BINDURA UNIVERSITY OF SCIENCE EDUCATION

DEPARTMENT OF ENVIRONMENTAL SCIENCE

BIOLOGICAL DENITRIFICATION AS A REMEDIATION OPTION FOR HIGH NITRATE INDUSTRIAL EFFLUENT FROM SABLE CHEMICAL INDUSTRIES USING RAW MUNICIPAL SEWAGE

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DEDICATION

This work is dedicated to my loved ones whose unwavering support and encouragement fuelled my pursuit of knowledge.

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Lastly, I offer my heartfelt thanks to the Divine for granting me the strength, wisdom, and perseverance to navigate through the challenges encountered during this research journey. I am humbled by the blessings and guidance received throughout this endeavor, and I acknowledge the presence of a Higher power that has been instrumental in every step of the way.

DECLARATION

I, Tendai Govere, now affirm that the research work presented in this project report is entirely my own effort. A dissertation submitted in partial fulfillment of the requirements for a B.Sc. (Honours) in Environmental Science in Safety, Health and Environmental Management. I confirm that this work has never been submitted before for any other purpose at any other Academic institution. All additional sources of information has been acknowledged employing references.

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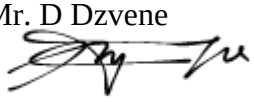
Signature:	
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Abstract

In Zimbabwe, industrial effluent, notably from processes at the Sable Chemical factory has been found to contain excessive nitrate levels and that these present a major environmental and health challenge. The project's objective is to assess the potential of biological denitrification as a viable remediation strategy for high nitrate effluent from Sable Chemicals using raw municipal sewage as an organic carbon source, promoting bacterial growth and activity essential for efficient denitrification. The study found that using raw municipal sewage as carbon source effectively removed nitrates from industrial effluent with ethanol and hydrazine enhancing the process, offering a sustainable and cost-effective solution for wastewater treatments and reducing environmental pollution. The study was initiated by determining the initial nitrate content in the effluent from Sable Chemicals by use of ultraviolet spectrometer, to act as ground zero for treatment efficacy monitoring as well as the physico-chemical characteristics of Kwekwe Municipality. Nitrates were high in all sampling points and the BOD from Kwekwe sewage was way above the legal limit. Sable process effluent, sampled from different sites (dam1 dam 2 dam 3) rich in nitrate was inoculated with various sewer treatments, including returning activated sludge, BNR liquor, and raw sewer. They were maintained under anaerobic conditions. Where effluent was inoculated with sewage, some samples were infused with distilled water to enable comparison. Other experimental treatments were conducted, including adding hydrazine as an oxygen scavenger to the sewage inoculum and adding an external carbon source as 5% ethanol, to assess the rate of denitrification. The amount of nitrates in the batch tests where sewer was infected decreased overall. The rate of removal for BNR returned activated sludge and raw sewage were 67%, 77% and 74% respectively, for a period of 16 days. In an experiment with distilled water the nitrate removal was 27%. Nitrate levels lowered from 90 mg/L to 23 mg/L overall in 16 days. Average amount of nitrate removal in all treatments was 3.7mg/L per day. During a ten-day timeframe, batch test inoculated with hydrazine and ethanol showed a 60% and 64% drop in nitrate-nitrogen. The results of the experiment demonstrated that total nitrogen in Sable effluent may be reduced or removed through denitrification using municipal sewage effluent as a bioremediation medium and shift from the red band of their effluent disposal license to a lower and less expensive band.

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CHAPTER 1: INTRODUCTION

This chapter introduces the whole research project. The main context includes background to the study, problem statement, general objectives, specific objectives, hypothesis, justification, and study area map, physical characteristics of study area and lastly socio-economic characteristics of the study area.

1.1 Background to the study

Nitrate pollution from industrial effluent is a growing environmental issue, exacerbated by various industrial processes that release significant amounts of nitrogen compounds into wastewater. Elevated nitrate levels in aquatic environments can result to detrimental effects such as eutrophication and pose major hazards to one's health when found in drinking water. Biological denitrification has emerged as a promising remediation strategy, leveraging microbial activity to convert nitrate into nitrogen gas. This study explores the use of raw municipal sewage as an organic carbon source to enhance biological denitrification in high nitrate industrial effluent.

Recent research highlights the efficacy of biological denitrification in treating nitrate-rich wastewater. This process relies on denitrifying bacteria that, in the absence of oxygen, use nitrate as an electron acceptor, transforming it to nitrogen gas (N_2), thus removing it from the water (Chen et al., 2019). Traditional techniques like physical separation, chemical reduction, and ion exchange are often expensive and generate secondary waste. Therefore, biological denitrification presents a more sustainable and cost-effective solution.

The role of organic carbon in enhancing denitrification has been extensively studied. Sources of organic carbon, like methanol, ethanol, and acetate are commonly used to facilitate the development and activity of denitrifying bacteria (Gao et al., 2020). However, these sources can be costly and may not be sustainable in the long term. Consequently, there is increasing interest in using alternative, low-cost carbon sources such as raw municipal sewage, which is rich in organic matter and readily available (Li et al., 2021).

Several studies have demonstrated the feasibility of using raw municipal sewage for denitrification. For instance, Xia et al. (2018) reported successful nitrate removal in synthetic wastewater using raw sewage as an organic carbon source. The study found that sewage provided a sufficient and continuous supply of carbon, which supported the denitrifying

bacteria and enhanced nitrate removal efficiency. Despite the progress made, there are still several areas requiring further research. One key area is the optimization of operational parameters such as the sewage-to-effluent ratio, temperature, pH, and retention time to maximize denitrification efficiency (Wang et al., 2020). Additionally, the microbial community dynamics in the presence of raw municipal sewage need to be better understood to ensure stable and effective nitrate removal.

Another area that warrants further investigation is the potential impact of using raw municipal sewage on the overall water treatment process. While sewage provides the necessary carbon source, it also introduces additional organic and inorganic contaminants that may affect the treatment efficiency and quality of the treated effluent (Zhang et al., 2022). Moreover, recent advancements in bioreactor designs, for example using sequencing batch reactors (SRBs) and moving bed film reactors (MBBRs), have shown promise in optimizing the denitrification process (Zhao et al., 2021). These systems facilitate better contact between microorganisms and substrates, improving the overall efficiency of nitrate removal.

Around the world, various methods have been employed to address nitrate pollution in industrial effluents, each tailored to the local context and available resources. In Europe, constructed wetlands have gained popularity due to their low operational costs and ability to integrate with natural landscapes. These systems leverage natural processes involving plants, soil, and microorganisms to remove nitrates and other contaminants from wastewater (Vymazal, 2018). In Asia, particularly in China, advancements in membrane bioreactor (MBR) technology have shown significant promise. These systems combine biological treatment with membrane filtration, offering high efficiency in nitrate removal and producing high-quality effluent suitable for reuse (Li et al., 2019).

In North America, particularly in the United States, the application anammox or anaerobic ammonium oxidation has been explored extensively. Anammox is an energy-efficient process that converts ammonium and nitrate directly into nitrogen gas, reducing the need for external carbon sources and operational costs (Kartal et al., 2018). These diverse approaches underscore the global efforts to find effective and sustainable solutions for nitrate pollution, highlighting the need for continued innovation and adaptation to local conditions.

In the Middle East, countries facing severe water scarcity have turned to advanced desalination technologies coupled with MBR systems to treat and reuse industrial wastewater. These integrated systems, such as MBR combined with reverse osmosis (RO) and Nano

filtration (NF), are designed to help eliminate a variety of pollutants, including nitrates, and provide high-quality reclaimed water suitable for various uses. Furthermore, the implementation of anaerobic MBRs (AnMBRs) combined with RO has shown promise in producing potable and non-potable water, while minimizing sludge production and enhancing energy efficiency

Southern African countries, because of a lack of equipment and skilled personnel, mainly treat their industrial effluent waste rich in nitrate with natural means like the use of retention dams, reactive dams and constructed wetlands like what is being done at Sable Chemicals. Because of the underdeveloped economies, organizations value production more than they value the environment. Some regulations for example in Zimbabwe, the Environmental Management Act penalize organizations through fines but do not strictly restrict organizations from discharging effluent into the environment.

Overall, while there are common challenges like membrane fouling and high operational costs, on-going research and technological advancements continue to improve the feasibility and effectiveness of these methods, suggesting a global trend towards more sustainable and efficient wastewater treatment solutions. The application of biological denitrification using raw municipal sewage as a remediation option for high nitrate industrial effluent presents a promising and sustainable approach. Current research supports its feasibility and potential benefits, but further studies are necessary to optimize the process and address the associated challenges. By exploring these aspects, this study aims to aid in the growth of an effective and environmentally friendly method for managing high nitrate industrial effluent.

