URINE STABILISATION USING LACTIC ACID FROM FRUIT AND VEGETABLE PEELS FERMENTATION: A POTENTIAL SOURCE OF NITROGEN FERTILISER

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A Thesis Submitted in Partial Fulfilment of the Requirements for Conferment of the Master of Science Degree in Sanitation of Meru University of Science and Technology

2025

DECLARATION

This thesis is my original work and has not been presented for a degree award in any other institution.

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DECLARATION BY UNIVERSITY SUPERVISORS

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis Of Variance
CFU/g	Colony-forming unit per gram
COVID	Coronavirus Disease
DNA	Deoxyribonucleic Acid
E. coli	Escherichia coli
FS	Faecal Sludge
GDP	Gross Domestic Product
KEBS	Kenya Bureau of Standards
KESH Policy	Kenya Environmental Sanitation and Hygiene Policy
KJ	Kjeldahl method
LA	Lactic Acid
LAB	Lactic Acid Bacteria
LAF	Lactic Acid Fermentation
LCMS	Liquid Chromatography Mass Spectrometry
MIRERC	Meru University Institutional Research and Ethics Review Committee
MS	Mass Spectrometry
NACOSTI	National Commission for Science, Technology and Innovation
PKa	acid Dissociation constant of a solution
PCR	Polymerase Chain Reaction
SDG	Sustainable Development Goals
SRI	Sanitation Research Institute
SSA	Sub-Saharan Africa
UDDT	Urine Diverting Dry Toilet

UNICEF	United Nations Children's Fund
WHO	World Health Organisation

SYMBOLS

B	Boron
Ca	Calcium
Cl	Chlorine
Cu	Copper
Fe	Iron
H^{+}	Hydrogen ion
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Ni	Nickel
P	Phosphorus
S	Sulphur
Zn	Zinc
OH^{-}	Hydroxyl ion
PO_4^{3-}	Phosphate
CH_3	Methyl
$CH_3CH(OH)COOH$	Lactic Acid
$CuSO_4$	Copper (II) Sulphate
$FeCl_3$	Iron (III) chloride
HCl	Hydrochloric acid
H_2O	Water

H_2CO_3	Carbamic acid
H_2SO_4	Sulphuric acid
H_3BO_3	Boric acid
K_2SO_4	Potassium sulphate
NaOH	Sodium hydroxide
NH_2COOH	Urea
NH_3	Ammonium ion
NH_4	Ammonia
$(NH_4)_2\ SO_4$	Ammonium sulphae
TiO_2	Titanium dioxide

OPERATIONAL DEFINITION OF TERMS

Anaerobic Fermentation	Is a metabolic process that converts carbohydrates (sugar) to organic acids, gases, or alcohols in an environment devoid of oxygen (Tang *et al.,* 2016).
Lactic Acid Bacteria (LAB)	Lactic acid bacteria are a diverse group of Gram-positive bacteria that produce lactic acid as a by-product of their fermentation process (Scheinemann *et al*., 2015).
Urea Hydrolysis	Is the process of breaking down urea into ammonia and carbon dioxide in the presence of urease enzyme (Sharma *et al.,* 2020).
Urine Stabilisation	is a technique for preventing or slowing down urea degradation in preserved human urine, primarily by inhibiting enzymatic urea hydrolysis, in order to retain nitrogen (Randall *et al*., 2016).
Treatment	The process in which a chemical or other substance is put on or in something to protect it, preserve it or clean it.

ABSTRACT

Poor sanitation costs Kenya about 1-2% of its GDP per year, emphasising the crucial need for sustainable solutions. Source separation of excreta is a viable option because it allows for nutrient recovery. However, its direct use as a fertiliser is limited by urea hydrolysis, which elevates pH, causes nitrogen loss, and generates ammonia. Stabilisation is thus essential to retain nitrogen while reducing microbial activity. Simultaneously, Kenya struggles with fruit and vegetable waste disposal, which contributes to environmental damage. The study addressed the stabilisation and treatment of human urine using lactic acid produced from the anaerobic fermentation of fruit and vegetable peels (mango, pineapple, banana, orange, and cabbage) as a sustainable and potential source of nitrogen fertiliser. Lactic acid was produced after three-day fermentation of samples prepared at 1:1 and 1:2 substrate-to-water ratios and incubated at 34°C, 37°C, and 40°C. Qualitative analysis of the sample using Liquid Chromatography-Mass Spectrometry (LCMS/MS) showed sharp, pronounced peaks at 3.691 minutes each, indicating high concentration of lactic acid (LA). The Lactic acid was then quantified spectrophotometrically at 410 nm. The highest LA concentration was found to be 1304.7 mg/L, for the sample fermented at 37°C and lowest for sample at 40°C (538 mg/L). Urine samples were then treated with the resulting lactic acid for 4, 7, and 10 days. The pH of the samples was measured to determine lactic acid's efficiency in inhibiting urea hydrolysis. The resulting pH of the urine samples ranged from 3.6-4.2, indicating lactic acid action on urine to inhibit hydrolysis. An ANOVA Tukey HSD test showed that both the *p*-values for Temperature difference (p = 0.00) for 37°C and 40°C and (p = 0.003) for 40°C and 34°C, and different days' interval (p = 0.001) for 4-10 days and (p = 0.00) for 7-10 days were statistically significant. The nitrogen content in the urine was determined using the Kjeldahl method while the MacConkey Agar tested pathogen inactivation through identifying *E. coli* growth. Lactic acid treatment significantly reduced the pH of urine samples across all settings, with the greatest pH reduction at 37°C (3.4). Nitrogen analysis revealed that samples treated at 37°C in a 1:1 ratio had the most nitrogen concentration (2450 mg/L). A Tukey's HSD test comparing the mean nitrogen concentrations across different treatment days and temperature (4, 7, and 10 days), showed significant difference in concentration over the number of days, especially at 10 days ($p = 0.001$) and for each of the temperature settings (p = 0.000, 0.001, 0.002). Furthermore, lactic acid had strong antibacterial action, inhibiting *E. coli* growth. This study revealed the ability of lactic acid from organic waste to stabilise urine, preserve nitrogen content, and ensure pathogen safety, supporting sustainable sanitation practices and resource recovery in agriculture.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The United Nations SDG 6.2 aims to ensure access to sufficient and equitable sanitation and hygiene for everyone by 2030, as well as to eliminate open defecation, with a focus on women and girls as well as those in vulnerable situations (United Nations, 2015). However, only 2.9 billion people (39% of the world's population) have access to safe sanitation services in 2015 (WHO, 2018; Yang *et al*., 2021). Over 1.5 billion people still need access to normal sanitation services like private toilets or pit latrines (United Nations Children's Fund & World Health Organisation, 2024).

