	97,75	97,64	97,98		Sample 6				
Sample 6	96,94	97,07	97,71	96,47	96,64		Sample 7			
Sample 7	97,84	97,76	98	97,68	98,4	97,05		Sample 8		
Sample 8	98,18	97,83	97,76	97,84	98,56	97,08	98,17		Sample 9	
Sample 9	97,79	97,93	98,55	97,64	98,31	97,56	98,22	98,19		Sample 10
Sample 10	97,78	97,83	97,97	97,76	98,16	97,04	98,04	99,02	98,15	

Figure 5.4: The Dice coefficient was determined by measuring the Euclidean distance between the microbial diversity in the abattoir effluent samples. The resulting percentage indicates the level of similarity. The samples marked in green exhibit a significant similarity rate as it exceeds 98%.

The remainder of the groups (samples 8, 5, 7, and 10) represented days when cattle and pigs had been slaughtered. Overall, the differences in the stomach and faecal microbiota between pigs and

cows reflected the adaptations of their respective digestive systems to the types of feed they had consumed. The stomachs of pigs are known to be relatively simple and more acidic compared to those of cows. Pigs are monogastric animals, which means they have a single-chambered stomach and the acidic environment helps to break down and digest food. The predominant bacteria in pigs' stomachs are usually acid-resistant microorganisms that can survive in a low-pH environment (Floridia et al., 2023; Rowan et al., 1997). On the other hand, cows are ruminant animals with a complex stomach that has four compartments. The rumen is the largest compartment and houses diverse and unique microbiota. It contains anaerobic microbes like bacteria, protozoa, and fungi that help ferment and break down the fibrous plant materials cattle consume. This symbiotic relationship allows cows/cattle to extract nutrients from cellulose-rich plant materials that would otherwise be indigestible for monogastric animals like pigs (Mizrahi & Jami, 2018; Xu et al., 2021; Mizrahi & Jami, 2018; Xu et al., 2021).

A significantly high similarity rate (above 98%) was detected in cluster 1 and 2 samples. The similarity rate was determined using the Dice coefficient as presented in Table 5.1, which involved measuring the Euclidean distance between the microbial diversity profiles of the samples. The Euclidean distance quantifies the dissimilarity or difference between two sets of data points, and it was therefore useful in assessing the similarity of microbial compositions in the samples. It was found that sample 6 showed a high similarity rate with the other samples as well, ranging from 96.47 - 97.71%.

Sample 6 represented cluster 3 and it was taken on the only day that only pigs had been slaughtered. It had a unique microbial composition that was different from that of the other samples, and this difference was mostly evident in the genetic fungal composition. This was the only sample containing Mucoromycota phyla, but the presence of Neocallimastigales was not detected in this sample while it was present in all the other samples. Mucoromycota fungi are found in the gastrointestinal tract of pigs and they are excreted in their faeces. Fungi are typically present in lower quantities in the pig gut compared to the bacterial population (Gutu et al., 2021; J. Li et al., 2022); however, in this effluent the Mucoromycota appeared very distinct. Generally, there are several bacteria and other microorganisms in the gut microbiota of pigs, and their relative abundance changes during the host growth which occurs mainly from the post-natal to the weaning period. This composition is affected by environmental and feeding factors during the entire life of pigs (Floridia et al., 2023).

The microbial content found in the effluent from the red meat abattoir exhibited a high degree of similarity across all the samples. The similarity rate was in the range of 96.64 - 98.56%, which

indicated that the microbial diversity among these samples was very similar. Based on the above findings, it was concluded that, regardless of the specific animals being slaughtered, the microbial content in the effluent was generally similar. This finding suggests that the microbiomes from the different animals' meat, stomach content, and hides overlapped substantially in terms of their composition, indicating a commonality in the microbial species present in the effluent. This result may have implications for understanding the microbial ecology in the abattoir setting and could be relevant for waste treatment and management in such facilities.

Chapter 6

Concluding Remarks

6.1 Introduction

The management of wastewater generated from food production and agricultural activities, particularly from red meat abattoirs, presents a significant challenge due to the high organic concentration in effluent and extensive water consumption in such facilities. South Africa's increasing demand for meat products has led to a proliferation of abattoirs, exacerbating water scarcity issues and straining water resources. The discharge of untreated abattoir effluent directly into municipal sewer systems is not only burdening sanitation infrastructure, but also causes downstream contamination and jeopardises precious water sources.

Despite monitoring the physicochemical qualities and perceived environmental effects of abattoir effluent according to national by-laws, there remains a critical gap in understanding the microbial diversity of this type of wastewater. Addressing this information deficit and fostering accountability and awareness regarding the environmental impact of abattoir wastewater are essential steps in mitigating the adverse effects on South Africa's water ecosystem. To safeguard the nation's water resources and promote sustainable practices within the agricultural sector, it is crucial to prioritise effective waste treatment and disposal measures for abattoir wastewater. Only through concerted efforts and responsible management can we alleviate the strain on water systems and protect the precious water sources that are vital to our ecosystems and future well-being.

6.2 Concluding Remarks

Initially, in the project proposal, a comprehensive sampling plan was devised with the intention to conduct two sampling cycles over 10 consecutive days during both winter and summer seasons. It was envisaged that samples would be collected each day every 2 hours, resulting in a total of 5 samples per day. This approach aimed to provide a detailed and robust dataset for analysis.

However, following the pilot study, it became evident that the initial sampling protocol was overly ambitious. The challenges faced during the pilot study, such as the difficulty of maintaining consistent and accurate collection intervals, led to the decision to modify the sampling strategy. Instead of two cycles, a single sampling cycle of 10 consecutive days was implemented. This adjustment ensured the collection of a composite sample totalling 5 L of sample each day, which was deemed more feasible and equally reliable. The revised approach was motivated by the recognition that the original sampling plan would have generated an extensive and complex dataset that would potentially have exceeded the resources available for analysis. Moreover, the comparison of peak and off-peak data may not have provided significant insights given the inherent variability in abattoir wastewater generation and management practices throughout the day.

The process of sampling proved to be more arduous than initially anticipated. This demanding task required rigorous dedication and persistence. Throughout the sampling period, challenges emerged concerning the consistency of wastewater collection and treatment practices within the abattoir. Notably, there were instances when the abattoir was non-operational and, as a consequence, water, sometimes heated, was sporadically flushed through the wastewater system. This irregularity underscored the criticality of obtaining a genuinely comprehensive and representative composite sample set. In light of these challenges, it is evident that future endeavours to investigate abattoir effluent must carefully account for the operational patterns of the facility under study. Adequate consideration of such factors is paramount to ensure the accuracy and reliability of the data gathered, as this will ultimately contribute to a more robust understanding of the environmental impact of red meat abattoir effluent and the need for the effective management of such effluent.

The analyses revealed that four of the eight physicochemical parameters (i.e., 37.5% of the total) that are supposed to be monitored by the Mangaung Municipality and that are addressed in its by-laws exhibited non-compliance with established limits. This finding underscores the importance of continued monitoring and persistent adherence to regulatory standards to ensure proper management and control of wastewater in the Mangaung area. Moreover, efforts to address and rectify non-compliant parameters are crucial for safeguarding the local environment and maintaining water quality within acceptable levels.

This study revealed elevated culturable bacterial and fungal populations in the examined samples, with bacteria being the more dominant microorganisms compared to fungi. Bacterial levels ranged from 1×10^6 - 6.8×10^6 CFU.mL⁻¹, with high contamination of faecal coliforms *Enterobacter aerogenes*

and *Escherichia coli*. The average value of the yeast count for the abattoir effluent was 5.32×10^3 , while it was 7.10×10^1 for mould. These findings align with previous research, highlighting the prevalence of bacteria in abattoir effluent and the significant presence of *E. coli* and coliforms.

The functional biodiversity expressed by the Shannon diversity index was moderately high in all samples, the Simpson index displayed a very diverse community, and the evenness values depicted the presence of a large abundance of species. The relative abundance of the metabolic activities grouped according to the chemical family was dominated by the metabolism of polymers and carbohydrates. These results showed that the functional biodiversity of the effluent was quite consistent, which occurred irrespective of the species of animals and numbers slaughtered on specific days.

Red meat abattoir effluent microbiota were also characterised by employing metagenomic analyses of the 16S rRNA and ITS gene sequences. Taxonomic diversity analysis showed that the three major bacterial phyla were Bacteroidetes (39.40%), Firmicutes (33.23%), and Proteobacteria (20.1%). Genus richness varied in dominance distribution. *Acinetobacter*, belonging to the Proteobacteria phylum, *Bacteroides*, and *Rikenellaceae_RC9_gut_group*, both belonging to the Bacteroidetes phylum, were the most abundant genera and their dominance varied in each sample. Fungal diversity was dominated by the phyla Neocallimastigomycota (52.61%) or Ascomycota (36.18%). Basidiomycota appeared in small, varied levels for each sample, except the one taken on day 6, as this day's sample contained an abundance of Mucoromycota. The fungal community was dominated by a particular genus in each sample, which included *Caecomyces*, *Neocallimastix*, and *Ciliophora*.

The culture-dependent and culture-independent microbial content observed in the effluent samples from the red meat abattoir exhibited a remarkably high degree of similarity across all samples. The detected similarity rate ranged from 96.64 - 98.56%, indicating a striking resemblance in microbial diversity among the samples. Regardless of the specific animals being processed, the effluent consistently contained a similar microbial content. This finding suggests a significant overlap in microbiomes among the different animals' meat, stomach content, and hides, implying a commonality in the microbial species present in the effluent. The only notable difference was observed in sample 6, which presented Mucoromycota. This finding may require further investigation, especially in high throughput abattoirs where pigs are the majority of the species slaughtered.

The implications of these results are noteworthy for understanding the microbial ecology within the abattoir environment and they hold relevance for waste treatment and management practices in such facilities. The substantial overlap in microbial composition in the effluent samples stresses the need for tailored and efficient strategies to address and control microbial contamination. By recognising this commonality, abattoirs can potentially optimise waste treatment processes and improve overall hygiene standards in the quest to contribute to the preservation of environmental health and public safety.