1.2 Problem statement

Sable chemicals industry in Kwekwe has been manufacturing ammonium nitrate fertilizer ever since 1969, discharging nitrate rich effluent resulting in the pollution of fresh surface water bodies and contamination of underground water. This has largely contributed to the deterioration of water quality over the years, affecting human lives and posing a risk to human health, livestock, and aquatic ecosystems. Vivid evidence is the eutrophication of the Munyati River, the receiving platform. The river supplies water to nearby farms, artisanal miners, industries like ZPC Munyati and Intrachem, nearby residents etc. This means people; their livestock, industries, and the environment depend on that contaminated water.

According to the Environmental Management Act, Statutory instrument 6 of 2007 (Effluents and Solid waste Disposal) total nitrogen in the effluent should not exceed 50ppm and Sable Chemicals is in the red zone with nitrogen content in process effluent as high as over 1500 ppm. Due to that reason, Sable Chemical Industries has been losing revenue as fines, quarterly discharge, monitoring fees and annual monitoring fees to the Environmental Management Agency (EMA) over the past years, close to $35,000 each year. To add on, in the year 2012, Sable chemicals effluent was the major suspect in the death of 73 cattle which died within a period of one month (Sable Chemicals 1stQ Report, 2012). Research indicates that animals experience abrupt death when exposed to nitrogen concentrations between 100 and 300mg N/L (Thompson, 2021)

Figure 1.1: The insert provides evidence of eutrophication of the Munyati River and insert 2 provides evidence of chemical precipitation forming along the drain.

1.3 Main Objective

- To assess the biological denitrification as a remediation option for high- nitrate industrial effluent from sable chemicals using raw municipal sewage.

1.4 Specific objectives

- To determine the physio-chemical quality of the process effluent and of the raw sewage
- To assess the raw sewage's and process effluent's physio-chemical quality.
- To determine the extent of ethanol (external carbon source) and hydrazine (oxygen scavenger) on nitrate reduction.

1.5 Hypothesis

- H_1- High Nitrate Industrial effluent at sable chemicals can be remediated by raw municipal sewage
- H_0 - High nitrate industrial effluent at sable chemicals cannot be remediated by raw municipal sewage

1.6 Justification

This is an experimental research project that might be of great significance for the remediation of the high nitrate rich industrial process effluent at sable chemicals and can be applied anywhere in Zimbabwe. It is a research project to bring industries one step closer to sustainable development, to a water pollution-free environment through the reduction of excess nitrogen in water bodies thus promoting water quality. This will greatly benefit people and reduce livestock poisoning as well as industries that depend on the water thereby reducing externalities. The research project may be of great importance for Sable Chemicals by assisting them to get a blue license or even a better one thereby reducing costs which are being paid as penalties. This project is a great initiative towards an environmental monitoring programme, and may be of great assistance to the organization towards accomplishing its ISO 14001 certification goal. Moreover, this approach might assist towards the achievement of Goal 6 of the Sustainable Development Goals which emphasizes that water and sanitation are accessible and managed sustainably for everyone (Sufiana, 2023).

1.7 Area of study

Sable Chemical Industries Ltd is located in the Kwekwe midlands province of Zimbabwe. It is located 16 kilometres away from Kwekwe central business district towards Kadoma along Harare Highway, specifically 400m from the highway (Sable SHEQ manual).

Figure 1.2: Sable Factory map

1.8 Physical and socio-economic characteristics

In Zimbabwe, Sable Chemical Industries Limited is the only company producing ammonium nitrate fertilizers. It began operations in 1969 after being incorporated in 1965. The company has four operational plants within its complex which are The Water Treatment Plant, Ammonium Nitrate Plant, The Nitric Acid Plant and The Sanitizer Manufacturing Plant. The company can manufacture 240,000 tons of ammonium nitrate per year.

Kwekwe, where the Sable Chemicals Industry is situated is in the central region of Zimbabwe. Geographically, Kwekwe is bounded by 18.9167-19.0000^{o}S; latitude and 29.7500-29.8167^{o}E, longitude (Opeolu, 2017). It is in the natural farming region 4. It is marked by high temperature, mid-season dry periods, and unevenly distributed average annual rainfall of 500- 750 mm (FAO, 2020). The factory was situated in the Middleveld zone where the relief is 900-1200m above sea level.

The area is dominated by sandy loams with greyish-brown sands made from granitic rocks (FAO, 2020). The pH of the soil ranges from 4.6 to 4.9. Moreover, the soil has a low water-holding capacity. The areas surrounding Sable Chemicals are dominated by intensive farming with wheat, maize, and cotton being the major grown crops. There has been an increase in the number of farmers who practice cattle ranching. Dairy farming is not done intensively around the area. The soil supports medium to dense indigenous hardwoods of mainly Brachystegia spiciformis family (Essettle Map Service, 1985).

Kwekwe River and Sebakwe are the two major rivers flowing north that dominate the district's drainage (William, 1994). About 8km from Kwekwe town there's the Munyati River, into which Sable contents are fed. Sable Chemical Industry is located on a plateau between Sebakwe and Munyati River.

1.9 Socio-Economic Characteristics

Sable Chemical Industries employs over 300 permanent employees and over 100 contract employees, the majority of which are Zimbabweans. There are shift employees who work 12 hours a day and those with regular or fixed schedules who work 8 hours a day. Sable Chemicals is located far from the CBD or residents, so employees are ferried to work every single day by the company bus. Some are offered residence by the company and some not depending on the employee's pay grade.

The industry is male-dominated. However, the number of female employees has been increasing. Females were dense in the office areas, but now a number of them are aggravating operations. The majority of employees are middle-aged, and only a few are almost at their retirement age. People are employed in terms of competence or education in their area of specialty.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter gives a review of previous studies that have been undertaken on the biological denitrification of high nitrate effluent. It also discusses industrial processes from Sable, the nitrogen cycle, anthropogenic sources of nitrogen pollution, impacts of nitrogen pollution both on humans and the environment, biological denitrification and factors and microorganisms involved in biological denitrification. It further discusses why municipal wastewater was preferred for enhancement of the process including its properties.

2.2 Nitrogen Pollution and the Nitrogen Cycle

Different countries have put up restricted requirements when it comes to the discharge of effluent into water bodies because water is a global common (Paulot, 2019). Excess nitrogen is a major threat to water sources, both underground and surface. Nitrogen pollution affects the environment, human health and the economy. Anthropogenic activities contribute mostly to nitrogen water pollution in Africa through industrial processes like fertilizer manufacturing, sewage treatment plant discharge, agriculture, detergents, failing septic tanks, and storm-water runoffs (Ramakrishna, 2015). This unpleasantly alters the natural environmental process like the nitrogen cycle therefore affecting other cycles for example, the carbon and the oxygen cycle (Li, 2020).

Nitrogen, constituting 78% of Earth's atmosphere is crucial for life as it forms a significant part of the protein in all organisms except certain microorganisms (Wang, 2023). These microorganisms utilize different forms of fixed nitrogen for protein synthesis rather than N2.

However, nitrogen must transform via the nitrogen cycle to become usable by plants and animals, converting it into various compounds (Hoffman, 2014).

Nitrogen exists as organic and inorganic. Inorganic nitrogen is made available to plants through bacteria fixation. Before the industrial revolution, bacterial nitrogen fixation was the primary method for producing biologically accessible nitrogen. However, human activities now exceed natural nitrogen fixation rates globally, controlling the nitrogen fixation process (Erisman, 2013; Fowler, 2013).

In water bodies, nitrogen fixation by symbiotic and free-living bacteria produces ammonia. Nitrification converts this into nitrite and then nitrate, mainly in oxygen-rich areas. Aquatic plants absorb nitrogen compounds through their roots. Ammonification from organic matter decomposition replenishes ammonia levels. Denitrification in anaerobic zones converts nitrate back to gaseous nitrogen, closing the cycle (Erisman, 2013). The difference between the nitrogen cycle from the terrestrial and aquatic environments as explained by Robertson and Groffman (2023) is that the mechanism may vary according to oxygen availability, water flow and microbial communities.

2.3 Anthropogenic Sources of Nitrogen Pollution

2.3.1 Agriculture activities

According to (Rauh, 2022) Agriculture activities contribute to natural nitrogen in the atmosphere and primarily in aquatic environments, leading to pollution of water bodies. Mainly through the use of synthetic fertilizers, large-scale animal grazing, poultry production

2.3.2 Fertilizer use

Fertilizer is the most subsidized inputs by the government (FAO). According to FAO report (2012), it has been predicted that from 2007 the global supply and demand for fertilizer has been rising gradually and is expected to continue for the next 50 years. The fertilisers, particularly nitrogen-based are applied to increase crop production.