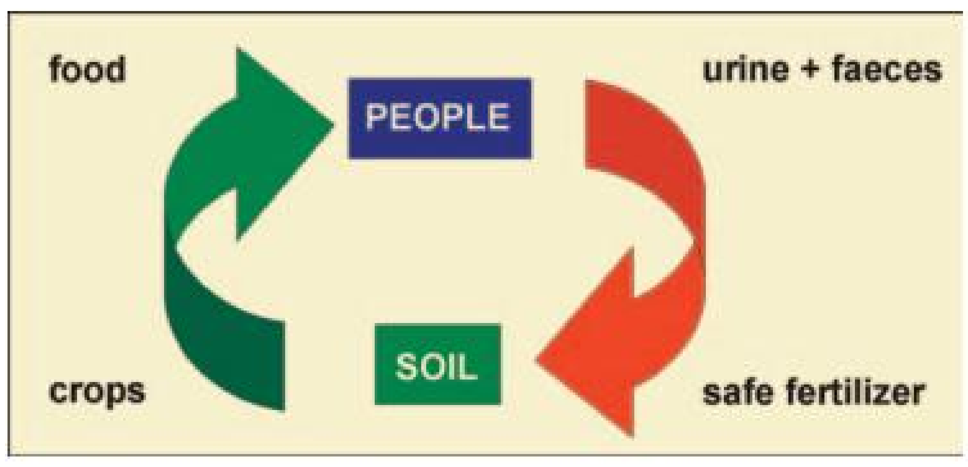
In Sub-Saharan Africa, sewers and flushable toilets serve only 7.6% of the total population and 17% of the urban population (Atangana & Oberholster, 2023). Furthermore, due to inadequate sanitation infrastructure, approximately 80% of sewer wastewater is directly disposed into water bodies, causing water contamination (Kilingo *et al*., 2021), and expanding sewer coverage is typically an expensive and logistically tricky solution, particularly in informal settlements. Poor Sanitation costs Kenya 1-2% of its Gross Domestic Product (GDP) each year, according to the Kenya Environmental Sanitation and Hygiene Policy 2016- 2030 (KESH Policy 1) (Toilet Board Coalition, 2020).

Human excreta source separation is a more resource-efficient alternative to conventional water-borne urban drainage and wastewater treatment (Randall *et al*., 2016). Urine can be separated from solid faeces and used as a liquid fertiliser (Malila *et al*., 2019). Ecological sanitation systems are an example of onsite sanitation and require little space with urine diversion. In this system, urine is separated from faeces, and the excreta is collected differently in sealable containers, which can be reused in agriculture (Bischel *et al*., 2019). The concept of urine diversion in sanitation systems, particularly ecological

sanitation systems, has recently been enhanced due to some advantages, such as preventing the spread of pathogen-containing material, safer and easier handling and use of excreta, and less odour. It also prevents eutrophication and waste-related pollution of natural water sources while enabling sustainable reuse of waste for agricultural purposes. Implementing diverting systems is a good substitute for the current sanitation options to improve circular sanitation and enhance sustainability as in Figure 1.1 (Malila *et al.*, 2019).

Figure 1.1:

Circular uirine-diverting ecological sanitation systems



Source: (Malila et al., 2019)

However, storage alone is ineffective for excreta treatment in source-separating toilets because of inadequate hygienisation and significant loss of nutrients (Andreev *et al.*, 2016), unless a system that prevents the loss of these nutrients is implemented.

Emphasis on innovations and interventions in which the outputs of sanitation interventions become inputs in agricultural farming, is growing (Toilet Board Coalition, 2020), especially with the Coronavirus Disease (COVID-19) pandemic (Mallory *et al.*, 2021) and disease caused by Monkeypox which brought to attention the risk of pathogens in urine (Xu *et al.*, 2022). The link between sanitation and stable ecological

and environmental systems has been strengthened. In rapidly growing cities, particularly in Sub-Saharan Africa, there is a large potential for source-separating systems that enable the recovery of nutrients that can be used as fertiliser while also preventing environmental pollution (Randall *et al*., 2016). Integration has been implemented to provide low-cost sanitation services focusing on nutrient recovery, which can help capture nitrogen and phosphorus from human excreta that would otherwise be lost to the environment (Bischel *et al*., 2019).

Historically, urine has been used as a fertiliser in many countries for various crops (Zerihun *et al*., 2021). Scientific studies and development projects have investigated and demonstrated the safety and effectiveness of its reuse in agriculture. However, recently, greater emphasis has been put on recovering nutrients from urine, which provides a sustainable approach to ease the strain between the high demand for food and fertiliser and the shortage of nutrients (Badeti *et al*., 2022; Fearn *et al*., 2015).

The macronutrients required for agriculture are nitrogen, phosphorus, and potassium. Urine contains approximately 90% nitrogen, 50-65% phosphorus, and 50-80% potassium, which comparatively can be equated to the amount of fertiliser required to produce 250 kg of cereal, which is equivalent to what one person can consume in a year (Mateo-Sagasta *et al*., 2015). Using urine as a nutrient substitute allows for excreta to be reused in a circular economy while also addressing the economic burden associated with fertiliser acquisition, especially for low-income earners. Human urine is already widely used as a fertiliser in cultivating tomatoes, cabbage, cucumber, cereal, and fruit crops (Adejumo *et al*., 2019).

While urine use is becoming more popular around the world, pathogen concerns have slowed its acceptance by many. Urine requires stabilisation to maximise nutrient recovery and prevent malodour. Stabilisation primarily refers to the prevention of

enzymatic urea hydrolysis. Urease, the enzyme responsible for enzymatic urea hydrolysis, is abundant in the environment (Randall *et al*., 2016), and causes nitrogen loss from the urine, making it less reliable as fertiliser (Boncz *et al.*, 2016).

While numerous methods for nutrient recovery have been proposed, the majority of these methods are complex and impractical for large-scale implementation. Stabilisation through acidification is one of the methods that has been experimented on in order to prevent nitrogen loss. Most acids used are expensive and dangerous to handle within local contexts. Biological methods have the potential to provide a feasible, low-energy method with low operation and maintenance costs, resulting in a less expensive method of nutrient retention and urine stabilisation (Boncz *et al*., 2016). An example of these biological methods would be using organic acids to stabilise urine, which helps reduce the pH in urine, lowering the environment for hydrolysis. These acids also have microbial compounds that help kill microorganisms and pathogens that may contaminate the urine, hence making them safe for reuse in farming (Zerihun *et al.*, 2021).

Utilising organic wastes to obtain these acids reduces cost and promotes sustainability because it makes it possible to manage waste disposal and create circular solutions that will enable locals to use urine for farming close to where they live. Although previous studies towards optimisation of Lactic acid production from organic wastes have been made, little research has been done towards direct application of LAF from fruit and vegetable wastes in urine medium for stabilisation, especially aiming towards nitrogen preservation. Only comparative studies have been made towards the same in faecal sludge (Andreev *et al.*, 2016).