References

- A, I., Talukder, S., & Selvakumar, K. (2015). Current Practices and Emerging Trends in Abattoir Effluent Treatment in India: A Review. *International Journal of Livestock Research*, 5(2), 13. <https://doi.org/10.5455/ijlr.20150225043513>
- A.B., R., B., O. S., B., M. S., G., H. L., & J., I. U. J. (2010). Microbiological And Physico-chemical Assessment Of Soil Contaminated With Abattoir Effluents In Sokoto Metropolis, Nigeria. *Science World Journal*, 5(3). <https://www.scienceworldjournal.org/article/view/6713>
- Abarenkov, K., Nilsson, R. H., Larsson, K. H., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010). The UNITE database for molecular identification of fungi--recent updates and future perspectives. *The New Phytologist*, 186(2), 281–285. <https://doi.org/10.1111/J.1469-8137.2009.03160.X>
- Abu-Zeid, M., & Shiklomanov, I. A. (2004). Water Resources as a Challenge of the Twenty-First Century. In *World Meteorological Organization* (Issue 959).
- Adams, R. I., Bhangar, S., Pasut, W., Arens, E. A., Taylor, J. W., Lindow, S. E., Nazaroff, W. W., & Bruns, T. D. (2015). Chamber Bioaerosol Study: Outdoor Air and Human Occupants as Sources of Indoor Airborne Microbes. *PLOS ONE*, 10(5), e0128022. <https://doi.org/10.1371/JOURNAL.PONE.0128022>
- Adesoji, A. T., Abubakar, F., & Ipinlaye, J. S. (2016). Occurrence of Antibiotic Resistant Bacteria in Faeces from Abattoir Waste, Processing Water and Products from Dutsin-Ma, Katsina State, Nigeria. *J Bacteriol Mycol*, 3(1). www.austinpublishinggroup.com
- Akhtar, N., Syakir Ishak, M. I., Bhawani, S. A., & Umar, K. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water (Switzerland)*, 13(19). <https://doi.org/10.3390/w13192660>
- Akhtar, N., Syakir Ishak, M. I., Muhammad Izzuddin, B., Bhawani, S. A., & Showkat Ahmad, B. (2021). Various natural and anthropogenic factors responsible for water quality degradation: A review. *Water (Switzerland)*.
- Akpor, O. B., & Muchie, M. (2011a). Challenges in Meeting the MDGs: The Nigerian Drinking Water Supply and Distribution Sector. *Journal of Environmental Sciences and Technology*, 4(5), 480–489. <https://doi.org/https://dx.doi.org/10.3923/jest.2011.480.489>

- Akpor, O. B., & Muchie, M. (2011b). Environmental and public health implications of wastewater quality. *African Journal of Biotechnology*, 10(13), 2379–2387. <https://doi.org/10.4314/ajb.v10i13>.
- Al-Rumaihi, A., McKay, G., Mackey, H. R., & Al-Ansari, T. (2020). Environmental impact assessment of food waste management using two composting techniques. *Sustainability (Switzerland)*, 12(4). <https://doi.org/10.3390/su12041595>
- Applequist, B. (2012). *A brief introduction to Kjeldahl Nitrogen Determination - Labconco*. <https://www.labconco.com/articles/a-brief-introduction-to-kjeldahl-nitrogen-determ>
- Ayilara, M. S., & Babalola, O. O. (2023). Bioremediation of environmental wastes: the role of microorganisms. *Frontiers in Agronomy*, 5, 1183691. <https://doi.org/10.3389/FAGRO.2023.1183691/BIBTEX>
- Balasaheb Gunwantrao, B., Patil, C. R., & Pasha, M. A. (2017). Bacterial Community Analysis of Soybean (Glycine max) Sprayed with Panchagavya Revealed by DGGE. *Int.J.Curr.Microbiol.App.Sci*, 6(4), 1207–1217. <https://doi.org/10.20546/ijcmas.2017.604.148>
- Bandaw, T., & Herago, T. (2017). *Review on Abattoir Waste Management 1*. 19(2), 517–524. <https://doi.org/10.5829/idosi.gv.2017.517.524>
- Barik, M. (2020). MewarJournal-October-December-2019-finalArticle. *Journal of Indian Research*.
- Barros, D. J., Carvalho, G. A., de Chaves, M. G., Vanzela, L. S., Kozusny-Andreani, D. I., Guarda, E. A., Neu, V., de Moraes, P. B., Tsai, S. M., & Navarrete, A. A. (2023). Microbial metabolic activity in Amazon floodplain forest and agricultural soils. *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1144062>
- Begmatov, S., Dorofeev, A. G., Kadnikov, V. V., Beletsky, A. V., Pimenov, N. V., Ravin, N. V., & Mardanov, A. V. (2022). The structure of microbial communities of activated sludge of large-scale wastewater treatment plants in the city of Moscow. *Scientific Reports* 2022 12:1, 12(1), 1–14. <https://doi.org/10.1038/s41598-022-07132-4>
- Benit, N., & Roslin, A. S. (2015). Physicochemical properties of wastewater collected from different sewage sources. In *IJSET-International Journal of Innovative Science, Engineering & Technology* (Vol. 2, Issue 11). www.ijiset.com
- Bhagwat, V., & Vinod, R. (2019). Safety of water used in food production. In *Food Safety and Human Health*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-816333-7.00009-6>
- Biolog EcoPlate™. (2023). *Microbial Community Analysis*.
- Biolog Inc. (n.d.). *Microbial Community Analysis with EcoPlates – Biolog*. Retrieved February 19, 2023, from <https://www.biolog.com/products-portfolio-overview/microbial-community-analysis-with-ecoplates/>

- Bitton, G. (2010). Wastewater Microbiology: Fourth Edition. In *Wastewater Microbiology: Fourth Edition*. <https://doi.org/10.1002/9780470901243>
- Botai, C. M., Botai, J. O., Dlamini, L. C., Zwane, N. S., & Phaduli, E. (2016). Characteristics of Droughts in South Africa: A Case Study of Free State and Northwest Provinces. *Water* 2016, Vol. 8, Page 439, 8(10), 439. <https://doi.org/10.3390/W8100439>
- Brosnan, J. A., & Iacobuzio-Donahue, C. A. (2012). *A New Branch on the Tree: Next-Generation Sequencing in the Study of Cancer Evolution*. <https://doi.org/10.1016/j.semcd.2011.12.008>
- Brown, T. (2016). Global Developmental Goals in 2025. House of Lords *Library Note*.
- Bustillo-Lecompte, C., & Mehrvar, M. (2017a). Slaughterhouse Wastewater: Treatment, Management and Resource Recovery. InTech. <https://doi.org/10.5772/65499>
- Bustillo-Lecompte, C., & Mehrvar, M. (2017b). Treatment Management. In R. Farooq & Z. Anmad (Eds.), *Physico-chemical wastewater treatment and resource recovery* (p. 153). InTech. <https://doi.org/DOL: 10.5772/65499>
- Calverley, C. M., & Walther, S. C. (2022). Drought, water management, and social equity: Analyzing Cape Town, South Africa's water crisis. *Frontiers in Water*, 4. <https://doi.org/10.3389/FRWA.2022.910149/FULL>
- Cardoso, A. M., Cavalcante, J. J. V., Cantão, M. E., Thompson, C. E., Flatschart, R. B., Glogauer, A., Scapin, S. M. N., Sade, Y. B., Beltrão, P. J. M. S. I., Gerber, A. L., Martins, O. B., Garcia, E. S., de Souza, W., & Vasconcelos, A. T. R. (2012). Metagenomic Analysis of the Microbiota from the Crop of an Invasive Snail Reveals a Rich Reservoir of Novel Genes. *PLOS ONE*, 7(11), e48505. <https://doi.org/10.1371/JOURNAL.PONE.0048505>
- Carles, L., Wulschleger, S., Joss, A., Eggen, R. I. L., Schirmer, K., Schuwirth, N., Stamm, C., & Tlili, A. (2022). *Wastewater microorganisms impact microbial diversity and important ecological functions of stream periphyton*. <https://doi.org/10.1016/j.watres.2022.119119>
- Carroll, G. A., Boyle, L. A., Teixeira, D. L., Van Staaveren, N., Hanlon, A., & O'Connell, N. E. (2015). Effects of scalding and dehairing of pig carcasses at abattoirs on the visibility of welfare-related lesions. *Animal*, 10(3), 460–467. <https://doi.org/10.1017/S1751731115002037>
- Cason, E. D., Mahlomaholo, B. J., Taole, M. M., Abong, G. O., Vermeulen, J. G., de Smidt, O., Vermeulen, M., Steyn, L., Valverde, A., & Viljoen, B. (2020). Bacterial and Fungal Dynamics During the Fermentation Process of Sesotho, a Traditional Beer of Southern Africa. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.01451>
- Challacombe, J. F., Hesse, C. N., Bramer, L. M., McCue, L. A., Lipton, M., Purvine, S., Nicora, C., Gallegos-Graves, L. V., Porras-Alfaro, A., & Kuske, C. R. (2019). Genomes and secretomes of

- Ascomycota fungi reveal diverse functions in plant biomass decomposition and pathogenesis. *BMC Genomics* 2019 20:1, 20(1), 1–27. <https://doi.org/10.1186/S12864-019-6358-X>
- Chandra, P., Enespa, Singh, R., & Arora, P. K. (2020). Microbial lipases and their industrial applications: A comprehensive review. In *Microbial Cell Factories* (Vol. 19, Issue 1). BioMed Central Ltd. <https://doi.org/10.1186/s12934-020-01428-8>
- Charlebois, S., & Summan, A. (2014). Abattoirs, meat processing and managerial challenges: A survey for lagging rural regions and food entrepreneurs in Ontario, Canada. *International Journal of Rural Management*, 10(1), 1–20. <https://doi.org/10.1177/0973005214526504>
- Chen, H., Cui, H., He, Z., Lu, L., & Huang, Y. (2021). Influence of chloride deposition rate on rust layer protectiveness and corrosion severity of mild steel in tropical coastal atmosphere. *Materials Chemistry and Physics*, 259. <https://doi.org/10.1016/j.matchemphys.2020.123971>
- Chiemchaisri, W., Chiemchaisri, C., Witthayaphirom, C., Mahavee, K., & Watanabe, T. (2023). Surveillance of antibiotic persistence adaptation of emerging antibiotic-resistant bacteria in wastewater treatment processes: Comparison between domestic and hospital wastewaters. *Environmental Technology and Innovation*, 31. <https://doi.org/10.1016/j.eti.2023.103161>
- Cholewińska, P., Wołoszyńska, M., Michalak, M., Czyż, K., Rant, W., & Janczak, M. (2020). Evaluation of Changes in the Levels of Firmicutes and Bacteroidetes Phyla of Sheep Feces Depending on the Breed. *Animals* 2020, Vol. 10, Page 1901, 10(10), 1901. <https://doi.org/10.3390/ANI10101901>
- Cholewińska, P., Wołoszyńska, M., Michalak, M., Czyż, K., Rant, W., Smoliński, J., Wyrstek, A., & Wojnarowski, K. (2021). Influence of selected factors on the Firmicutes, Bacteroidetes phyla and the Lactobacillaceae family in the digestive tract of sheep. *Scientific Reports* |, 11, 23801. <https://doi.org/10.1038/s41598-021-03207-w>
- Climate Policy Watcher. (2020). *Characteristics of Agricultural and Food Wastewater*. Climate Policy Watcher. <https://www.climate-policy-watcher.org/wastewater-treatment/characteristics-of-agricultural-and-food-wastewater.html>
- Cooper, G. M. (2000). *The Central Role of Enzymes as Biological Catalysts*. <https://www.ncbi.nlm.nih.gov/books/NBK9921/>
- Cotruvo, J. (2018). *Wastewater treatment challenges in food processing and agriculture*. Water Technology. <https://www.watertechnonline.com/wastewater/article/15550688/wastewater-treatment-challenges-in-food-processing-and-agriculture>
- CPW, C. P. W. (2023). *Important Microorganisms in Wastewater Bacteria and fungi - Wastewater Treatment*. <https://www.climate-policy-watcher.org/wastewater-treatment/important-microorganisms-in-wastewater-bacteria-and-fungi.html>

- Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, 17(1), 145–155. <https://doi.org/10.1007/s10311-018-0785-9>
- Cross, H. R., & Singh, R. P. (2020). *meat processing | Steps, Equipment, Meat Inspection, & Cutting* | Britannica. Britannica. <https://www.britannica.com/technology/meat-processing/Livestock-slaughter-procedures>
- Dabkowski, B., & White, M. (2016). Understanding the Different Phosphorus Tests. *Hach*, 1–4.
- Daskiran, C., Attiya, B., Altimemy, M., Liu, I. H., & Oztekin, A. (2019). Oxygen dissolution via pump-turbine – Application to wastewater treatment. *International Journal of Heat and Mass Transfer*, 131, 1052–1063. <https://doi.org/10.1016/J.IJHEATMASSTRANSFER.2018.11.130>
- de Beer, D. M., Botes, M., & Cloete, T. E. (2018a). The microbial community of a biofilm contact reactor for the treatment of winery wastewater. *Journal of Applied Microbiology*, 124(2), 598–610. <https://doi.org/10.1111/jam.13654>
- de Beer, D. M., Botes, M., & Cloete, T. E. (2018b). The microbial community of a biofilm contact reactor for the treatment of winery wastewater. *Journal of Applied Microbiology*, 124(2), 598–610. <https://doi.org/10.1111/jam.13654>
- Department of Agriculture and Rural Development. (2009). *GDARD Manual for Abattoir Waste Management GUIDELINE MANUAL FOR THE MANAGEMENT OF ABATTOIRS AND OTHER WASTE OF ANIMAL ORIGIN Department of Agriculture and Rural Development Guideline 2 GDACE Manual for Abattoir Waste Management GDARD Manual for Abattoir Waste Management*. www.gdard.gpg.gov.za
- Department of Agriculture, L. R. and R. D. (2021a). *A PROFILE OF THE SOUTH AFRICAN BEEF MARKET VALUE CHAIN 2021*. www.dalrrd.gov.za
- Department of Agriculture, L. R. and R. D. (2021b). *A PROFILE OF THE SOUTH AFRICAN BEEF MARKET VALUE CHAIN 2021*. www.dalrrd.gov.za
- Department of Environmental Affairs. (2014). *National Guideline for the Discharge of Effluent From Land-based Sources into the Coastal Environment*.
- Department of Environmental Affairs and Development Planning. (2015). *Annual Report 2014-2015* (p. 196).
- Department of Water Affairs. (2011). *Introduction to the Green Drop Programme and Handbook* (p. 210). DWA.
- Department of Water Affairs. (2012a). *Annual Report: 1 April 2011-31 March 2012*. 1–33.
- Department of Water Affairs. (2012b). *National Water Resource Strategy* (p. 8).

- Department of Water and Sanitation. (2017). *National NORMS AND STANDARDS FOR DOMESTIC WATER AND SANITATION SERVICES* (41100; 3).
- Dias, V. C. F., Durand, A.-A., Constant, P., Prévost, M., & Bédard, E. (2019). *Identification of Factors Affecting Bacterial Abundance and Community Structures in a Full-Scale Chlorinated Drinking Water Distribution System*. <https://doi.org/10.3390/w11030627>
- Diyantoro, & Wardhana, D. K. (2019). Risk Factors for Bacterial Contamination of Bovine Meat during Slaughter in Ten Indonesian Abattoirs. *Veterinary Medicine International*, 2019. <https://doi.org/10.1155/2019/2707064>
- Dos Santos, Á., Cook, A. W., Gough, R. E., Schilling, M., Olszok, N. A., Brown, I., Wang, L., Aaron, J., Martin-Fernandez, M. L., Rehfeldt, F., & Toseland, C. P. (2021). DNA damage alters nuclear mechanics through chromatin reorganization. *Nucleic Acids Research*, 49(1), 340–353. <https://doi.org/10.1093/nar/gkaa1202>
- DWA. (1998). *National Water Act, Act No 36 of 1998*.
- DWS. (2023a). *BLUE DROP WATCH REPORT 2023: TECHNICAL ASSESSMENT OF THE CONDITION OF MUNICIPAL DRINKING WATER TREATMENT AND DISTRIBUTION SYSTEMS IN SOUTH AFRICA FOREWORD* by HONOURABLE MINISTER.
- DWS. (2023b). *GDPAT_2023_Report*.
- Eberhard, R. E. (n.d.). *Administered Prices Water* (p. 50).
- Ediene, V., Ediene, V. F., Iren, O., Bartosz, A., & Munnoli, P. M. (2017). Article no.AJEE.33341 SCIEDOMAIN international Reviewers: (1) Mustapha Umar, Nigerian Institute of Leather and Science Technology. *Article in Asian Journal of Environment & Ecology*, 2(3), 1–10. <https://doi.org/10.9734/AJEE/2017/33341>
- Edokpayi, J. N., Odiyo, J. O., & Durowoju, O. S. (2017). Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. In *Water Quality*. InTech. <https://doi.org/10.5772/66561>
- Edokpayi, N. J., Odiyo, O. J., & Durowoju, S. O. (2017). Impact of Wastewater on Surface Water Quality in \developing Countries: A case Study of Southa Africa. *Intech*, 32, 137–144. <http://www.intechopen.com/books/trends-in-telecommunications-technologies/gps-total-electron-content-tec-prediction-at-ionosphere-layer-over-the-equatorial-region%0AInTec>
- Ene, I. V., Brunke, S., Brown, A. J. P., & Hube, B. (2014). Metabolism in Fungal Pathogenesis. *Cold Spring Harbor Perspectives in Medicine*, 4(12). <https://doi.org/10.1101/CSHPERSPECT.A019695>
- Esterhuizen, L. (2014). *Drinking water quality and farming practices on dairy farms in the greater Mangaung Metro, South Africa*.

- Fakruddin, M. (2015). Methods for analyzing diversity of microbial communities in natural environments. *Microbes in Soil and Their Agricultural Prospects*, 42(1), 123–151. <https://doi.org/10.4038/cjsbs.v42i1.5896>
- Fang, D., Zhao, G., Xu, X., Zhang, Q., Shen, Q., Fang, Z., Huang, L., & Ji, F. (2018). Microbial community structures and functions of wastewater treatment systems in plateau and cold regions. *Bioresource Technology*, 249, 684–693. <https://doi.org/10.1016/J.BIORTECH.2017.10.063>
- FDA. (2014). *ANNUAL REPORT 2013/14 MANGAUNG METROPOLITAN MUNICIPALITY 2 TABLE OF CONTENTS ANNUAL REPORT 2013/2014-MANGAUNG METROPOLITAN MUNICIPALITY CONTENTS*.
- Feigl, V., Ujaczki, É., Vaszita, E., & Molnár, M. (2017). Influence of red mud on soil microbial communities: Application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. *Science of The Total Environment*, 595, 903–911. <https://doi.org/10.1016/J.SCITOTENV.2017.03.266>
- Ferrera, I., & Sánchez, O. (2016). Insights into microbial diversity in wastewater treatment systems: How far have we come? In *Biotechnology Advances* (Vol. 34, Issue 5, pp. 790–802). Elsevier Inc. <https://doi.org/10.1016/j.biotechadv.2016.04.003>
- Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, 3(4). <https://doi.org/10.4161/GMIC.19897>
- Floridia, V., Giuffrè, L., Giosa, D., Arfuso, F., Aragona, F., Fazio, F., Chen, C., Song, C., Romeo, O., & D'Alessandro, E. (2023). Comparison of the Faecal Microbiota Composition Following a Dairy By-Product Supplemented Diet in Nero Siciliano and Large White × Landrace Pig Breeds. *Animals*, 13(14), 2323. <https://doi.org/10.3390/ani13142323>
- Flugaur, N. J. (2003). *Wastewater Effluent Treatments and Control Technologies In the Beef Processing Industry*.
- Forcina, G., Pérez-Pardal, L., Carvalheira, J., & Beja-Pereira, A. (2022). Gut Microbiome Studies in Livestock: Achievements, Challenges, and Perspectives. In *Animals* (Vol. 12, Issue 23). MDPI. <https://doi.org/10.3390/ani12233375>
- Furtak, K., Gałazka, A., Grzadziel, J., & Nledzwlechl, J. (2019). *Changes in the soil microorganism community as a result of water stagnation*. <https://www.researchgate.net/publication/336686222>
- Gałazka, A., & Furtak, K. (2019). Functional Microbial Diversity in Context to Agriculture. In *Microbial Diversity in the Genomic Era* (pp. 347–358).