Fertilizers contribute to aquatic pollution through the leaching of nutrients into the soil, lowering plant bioavailability and damaging environmental quality (Bechmann 2014). Nitrogen moves horizontally or vertically (Yan, 2023), with vertical movement contributing more to pollution than lateral movement. Factors such as soil type, crop type, cultivation

method, and weather conditions also affect nutrient leaching (Yan, 2023). According to reports from the World Resource Institute (2014), China is among the biggest agricultural countries in the world, and in 2011 it used a total of 54 million tons of fertilizer (one-fourth) as compared to the entire World. Other significant users of nitrogen fertilizer are India, the United States and Brazil. Meanwhile, those countries are leading in terms of eutrophication (Gao, 2023). However, they are also putting in place sustainable agricultural practices and proper monitoring to reduce the environmental impacts

2.3.2 Farming Technique

Tile drainage systems in agriculture contribute to excess nitrogen pollution by removing subsurface water from the soil, which often contains nitrate salts. This system improves crop yield in poorly drained soils but also facilitates the movement of nitrate into drainage ditches, streams, and larger water bodies. Uncontrollable elements like precipitation and soil mineralization influence drainage volume and nitrate loads.

2.3.3 Livestock and poultry production

The high demand for food has changed how farming is carried out. The study conducted by Phang (2019) stated that the concentrated animal feeding operation (CAFO) which is done in developed countries, allowed for millions of animals to be fed on a strict nitrogen rich diet. Undoubtedly this leads to large amounts of manure production containing nitrogen compounds contributing to nitrate underground water pollution by leaching nutrients, and surface runoff. Furthermore, unsafely managed lagoons can fail leading to direct manure discharge from feed locks to water bodies or volatilization of ammonia gas from feed locks to aquatic environments.

2.3.4 Farming processes

Post-harvest nitrogen pollution occurs when crop residues decompose, releasing nitrates into the soil, and polluting underground water sources (Phang, 2019). Overwatering during irrigation and ineffective irrigation can also contribute to this issue.

2.4 Industrial processes

Industrial processes contribute to nitrogen pollution in aquatic environments through various pathways, including direct discharge of wastewater, runoff from industrial sites, leakage and

spills, atmospheric deposition and emissions from industrial agriculture, and fossil fuel combustion. The industrial nitrogen fixation by the Harber-Borsch process has been adapted globally. Atmospheric nitrogen is fixed to form ammonia which is then used as a raw material to produce ammonia products such as ammonium nitrate fertilizer, industrial chemicals and explosives. The Harber process leads to large quantities of nitrogen being released into the environment.

Figure 2.1: Sable Chemicals ammonium nitrate manufacturing process flow chart

2.5 Effects of Nitrogen Pollution

2.5.1 Eutrophication

INA (2019) stated that in the past years, eutrophication was considered to be the natural ageing of aquatic environments and Akinnawo (2023) suggested that in the present day, it is the major factor contributing to the accelerated ageing of water bodies. Akinnawo (2023) pointed out that, the use of fertilizer in agro ecosystems caused non-point source pollution, accounting for 17 -92% of the yearly N in the world's aquatic environments. Another source of nutrients in water bodies is wet and dry nitrous oxide deposition. Hodgkiss (2019) pointed out that an inorganic nitrogen concentration of 0.30mg/L in a water body is critical for the stimulation of algae growth. A study conducted by (EEA, 2023) stated that 50-100% of harmful algae blooms that are primarily produced in the Yellow Sea in China are generated by precipitation containing nitrogen and phosphorus. Eutrophication is one of the greatest

impacts which causes excessive algae bloom and growth of aquatic weeds leading to a decrease in aesthetic value, unpleasant odor, depletion of DO and destruction of the aquatic ecosystem due to the formation of toxins.

2.5.2 Health impacts

Health effects associated with the consumption and general use of nitrogen-contaminated water include carcinogenesis, methemoglobinemia, birth defects and blue baby syndrome. Studies show that vegetables account for 70% of nitrate that a human can take, 6% comes from meat products and 21% from drinking water (Mathewson, 2020). Nitrates are reduced to nitrite by the bacteria found in the human body (Mathewson, 2020). The acute toxic effects of ingestion of nitrate compounds are severe gastroenteritis characterised by dyspepsia, mental health, and hemorrhage, blood in the urine and stools, and abdominal pain (Pennino, 2017). Chronic effects include methaemoglobinemea. Nitrite oxidises the ferrous iron of haemoglobin to form methaemoglobin (Blaisdell, 2019). Infants are more susceptible to the toxicity of nitrates because of higher pH in the stomach (Pennino, 2017). More than 10% Methemoglobin of haemoglobin in human blood causes clinical cyanosis and cellular anoxia (Blaisdell, 2019).

Furthermore, nitrates react with other chemicals to form carcinogenic compounds which are cancer-causing compounds (Jones et al….2016). Examples according to (Jones et al….2016) are cancers of the stomach, oesophagus bladder, and nasopharynx and colon cancer. Nitrite ion readily reacts with secondary amines to form carcinogenic nitrosamines. In pregnant women, nitrates cause stillbirth, low weight or death of the foetus. Skin contact with some of the algae toxins produced due to the presence of nitrates may lead to dermal irritation, and eye or ear infection.

2.6 Process Mechanisms for Biological Denitrification

Nitrates (NO_3^-) have to be reduced to volatile nitrogen compounds (N_2) via nitrite (NO_2^-) by microbial processes in the process of denitrification.
$2NO_3^- + 1{\cdot}25CH_3COOH \rightarrow N_2 + 2{\cdot}5CO_2 + 1{\cdot}5\,H_2O + 2OH^-$ (Mishra et al…2022). the denitrifying bacteria are widely distributed in aquatic environments and the soil such as *Pseudomonas* and *paracoccus*. Mishra et al (2022) highlight that the bacteria utilise nitrate as an electron acceptor for respiration ultimately transforming it to nitrogen gas in the absence of readily available molecular dissolved oxygen (heterotrophic denitrification). The process is

catalysed by enzymes found in bacteria. Nitrate reductase reduces nitrate (NO_3^-) to nitrite (NO_2^-), Nitrite reductase converts nitrite to nitrous oxide (N_2O), Nitrous oxide reductase reduces nitric oxide to nitrogen gas (N_2), and Nitrous oxide reductase transforms nitrous oxide to nitrogen gas. However, for processes, an external organic carbon source must be included such as methanol and ethanol.

2.7 Environmental Factors Affecting Biological Denitrification

2.7.1 Temperature and pH

According to (Rassamee, 2014) the ideal pH and temperature for denitrification activity are 7-9 and 20 to 30 degrees Celsius respectively. However, denitrifying bacteria can be classified according to temperature or pH favourability. Temperature and pH affect enzyme activities, with higher temperatures accelerating denitrification and lower temperatures slowing it down. Optimal denitrification levels range from neutral to slightly alkaline, as extreme acidity can hinder enzyme function. In a research done by (Pen et al, 2012) it was noted that nitrite and nitrous oxide accumulated at suboptimal pH. Pen et al (2012) also showed that the N_2O reduction rate was pH dependent and much greater than the nitrite and nitrate reduction rates in methanol enriched denitrifying culture. Several studies have pointed out that most denitrifying bacteria are more sensitive to temperature rather than pH. In regards to the current study, the average temperature of Kwekwe is 26.25 degrees Celsius which is almost close to the optimum temperature and favourable for the study.

2.7.2 Concentration of dissolved oxygen

Dissolved oxygen inhibits dissimilation but does not change assimilation in wastewater denitrification systems (Mishra et al…2022. Oxygen provides a better electron acceptor for denitrifying populations, inhibiting denitrification. High dissolved oxygen concentrations reduce the available quantity of electron donors for denitrification and can decrease the abundance and activity of facultative anaerobes, reducing the overall denitrification capacity of the ecosystem (Rassamee, 2014).

2.7.3 Carbon source

Carbon substrate availability and quality influence the denitrification process. Organic carbon sources including glucose, acetate, or plant residues can stimulate denitrification by providing

energy and reducing power for denitrifying bacteria. These carbon sources serve as electron donors, fuelling the reduction of nitrate to nitrogen gas (Elefsiniotis, 2006). Conversely, carbon limitation can impede denitrification rates, constraining microbial activity and nitrate reduction. The type and concentration of carbon source also impact denitrification efficiency and production of nitrogenous compounds. Lu et al (2011) carried out investigations using various carbon sources to establish the regulatory relationship between the metabolism of carbon and nitrogen in denitrification. Organic carbon-induced denitrification was well correlated with denitrification activities (Lu et al…. 2011).