The use of fruit and vegetable peels in LAF for urine treatment and stabilisation is a promising methodology. This study sought to incorporate the LA obtained from fermentation of mango, pineapple, banana, orange and cabbage peelings in urine medium.

The LAF procedure effectively stabilised the urine, eliminated pathogens, and preserved nitrogen, a crucial component for plant growth in the urine. In this research, Lactic acid production was focused on above other organic acids since lactic acid fermentation is more energy-efficient and yields fewer toxic by-products than other organic acid production methods. It is a more environmentally friendly choice that fits with the growing trend of sustainable manufacturing practices (Rachwał & Gustaw, 2024).

1.2 Statement of the Problem

Fruit and vegetable wastes are produced and discarded in tremendous amounts during industrial processing and household usage. They are extremely susceptible to microbial deterioration due to high moisture and total soluble sugar concentrations, posing significant environmental risk, especially in tropical and subtropical countries whose conditions favour the growth of spoilage-causing microorganisms (Khattak & Rehman, 2017). This comes with significant odour and pollution, necessitating a means to contain them.

Due to inadequate space, filled-up latrines in low-income areas become health hazards as they are abandoned for open defecation alternatives due to odour and the unsanitary state of these latrines. Human excreta has also been discharged into the environment without treatment, leading to degradation and eutrophication in water bodies (Okem & Odindo, 2020). There are numerous issues with the storage, transportation, and use of source-separated urine in urine diverting toilets.

Urea is hydrolysed by urease during urine storage, resulting in odour, precipitation, and ammonia loss, which is a challenge to overcome in urine recycling (Xu *et al*., 2022). The excreted urine has an initial pH of 4.8-7.5, and nitrogen mainly exists as urea, which only produces a slightly peculiar smell. The pH however rises to 9.0-9.3 during hydrolysis,

accompanied by precipitation and ammonia gas with a pungent odour (Yang *et al.*, 2021).

This process is catalysed by urease, resulting in low utilisation efficiency and severe environmental contamination as well as in significant nutrient loss. The loss of nitrogen due to its transformation into gaseous ammonia can reach 50%. Large amounts of ammonia are produced when urease is not deactivated, putting the operator at risk. Trials at treating and stabilising urine have been made, and various techniques innovated to help preserve the nitrogen in urine for its reuse as fertiliser. However, a lot of these techniques are expensive and technically complicated (Boncz *et al.*, 2016).

The rising cost of chemical fertilisers because of the scarcity of nutrients used in producing them has endangered their continuous availability for farming. This has increased food prices, shortages, and geopolitical rifts within a century (Okem & Odindo, 2020). Because these fertilisers having a high proportion of nitrogen have a short-term fertilising effect, there is need to pursue new solutions for long-term fertiliser production. Cheaper urine fertilisers with a higher potential for increasing agricultural productivity could help to solve this problem (Jurga *et al.*, 2021).

1.3 Research Questions

i. Does a three-day anaerobic fermentation of fruit and vegetable waste at different temperatures and different substrate to water ratio effectively yield lactic acid?

ii. Can lactic acid obtained from organic waste fermentation stabilise fresh urine over different time intervals (4, 7, and 10 days)?

iii. To what extent does the lactic acid-stabilised urine inhibit urea hydrolysis compared to untreated urine, based on its total nitrogen content analysis?

iv. What is the effect of lactic acid stabilisation on *E. coli* presence in urine samples?

1.4 Study Objectives

The study was guided by the following objectives.

1.4.1 General objective

To stabilise urine using lactic acid from fruit and vegetable peel fermentation as a potential source of nitrogen fertiliser.

1.4.2 Specific objectives

i. To evaluate lactic acid from fruit and vegetable waste through anaerobic fermentation at three different temperatures, with different substrate to water ratios over a three-day fermentation period.
ii. To stabilise the fresh urine using the extracted lactic acid and monitor the changes over 4, 7 and 10 days.
iii. To assess the inhibition of urea hydrolysis by comparing the pH levels and the total nitrogen content (mg/L) in stabilised urine with the untreated urine sample.
iv. To evaluate the presence of *E. coli* in stabilised urine by carrying out a pathogen analysis using MacConkey Agar technique.

1.5 Justification of the Study

Several studies have shown that urine is an effective fertiliser, especially for crops limited in nitrogen (Fearn *et al*., 2015; Moya *et al*., 2019; Sugihara, 2020). Due to the high economic value of fertilisers, urine reuse is viewed as a workable business model which can be used by everyone, including low-income earners (Fearn *et al*., 2015). The reuse of urine would help solve the demand for food production to sustain growing populations, as well as help manage sanitation systems that are already outdated (Sugihara, 2020). Urine diversion through ecosan toilets provides a renewable source of urea, transforming a would-be costly contaminant into an economically valuable asset. Urine diversion is a solution to wastewater management issues since it is urine that

contributes the most nutrients and micropollutants to wastewater. Nitrogen recovered from urine which is collected separately saves the cost and energy needed to remove nitrogen from wastewater and create fertiliser (Tarpeh *et al*., 2018). The volume of excreta carelessly disposed of in the environment is significantly reduced by using urine source separation, thereby reducing health risks (Viskari *et al*., 2018).

Reusing human excrement products generated by sanitation technologies also boosts local agricultural production while amplifying the impact of local wastewater treatment efforts. Urine contains significantly more nutrients than faeces, grey water, and biodegradable suspended solids compared to other household waste types. Significant research on the direct use of urine on crops has been conducted, and it appears to be a viable option (Fearn *et al.*, 2015; Sugihara, 2020).

Compared to mineral fertilisers, urine fertilisers use less energy and have a lower environmental impact. From an environmental perspective, the use of urine fertiliser advances the soil's physical and chemical properties, hence restoring soil fertility. Urine fertilisation is also inexpensive and straightforward (Medeiros *et al*., 2020).

Urine stabilisation using fruit and vegetable waste makes use of local resources, hence providing more positive ways of managing waste and reducing the risk associated with using strong commercial acids such as sulphuric acid and hydrochloric acid. In impoverished communities, incorporating sanitation and agricultural technologies for crop production combats malnutrition and hunger. When urine is used as a fertiliser, it helps food production and provides reasonable sanitation solutions for many people worldwide due to reduced discharge to the environment (Sugihara, 2020).

For this reason, the utilisation of fruits and vegetables waste in stabilisation of urine was investigated.