- Gao, F., Liu, G., She, Z., Ji, J., Gao, M., Zhao, Y., Guo, L., & Jin, C. (2021). Effects of salinity on pollutant removal and bacterial community in a partially saturated vertical flow constructed wetland. *Bioresource Technology*, 329, 124890. <https://doi.org/10.1016/J.BIORTECH.2021.124890>
- García-Díez, J., Saraiva, S., Moura, D., Grispoldi, L., Cenci-Goga, B. T., & Saraiva, C. (2023). The Importance of the Slaughterhouse in Surveilling Animal and Public Health: A Systematic Review. *Veterinary Sciences*, 10(2), 1–42. <https://doi.org/10.3390/vetsci10020167>
- GDARD, G. D. of A. and R. D. (2009). *GDARD Manual for Abattoir Waste Management GUIDELINE MANUAL FOR THE MANAGEMENT OF ABATTOIRS AND OTHER WASTE OF ANIMAL ORIGIN*. www.gdard.gpg.gov.za
- GDARD (Gauteng Department of Agriculture and Rural Development). (2009). *Guideline Manual for the Management of Abattoirs and Other Waste of Animal Origin*. March, 1–214. [http://www.gdard.gpg.gov.za/Services/Documents/Guideline Manual for the Management of Abattoirs.pdf](http://www.gdard.gpg.gov.za/Services/Documents/Guideline%20Manual%20for%20the%20Management%20of%20Abattoirs.pdf)
- Gordon, G. L. R., & Phillips, M. W. (1998). The role of anaerobic gut fungi in ruminants. *Nutrition Research Reviews*, 11(1), 133–168. <https://doi.org/10.1079/NRR19980009>
- Gryta, A., Frąc, M., & Oszust, K. (2014). The Application of the Biolog EcoPlate Approach in Ecotoxicological Evaluation of Dairy Sewage Sludge. *Applied Biochemistry and Biotechnology*, 174(4), 1434–1443. <https://doi.org/10.1007/s12010-014-1131-8>
- Guedes, R. (2008). *Known facts about the epidemiology of porcine proliferative enteropathy*. https://www.researchgate.net/publication/287636187_Known_facts_about_the_epidemiology_of_porcine_proliferative_enteropathy
- Gupta, N. (2019). DNA Extraction and Polymerase Chain Reaction. *Journal of Cytology*, 36(2), 116–117. https://doi.org/10.4103/JOC.JOC_110_18
- Gutu, L., Basitere, M., Harding, T., Ikumi, D., Njoya, M., & Gaszynski, C. (2021a). Multi-integrated systems for treatment of abattoir wastewater: A review. In *Water (Switzerland)* (Vol. 13, Issue 18). MDPI. <https://doi.org/10.3390/w13182462>
- Gutu, L., Basitere, M., Harding, T., Ikumi, D., Njoya, M., & Gaszynski, C. (2021b). Multi-integrated systems for treatment of abattoir wastewater: A review. *Water (Switzerland)*, 13(18). <https://doi.org/10.3390/W13182462>
- HACH. (2016). *HQD Meters & electrodes*.
- HACH. (2017). *sensION™ + DL Portable Meter 07/2017, Edition 2 User Manual*.
- HACH. (2021). *DR 6000 DOC022.53.90367_7ed*.

- Hailu, A. M., Ayenew, T., & Mulu, A. (2015). Characterization of Abattoir Wastewater and Evaluation of the Effectiveness of the Wastewater Treatment Systems in Luna and Kera Abattoirs in Central Ethiopia Grofutures (part of the UPGRO programme) View project Anaerobic Codigestion View project Characterization of Abattoir Wastewater and Evaluation of the Effectiveness of the Wastewater Treatment Systems in Luna and Kera Abattoirs in Central Ethiopia. *International Journal of Scientific & Engineering Research*, 6(4). <http://www.ijser.org>
- Hajibabaei, M., Singer, G. A. C., Clare, E. L., & Hebert, P. D. N. (2007). Design and applicability of DNA arrays and DNA barcodes in biodiversity monitoring. *BMC Biology*, 5(1), 24. <https://doi.org/10.1186/1741-7007-5-24>
- Halder, J. N., & Islam, M. N. (2015). Water Pollution and Its Impact on the Human Health. *Journal of Environment and Human*, 2, 36–46. <https://doi.org/10.15764/EH.2015.01005>
- Hamawand, I., Ghadouani, A., Bundschuh, J., Hamawand, S., Al Juboori, R. A., Chakrabarty, S., & Yusuf, T. (2017). A Critical Review on Processes and Energy Profile of the Australian Meat Processing Industry. *MDPI*, 10(731), 29.
- Hamilton, D., & Zhang, H. (n.d.). *Oklahoma Cooperative Extension Service*. Retrieved February 28, 2023, from <http://osufacts.okstate.edu>
- Hande Gursoy-Haksevenler, B., & Arslan-Alaton, I. (2020). Effects of treatment on the characterization of organic matter in wastewater: a review on size distribution and structural fractionation. *Water Science and Technology*, 82(5), 799–828. <https://doi.org/10.2166/WST.2020.403>
- Hardy Diagnostics. (2019). *ROSE BENGAL AGAR WITH CHLORAMPHENICOL*. www.cdc.gov/ncidod/dhqp/gl_isolation.html.
- He, Y., Maltecca, C., & Tiezzi, F. (2021). Potential use of gut microbiota composition as a biomarker of heat stress in monogastric species: A review. In *Animals* (Vol. 11, Issue 6). MDPI AG. <https://doi.org/10.3390/ani11061833>
- Hearle, H. (2016). The Layout and Processes of an Abattoir. *Food Safety: Meat Inspections*, 5. https://www.researchgate.net/publication/311065368_The_Layout_and_Processes_of_an_Abattoir
- Hess, M., Paul, S. S., Puniya, A. K., van der Giezen, M., Shaw, C., Edwards, J. E., & Fliegerová, K. (2020). Anaerobic Fungi: Past, Present, and Future. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/FMICB.2020.584893/FULL>
- Hibbett, D. S., Ohman, A., Glotzer, D., Nuhn, M., Kirk, P., & Nilsson, R. H. (2011). *Plenary Paper Progress in molecular and morphological taxon discovery in Fungi and options for formal classification of environmental sequences*. <https://doi.org/10.1016/j.fbr.2011.01.001>

- Hyde, K. D., Xu, J., Rapior, S., Jeewon, R., Lumyong, S., Niego, A. G. T., Abeywickrama, P. D., Aluthmuhandiram, J. V. S., Brahamanage, R. S., Brooks, S., Chaiyasen, A., Chethana, K. W. T., Chomnunti, P., Chepkirui, C., Chuankid, B., de Silva, N. I., Doilom, M., Faulds, C., Gentekaki, E., ... Stadler, M. (2019). The amazing potential of fungi: 50 ways we can exploit fungi industrially. *Fungal Diversity* 2019 97:1, 97(1), 1–136. <https://doi.org/10.1007/S13225-019-00430-9>
- Idrees, M., Mohammad, A. R., Karodia, N., & Rahman, A. (2020). Multimodal Role of Amino Acids in Microbial Control and Drug Development. *Antibiotics*, 9(6), 1–23. <https://doi.org/10.3390/ANTIBIOTICS9060330>
- Ijah, U. J. J., Musa, I. O., Amao, F., Maude, A. M., & Victor-Ekwebelem, M. O. (2022). WASTEWATER QUALITY AND UNHYGIENIC PRACTICES IN MINNA ABATTOIR, NORTH CENTRAL NIGERIA. *Science World Journal*, 17(2), 2022. www.scienceworldjournal.org
- Illumina. (2019). *MiSeq System Guide*. www.illumina.com/company/legal.html.
- Iloms, E. C. (2018). *Investigating industrial effluent impacts on municipal wastewater treatment plant*. July, 1–148.
- Iloms, E., Ololade, O. O., Ogola, H. J. O., & Selvarajan, R. (2020a). Investigating Industrial Effluent Impact on Municipal Wastewater Treatment Plant in Vaal, South Africa. *International Journal of Environmental Research and Public Health*, 17(3). <https://doi.org/10.3390/IJERPH17031096>
- Iloms, E., Ololade, O. O., Ogola, H. J. O., & Selvarajan, R. (2020b). Investigating industrial effluent impact on municipal wastewater treatment plant in vaal, South Africa. *International Journal of Environmental Research and Public Health*, 17(3), 1–18. <https://doi.org/10.3390/ijerph17031096>
- Ingrao, C., Strippoli, R., Lagioia, G., & Huisinigh, D. (2023). Water scarcity in agriculture: An overview of causes, impacts and approaches for reducing the risks. *Heliyon*, 9(8). <https://doi.org/10.1016/j.heliyon.2023.e18507>
- Itumeleng Phyllis Molobela. (2011). Management of water resources in South Africa: A review. *African Journal of Environmental Science and Technology*, 5(12), 993–1002. <https://doi.org/10.5897/ajest11.136>
- Jabari, L., Gannoun, H., Khelifi, E., Cayol, J. L., Godon, J. J., Hamdi, M., & Fardeaub, M. L. (2016a). Bacterial ecology of abattoir wastewater treated by an anaerobic digester. *Brazilian Journal of Microbiology*, 47(1), 73–84. <https://doi.org/10.1016/J.BJM.2015.11.029>
- Jabari, L., Gannoun, H., Khelifi, E., Cayol, J. L., Godon, J. J., Hamdi, M., & Fardeaub, M. L. (2016b). Bacterial ecology of abattoir wastewater treated by an anaerobic digester. *Brazilian Journal of Microbiology*, 47(1), 73–84. <https://doi.org/10.1016/J.BJM.2015.11.029>

- Jack, U., de Souza, P., & Moorgas, S. (2016). *Guidelines on using the Wastewater Treatment Technology Selection Decision Support Tool*.
- Jałowiecki, Ł., Chojniak, J. M., Dorgeloh, E., Hegedusova, B., Ejhed, H., Magnér, J., & Plaza, G. A. (2016). *Microbial Community Profiles in Wastewaters from Onsite Wastewater Treatment Systems Technology*. <https://doi.org/10.1371/journal.pone.0147725>
- Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M. A., Hussain, T., Ali, M., Rafiq, M., & Kamil, M. A. (2018). Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association*, 81(1), 7–11. <https://doi.org/10.1016/J.JCMA.2017.07.012>
- Jha, R., Fohse, J. M., Tiwari, U. P., Li, L., & Willing, B. P. (2019). Dietary fiber and intestinal health of monogastric animals. In *Frontiers in Veterinary Science* (Vol. 6, Issue MAR). Frontiers Media S.A. <https://doi.org/10.3389/fvets.2019.00048>
- Joblin, K. N. (2010). *Ruminal acetogens and their potential to lower ruminant methane emissions*. <https://agris.fao.org/agris-search/search.do?recordID=US201302935569>
- Kazemi Shariat Panahi, H., Dehghani, M., Guillemin, G. J., Gupta, V. K., Lam, S. S., Aghbashlo, M., & Tabatabaei, M. (2022). A comprehensive review on anaerobic fungi applications in biofuels production. *Science of The Total Environment*, 829, 154521. <https://doi.org/10.1016/J.SCITOTENV.2022.154521>
- Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *BIOINFORMATICS APPLICATIONS NOTE*, 26(11), 1463–1464. <https://doi.org/10.1093/bioinformatics/btq166>
- Khanna Kapur, M., Das, P., Kumar, P., Kumar, M., & Solanki, R. (2018). Role of Microbial Extracellular Enzymes in the Biodegradation of Wastes. *J Pharm Chem Biol Sci*, 6(3), 237–249. <https://www.jpcbs.info>
- Kink, M. (2016). *Fine screening system-the more intelligent primary clarifier Development and integration of innovative sewage treatment plant technologies for the transformation process towards the technology turnaround*.
- Kinzelman, J. L., Dufour, A. P., Wymer, L. J., Rees, G., Pond, K. R., & Bagley, R. C. (2006). *Lake and Reservoir Management Comparison of Multiple Point and Composite Sampling for Monitoring Bathing Water Quality* Comparison of Multiple Point and Composite Sampling for Monitoring Bathing Water Quality. 22(2), 95–102. <https://doi.org/10.1080/07438140609353887>
- Kirby, T. O., Brown, M., Ochoa-Repáraz, J., Rouillet, J. B., & Gibson, K. M. (2019). Microbiota Manipulation as a Metagenomic Therapeutic Approach for Rare Inherited Metabolic Disorders. *Clinical Pharmacology & Therapeutics*, 106(3), 505–507. <https://doi.org/10.1002/CPT.1503>