2.8 Advantages of biological denitrification

Biological denitrification is an environmentally friendly, cost-effective, and versatile process that contributes to ecosystem diversity (Zhengzhe, 2020)... It relies on naturally occurring microorganisms, reducing secondary pollution and energy requirements. The process is also versatile, as it can adapt to various environmental conditions, reducing the formation of by-products. This makes it an attractive option for treating wastewater (Zhengzhe, 2020).

2.9 Challenges and limitations of biological denitrification

Biological denitrification is slower than chemical processes, limiting its significance in areas needing rapid nitrogen removal (Rassamee, 2014). Extreme conditions can inhibit denitrifying bacteria, leading to incomplete denitrification and environmental risks. Insufficient nutrients and carbon, controlling anoxic conditions, and competition with other microbial communities also impact denitrification (Mishra et al…2022).

2.10 Potential of raw sewage as a substrate for denitrification

Several studies have been carried out to enhance the biological denitrification of wastewater from industries using different methods, most indicating the need for an organic carbon source. Some external carbon sources that have been used include methanol, glucose, ethanol, and acetate. Methanol takes a long span during the start-up of the system whereas ethanol and acetate are costly (Brown, 2021).

Raw or primarily treated municipal wastewater could be used as an alternative. It is a complex of mixed organic and inorganic compounds. Sewage contains relatively high levels of organic compounds in the form of lipids, proteins and carbohydrates, and may be a good

carbon source. The compounds serve as substrates for microbial growth and denitrification. Moreover, raw sewage contains suspended solids, nutrients, and microbial populations, all of which can contribute to its sustainability as a source of carbon for denitrification. The major advantage of utilizing raw sewage is that it eliminates the need for additional carbon supplements thereby lowering costs for treatment. It is a resource that is readily available and generated every day by municipalities thus a sufficient carbon source. It also contains multiple diversities of microbial communities which might enhance the process of denitrification. Nevertheless, the contents of sewage vary, depending on some factors like industrial activity, diet and population density (Sun et al…2013). This might influence the rates of denitrification and efficiency. Moreover, raw municipal sewage might contain inhibitory substances such as xenophobic compounds and heavy metals that might interfere with microbial activities and hinder denitrification.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

This chapter provides the design of the research. It takes a look at the sampling procedure, which was explained as well. It explains the sampling techniques and the research analysis tools used for the data. It also explains the research ethics that were observed during the conduct of the study and the limitations encountered.

3.2 Research design

The topic under study is quantitative research because the phenomena can be expressed in terms of observable or measurable variables. The researcher used an experimental research design because it allows the researcher to establish a cause-effect relationship (Taherdoost,

2017). The flow chart below illustrates the research design.

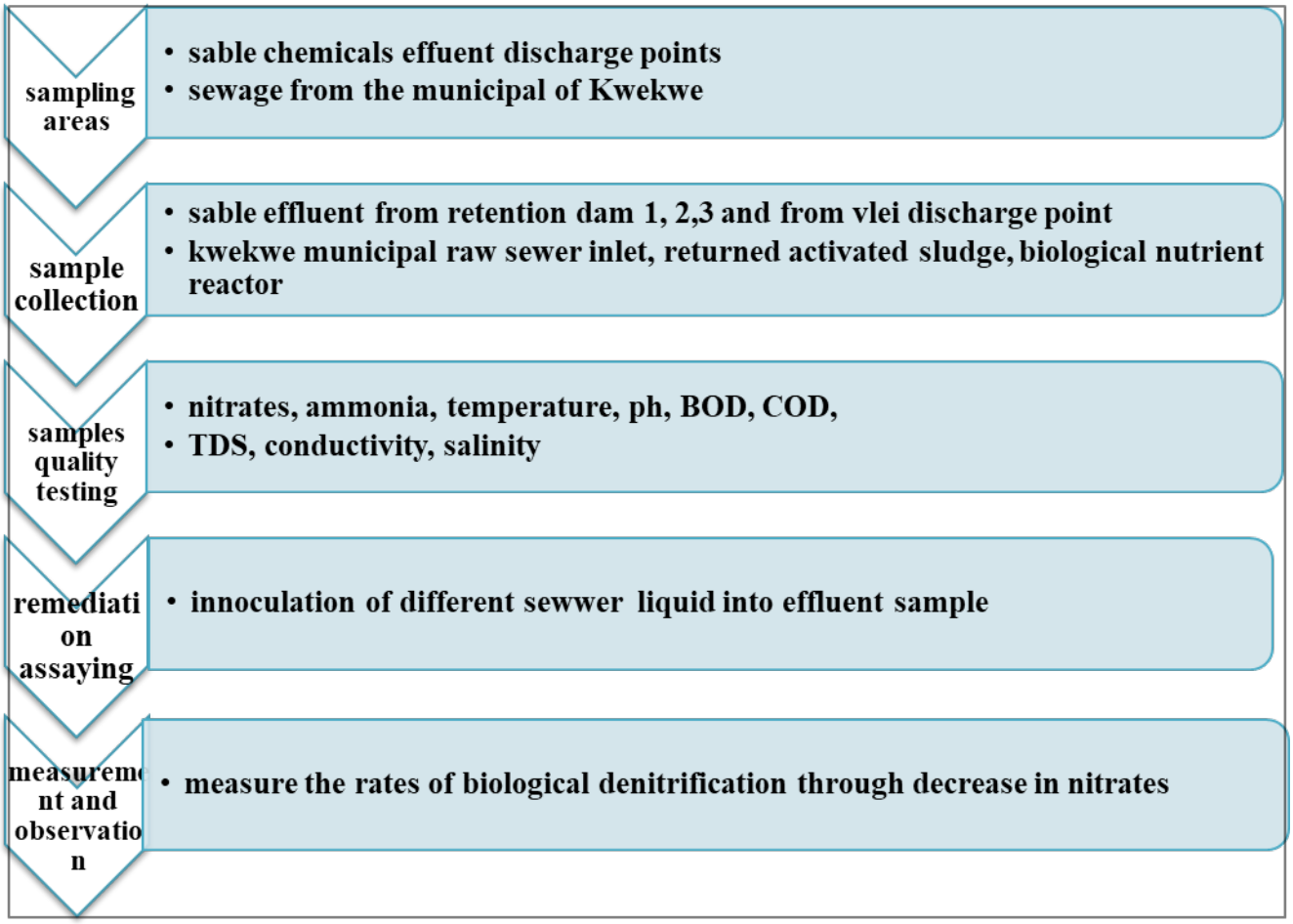

Figure 3.1: Study Research Design

The experiments aimed at measuring denitrification rates on effluent rich in nitrates. Some control experiments were put in place for clear comparisons. Other researchers used other materials such as ethanol and hydrazine to be able to compare results with the study as well.

3.3 Sampling procedure

3.3.1 Sampling techniques

The researcher used purposive sampling with non-probability sampling. This was because the researcher deliberately chose samples and sample points based on specific characteristics and qualities relevant to the research being undertaken (Stratton, 2021). Smith and Jones (2021) used purposive sampling to research how changing land use affects the quality of water in urbanizing watersheds. This method ensured the relevance, accuracy and variability of data, allowing a comprehensive analysis of overall water quality, and enhancing the validity of

their finding. The triangulation method was used to minimize bias, through assistance from several lab samplers who sampled from different sites (Bhandari, 2023). The researcher identified sampling sites and prepaid materials, cleaned containers, and collected samples from various locations, which were then taken to the Sable Chemicals laboratory.

3.3.2 Process Effluent Sample Collection

Samples for Sable Chemicals effluent were collected from the outlet of dams 1, 2 and 3 and from Vlei stream which discharges water into the Munyati River. 2 liter plastic bottles were used. 1 sample was collected from each sampling point and stored in the laboratory fridge at approximately 4^0C.

3.3.3 Municipal Sewage Sample Collection

Sewage effluent was collected from the water treatment of the Kwekwe City Council. The sampling points were the inlet of raw sewage, returned activated sludge and the biological nutrient reactor. They were collected in thoroughly sterilized 2-litre plastic bottles. Six litres of samples were collected, 2 liters from each collection point.

3.4 Primary Data Sources Analytical Methods

3.4.1 Nitrates

Nitrate nitrogen in the industrial effluent and the sewer was determined by the use of the ultraviolet spectrophotometer procedure (Appendix 2)

3.4.2 Ammonia

Ammonia was determined from the sewer and the industrial effluent using the method of measurement by calorimetric method (Appendix 3)

3.4.3 BOD

BOD was measured using the 5-day Biochemical Oxygen Demand test. The samples were tested for initial dissolved oxygen concentration by a DO meter. A seed inoculum was added in to the sample. The bottle was bottled and closed tightly to prevent oxygen from entering, and it was kept in cardboard at room temperature. After 5 days, the final dissolved oxygen was measured again

Calculation: BOD_5 mg/L =Initial DO –Final DO × dilution factor

P

3.4.4 COD

COD was measured using the closed reflux method. The sample was added to a COD digestion tube and potassium dichromate and sulfuric acid (H2SO4) were added to the sample. The COD digestion tube was heated in an oven at 150 degrees Celsius for 2 hours. The sample was then removed and cooled to room temperature. A reagent blank was used as a reference to measure the absorbance of the sample using the Colorimeter spectrophotometer. A calibration curve was used to correlate the absorbance reading to COD concentration.