1.6 Limitations of the Study

Some of the limitations to the study are addressed below. The study was conducted on a small scale, laboratory-based setup with monitored conditions that may not accurately reflect real-world environmental conditions which can impact the performance of lactic acid extraction and urine stabilisation when scaled up. The study used established laboratory methods for pathogen detection and chemical analysis. However, constraints in equipment and technical capabilities limited the amount, range and quality of data collected. The study did not conduct field-level agronomic tests to assess the efficacy of stabilised urine as a fertiliser. Future research, such as soil application tests and plant development studies, would be critical in assessing the practical application in agriculture.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sustainable Sanitation in Sub-Saharan Africa (SSA)

Rapid urbanisation has brought the need to improve the expansion of sanitation coverage in emerging countries in developing nations (Ferreira *et al*., 2021). While great progress towards SDG 6.1, which calls for everyone to have equal access to safe and cheap drinking water has been made, SDG 6.2, which calls for access to Sanitation, has yet to make as much headway (United Nations Children's Fund & World Health Organisation, 2024). The problem is caused by insufficiency in sanitation facilities as well as by ineffective treatment of excreta after collection. Among the 2.8 billion people having access to sewers, only 1.9 billion have proper Sanitation that treats human waste. Because of their high price and challenging topography, sewer systems are rarely practicable (Zerihun *et al*., 2021).

In Sub-Saharan Africa, the statistics of urban residents living in informal settlements is over 60%; with most of these residents being especially susceptible to water and excreta-related diseases (Zerbo *et al*., 2021). Effective collection and treatment of excreta can benefit human healthiness and the environment by reducing disease transmission and avoiding nitrogen discharge into waters, which alters aquatic environments (Tarpeh *et al*., 2018). As a result, it can be a valuable tool in achieving clean water and Sanitation and sustainable cities and communities in the SDGs goals (Xu *et al*., 2022).

Urine-diverting dehydrating toilets (UDDTs) have been implemented to improve sanitary conditions in informal settlements in low-income countries (Riungu *et al*., 2019). This source-separated urine system is used to achieve sanitation goals, which include effective nutrient recovery, simple micro pollutant control, and reduced health menaces from faecal pathogens (Xu *et al*., 2022).

Onsite sanitation systems, which require emptying and suitable treatment and disposal to avert public health and ecological hazards, are estimated to provide between 65% and 100% of sanitation in sub-saharan Africa (Moya *et al*., 2019). Most of these systems are container-based and urine-diverting, and they can be reused. They enable the complete recovery of nutrients in water bodies from human activities, predominantly excreta, hence benefiting agriculture (Adejumo *et al.*, 2019). Inadequate Sanitation is estimated to cost around $260 billion per annum for health care, infant and premature deaths, and productivity losses (Hans, 2023).

A strategy where sanitation providers strive to better public health, environmental quality, and profit will help to speed up and sustain sanitation access. This work focused on onsite, container-based sanitation and sought to utilise source-separated urine in agriculture. This needed a means of stabilising it to prevent nutrient loss.

2.2 Nutrients in Human Urine

Human excreta consist of urine and faeces. Each year, one person generates approximately 5.7 kilograms of nitrogen, 0.6 kilograms of phosphorus, and 1.2 kilograms of potassium from excreta refuse (Mateo-Sagasta *et al*., 2015). Urine comprises more than 50-70% of the nitrogen (N) and 90% of the phosphorus (P) excreted (Krishnamoorthy *et al*., 2020).These nutrients, particularly nitrogen, are crucial for plant growth and agricultural productivity (Boness *et al*., 2024). Human urine is a multifaceted milieu of chemicals since it contains metabolic breakdowns from food, drink, and pharmaceuticals as well as endogenic waste metabolites, external pollutants, and bacterial by-products. It has recently been found to include about 400 volatiles, which come from over 15 different chemical classes and include alcohols, carbonyl, hydrocarbons, and carboxylic acids, among others (Llambrich *et al.*, 2022).

Human urine is particularly valuable since it contains all the necessary trace elements (e.g., Cl, Fe, Zn, Cu, B, Mn, Ni, and Mo) and major nutrients (e.g., Ca, Mg, and S), in addition to three significant nutrients, N, PO_4^{3-} and K (Ray, 2020). Nitrogen (N) is the most abundant nutrient in urine and the most desirable nutrient to target for recovery over the other nutrients in urine. The nutrients in urine are in a form readily available to plants, similar to ammonium nitrate-based fertilisers, and with comparable results on plant growth (Tarpeh *et al.*, 2018).

2.3 Urine Treatment Technology

Urine treatment is crucial in sustainable sanitation practices, especially in resource-constrained areas. The urine treatment process involves removing pathogens and recovering valuable nutrients, such as nitrogen, for agricultural purposes. The unique chemistry of urine must be considered when selecting a treatment method. Fresh urine's pH ranges from 4 to 8 when it initially leaves the body because the nitrogen exists in the form of urea (Fearn *et al*., 2015) and calcium and magnesium are present. However, urea in urine spontaneously undergoes urea hydrolysis when bacteria are present, particularly when it comes into touch with the urease enzyme. Every toilet has urease, a universal enzyme found in plants, fungi, and bacteria (Sharma *et al*., 2020).

The hydrolysis of urea during the collection, storage, transport, and utilisation of urine results in ammonia volatilisation (Harder *et al*., 2019; Sharma *et al*., 2020)**,** formation of bicarbonate, an elevation of the pH from 6 to 9-9.3, and the struvite precipitation and hydroxyapatite13-16, leading to the formation of ammonium (Fearn *et al*., 2015). As a result of becoming more alkaline and increasing the bicarbonate and ammonia concentration, the buffering capacity of urine increases as well. Since nitrogen is in the form of ammonia, it can evaporate into the air, leading to the loss of vital nutrients that

are needed for plant growth (Nagy *et al.*, 2019)., and resulting in low utilisation efficiency and severe environmental contamination.

Aside from influencing the recovery efficiency of nitrogen, hydrolysis triggers undesirable odour emissions, which are intensified by other putrid components, such as volatile fatty acids released by bacteria and are a nuisance to toilet users along with those reusing the urine for cultivation (Zhang *et al*., 2022). The nitrogen present in urine can only be used for fertiliser production after developing a mechanism to prevent it from hydrolysis (Boncz *et al.*, 2016). Consequently, depending on the urine state, nitrogen can be recovered from urea or ammonia, which are forms through which urine exists (Ray, 2020). The urine can be converted into a more vegetable-available nitrate with competitive NPK values with a small amount of stabilisation. It, therefore, has a high potential to substitute chemical fertiliser due to its large amount of these plant nutrients (Adejumo *et al*., 2019). In recent times, researchers have been exploring and developing innovative and cost-effective technologies to enhance reuse and nutrient recovery from urine, reduce ammonia volatilisation, prevent decomposition of urea in the urine and to come up with hygienic end products obtained from human excreta for reuse or disposal (Liu *et al*., 2022; Zerihun *et al*., 2021).