- Kirk, P. M., Cannon, P. F., & Stalpers, J. A. (2008). *Dictionary of The Fungi 10th ed.*-CABI. <https://www.scribd.com/document/474722518/Paul-M-Kirk-Paul-F-Cannon-J-A-Stalpers-Dictionary-of-the-Fungi-10th-ed-CABI-2008#>
- Koh, L. M., & Khor, S. M. K. (2023). Concept and Significance of Microbial Consortium in the Biodegradation Process. In *Handbook of Biodegradable Materials*. (pp. 137–177).
- Kongsted, H., Foldager, L., & Sørensen, J. T. (2020). Data from routine meat inspection is a poor indicator of the prevalence of tail lesions in undocked pigs. *Porcine Health Management*, 6(1), 1–4. <https://doi.org/10.1186/S40813-020-00149-Z/FIGURES/2>
- Kroiss, H., Rechberger, H., & Egle, L. (2011). Phosphorus in Water Quality and Waste Management. *Integrated Waste Management - Volume II*. <https://doi.org/10.5772/18482>
- Kurten, G. L., & Barkoh, A. (2014). *Evaluation of Community-Level Physiological Profiling for Monitoring Microbial Community Function in Fish Hatchery Ponds*.
- Li, J., Chen, D., Yu, B., He, J., Huang, Z., Zheng, P., Mao, X., Li, H., Yu, J., Luo, J., Yan, H., & Luo, Y. (2022). Batch and sampling time exert a larger influence on the fungal community than gastrointestinal location in model animals: A meaningful case study. *Frontiers in Nutrition*, 9. <https://doi.org/10.3389/FNUT.2022.1021215/FULL>
- Li, J., Gu, S., Zhao, Z., Chen, B., Liu, Q., Sun, T., Sun, W., & Tian, C. (2019). Dissecting cellobiose metabolic pathway and its application in biorefinery through consolidated bioprocessing in *Myceliophthora thermophila*. *Fungal Biology and Biotechnology*, 6(1), 1–12. <https://doi.org/10.1186/S40694-019-0083-8/FIGURES/6>
- Li, Y. hua, Li, H. bo, Xu, X. yang, Xiao, S. yao, Wang, S. qi, & Xu, S. cong. (2017). Fate of nitrogen in subsurface infiltration system for treating secondary effluent. *Water Science and Engineering*, 10(3), 217–224. <https://doi.org/10.1016/J.WSE.2017.10.002>
- Li, Z., Yu, E., Zhang, K., Gong, W., Xia, Y., Tian, J., Wang, G., & Xie, J. (2020). Water Treatment Effect, Microbial Community Structure, and Metabolic Characteristics in a Field-Scale Aquaculture Wastewater Treatment System. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/FMICB.2020.00930>
- Liu, Y. Y., & Haynes, R. J. (2011). Origin, nature, and treatment of effluents from dairy and meat processing factories and the effects of their irrigation on the quality of agricultural soils. In *Critical Reviews in Environmental Science and Technology* (Vol. 41, Issue 17). <https://doi.org/10.1080/10643381003608359>
- Lojkova, L., Vranová, V., Formánek, P., Drápelová, I., Brtnický, M., & Datta, R. (2020). Enantiomers of Carbohydrates and Their Role in Ecosystem Interactions: A Review. *Symmetry* 2020, Vol. 12, Page 470, 12(3), 470. <https://doi.org/10.3390/SYM12030470>

- Louise Glass, N., Schmoll, M., Cate, J. H. D., & Coradetti, S. (2013). Plant cell wall deconstruction by ascomycete fungi. *Annual Review of Microbiology*, 67, 477–498. <https://doi.org/10.1146/ANNUREV-MICRO-092611-150044>
- Ma, J., Wu, S., Shekhar, N. V. R., Biswas, S., & Sahu, A. K. (2020). Determination of Physicochemical Parameters and Levels of Heavy Metals in Food Waste Water with Environmental Effects. *Bioinorganic Chemistry and Applications*, 2020. <https://doi.org/10.1155/2020/8886093>
- Ma, Q., Qu, Y. Y., Zhang, X. W., Shen, W. L., Liu, Z. Y., Wang, J. W., Zhang, Z. J., & Zhou, J. T. (2015). Identification of the microbial community composition and structure of coal-mine wastewater treatment plants. *Microbiological Research*, 175, 1–5. <https://doi.org/10.1016/J.MICRES.2014.12.013>
- Macherey-Nagel. (2023). *Genomic DNA from stool samples*. www.mn-net.com
- Madoroba, E., Magwedere, K., Chaora, N. S., Matle, I., Muchadeyi, F., Mathole, M. A., & Pierneef, R. (2021). *microorganisms Microbial Communities of Meat and Meat Products: An Exploratory Analysis of the Product Quality and Safety at Selected Enterprises in South Africa*. <https://doi.org/10.3390/microorganisms9030507>
- Maguvu, T. E., Bezuidenhout, C. C., Kritzing, R., Tsholo, K., Plaatjie, M., Molale-Tom, L. G., Mienie, C. M., & Coertze, R. D. (2020). Combining physicochemical properties and microbiome data to evaluate the water quality of South African drinking water production plants. *PLoS ONE*, 15(8 August). <https://doi.org/10.1371/journal.pone.0237335>
- Mahgoub, S., Samaras, P., Abdelbasit, H., & Abdelfattah, H. (2016). Seasonal variation in microbiological and physicochemical characteristics of municipal wastewater in Al-Sharqiya province, Egypt (case study). *Desalination and Water Treatment*, 57(5), 2355–2364. <https://doi.org/10.1080/19443994.2015.1012342>
- Marietta, M. (2014). The Role Of Alkalinity In Aerobic Wastewater Treatment Plants : Magnesium Hydroxide vs . Caustic Soda. *Magnesium*, 1–3. <http://magnesiumspecialties.com/wp-content/uploads/Role-of-Alkalinity-in-Aerobic-Wastewater-Treatment-Plants-MgO-vs-Caustic-Soda1.pdf>
- Martin Zhuwakinyu., the R. U. of CreamerMedia. (2012). Review of South Africa's water sector. *Powertech*.
- Martínez-Gallardo, M. R., López, M. J., López-González, J. A., Jurado, M. M., Suárez-Estrella, F., Pérez-Murcia, M. D., Sáez, J. A., Moral, R., & Moreno, J. (2021). Microbial communities of the olive mill wastewater sludge stored in evaporation ponds: The resource for sustainable

- bioremediation. *Journal of Environmental Management*, 279, 111810.
<https://doi.org/10.1016/J.JENVMAN.2020.111810>
- Martínez-Gallardo, M. R., L'opeza, M. J., L'opez-Gonz'aleza, J. A., Jurado, M. M., Su'arez-Estrella, F., P'erez-Murciab, M. D., S'aezb, J. e A., Moralb, R., & Morenoa, J. (2021). Microbial communities of the olive mill wastewater sludge stored in evaporation ponds: The resource for sustainable bioremediation. *Journal of Environmental Management*.
- Mavasa, W. (2023, December 5). *Water and Sanitation releases 2023 full Blue Drop Report | South African Government*. <https://www.gov.za/news/media-statements/water-and-sanitation-releases-2023-full-blue-drop-report-05-dec-2023>
- McGinnis, M. R., & Tying, S. K. (1996). Introduction to Mycology. *Methods for General and Molecular Microbiology*, 925–928. <https://doi.org/10.1128/9781555817497.part6>
- McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One*, 8(4).
<https://doi.org/10.1371/JOURNAL.PONE.0061217>
- Mema, V. (n.d.-a). *IMPACT OF POORLY MAINTAINED WASTEWATER AND SEWAGE TREATMENT PLANTS: LESSONS FROM SOUTH AFRICA*.
- Mema, V. (n.d.-b). *IMPACT OF POORLY MAINTAINED WASTEWATER AND SEWAGE TREATMENT PLANTS: LESSONS FROM SOUTH AFRICA*.
- Meng, F., Yang, A., Zhang, G., Li, J., Li, X., Ma, X., & Peng, M. (2019). Effects of dissolved oxygen on key enzyme activities during photosynthetic bacteria wastewater treatment. *Process Biochemistry*, 76, 165–170. <https://doi.org/10.1016/J.PROCBIO.2018.09.025>
- Meng, Q., Yang, W., Men, M., Bello, A., Xu, X., Xu, B., Deng, L., Jiang, X., Sheng, S., Wu, X., Han, Y., & Zhu, H. (2019). Microbial community succession and response to environmental variables during cow manure and corn straw composting. *Frontiers in Microbiology*, 10(MAR).
<https://doi.org/10.3389/FMICB.2019.00529/FULL>
- Metcalf, & Eddy. (2003). *Wasterwater Engeneering - Treatment and Reuse*. www.mhhe.com
- Michael, S., Paschal, C., Kivevele, T., Rwiza, M. J., & Njau, K. N. (2020). Performance investigation of the slaughterhouse wastewater treatment facility: A case of Mwanza city Slaughterhouse, Tanzania. *Water Practice and Technology*, 15(4), 1096–1110.
<https://doi.org/10.2166/wpt.2020.085>
- Mitchell, S. A. (Stephen A., de Wit, M. P., Blignaut, J. N., & Crookes, D. (2014). *WASTE WATER TREATMENT PLANTS: THE FINANCING MECHANISMS ASSOCIATED WITH ACHIEVING GREEN DROP RATING*. 116.