3.4.5 pH

The pH was determined by the pH meter from all the samples that were collected and recorded. This was done by immersing the electrode in the sample and taking note of the displayed reading. Electrodes were cleaned thoroughly with distilled water and were kept in a 3-4 M potassium chloride solution

3.4.6 Conductivity

Conductivity was determined by the use of a conductivity meter. The electrode was dipped into the sample and it was rinsed with the sample before measuring the conductivity. The reading was taken from the display. The cell was stored in stilled water after use.

3.4.7 Temperature

Temperature was determined from all the samples by dipping a thermometer in the samples. The thermometer was rinsed before and after the readings was taken.

3.4.8 Total dissolved solids (TDS)

Total dissolved solids were determined by filtering water samples using pulp paper under vacuum or filter paper and funnel into the pre-weighted, clean and dry evaporating dish. the same sample size of the unfiltered sample is pipetted into the evaporation dish. The evaporation dish was put in a water bath till almost dry. Final evaporation is carried out in an

oven at 110^0C for a few minutes. The dish was placed in a desiccator and allowed to cool and reweighed after.

Calculation: ppm TDS = (gm wt of dish after drying – g wt of empty)

The volume of sample use

3.4.9 Daily Effluent Analysis

The researcher used the Kjeldahl method of total nitrogen determination in HNO_3, NH_3, and NH_4NO_3 using Devada's alloy by distillation. 50mls were pipetted into a 500ml Kjeldahl flask. ½ spatula of the Devadas alloy, which is the catalyst was added to the sample. In another beaker to be put at the receiving end, 50mls of 4% Boric acid along with 2-3 drops of mixed indicator were added. The apparatus was connected tightly for distillation. To the K about flask 300mls of hot distilled water was added. And finally, 20% of sodium hydroxide was added to the solution. Bubbles were observed on the receiving end. The solution was left for 10 minutes to allow for digestion before applying heat. 300mls was collected from the receiving end which was titrated using 0.05 N_{HCL} from the blue endpoint to the brown endpoint.

Calculations: $\frac{\text{Titer} \times \text{Normality} \times 14 \times 1000}{50\ (\text{vol})} = ?\ \text{ppm}$

$= \frac{\text{ppm} \times \text{flow rate}}{1000} = ?\ \text{Kg/h}$

3.5 Experiment 1: Preparation for denitrification with Sewage

This experimentation was carried out to measure denitrification rates from process effluent by measuring daily changes in nitrates and initial and final levels available in the samples after 16 days. An initial measurement of parameters was done. 1000ml plastic batch reaction containers were prepared, and the containers were labeled according to treatment. In each container 500mls of process effluent was added from each retention dam (three samples from each dam). The samples from the dams of the process effluent were inoculated with300mls of different sewer effluent from the municipality. The initial nitrate levels were also measured

after the inoculation and recorded. The containers were closed tightly and shaken vigorously to allow the solutions to mix before they were placed in dark cardboard, hindering exposure to direct sunlight to avoid algae blooming. They were placed in the cardboard for 24 hours before changes were measured. Some other solutions were control experiments, in which the same procedure was applied but effluent waste was inoculated with distilled water.

Table 3.1: Preparation for denitrification with Sewage

X1000ml industrial process effluent Container (500mls)	**X300mls Inoculant**	**Comment**
Treatment 1 (Dam 1)	Raw Sewage	Tightly sealed container
Treatment 2(Dam 2)	Returned Activated Sludge	Tightly sealed container
Treatment 3(Dam 3)	Bio Nutrient Reactor	Tightly sealed container
Treatment 4 (Vlei point)	Distilled Water	Control Experiment

3.5.1 Rate of denitrification from the experiment

The rate of denitrification from the experiment was determined by calculating the initial nitrates and the final nitrates that were found in the samples. The following formulae were used

$$= \frac{\textit{Initial Nitrates} - \textit{Final Nitrates}}{\textit{Time in days}}$$

3.5.2 Experimental repeatability

After 24 hours of keeping the samples in closed cardboard, the nitrates were measured repeatedly for 16 days to average denitrification rates. The samples were made sure not to be open to oxygen for more than 25-30 seconds during measurement, to maintain anoxic conditions for accurate results.

3.6 Experiment 2: Preparation for denitrification with Ethanol

The experimental procedure of experiment two was almost similar to that of experiment 1. In this experiment, 10mls of ethanol, which was used as an external source of carbon, were

added to different sample containers (200mls of sample). The samples were air-tightly closed and kept in cardboard for 24 hours. The initial nitrate composition levels were measured and recorded. After 24 hours the denitrification rates were measured. The nitrate levels were repeatedly measured for 10 days. During measurement containers containing samples were not left open for more than 30 seconds to avoid oxygen interference. The ethanol will act as an external source of carbon to complement the internal carbon source contained in the sewer mixed liquor.

Table 3.2: Preparation for denitrification with Ethanol

Treatment	**Inoculant**
1. Dam1 effluent + Distilled water	Ethanol
2. Dam 1 effluent + Distilled water	Ethanol
3. Dam 1 effluent + raw sewage	Ethanol

3.7 Experiment 3: Preparation for denitrification with Hydrazine

For experiment 3, hydrazine was used as an oxygen scavenger for nitrate reduction. 2 grams of hydrazine was added to the solution of 200mls of industrial process effluent with different sewage sample. The hydrazine was added to the control experiments as well. The same procedure of shaking to allow proper mixing of the solutions was applied. The containers were kept air-tight and stored in cardboard at room temperature and kept away from the sun to avoid interference. The initial nitrate levels were measured and recorded. After 24 hours nitrate reduction rates were measured.

Table 3.3: Preparation for denitrification with Hydrazine

Treatment	**Inoculant**
1. Effluent from Dam 1	Hydrazine
2. Distilled water	Hydrazine
3. Effluent from Dam 1+Raw sewage	Hydrazine

3.8 Data analysis and presentation

For quantitative data that was obtained during the research, the researcher used Microsoft Excel software for analysis and presentations, which was presented through line and bar graphs. Descriptive statistics were also employed.

3.9 Ethical considerations

The study on treating high nitrate effluent from Sable Chemicals involves using raw municipal sewage, a scientific solution which requires ethical considerations. The researcher obtained permission, and informed consent, and complied with research ethics approved by BUSE. The study assessed environmental impacts, prioritized the safety and health of all involved, and upheld scientific integrity. Data privacy was practiced, and transparency was maintained to avoid bias or conflicts of interest. The long-term sustainability of the denitrification process was evaluated, considering factors like resource availability, energy consumption, and scalability.

3.10 Delimitations

The study was carried out over a predefined timeframe and predefined environmental conditions. It did not allow for recording seasonal differences in denitrification efficiency and effluent characteristics. Keeping environmental factors under control in a laboratory setting, where different analyses from different departments were done, made the study experiment almost difficult. It was difficult to maximize resource efficiency because of different processes requiring different methods. There was a shortage of other apparatus such as the Kjeldahl flask to analyze all the samples concurrently so study components were put in order of importance.

CHAPTER 4: RESULTS

The following chapter lays out the research findings from the experimentation into the efficacy of biological denitrification for treating high nitrate industrial effluent from Sable Chemicals using raw municipal sewage. It details nitrate reduction rates, effluent quality changes, under various conditions and percentage of removal by different treatments.

4.1 Industrial Process effluent quality (Sable Chemicals)

Table 4.1: Sable Chemicals Effluent Physico-chemical Quality

						Legal limits (Si 6 of 2007)			
Parameter	**Dam 1**	**Dam 2**	**Dam 3**	**Vlei Point**	**Units of measurement**	**Blue**	**Green**	**Yellow**	**Red**
Ammonia Nitrogen	70	62	45	30	mg N/L	≤0,5	≤1.0	≤1.50	≤2.0
Nitrate Nitrogen	90	83	75	90	mg N/L	≤10	≤20	≤30	≤50
TN	240	200	170	140	mg N/L	≤10	≤20	≤30	≤50
BOD	4	2.8	5.5	3	mg/L	≤15	≤30	≤50	≤100
DO	13	12.9	11	8	mg/L	≤75	≤60	≤50	≤30

COD	46.6	30.3	22	19	mg/L	≤60	≤90	≤150	≤200
PH	8.4	8.12	6.7	7		6−9	5−6 9−10	4−5 10−12	0−4 12−14
Conductivity	277	260	267	240	µS/cm	≤1000	≤2000	≤3000	≤3500
TDS	1.8	1.8	1.58	1.58	Ppm	≤500	≤1500	≤2000	≤3000

Table 4 shows industrial effluent parameters that were recorded after being analyzed in the laboratory. The outcomes were compared with the legal limit according to Zimbabwe's Environmental Management Act, Si 6 of 2007 which includes multiple categories (Blue, Green, and Yellow) representing different threshold values. The values for Ammonia, nitrates and total nitrogen exceed the highest recommended legal limits at all locations. All other parameters are within the recommended threshold value.