Struvite precipitation, nitrification, ion exchange, and ammonia air stripping are the most common and studied processes (Ray, 2020). However, most of these methods are expensive, and are aimed explicitly at either nutrient recovery or elimination of pathogens. The most popular procedures for stabilising liquid streams are biological processes like partial nitrification and LAF and chemical processes like acidification and alkalinisation (Harder *et al*., 2019).Provision needs to be made to utilise local methods that combine the conversion of ammonia and remove complex agents that may prevent nutrient recovery (Sharma *et al.*, 2020).

Based on a research by Zerihun *et al*., 2021, maintaining a pH below 4 by incorporating acetic and sulphuric acids (2.9 g/L) prevents the breakdown of urea and renders bacteria inactive. However, it is expensive and may create health risks during the handling of the acids. It is therefore essential to develop a simple and cost-effective method to acidify urine and minimise urea decomposition to reduce nitrogen loss. This will make it possible to safeguard its fertiliser value for agricultural applications (Andreev *et al*., 2016).

Using lactic acid to stabilise urine over the course of treatment is one promising approach. This is why the study investigated urine stabilisation using lactic acid fermentation of peels from orange, pineapple, lemon, and mango along with cabbage waste. Doing this focused on techniques for recovering nitrogen optimally through acidification while making use of locally available resources. The recovered urea produces slow-release nitrogen fertiliser which allows effective utilisation of nutrients in agricultural production (Oishi *et al*., 2023).

2.4 Urine Stabilisation

Urine stabilisation is the process of treating urine chemically, mechanically, or biologically to preserve its components and qualities for a predetermined amount of time. When urine is stabilised effectively, all of its naturally occurring components remain dissolved in the liquid phase and no solid or gas phases are produced (Yang *et al*., 2021). Urine's high biological activity potential has led to an emphasis on bacterial growth management during urine collection, transit, and storage to stabilise the urine (Brunzel, 2022).

Urea is a stable compound with a half-life of 3.6 years when it is chemically hydrolysed without enzymes at 38^0C. Urine contaminated with faeces usually shows favourable hydrolysis properties, which can reach 104 times that of chemical hydrolysis (Yang *et al*.,

2021). Since urease has bi-nickel active sites when interacting with urea, one binds and activates the urea, and the other binds and activates water molecules. It is generally believed that the reaction of urea hydrolysis by urease is composed of four steps. The overall reaction equation and stepwise equations are as follows (Yang *et al*., 2021).

Overall reaction:

$(NH_2)_2CO + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^-$ (2.1)

Urea

Stepwise reactions:

$(NH_2)_2CO + H_2O \rightarrow NH_3 + NH_2COOH$ (2.2)

Urea

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$ (2.3)

Carbamic acid

$H_2CO_3 \rightarrow H^+ + HCO_3^-$ (2.4)

$NH_3 + H_2O \rightarrow NH_4^+ + OH^-$ (2.5)

Urease converts urea and water into ammonia and carbamate, which then spontaneously hydroxylates to ammonia and carbonic acid. The formation of ammonia in the hydrolysis of urea causes an increase in pH. There is a direct connection between the increased ammonia concentration and the electrical conductivity, indicating that the hydrolysis of urea in the storage container can be tracked by the changes in urea concentration, ammonia concentration, conductivity, and pH over time (Yang *et al*., 2021).

Urine stabilisation is not an isolated method but is usually applied within the cycle of source separation and recycling of urine. It is vital in urine diversion systems for nutrient recovery (Yang *et al*., 2021). It inhibits the catalysis functions of the urease enzyme to ensure urea hydrolysis does not occur. Although hydrolysis promotes the pathogens' inactivation, it also brings nitrogen loss due to ammonia volatilisation and phosphorus

loss due to phosphate precipitation (Xu *et al*., 2022). A pH decrease is needed to shift the ammonia-ammonium equilibrium towards the non-volatile ammonium to inhibit urea hydrolysis. This can be achieved by direct acid addition (Randall *et al*., 2016), which, as research has found, lowers the pH to 4–4.5 (Ray, 2020). Acidification (pH < 5 (Moharramzadeh *et al*., 2022; Saetta *et al*., 2020), inactivates the urease-producing bacteria. The stabilisation process has the potential to inactivate some pathogens. Certain organic contaminants can also be degraded by biological processes (Harder *et al*., 2019).

Urine has been stabilised using various strong acids. In 2012, to eliminate excess sulphate ions in pre-treated urine, Dean Muirhead replaced the sulphuric acid (H_2SO_4) in the urine stabiliser solution with three different mineral acids (phosphoric acid, hydrochloric acid, and nitric acid). He controlled the dose to stabilise the urine and reduce the risk of mineral precipitation during the distillation process (Muirhead & Carrier, 2012). Saetta & Boyer, 2017 carried out an experiment where they added 2.5 mL of 2,500 meq/L acetic acid to an anhydrous urinal after each urination to reduce the pH to inhibit the hydrolysis of urea in artificial urine and real urine.

Ray *et al*.,2018, designed an experiment to study the inhibitory effect of three types of urease inhibitors: metal, fluoride, and acids on urea hydrolysis. The results were citric acid> acetic acid> vinegar> sulphuric acid> ionic silver> ionic zinc> sodium fluoride. Acetic acid, citric acid, and vinegar can effectively inhibit urea hydrolysis when the concentration is between 3.2×10^1 and 1.6×10^2 meq L^{-1} (Ray *et al*., 2018). The order of addition of urease and acid affects the hydrolysis of urea. The pre-adjusted acidic environment can inhibit the activity of urease more (Yang *et al*., 2021). Since adding acid is a reversible pH-dependent inhibition strategy, feeding regularly to maintain a low pH is an effective way to stabilise urine. Batch chemical addition experiments also

proved this method. In addition, acidification has a positive effect on hygiene (Harder *et al*., 2019).

In the case of low pH, the number of microorganisms and bacteria in the urine is greatly reduced, and it is difficult for pathogens to survive, which, to a certain extent, curbs the spread of diseases. Therefore, acidification is a good choice for underdeveloped areas (Yang *et al*., 2021). However, since the stabilised urine is aimed for reuse in agriculture, strong acids may not be suitable as they may impact the soils and crops negatively (Liu *et al*., 2024). Organic, locally made acids are a suitable substitute for these commercial strong acids as they are cheaper and have fewer chemicals. An example of this is Lactic acid obtained from fruit and vegetable waste. Being acidic, it still aids in stabilising urine, at a cheaper cost and with little effect to the soil and crops (Andreev *et al*., 2016).

2.5 Nutrient Recovery Methods

In the past, the WHO advised keeping urine in storage containers for six months at a temperature of 22°C to be considered hygienic (WHO, 2018). However, this has created issues with where to keep it and how much odour will be released while it is in storage. Due to vaporisation, the nutrients are also easily lost to the environment. Any temperature fluctuation also impacts the process, making it unpredictable and unreliable. The urine nutrients can be fixed and reused instantly to drastically cut down on that time (Saliu *et al*., 2024). Recovery of nutrients from human urine promotes water conservation. If implemented on a broader scale, it can help boost the economy by supplying households with a direct fertiliser supply or creating a local market inside the nation (Maiza *et al*., 2025). There are certain benefits to separating faeces and urine at the source for nutrient reuse. Most nutrients that individuals excrete are found in their urine. There are several ways to recover nutrients from urine that has had its source separated, primarily nitrogen and phosphorus (Fearn *et al*., 2015).