- Mittal, G. S. (2004a). Characterization of the effluent wastewater from abattoirs for land application. *Food Reviews International*, 20(3), 229–256. <https://doi.org/10.1081/FRI-200029422>
- Mittal, G. S. (2004b). Characterization of the effluent wastewater from abattoirs for land application. *Food Reviews International*, 20(3), 229–256. <https://doi.org/10.1081/FRI-200029422>
- Mittal, G. S. (2006). Treatment of wastewater from abattoirs before land application—a review. *Bioresource Technology*, 97(9), 1119–1135. <https://doi.org/10.1016/J.BIORTECH.2004.11.021>
- Mizrahi, I., & Jami, E. (2018). Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission. *Animal*, 12(s2), s220–s232. <https://doi.org/10.1017/S1751731118001957>
- Modin, O., Persson, F., Wilén, B. M., & Hermansson, M. (2016). Nonoxidative removal of organics in the activated sludge process. *Critical Reviews in Environmental Science and Technology*, 46(7), 635–672. https://doi.org/10.1080/10643389.2016.1149903/SUPPL_FILE/BEST_A_1149903_SM8369.DOCX
- Mølbak, L., Johnsen, K., Boye, M., Jensen, T. K., Johansen, M., Møller, K., & Leser, T. D. (2008). The microbiota of pigs influenced by diet texture and severity of infection. *Veterinary Microbiology*, 128(2). <https://doi.org/10.1016/j.vetmic.2007.09.012>
- Molelekoa, T. B. J., Regnier, T., da Silva, L. S., & Augustyn, W. (2021). Production of Pigments by Filamentous Fungi Cultured on Agro-Industrial by-Products Using Submerged and Solid-State Fermentation Methods. *Fermentation* 2021, Vol. 7, Page 295, 7(4), 295. <https://doi.org/10.3390/FERMENTATION7040295>
- Montserrat García-Alegría, A., Iv, I., Anduro-Corona, I., Jhovanna Pérez-Martínez, C., Alba, M., Corella-Madueño, G., Rascón, M. L., Rascón-Durán, R., & Astiazaran-Garcia, H. (2020). Quantification of DNA through the NanoDrop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. <https://doi.org/10.1155/2020/8896738>
- Moreroa, M., & Basitere, M. (2022a). The challenges and treatment of abattoir effluents: a South African perspective. *Water Practice and Technology*, 17(12), 2598–2613. <https://doi.org/10.2166/wpt.2022.147>
- Moreroa, M., & Basitere, M. (2022b). The challenges and treatment of abattoir effluents: a South African perspective. *Water Practice and Technology*, 17(12), 2598–2613. <https://doi.org/10.2166/WPT.2022.147>

- Mothetha, M. L. (2016). *EVALUATION OF THE IMPACTS OF MUNICIPAL WASTEWATER TREATMENT ON THE RECEIVING ENVIRONMENT: A CASE STUDY OF THE OLIFANTSVLEI WASTEWATER TREATMENT PLANT IN THE GAUTENG PROVINCE, SOUTH AFRICA* [MASTER OF SCIENCE]. UNIVERSITY OF SOUTH AFRICA.
- Motta, P., Molla, G., Pollegioni, L., & Nardini, M. (2016). Structure-Function Relationships in L-Amino Acid Deaminase, a Flavoprotein Belonging to a Novel Class of Biotechnologically Relevant Enzymes. *The Journal of Biological Chemistry*, 291(20), 10457. <https://doi.org/10.1074/JBC.M115.703819>
- Muller, A. M. (2004). *Revision of General Authorisations in terms of section 39 of National Water Act, 1998*. 26187.
- Müller, J. R. (2017). Water and wastewater management in the red meat abattoir industry. In *WRC Report*.
- Muralikrishna, I. V., & Manickam, V. (2017). Industrial Wastewater Treatment Technologies, Recycling, and Reuse. In *Environmental Management* (pp. 295–336). Elsevier. <https://doi.org/10.1016/b978-0-12-811989-1.00013-0>
- Murugesan, S. (2017). Sustainable food security: Edible and medicinal Mushroom. *Sustainable Agriculture towards Food Security*, 185–196. https://doi.org/10.1007/978-981-10-6647-4_11
- Nafarnda, W. D., Ajayi, I. E., Shawulu, J. C., Kawe, M. S., Omeiza, G. K., Sani, N. A., Tenuche, O. Z., Dantong, D. D., Tags, S. Z., Balkema-Buschmann, A., Butaye, P., & Kogut, M. H. (2012). *Bacteriological Quality of Abattoir Effluents Discharged into Water Bodies in Abuja, Nigeria*. 2012. <https://doi.org/10.5402/2012/515689>
- Naidoo, S., & Olaniran, A. O. (2013). Treated wastewater effluent as a source of microbial pollution of surface water resources. *International Journal of Environmental Research and Public Health*, 11(1), 249–270. <https://doi.org/10.3390/ijerph110100249>
- Naidoo, V. (1999). *MUNICIPAL WASTEWATER CHARACTERIZATION Application of denitrification batch tests*.
- Naranjo-Ortiz, M. A., & Gabaldón, T. (2019). Fungal evolution: major ecological adaptations and evolutionary transitions. *Biological Reviews of the Cambridge Philosophical Society*, 94(4), 1443. <https://doi.org/10.1111/BRV.12510>
- Naylor, D., McClure, R., & Jansson, J. (2022). Trends in Microbial Community Composition and Function by Soil Depth. *Microorganisms* 2022, Vol. 10, Page 540, 10(3), 540. <https://doi.org/10.3390/MICROORGANISMS10030540>
- NEMW Amendment Act. (2014). *Government Gazette REPUBLIC OF SOUTH AFRICA*.
- NEMWA. (2009). *National Environmental Management: Waste Act 59 of 2008*.

- Nickel, J. P., & Fuchs, S. (2021). Large-volume samplers for efficient composite sampling and particle characterization in sewer systems. *Water (Switzerland)*, 13(20), 2831. <https://doi.org/10.3390/W13202831/S1>
- Niestępski, S., Harnisz, M., Ciesielski, S., Korzeniewska, E., & Osińska, A. (2020). Environmental fate of Bacteroidetes, with particular emphasis on Bacteroides fragilis group bacteria and their specific antibiotic resistance genes, in activated sludge wastewater treatment plants. *Journal of Hazardous Materials*, 394, 122544. <https://doi.org/10.1016/J.JHAZMAT.2020.122544>
- Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K.-H. (2008). Intraspecific ITS Variability in the Kingdom Fungi as Expressed in the International Sequence Databases and Its Implications for Molecular Species Identification. In *Evolutionary Bioinformatics*.
- Nishijima, S., Suda, W., Oshima, K., Kim, S. W., Hirose, Y., Morita, H., & Hattori, M. (2016). The gut microbiome of healthy Japanese and its microbial and functional uniqueness. *DNA Research*, 23(2), 125–133. <https://doi.org/10.1093/dnares/dsw002>
- Noukeu, N. A., Gouado, I., Priso, R. J., Ndongo, D., Taffouo, V. D., Dibong, S. D., & Ekodeck, G. E. (2016). Characterization of effluent from food processing industries and stillage treatment trial with Eichhornia crassipes (Mart.) and Panicum maximum (Jacq.). *Water Resources and Industry*, 16, 1–18. <https://doi.org/10.1016/j.wri.2016.07.001>
- Ntuli, F. (2012). *Management and Control of Industrial Effluents Discharged to Public Sewers : A Case Study*. 121–126.
- O'Dwyer, E., Chen, K., Wang, H., Wang, A., Shah, N., & Guo, M. (2020). Optimisation of wastewater treatment strategies in eco-industrial parks: Technology, location and transport. *Chemical Engineering Journal*, 381(August 2019), 122643. <https://doi.org/10.1016/j.cej.2019.122643>
- Oliveira, S. C., & Von Sperling, M. (2008). Reliability analysis of wastewater treatment plants. *Water Research*, 42(4–5), 1182–1194. <https://doi.org/10.1016/j.watres.2007.09.001>
- Osibanjo, O., & Adie, G. U. (2007). Impact of effluent from Bodija abattoir on the physicochemical parameters of Oshunkaye stream in Ibadan City, Nigeria. *African Journal of Biotechnology*, 6(15), 6.
- Osunmakinde, C. O., Selvarajan, R., Mamba, B. B., & Msagati, T. A. M. (2019). Profiling bacterial diversity and potential pathogens in wastewater treatment plants using high-throughput sequencing analysis. *Microorganisms*, 7(11). <https://doi.org/10.3390/microorganisms7110506>
- Otsuka, T., Yamazaki, H., Ankyu, E., Ahamed, T., Anda, M., & Noguchi, R. (2020). Elucidation of the Mechanism of Blockage in Sewer Pipes by Fatty Acid Deposition and Suspended Solid. *Water* 2020, Vol. 12, Page 2291, 12(8), 2291. <https://doi.org/10.3390/W12082291>

- Paruch, L., & Paruch, A. M. (2021). Cross-tracking of faecal pollution origins, macronutrients, pharmaceuticals and personal care products in rural and urban watercourses. *Water Science and Technology*, 83(3), 610–621. <https://doi.org/10.2166/wst.2020.603>
- Paruch, L., Paruch, A. M., Blankenberg, A. G. B., Haarstad, K., & Mæhlum, T. (2017). Norwegian study on microbial source tracking for water quality control and pollution removal in constructed wetland treating catchment run-off. *Water Science and Technology: A Journal of the International Association on Water Pollution Research*, 76(5–6), 1158–1166. <https://doi.org/10.2166/WST.2017.303>
- Paul, D., Kumbhare, S. V., Mhatre, S. S., Chowdhury, S. P., Shetty, S. A., Marathe, N. P., Bhute, S., & Shouche, Y. S. (2016). Exploration of microbial diversity and community structure of Lonar Lake: The only hypersaline meteorite crater lake within basalt rock. *Frontiers in Microbiology*, 6(JAN), 1553. <https://doi.org/10.3389/FMICB.2015.01553/XML/NLM>
- Paul, S., Cortez, Y., Vera, N., Villena, G. K., & Gutiérrez-Correa, M. (2016). Metagenomic analysis of microbial community of an Amazonian geothermal spring in Peru. *Genomics Data*, 9, 63–66. <https://doi.org/10.1016/j.gdata.2016.06.013>
- Periyannan, G. R. (2019). Bacterial cellobiose metabolism: An inquiry-driven, comprehensive undergraduate laboratory teaching approach to promote investigative learning. *Biochemistry and Molecular Biology Education*, 47(4), 438–445. <https://doi.org/10.1002/BMB.21237>
- Phungela, T., Gqomfa, B., Maphanga, T., & Shale, K. (2022). THE IMPACT OF WASTEWATER TREATMENT EFFLUENT ON WATER RESOURCES: A SOUTH AFRICAN PERSPECTIVE. *Water Law*.
- Pinto, J., Boavida-Dias, R., Matos, H. A., & Azevedo, J. (2022). Analysis of the Food Loss and Waste Valorisation of Animal By-Products from the Retail Sector. In *Sustainability (Switzerland)* (Vol. 14, Issue 5). MDPI. <https://doi.org/10.3390/su14052830>
- Pluciennik-Koropczuk, E., & Myszograj, S. (2019). New Approach in COD Fractionation Methods. *Water* 2019, Vol. 11, Page 1484, 11(7), 1484. <https://doi.org/10.3390/W11071484>
- Preston-Mafham, J., Boddy, L., & Randerson, P. F. (2002). Analysis of microbial community functional diversity using sole-carbon-source utilisation profiles – a critique. *FEMS Microbiology Ecology*, 42(1), 1–14. <https://doi.org/10.1111/J.1574-6941.2002.TB00990.X>
- Qin, F., Li, J., Mao, T., Feng, S., Li, J., & Lai, M. (2023). 2 Hydroxybutyric Acid-Producing Bacteria in Gut Microbiome and *Fusobacterium nucleatum* Regulates 2 Hydroxybutyric Acid Level In Vivo. *Metabolites*, 13(3), 451. <https://doi.org/10.3390/METABO13030451/S1>