4.2 Municipal Sewage Quality Results (Kwekwe Municipality)

Table 5 below shows the parameters from the Kwekwe Municipal which were measured to determine the quality of the sewage from different points before it was used for experimentation. The parameters are within the legal limit except for BOD which suggests a high presence of organic carbon.

Table 4.2: Kwekwe Municipality Sewage physico-chemical quality Results

					legal limits (Si 6 of 2007)			
Parameter	**Raw Sewage**	**Returned Activated Sludge**	**Bio Nutrient Reactor**	**Units of Measurement**	**Blue**	**Green**	**Yellow**	**Red**
Ammonia Nitrogen	0.7	0.6	0.4	mgN/L	≤0,5	≤1.0	≤1.50	≤2.0
Nitrate Nitrogen	0.8	0.7	0.5	mgN/L	≤10	≤20	≤30	≤50
DO	13	11.7	10	mg/L	≤75	≤60	≤50	≤30
BOD	100	80	73	mg/L	≤15	≤30	≤50	≤100

4.3 Rate of Nitrate Removal

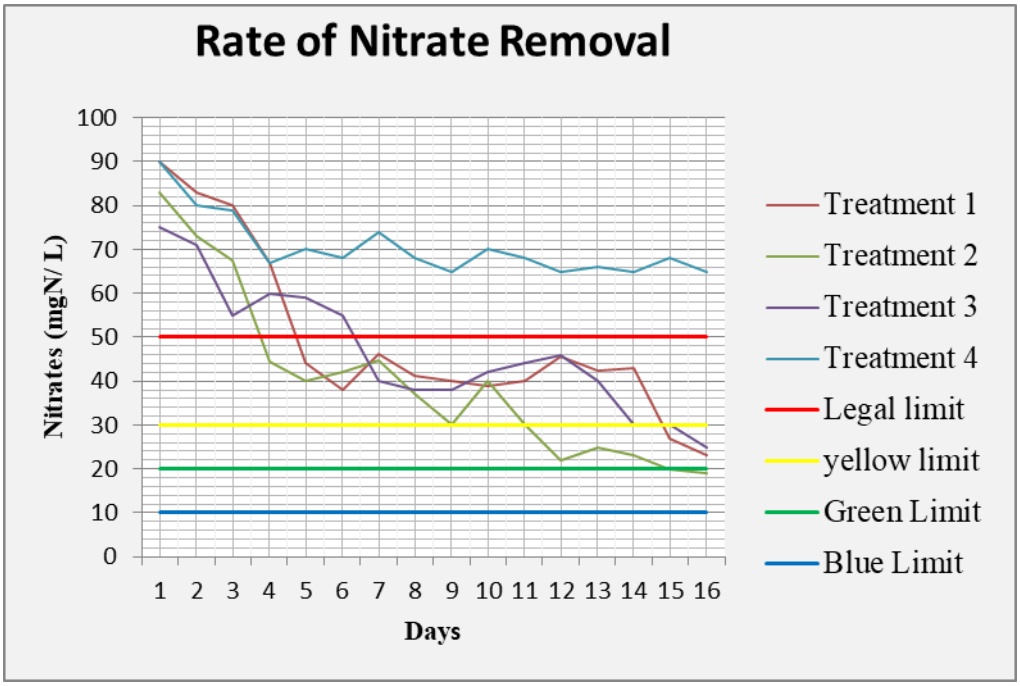

Figure 4.1: Comparison on the amount of nitrate removed from various sewage

Rates of effluent nitrate (NO_3^-) removal by different forms of sewage were compared as shown in Fig 4.3, comparing levels of nitrate in different treatments over the 16 days (at room temperature). Nitrate levels reduced from 90 mg/L to 23 mg/L during the course of two weeks. The average amount of nitrate removal for all treatments was 3.7 mg/L/day. The treatments except control which was treated with distilled water overall produced over 70% removal over the period of 16 days. Most treatments are compiled with the legal limit (50mg/L) by day 10. Neither the treatments reached the stricter blue (20mg/l) or green (10mg/L) at least for the annotated period. The control experiment showed the lowest rate of nitrogen removal. However, the graph showed a uniform pattern of the levels of nitrates decreasing inconsistently, with fluctuations. This might be due to consecutive nitrification and denitrification processes (Tabassum, 2019).

4.4 Results of ethanol as an external carbon source

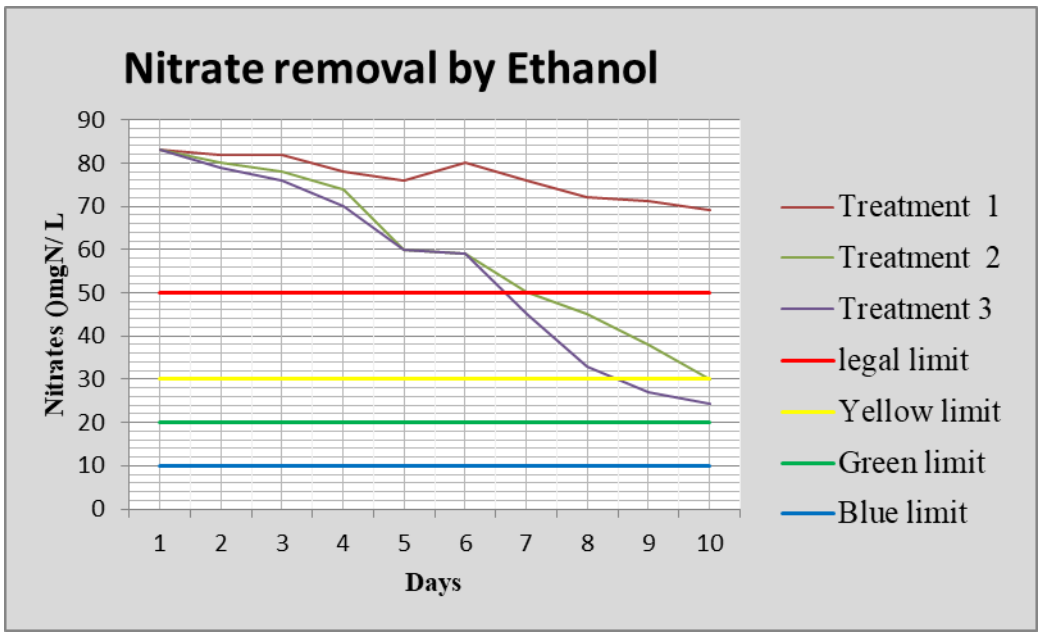

Figure 4.2: Impact of external carbon source (ethanol) on Denitrification

Fig 6 shows the result of using ethanol as an external source of carbon for nitrate **(**NO_3^-**)** removal over 10 days across three samples all starting with 83mg/L of nitrate. Initial nitrate levels across all three samples start at 83mg/L. The daily removal rate recorded ranged from 1.4 to 5.86mg/L across the samples. Ethanol exhibited an average removal of 41.87mg/L of nitrates and an overall removal efficiency of over 60%. Sample 1 exhibited a moderate decrease, to 69mg/L by day 10. Sample 2 showed a significant reduction, reaching 30mg/L, while the third sample demonstrated the highest efficiency, dropping to 24.4 mg/L. Both samples (1 &2) fell below the legal limit of 50mg/L by days 6 and 5, respectively, with sample 2 hitting the yellow limit (30mg/L) by day 10. However, none met the stricter limit (10 and 20 mg/L). These results suggest ethanol can effectively reduce nitrate levels making it potentially useful for meeting more stringent environmental standards.