Table 2.1:

NPK composition in urine and faeces

Type of waste	N (kg)	P (kg)	K (kg)
Urine	2.5-4.3	0.7-1.0	0.9-1.90
Faeces	0.5-0.7	0.3-0.5	0.1-0.2

Source: (Fearn et al., 2015)

By converting ammonia into nitrates, which are more accessible to plants and have NPK values that rival those of other fertilisers, nitrifying bacteria can turn urine into nitrate. Techniques involving pH change in such as utilising urease inhibitors, acidification, alkalisation, or both can stop nitrogen loss. (Boncz *et al., 2016)*.

2.6 Lactic Acid among other Organic Acids

Organic acids occur naturally and have been used by people since prehistoric times. Organic acids are conventional products of food and biotechnology. For centuries, food preparation has relied on fermentation processes that produce acetic and lactic acids (Sun *et al*., 2020). Organic acids are distinguished by the presence of a carboxyl group, which can donate a proton (H^+) in aqueous solutions, resulting in their acidic characteristics. These acids are key metabolites that promote plant development and are abundant in the metabolic pathways of all plants, particularly fruits and vegetables. Examples of organic acids in nature include: acetic acid, citric acid, lactic acid, malic acid, oxalic acid, formic acid, tartaric acid, butyric acid, ascorbic acid (vitamin C), benzoic acid, α-ketoglutaric acid and succinic acid (Vargas, 2017). Most organic acids have long been recognised as potent antimicrobials, and their effectiveness against microorganisms has been used in food preservation. The length of the carbon chain and the degree of unsaturation affect the acid's action against microorganisms, but the pKa of the acid also has an impact on its antimicrobial mechanism (Dutoit *et al*., 2024).

Lactic acid ($CH_3CH(OH)COOH$), with a molar mass of 90.08 g/mol, is a chiral molecule having two optical isomers: Levo (L-lactic Acid) and Dextro (D-lactic acid) isomers. Lactic acid can exist in either of its optically active forms (L (+) or D (-) giving it distinct chemical representations (Ojo & De Smidt, 2023). Figure 2.1 shows the chemical representation of the D(-) and L(+) lactic acid (Pohanka, 2020). Pure anhydrous lactic acid is a white crystalline solid with a low melting point of 53℃. It is typically found in concentrated aqueous solutions.

Lactic acid is considered both stable and flammable. Lactic acid is compatible with strong oxidising agents and is normally a clear to slightly yellowish liquid that is delivered to formulators in concentrations ranging from 88 to 92%. Lactic acid is generally found in diluted or concentrated aqueous solutions (Komesu *et al.*, 2017). This chirality adds another layer of uniqueness to lactic acid's chemical properties and applications. The core carbon is chiral, and the other two substituent groups are composed of a hydrogen atom on a methyl group ($-CH_3$). The α-hydroxyl and carboxylate groups form intramolecular hydrogen bonds, resulting in enhanced acidity (Ameen & Caruso, 2017).

Figure 2.1:

Three-Dimensional Configuration of Optical L-Lactic Acid and D-Lactic Acid

COOH
HO — H
CH_3

L(+) lactic acid

COOH
H — OH
CH_3

D(-) lactic acid

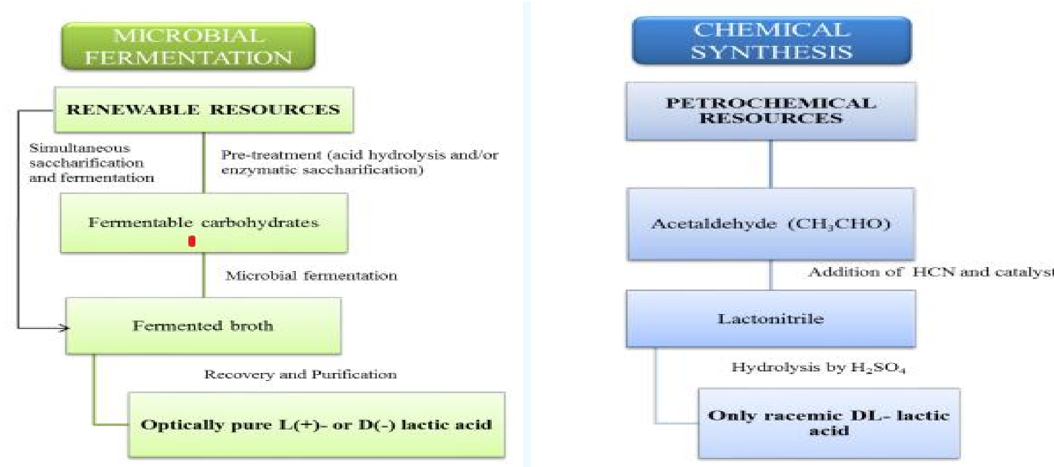
Source: (Pohanka, 2020)

Lactic acid (LA) is distinct from other organic acids due to its structure and the presence of a hydroxyl group (-OH) adjacent to the carboxyl group, a feature other organic acids typically lack. The hydroxyl group is highly soluble in water and can establish hydrogen bonds with water molecules. This structural aspect, along with its single dissociation step, gives lactic acid a simple buffering range around its pKa of 3.86 (Papadopoulou, 2023). The electron-withdrawing effect of the hydroxyl group stabilises the carboxylate ion formed upon dissociation, making lactic acid more acidic than similar acids lacking the hydroxyl group. The hydroxyl group also enhances solubility and reactivity, allowing lactic acid to engage in chemical processes like esterification, which is useful in chemical synthesis (Oguche *et al*., 2023).

In contrast, organic acids with multiple carboxyl groups, such as citric and malic acids, have greater buffering capacity due to multiple dissociation processes. Lactic acid has a lower pH compared to many other organic acids primarily due to its relatively low pKa, indicating it is a stronger acid and more readily donates protons in solution (Niaz *et al*., 2022). As a monocarboxylic acid, it has one -COOH group which dissociates to release hydrogen ions, contributing to the solution's acidity. The hydroxyl group on the second carbon stabilises the conjugate base (lactate ion) and increases the stability of the lactate ion in solution, enhancing the acid strength of lactic acid (Yadav *et al*., 2022). Lactic acid can be made by chemical synthesis or microbial fermentation as shown in Figure 2.2. Lactic acid plays a significant role in several metabolic processes. Due to the bifunctional reactivity of its carboxyl and hydroxyl groups, lactic acid exhibits considerable reaction flexibility (Ojo & De Smidt, 2023).