- Qiu, M., Hu, J., Peng, H., Li, B., Xu, J., Song, X., Yu, C., Zhang, Z., Du, X., Bu, G., Huang, A., Han, X., Zeng, X., Yang, C., & Kong, F. (2022). *Research Note: The gut microbiota varies with dietary fiber levels in broilers*. <https://doi.org/10.1016/j.psj.2022.101922>
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(Database issue). <https://doi.org/10.1093/NAR/GKS1219>
- R Core Team. (2013). *R: The R Project for Statistical Computing*. <https://www.r-project.org/>
- Racké, K., & Warnken, M. (2010). L-Arginine Metabolic Pathways. *The Open Nitric Oxide Journal*, 2, 9–19.
- Rajhans, G., Barik, A., Sen, S. K., & Raut, S. (2021). Degradation of dyes by fungi: an insight into mycoremediation. *BioTechnologia*, 102(4), 445. <https://doi.org/10.5114/BTA.2021.111109>
- Red Meat Abattoir Association (RMAA). (2011). *By-Products Management – Red Meat Abattoirs*.
- Red Meat Producers Organisation. (2020). *Red-Meat-Producers*.
- Regueira, A., Lema, J. M., & Mauricio-Iglesias, M. (2021). Microbial inefficient substrate use through the perspective of resource allocation models. *Current Opinion in Biotechnology*, 67, 130–140. <https://doi.org/10.1016/J.COPBIO.2021.01.015>
- Ries, L. N. A., Beattie, S., Cramer, R. A., & Goldman, G. H. (2018). Overview of carbon and nitrogen catabolite metabolism in the virulence of human pathogenic fungi. *Molecular Microbiology*, 107(3), 277. <https://doi.org/10.1111/MMI.13887>
- Ritchie, H., & Roser, \max. (2017). *Water use and stress*. <https://ourworldindata.org/water-use-stress>
- Roberts, H., de Jager, L., & Blight, G. (2009). Waste-handling practices at red meat abattoirs in South Africa. *Waste Management and Research*, 27(1), 25–30. <https://doi.org/10.1177/0734242X07085754>
- Rowan, J., Durrance, K., Combs, G., & Fisher, L. (1997). *The digestive tract of the pig*.
- Rytioja, J., Hildén, K., Yuzon, J., Hatakka, A., de Vries, R. P., & Mäkelä, M. R. (2014). Plant-polysaccharide-degrading enzymes from Basidiomycetes. *Microbiology and Molecular Biology Reviews : MMBR*, 78(4), 614–649. <https://doi.org/10.1128/MMBR.00035-14>
- Safford, H. R., Shapiro, K., & Bischel, H. N. (2022). Wastewater analysis can be a powerful public health tool—if it's done sensibly. In *Proceedings of the National Academy of Sciences of the United States of America* (Vol. 119, Issue 6). National Academy of Sciences. <https://doi.org/10.1073/pnas.2119600119>

- Saini, A., Varshney, A., Saini, A., & Mani, I. (2023). Insight into epigenetics and human diseases. *Progress in Molecular Biology and Translational Science*. <https://doi.org/10.1016/bs.pmbts.2023.01.007>
- Samer, M. (2015). Biological and Chemical Wastewater Treatment Processes. *Web of Science*, 32, 137–144. <http://www.intechopen.com/books/trends-in-telecommunications-technologies/gps-total-electron-content-tec-prediction-at-ionosphere-layer-over-the-equatorial-region%0AInTec>
- Sanger, F., Nicklen, S., & Coulson, A. R. (1977a). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, 74(12), 5463–5467. <https://doi.org/10.1073/pnas.74.12.5463>
- Sathya, K., Nagarajan, K., Carlin Geor Malar, G., Rajalakshmi, S., & Raja Lakshmi, P. (2022). A comprehensive review on comparison among effluent treatment methods and modern methods of treatment of industrial wastewater effluent from different sources. *Applied Water Science*, 12(4), 1–27. <https://doi.org/10.1007/s13201-022-01594-7>
- Saye, L. M. G., Navaratna, T. A., Chong, J. P. J., O'malley, M. A., Theodorou, M. K., & Reilly, M. (2021). The Anaerobic Fungi: Challenges and Opportunities for Industrial Lignocellulosic Biofuel Production. *Microorganisms*, 9(4). <https://doi.org/10.3390/MICROORGANISMS9040694>
- Seiyaboh, E., & Izah, S. (2017). Bacteriological Assessment of a Tidal Creek Receiving Slaughterhouse Wastes in Bayelsa State, Nigeria. *Journal of Advances in Biology & Biotechnology*, 14(1), 1–7. <https://doi.org/10.9734/jabb/2017/34593>
- Seyedalmoosavi, M. M., Mielenz, M., Veldkamp, T., Daş, G., & Metges, C. C. (2022). Growth efficiency, intestinal biology, and nutrient utilization and requirements of black soldier fly (*Hermetia illucens*) larvae compared to monogastric livestock species: a review. *Journal of Animal Science and Biotechnology* 2022 13:1, 13(1), 1–20. <https://doi.org/10.1186/S40104-022-00682-7>
- Sharma, I., & Sharma, I. (2020). Bioremediation Techniques for Polluted Environment: Concept, Advantages, Limitations, and Prospects. *Trace Metals in the Environment - New Approaches and Recent Advances*. <https://doi.org/10.5772/INTECHOPEN.90453>
- Sharma, M., Agarwal, S., Agarwal Malik, R., Kumar, G., Pal, D. B., Mandal, M., Sarkar, A., Bantun, F., Haque, S., Singh, P., Srivastava, N., & Gupta, V. K. (2023). Recent advances in microbial engineering approaches for wastewater treatment: a review. *Bioengineered*, 14(1), 2184518. <https://doi.org/10.1080/21655979.2023.2184518>
- Shen, Z., Bisset, A., & Rossel, R. (2021). *Estimating soil fungal abundance and diversity at a macroecological scale with deep learning spectrotransfer functions*.

- https://www.researchgate.net/publication/354554232_Estimating_soil_fungal_abundance_and_diversity_at_a_macroecological_scale_with_deep_learning_spectrotransfer_functions
- Sherman, F. (2002). Getting started with yeast. *Methods in Enzymology*, 350, 3–41. [https://doi.org/10.1016/S0076-6879\(02\)50954-X](https://doi.org/10.1016/S0076-6879(02)50954-X)
- Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. In *Molecular Ecology* (Vol. 21, Issue 8, pp. 1794–1805). <https://doi.org/10.1111/j.1365-294X.2012.05538.x>
- Shukri, A. A., Kyambadde, J., & Hawumba, J. F. (2017). The Impact of Kalerwe Abattoir Wastewater Effluent on the Water Quality of the Nsooba Channel. *Agricultural Research & Technology: Open Access Journal*, 6(1), 8.
- Silva, C., & Rosa, M. J. (2021). A Practical Methodology for Forecasting the Impact of Changes in Influent Loads and Discharge Consents on Average Energy Consumption and Sludge Production by Activated Sludge Wastewater Treatment. *Sustainability 2021, Vol. 13, Page 12293, 13(21), 12293*. <https://doi.org/10.3390/SU132112293>
- Silva, T. R., Valdman, E., Valdman, B., & Leite, S. G. F. (2007). SALICYLIC ACID DEGRADATION FROM AQUEOUS SOLUTIONS USING PSEUDOMONAS FLUORESCENS HK44: PARAMETERS STUDIES AND APPLICATION TOOLS. *Brazilian Journal of Microbiology*, 38, 39–44.
- Singh, A. (2018). *Improving efficiency and reducing waste for sustainable beef supply chain by Akshit Singh for degree of Doctor of Philosophy Norwich Business School*.
- Skouteris, G., Rodriguez-Garcia, G., Reinecke, S. F., & Hampel, U. (2020). *The use of pure oxygen for aeration in aerobic wastewater treatment: A review of its potential and limitations*. <https://doi.org/10.1016/j.biortech.2020.123595>
- Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., Arrieta, J. M., & Herndl, G. J. (2006). Microbial diversity in the deep sea and the underexplored “rare biosphere.” *Proceedings of the National Academy of Sciences of the United States of America*, 103(32), 12115–12120. <https://doi.org/10.1073/pnas.0605127103>
- South African Pork Producers Organisation. (2022). *African swine fever (ASF) update - SAPPO*. <https://sappo.org/lets-talk-indepth/african-swine-fever-asf-update/>
- Stare, A., Vrecko, D., Hvala, N., & Strmcnik, S. (2007). Comparison of control strategies for nitrogen removal in an activated sludge process in terms of operating costs: a simulation study. *PubMed*, 41(9). <https://doi.org/https://doi.org/10.1016/j.watres.2007.01.029>
- Swanepoel. (2014a). *Waste Management at Free State Abattoirs*.
- Swanepoel, J. (2014b). *WASTE MANAGEMENT AT FREE STATE ABATTOIRS*.

- Szeligowska, N., Cholewińska, P., Czyż, K., Wojnarowski, K., & Janczak, M. (2021). Inter and intraspecies comparison of the level of selected bacterial phyla in cattle and sheep based on feces. *BMC Veterinary Research*, 17(1). <https://doi.org/10.1186/s12917-021-02922-w>
- Talukder, S., & Selvakumar, K. (2015).) Feb'15 Hosted@www.ijlr.org International Journal of Livestock Research ISSN. *International Journal of Livestock Research*, 5(2). <https://doi.org/10.5455/ijlr.20150225043513>
- Tang, M. L. Y., & Lau, S. C. K. (2022). Strategy to Evaluate Changes in Bacterial Community Profiles and Bacterial Pathogen Load Reduction After Sewage Disinfection. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.919207>
- Teng, Z., Fan, W., Wang, H., Cao, X., & Xu, X. (2019). Monitoring soil microorganisms with community-level physiological profiles using Biolog EcoPlates™ in Chaohu lakeside wetland, east China. *BioRxiv*. <https://doi.org/10.1101/616821>
- Thajudeen, A. (2017). *What Is Screening in Wastewater Treatment and How Does It Work? - LA Progressive*. EngineeringCivil.Org. <https://www.laprogressive.com/sponsored/screening-in-wastewater-treatment>
- To, B. R., Discharge, T. H. E., & Industrial, O. F. (2008). *Mangaung Metropolitan Municipality By-Laws Relating To the Discharge of Industrial*. 60, 1–21.
- Tolera, S. T., Alemu, F. K., & Mengistu, D. A. (2022). Knowledge, Attitude, and Practice of Abattoir Workers Toward Abattoirs Waste Management in Eastern Ethiopia. *Environmental Health Insights*, 16. <https://doi.org/10.1177/11786302221075450>
- Toxopeus, M. of water and S. (2019). *Understanding water issues and challenges I*. hsf.org.za/publications/hsf-briefs/understanding-water-issues-ans-challenges-i-department-of-water-and-sanitation
- Turano, E., Curcio, S., De Paola, M. G., Calabrò, V., & Iorio, G. (2002). An integrated centrifugation–ultrafiltration system in the treatment of olive mill wastewater. *Journal of Membrane Science*, 209(2), 519–531. [https://doi.org/10.1016/S0376-7388\(02\)00369-1](https://doi.org/10.1016/S0376-7388(02)00369-1)
- Ungureanu, N., Vladut, V., & M, I. P. (2020). *Aspects regarding the treatment of slaughterhouse wastewater*. October.
- Van der Merwe-botha, M., & Manus, L. (2011). Wastewater Risk Abatement Plan, A W2RAP GUIDELINE - To plan and manage towards safe and complying municipal wastewater collection and treatment in South Africa. In *Water Research Commission Report No. TT 489/11* (Vol. 1, Issue 1).