4.5 Effects of an external oxygen scavenger on Denitrification

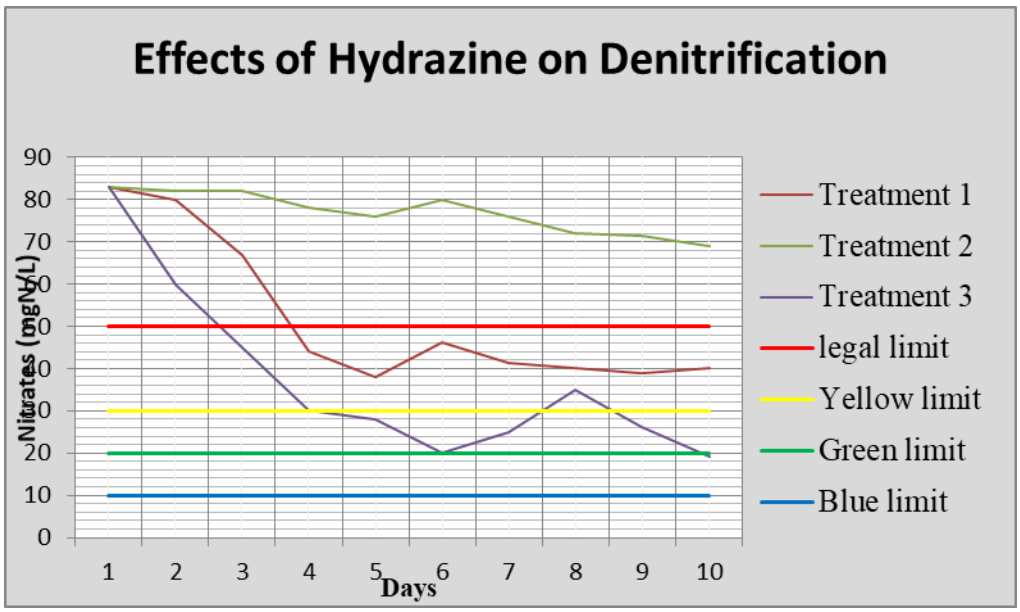

Figure 4.3: Impact of Hydrazine on denitrification

Fig 7 shows data on hydrazine denitrification over ten days indicating a general trend of decreasing concentrations across three samples, though with varying rates of reduction. The overall daily rate of hydrazine denitrification across all samples was 12.1mg/L. Sample 1 decreases gradually and stabilizes around the legal limit by day 10. Sample 2 shows a slower, more consistent decline, remaining above the legal throughout. The last sample demonstrates a rapid reduction, falling below the legal limit by day 3 and nearing the green limit by day 10. Overall the data suggest effective denitrification with varying efficiencies across samples.

4.6 Percentage of Removal by Different Treatments

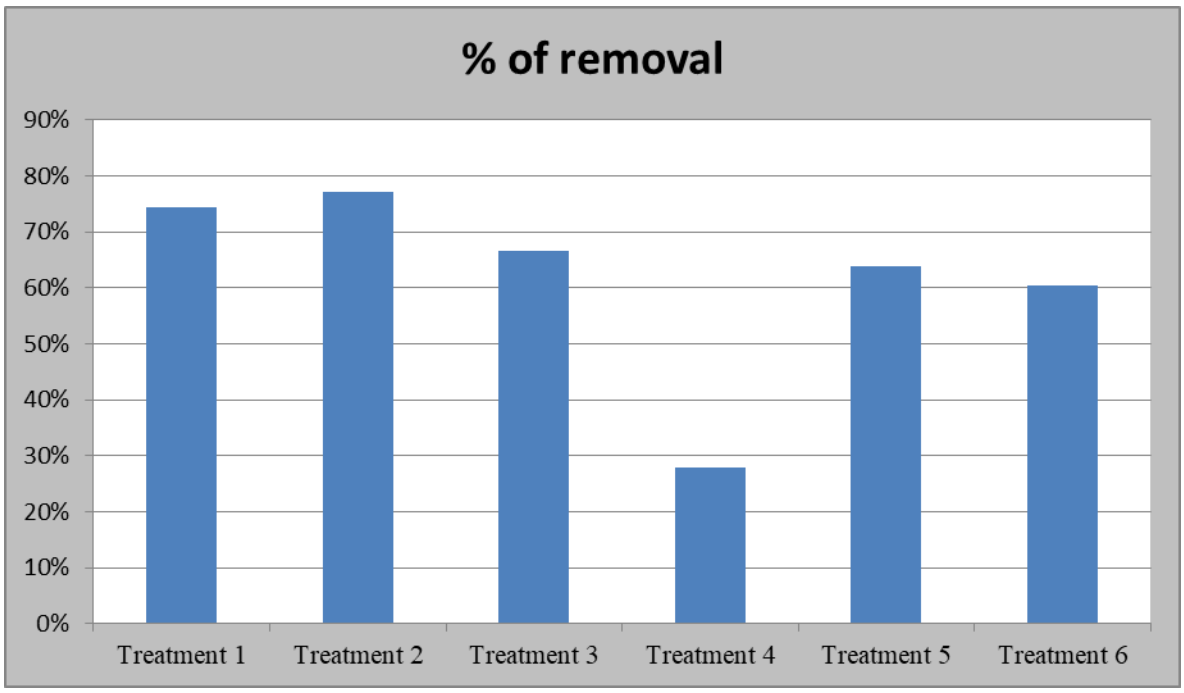

Figure 4.4: percentage of nitrate reduction due to different treatments

Fig 8 above presents the percentage of removal nitrate for various treatments over a set period of days, highlighting their effectiveness in nitrate removal. Data shows Treatment 2 (Effluent + Returned Activated Sludge) 77% removal rate, followed by Treatment 1 (Effluent + Raw Sewage) at 74%, and Treatment 3 (Effluent + Bio Nutrient Reactor Sludge) at 67%, Treatment 4 (Effluent + Distilled Water) at 28%. Despite a shorter duration of 10 days, Treatments 5 (Effluent + Ethanol) and 6 (Effluent + Hydrazine) achieved 64% and 60% removal rates, respectively, suggesting the potential for higher efficacy with extended time.

CHAPTER 5: DISCUSSIONS

5.1 Introduction

This chapter discusses the results of using biological denitrification with raw municipal sewage to treat high nitrate industrial effluent from Sable Chemicals. The chapter compares the study's results with existing studies, evaluates the method's effectiveness, considers its environmental, economic, and regulatory implications and also examines the study's outcomes and limitations, to assess the feasibility and sustainability of this remediation approach.

5.2 Sable Chemicals Effluent Physico-chemical Qualities

The results indicated that nitrate nitrogen at all discharge points (1st, 2nd, 3rd), were 90mgN/L, 83 and 75 respectively which is far above the legal limit (≤50mg/L), Same for ammonia and total nitrogen, indicating a pervasive issue with nitrogen pollution. BOD, OD and COD falling in the blue category suggests organic matter decomposition rate under control, reasonable oxygen availability and organic pollution not being a major issue (Du, 2020). The results were recorded when the plant has been offline for several months indicating that the results would probably be intense if the situation is reversed. In the year 2019, a report by EMA (unpublished) stipulated that the weekly total nitrogen from Sable Chemicals ranged from 450-675mgN/L when the plant was operating 24/7. This was during the rainy season thus supporting an argument by Mungwari, 2017 (unpublished) that the excess nitrogen would be probably a result of combined sources of nitrogen. Leading to the deposition of nitrogen in the form of precipitation. The process effluent is passed through several natural treatments (retention ponds, limestone, dams, and artificial wetlands) but still does not reach the environmentally friendly limit (≤30, 20,10mg/L). This indicates the necessity for another technique to aid the process of denitrification thus reducing the release of nitrates into aquatic environments. It is because of the inefficiency of the treatment process and the unreliability of the plant due to old age leading to ammonium and nitrogen nitrates finding their way to the water bodies.

5.3 Municipal sewage physicochemical Qualities

The use of raw municipal sewage and other sewage treatments for biological denitrification has proved efficient as the treatments accomplished over 70% removal. The concept of using raw municipal sewage was to utilize the high carbon in sewage as indicated by the results sampled from the Municipality of Kwekwe with BOD in the red legal limit. Raw municipal sewage proved to be efficient and effective because it was compared with control treatment without sewage. This was because of the presence of a diverse microbial community. The method of using primarily raw or treated sewage is much more economical and can achieve the desired outcome as compared to several other biological studies that involve the use of synthetic materials for denitrification. In a study conducted by Bariketal (2013), ammonia nitrogen was reduced to minimal detectable levels on the 5th day only (from 10 mg l^{-1} to 0.34 mg l^{-1}). This was due to specifically inoculated nitrifying bacteria (2.5µl^{-1} and 5µl^{-1}) and all treatments were continuously aerated through air flow passages. Compared to the present study, it did not achieve such results because microorganisms in the sewage were speculated rather than being specified. Furthermore, mechanical aeration and the biotechnology of inoculation of microorganisms require special expertise hence costly. Several biological methods can be explored for the treatment of nitrogen pollution. Li and Tabussum (2021) investigated the remediation of nitrate contaminated groundwater through the use of natural commercial paper plates and organic rice straws as solid organic carbon sources. The study recorded over 50% nitrate removal rate.