Figure 2.2:

Overview of the Manufacturing Methods of Lactic Acid-Chemical Synthesis and Microbial Fermentation



Source: (Umesh & Preethi, 2014)

In recent years, the fermentation approach has been focused on more in comparison to chemical approaches (Hassan *et al*., 2019). Lactic acid is produced primarily by microbial fermentation of carbon sources, which produces over 90% of the acid (Ojo & De Smidt, 2023). This is due to the growing demand for optimum, naturally produced lactic acid as well as its sustainable utilisation of inexpensive agricultural residues in bioprocess, thus serving as an alternative way to replace costly raw materials. It also produces pure lactic acid and makes use of affordable, renewable waste (Hassan *et al*., 2019).

During fermentation, lactic acid can be generated as the two isomers mentioned above. Usually, small-scale fermented vegetable products contain both lactic acid isomers. However, the D (-) lactic acid isomer concentrations are lower in LA-fermented vegetables. Bacteriocins, which are tiny proteins made by Lactic Acid Bacteria (LAB), are frequently inhibitory toward a wide range of harmful bacteria, including food-borne diseases (Montet *et al*., 2014).

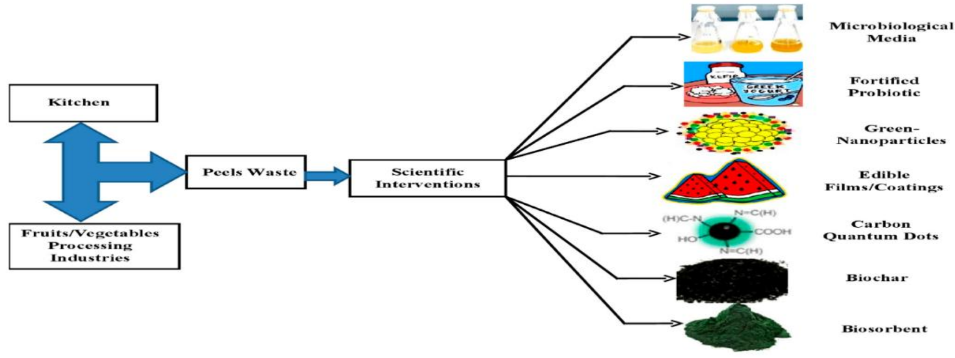
LAB are the main bacteria used to produce lactic acid; among these, *Lactobacillus* spp. has shown interesting fermentation capacities (Abedi & Hashemi, 2020). Lactic Acid bacteria (LAB) make lactic acid as their primary fermentation product by fermenting sugar (i.e., glucose). They are a phylogenetically varied group of Gram-positive bacteria that also create amines, organic acids, and short-chain fatty acids metabolite (Montet *et al*., 2014). They mostly grow anaerobically, though they are also aero-tolerant. Lactic acid can be produced by LAB as the only fermentation product or in combination with significant amounts of ethanol, acetate, and CO_2. To yield lactic acid singly, the bacteria *Lactobacillus delbrueckii ssp. delbrueckii* has been utilised and yields around 88% lactic acid from fruit peel wastes (Bustamante *et al.*, 2019).

2.7 Production of Lactic Acid from Vegetable and Fruit Peels

Scientists have discovered that fruit peels typically exhibit more significant biological and pharmacological applications than other parts of the fruit (Figure 2.3). This has led to an upsurge in the proposal of using fruit by-products, especially fruit peels. Fruit wastes are a great source of vitamins, minerals, and natural sugars (Jahid *et al*., 2018; Montet *et al*., 2014).

Figure 2.3:

Different Potential Ways That Waste has been Used



Source:(Kumar et al., 2020)

Food waste have simple sugars, which exhibit great potential for use as a medium for the manufacture of organic acids (Abdullahi *et al*., 2020). Conversely, because they have high mineral and vitamin content and a neutral pH, vegetables serve as a natural ground for lactic acid bacteria (LAB) fermentation. Lactic acid fermentation can happen naturally in fresh fruits and vegetables when the environment—including moisture content, temperature, anaerobic conditions, water activity, and salt concentration—is conducive to the development of autochthonous LAB (Di Cagno *et al*., 2015).

Fruits and vegetables can "spontaneously" ferment because of the naturally occurring lactic acid bacterial surface microflora, such as Lactobacillus, Leuconostoc, and Pediococcus (Montet *et al*., 2014). Among the most prevalent bacteria in food wastes are Lactobacillus plantarum, Lactobacillus brevis, and their respective *Lactobacillus mudanjiangensis, Lactobacillus yonginensis, Lactobacillus paucivorans, Lactobacillus malefermentans, and Lactobacillus mixtipabuli* (Tsapekos *et al*., 2020). Back slopping, a popular sauerkraut-making method that entails inoculating the raw material with a little amount of a previously completed, successful fermentation, can be used to maximise spontaneous fermentation (Di Cagno & Coda, 2014)

Fruit and vegetable peels are lingo cellulosic and can produce Lactic Acid (LA) since they contain various nutrients and are high in carbohydrates. They are also locally available and ferment quickly, making them suitable for optimal lactic acid production. Most fruit and vegetable wastes, like pomaces, pulps, cabbage leaves, pineapple waste, pineapple bran, etcetera, are perishable and highly fermentable, mainly because of their high moisture, crude protein, and total soluble sugar contents. Many organic acids, such as lactic acid, occur naturally in various plants and vegetables (Table 2.2) and are produced when these wastes ferment (Pratiwi *et al*., 2017).

Table 2.2:

Scientific Names of Fruits and Vegetables

Common Name	Botanical/Scientific Name
Lemon	*Citrus limon*
Papaya	*Carica papaya*
Cabbage	*Brasicca chinensis*
Lime	*Citrus aurantifolia*
Mango	*Mangifera indica*
Pineapple	*Piper nigrum*

Source: (Food and Agriculture Organisation [FAO], 2005)

Several studies have been made towards extracting organic acids from natural plant sources. In a research by Zerihun *et al*., (2021)., lactic acid was produced using cabbage waste, potato peels, and teff flour extracts for use in pathogen inactivation in faecal sludge. The Lactic Acid Bacteria (LAB) that were produced by fermenting cabbage were also utilised by (Andreev *et al*., 2016) to cultivate LA using urine as a growth medium. Numerous fruits have also been utilised to produce organic acids, such as lemons, tangerines, pomelo, and sweet oranges, which yield citric acid (Pratiwi *et al*., 2017), mango, orange, banana, and pineapple peels for lactic acid production(Umesh & Preethi, 2014), rotting tropical fruits are used for isolating LAB and using it to yield lactic acid (Ngouénam *et al*., 2021).