- Verlicchi, P., & Grillini, V. (2020). Surfacewater and groundwater quality in South Africa and mozambique-analysis of the most critical pollutants for drinking purposes and challenges in water treatment selection. *Water (Switzerland)*, 12(1). <https://doi.org/10.3390/w12010305>
- Viegas, C., Faria, T., dos Santos, M., Carolino, E., Sabino, R., Gomes, A. Q., & Viegas, S. (2016). Slaughterhouses Fungal Burden Assessment: A Contribution for the Pursuit of a Better Assessment Strategy. *International Journal of Environmental Research and Public Health*, 13(3). <https://doi.org/10.3390/IJERPH13030297>
- Vila-Santa, A., Mendes, F. C., Ferreira, F. C., Prather, K. L. J., & Mira, N. P. (2021). Implementation of synthetic pathways to foster microbe-based production of non-naturally occurring carboxylic acids and derivatives. *Journal of Fungi*, 7(12), 1020. <https://doi.org/10.3390/JOF7121020/S1>
- Wang, B. T., Hu, S., Yu, X. Y., Jin, L., Zhu, Y. J., & Jin, F. J. (2020). Studies of Cellulose and Starch Utilization and the Regulatory Mechanisms of Related Enzymes in Fungi. *Polymers 2020*, Vol. 12, Page 530, 12(3), 530. <https://doi.org/10.3390/POLYM12030530>
- Wang, X., Zhang, Y., Li, C., Li, G., Wu, D., Li, T., Qu, Y., Deng, W., He, Y., Penttinen, P., Zhang, H., Huang, Y., Zhao, K., & Zou, L. (2022). Antimicrobial resistance of *Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae* and *Enterococcus* spp. isolated from the feces of giant panda. *BMC Microbiology*, 22(1). <https://doi.org/10.1186/s12866-022-02514-0>
- Wang, Y., Yan, Y., Thompson, K. N., Bae, S., Accorsi, E. K., Zhang, Y., Shen, J., Vlamakis, H., Hartmann, E. M., & Huttenhower, C. (2021). Whole microbial community viability is not quantitatively reflected by propidium monoazide sequencing approach. *Microbiome*, 9(1). <https://doi.org/10.1186/s40168-020-00961-3>
- Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J. R., Vázquez-Baeza, Y., Birmingham, A., Hyde, E. R., & Knight, R. (2017). Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5(1), 1–18. <https://doi.org/10.1186/S40168-017-0237-Y/FIGURES/8>
- Wilkinson, M. J., Szabo, C., Ford, C. S., Yarom, Y., Croxford, A. E., Camp, A., & Gooding, P. (2017). Replacing Sanger with Next Generation Sequencing to improve coverage and quality of reference DNA barcodes for plants. *Scientific Reports*, 7. <https://doi.org/10.1038/srep46040>
- WRC. (2016). Wastewater Treatment Technologies - A Basic Guide (WRC Project No. K8/1106). In *Water Research Commission* (Issue 55). <http://www.wrc.org.za/wp-content/uploads/mdocs/TT651-15.pdf>
- WRC, W. R. C. (2017). *Water and Wastewater management in the red meat abattoir industry*.

- Wu, P. F., & Mittal, G. S. (2012). Characterization of provincially inspected slaughterhouse wastewater in Ontario, Canada. *Canadian Biosystems Engineering / Le Genie Des Biosystems Au Canada*, 54.
- WWB, W. B. (2017). *Total Kjeldahl Nitrogen*. <https://www.thewastewaterblog.com/single-post/2017/11/01/total-kjeldahl-nitrogen>
- Xu, P., Zhao, M., Fu, X., & Zhao, C. (2022). *Effect of chloride ions on the corrosion behavior of carbon steel in an iron bacteria system*. <https://doi.org/10.1039/d2ra02410a>
- Xu, Q., Qiao, Q., Gao, Y., Hou, J., Hu, M., Du, Y., Zhao, K., & Li, X. (2021). Gut Microbiota and Their Role in Health and Metabolic Disease of Dairy Cow. *Frontiers in Nutrition*, 8, 701511. <https://doi.org/10.3389/FNUT.2021.701511>
- Yang, R. H., Su, J. H., Shang, J. J., Wu, Y. Y., Li, Y., Bao, D. P., & Yao, Y. J. (2018). Evaluation of the ribosomal DNA internal transcribed spacer (ITS), specifically ITS1 and ITS2, for the analysis of fungal diversity by deep sequencing. *PLOS ONE*, 13(10), e0206428. <https://doi.org/10.1371/JOURNAL.PONE.0206428>
- Youssef, N. H., Couger, M. B., Struchtemeyer, C. G., Ligginstoffer, A. S., Prade, R. A., Najar, F. Z., Atiyeh, H. K., Wilkins, M. R., & Elshahed, M. S. (2013). The genome of the anaerobic fungus *Orpinomyces* sp. strain C1A reveals the unique evolutionary history of a remarkable plant biomass degrader. *Applied and Environmental Microbiology*, 79(15), 4620–4634. <https://doi.org/10.1128/AEM.00821-13>
- Zaborowska, E., Czerwionka, K., & Maćkonia, J. (2021). Integrated plant-wide modelling for evaluation of the energy balance and greenhouse gas footprint in large wastewater treatment plants. *Applied Energy*, 282(November 2020). <https://doi.org/10.1016/j.apenergy.2020.116126>
- Zeitz, M. A., Tanveer, Z., Openshaw, A. T., & Schmidt, M. (2019). Genetic Regulators and Physiological Significance of Glycogen Storage in *Candida albicans*. *Journal of Fungi*, 5(4). <https://doi.org/10.3390/JOF5040102>
- Zhang, H., Feng, J., Chen, S., Li, B., Sekar, R., Zhao, Z., Jia, J., Wang, Y., & Kang, P. (2018). Disentangling the drivers of diversity and distribution of fungal community composition in wastewater treatment plants across spatial scales. *Frontiers in Microbiology*, 9(JUN), 1291. <https://doi.org/10.3389/FMICB.2018.01291/BIBTEX>
- Zhang, J., Shao, Y., Liu, G., Qi, L., Wang, H., Xu, X., & Liu, S. (2021). Wastewater COD characterization: RBCOD and SBCOD characterization analysis methods. *Scientific Reports*, 11(1), 691. <https://doi.org/10.1038/S41598-020-80700-8>

Supplementary Table 1: Physicochemical parameters tested, including compliance to Mangaung Effluent Discharge By-Laws. Values with red font mean non-compliance.

Sample Day	Temp °C	pH	EC mS/m	TS mg.L ⁻¹	TDS mg.L ⁻¹	TSS mg.L ⁻¹	STS mg.L ⁻¹	TVS mg.L ⁻¹	FOG mg.L ⁻¹	TOC mg.L ⁻¹	NH ₃ mg.L ⁻¹	NO ₃ ⁻ mg.L ⁻¹	TKN mg.L ⁻¹	PO ₄ ³⁻ mg.L ⁻¹	SO ₄ ²⁻ mg.L ⁻¹	Cl ⁻ mg.L ⁻¹	CaCO ₃ mg.L ⁻¹	tCOD mg.L ⁻¹	sCOD mg.L ⁻¹
Limits		6-10	500			1000			400					100	1500		1000	5000	5000
1	25,97	7,29	103,25	3430	826,5	710	13,25	2693,3	<1	343	31	60	346	35,7	110	164	1090	5770	5240
2	24,875	7,47	93,25	2533,33	746	557	12,5	2460	<1	38	20	50	331	26,8	100	200	1310	4920	4150
3	25,7	7,555	152,15	7050	1217	1250	22,5	5450	<1	600	98	75	701	36,3	90	141	1600	9160	8270
4	25,15	7,5	112,15	4183,3	897,5	712	13,5	3290	<1	398	42	69	438	23,9	60	175	1480	5350	4430
5	26,25	7,19	101,95	3480	815,5	1430	15,5	2903,3	<1	490	45	70	4456	56,2	110	213	1470	6180	4200
6	28,2	7,37	78,6	1326,6	628	176	2,75	1166,6	<1	130	8	5	136	15,5	10	193	890	1220	740
7	26,525	7,565	104	4993,3	832	1030	15,75	4403,3	<1	443	22	40	491	43,2	130	163	1640	6690	5580
8	24,85	7,705	91,3	3953,3	730	476	13,5	3696,6	<1	485	15	78	369	29,1	60	173	1530	4570	4310
9	24,95	7,55	105,6	3986,6	845	499	7,5	3690	<1	433	48	10	334	21,8	40	193	1580	4790	4210
10	25,15	7,63	89,4	3546,6	715	552	7,5	3026,6	<1	253	11	44	151	30,4	40	202	1340	2790	1810
Mean	25,762	7,482	103,165	3848,33	825,25	739,2	12,425	3278	<1	341,3	34	50,1	775,3	31,89	75	181,7	1393	5144	4294
Min	24,85	7,19	78,6	1326,67	628	176	2,75	1166,6	<1	38	8	5	136	15,5	10	141	890	1220	740
Max	28,2	7,705	152,15	7050	1217	1430	22,5	5450	<1	600	98	78	4456	56,2	130	213	1640	9160	8270
SD	1,045	0,15	19,78	1497,91	158,3	385,3	5,44	1154,7	0	176,20	26,6	25,8	1303,31	11,6	38,6	22,1	241	2146,5	2033,4
% Non-Compliance		0	0			30			0					0	0		90	50	30