5.4 Percentage of Nitrate Removal by Different Treatments

The observed results demonstrate varying nitrate removal efficiencies across different treatments, with Treatment 2 (Effluent + Returned Activated Sludge) being the most effective due to its high microbial activity, aligning with findings from Wang et al. (2019). The low efficacy of Treatment 4 (Effluent + Distilled Water) highlights the need for active biological or chemical agents, as supported by Chen et al. (2020). Treatments involving ethanol and hydrazine also showed significant removal rates even over shorter durations, suggesting that chemical treatments can achieve rapid nitrate reduction, consistent with Johnson and Lee (2017). These results are in line with broader literature, such as Brown et al. (2021), and underscore the effectiveness of both biological and chemical methods for nitrate removal, indicating that active components and treatment duration significantly impact overall efficacy.

CHAPTER 6: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In as much as there is a potential risk of introduction of pathogens into the environment through the use of raw sewage, there is also potential for the formation of secondary pollutants through the application of inorganic carbon containing substances such as ethanol and hydrazine. The compounds indicated that they can be a very effective way of reducing nitrogen pollution in the environment through nitrate removal rate of 64% and hydrazine's 60% by day ten. The external carbon source added provided the bacteria with way more carbon for metabolism hence the accelerated rate of nitrate removal. Du (2020) pointed out that ethanol acts as a source of food and an electron donor for bacteria to enhance denitrification. This was seconded by the study carried out by Brown (2021) in which they observed an 80% removal rate through the use of ethanol. It is essential for a redox reaction, enabling nitrate reduction due to the loss of oxygen molecules during the reaction (Brown, 2018).

In conclusion, the study highlighted the potential of using raw municipal sewage as a sustainable and cost-effective option for treating high nitrate industrial effluent. The approach not only addresses the issue of nitrate pollution but also promotes the reuse of municipal waste, aligning with economic principles. At large, it can significantly contribute to sustainable wastewater management practices and environmental protection by reducing the environmental footprint of industrial activities.

6.2 Recommendations

- Sable Chemicals Industry might consider the use municipal sewage for the treatment of high nitrate effluent from its processes.
- Further studies have to be taken to assess the potential risk of use of raw municipal waste to the environment.
- Explore different types of municipal sewage such as primary versus secondary to determine the most effective carbon source for denitrification.
- Long-term studies should also be conducted to assess the sustainability of the use of municipal sewage in the treatment of industrial waste.

- Further studies should be undertaken to assess the practical viability and scalability of the use of municipal sewage to treat nitrate rich effluent.
- Studies should be conducted to examine the most effective ratio of process effluent to municipal sewage which helps achieve the best results.

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APPENDICES

APPENDIX 1: RESULTS FROM EXPERIMENTATION

1. Rate of removal for nitrates																
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Treatment 1	90	83	80	67	44	38	46.3	41.3	40	38.8	40	45.5	42.3	43	27	23
Treatment 2	83	73	67.5	44.4	40.12	42	44.6	37	30	40	30	22	25	23.1	20	19
Treatment 3	75	71	55	60	59	55	40	38	38	42	44	46	40	30	30	25
Treatment 4	90	80	79	67	70	68	74	68	65	70	68	65	66	65	68	65
Legal limit	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
yellow limit	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Green Limit	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Blue Limit	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

KEY

KEY					
Treatment 1	=	Effluent from Dam 1+ Raw Sewage			
Treatment 2	=	Effluent from Dam 2 + Return Activated Sludge			
Treatment 3	=	Effluent from Dam 3 + Bio Nutrient Reactor			
Treatment 4	=	Effluent from Vlei Point + Distilled Water			

2. results of ethanol as external carbon source										
Days	1	2	3	4	5	6	7	8	9	10
Treatment 1	83	82	82	78	76	80	76	72	71.3	69
Treatment 2	83	80	78	74	60	59	50	45	38	30
Treatment 3	83	79	76	70	60	59	45	33	27	24.4
legal limit	50	50	50	50	50	50	50	50	50	50
Yellow limit	30	30	30	30	30	30	30	30	30	30
Green limit	20	20	20	20	20	20	20	20	20	20
Blue limit	10	10	10	10	10	10	10	10	10	10

Key	Treatment 1= Effluent from Dam 1 + Distilled water
	Treatment 2 =Dam1 effluent + Disstilled Water +Ethanol
	Treatment 3 = Dam 1 effluent + Raw Sewage+ Ethanol

Hydrazine on Denitrification										
Days	1	2	3	4	5	6	7	8	9	10
Treatment 1	83	80	67	44	38	46.3	41.3	40	38.8	40
Treatment 2	83	82	82	78	76	80	76	72	71.3	69
Treatment 3	83	60	45	30	28	20	25	35	26	19
legal limit	50	50	50	50	50	50	50	50	50	50
Yellow limit	30	30	30	30	30	30	30	30	30	30
Green limit	20	20	20	20	20	20	20	20	20	20
Blue limit	10	10	10	10	10	10	10	10	10	10

Key						
Treatment 1=	effluent from Dam 1 + Raw Municipal Sewage		Hydrazine			
Treatment 2=	hydrazine+ distilled water					
Treatment 3=	Effluent from Dam 1+ Hydrazine+ Raw Municipal Sewage					

Percentage of Removal by Various Treatment

	% or removal	Number of days
Treatment 1	74%	16
Treatment 2	77%	16
Treatment 3	67%	16
Treatment 4	28%	16
Treatment 5	64%	10
Treatment 6	60%	10

APPENDIX 2: Determining nitrate nitrogen using ultraviolet spectrophotometry

Reagents

Nitrate standard solution (e.g., KNO3), Distilled or deionized water

Equipment

UV-Vis spectrophotometer, Quartz cuvettes, volumetric flasks, Pipettes and pipette tips, Beakers

Procedure

1. Preparation of Standards

Prepare a series of nitrate standard solutions of known concentrations. For instance, make solutions of 1, 5, 10, 20, 50 mg/L nitrate nitrogen by diluting a stock nitrate solution with distilled water.

2. Calibration Curve

Measure the absorbance of each standard solution at 220 nm and 275 nm using the UV-Vis spectrophotometer.

Record the absorbance values for each standard.

Plot a calibration curve of absorbance at 220 nm (corrected by the absorbance at 275 nm to account for organic interference) versus the concentration of nitrate nitrogen. This curve will be used to determine the nitrate concentration in the unknown samples.

3. Sample Preparation

Filter the water samples if they contain any particulate matter using a 0.45 μm filter.

If the nitrate concentration is expected to be high, dilute the sample appropriately with distilled water.

4. Measurement

Measure the absorbance of the filtered (and diluted, if necessary) samples at 220 nm and 275 nm using the UV-Vis spectrophotometer.

Record the absorbance values.

5. Calculation

Correct the absorbance at 220 nm by subtracting twice the absorbance at 275 nm:

Using the calibration curve, determine the nitrate nitrogen concentration corresponding to the corrected absorbance values for each sample.

6. Quality Control

Include a blank sample (distilled water) to zero the spectrophotometer.

Analyze quality control samples with known nitrate concentrations to ensure accuracy and precision of the measurements.

APPENDIX 3: DETERMIATION OF AMMONIA BY CALOMETRIC METHOD

Procedure: Preparation of Reagents:

Nessler's Reagent: Dissolve 50 g of potassium iodide in 50 mL of water. Add a saturated solution of mercuric chloride until a slight permanent precipitate forms. Add 400 mL of 50% potassium hydroxide solution and dilute to 1 liter with distilled water. Filter before use.

Ammonium Chloride Standard Solution: Prepare a standard solution with a known concentration of ammonium chloride (e.g., 1 mg/mL).

Sample Preparation:

Collect the sample and filter if necessary to remove any particulate matter.

Dilute the sample if the ammonia concentration is expected to be high.

Calibration Curve:

Prepare a series of standard ammonium chloride solutions with varying concentrations (e.g., 0.1, 0.2, 0.5, 1.0 mg/mL).

Add a fixed volume (e.g., 1 mL) of Nessler's reagent to each standard solution in separate test tubes or flasks.

Dilute each solution to a fixed volume (e.g., 50 mL) with distilled water.

Allow the solutions to stand for a fixed time (e.g., 10 minutes) for the colour to develop.

Measure the absorbance of each solution using a colorimeter or spectrophotometer at the wavelength specified for Nessler's reagent (typically around 425-450 nm).

Plot the absorbance against the ammonia concentration to create a calibration curve.

Sample Analysis:

Add a fixed volume (e.g., 1 mL) of Nessler's reagent to a known volume of the sample solution.

Dilute to the same volume as used for the standards (e.g., 50 mL) with distilled water.

Allow the solution to stand for the same fixed time as used for the standards for color development.

Measure the absorbance of the sample solution using the colorimeter or spectrophotometer.

Calculation:

Use the calibration curve to determine the ammonia concentration in the sample solution based on its absorbance.