This research work evaluated the fermentative utilisation of four fruit peel wastes (mango, orange, banana, and pineapple) mixed with cabbage waste as substrates and carbon sources for lactic acid production for use in nitrogen retention hence urine stabilisation, pathogen inactivation, and odour removal in urine. The cabbage waste was

used because of its rapid ability to reduce the pH and sustain it at that low level (Zerihun *et al*., 2021).

This was confirmed in an experiment where potato peels, teff flour, and cabbage waste were comparatively tested for their capability to produce lactic acid and reduce pH in urine for pathogen inactivation across several days. The cabbage had a higher LAB count and the lowest pH and also eliminated detectable *E. coli* counts faster than the rest (Zerihun *et al*., 2021). These four fruits were chosen because they are some of the most locally consumed tropical fruits known for their high nutritive values (Wongkaew *et al*., 2021). The fact that they are good carbon sources due to their high carbohydrate content also makes them suitable for lactic acid production (Ngouénam *et al*., 2021).

From literature, the orange peel contains 23% sugar, 22% cellulose, 25% pectin, and 11% hemicellulose, which make chemical–biological treatments used to produce reagents feasible in form of lactic acid, propionic acid, and butyric acid (Ayala *et al*., 2021). Banana peels are a good source of lignin (6-12%), pectin (10-21%), cellulose (7.6-9.6%), hemicellulose (6.4-9.4) (Osman *et al*., 2016). The mango peel has substantial levels of lignin (16%), cellulose (30%), and pectin (5–20%) (Wongkaew *et al*., 2021). According to recent research, pineapple waste contained 19.4% lignin, 32.4% cellulose, and 23.2% hemicellulose. Literature indicates that the orange peel contains 19.8% lignin, 22% cellulose, 25% pectin, and 11% hemicellulose. The high lignin, cellulose, and hemicellulose content in these fruits also makes them suitable as feed stocks to produce fermentable sugar (Aili Hamzah *et al*., 2021).

The fermentation process entails using microorganisms to break down organic molecules into simpler ones. Fruit and vegetable peelings are among the various carbohydrate waste sources that can be used by the various microorganisms that cause fermentation. These wastes can be tuned as valuable substrates in the commercial production of organic acids

like lactic acid (Saadoun *et al*., 2021). Many other microorganisms can also be used for the same, including *Penicillium janthinellum, Penicillium restrictum, Trichoderma viride, Mucor pirifromis, Ustulina vulgaris*, and various species of the genera *Botrytis, Ascochyta, Absidia, Talaromyces, Acremonium, and Eupenicillium* (Odey *et al*., 2018). Not all microorganisms can produce lactic acid in urine or faeces, which renders them useless for inactivating pathogens.

Peels from fruits and vegetables are a rich supply of minerals and carbohydrates that can be utilised to make lactic acid. They are used as carbon and nitrogen source in fermentation for producing lactic acid. The Lactic acid bacteria (LAB) converts the carbohydrate contents of the vegetables and fruit peels into lactic acid (Montet *et al*., 2014), consequently, bringing the pH down to below 4°C and stopping the formation of harmful organisms (Sirisha *et al*., 2021).

In addition to lactic acid, other organic acids can also be created by the anaerobic fermentation of fruit and vegetable waste. The particular kinds and amounts of organic acids that are produced are determined by variables such the microbial community, fermentation conditions, and substrate composition. Among these are propionic acid, citric acid, acetic acid, butyric acid, and formic acid (Kuley *et al*., 2020). However, to ensure the lactic acid is dominant over other acids, the following conditions including temperature regulation (three set ups ranging between 30 and 40°C), anaerobic conditions, using carbohydrate rich substrates and ensuring fermentation time was maintained for specific growth of LAB action over other bacteria while avoiding substrate depletion (Garcia *et al*., 2020). Key fermentation parameters above were monitored.

2.8 Lactic Acid Fermentation and Stabilisation of Urine

The acidic environment generated by the production of lactic acid exerts a toxic effect on other microorganisms and prevents the growth of pathogenic microbes (Sharma *et al*., 2020). Urine that has been fermented to produce lactic acid has a low population and dispersion of *E. coli* and other pathogens, making it safe and ideal for farming (Raman *et al.,* 2022).

Because lactic acid suppresses pathogens during fermentation and can function as a nitrogen fertiliser in agriculture, it has been found to be appropriate for urine treatment and stabilisation. In the past, lactic acid fermentation (LAF) has been shown to effectively suppress microorganisms in organic material preservation and fast-food storage (Zerihun *et al*., 2021). Moreover, it is a natural process that can be easily achieved using fruit and vegetable peels waste. Lactic acid is a potential technology for stabilising and treating urine. Using lactic acid in urine treatment and stabilisation can eradicate odour from human urine, kill bacteria, and retain nitrogen. This makes it sustainable and environmentally friendly for urine treatment, predominantly in areas where centralised wastewater treatment is not feasible, and nutrient recovery from human excreta is desirable (Anderson *et al*., 2015).

Lactic acid fermenting organisms (LAB) convert easily digestible carbohydrates to lactic acid through a metabolic process known as LAF (Scheinemann *et al*., 2015) found that bacterium pathogens, including *salmonella spp., Staphylococcus aureus,* and *Escherichia coli* (*E. coli*), were eliminated from cow manure after a few days of lactic acid fermentation. A study by Anderson *et al*., 2015, detected that within a week of receiving FS treatment with LAB produced by combining fermented milk containing *lactobacillus casei* with pasteurised whole milk, faecal coliform, and *E. coli* were dropped below the detection limit. He also noted that lactic acid fermentation (LAF) successfully inactivates

pathogens in faeces and urine and preserves valuable organic material during reuse. Treatment techniques have been developed to create hygienic end products from human excreta for reuse or disposal (Anderson *et al.*, 2015; Magri *et al*., 2015).

Lactic acid bacteria (LAB) quickly convert degradable carbohydrates to lactic acid with LAF. Various approaches have been proposed to limit the breakdown of urea in the urine and minimise ammonia volatilisation. Differences in temperature, pH, substrate composition, and nitrogen supplies all affect how well lactic acid is formed by LAF (Arekemase *et al*., 2020; Jörissen *et al*., 2015). However, studies have shown that LAF can effectively stabilise urine and reduce pathogen levels. The use of LAF in urine treatment and stabilisation can contribute to sustainable sanitation systems and nutrient recovery (Andreev *et al*., 2018).

2.9 Pathogen Removal

In the bladder of a healthy person, urine is typically pathogen-free. However, during excretion and the collection of urine in the toilet bowl, cross-contamination from faeces occurs, even in urine-diverting toilets. Cross-contamination from faeces presents a risk of up to an average rate of 9.1 mg of faeces per litre of urine, as assessed by faecal sterols (Senecal & Vinnerås, 2017). According to the Kenya Bureau of Standards (KEBS) Organic fertiliser specifications, Pathogenic organisms should not be present in organic fertilisers. (*Kenya Bureau of Standards*, 2023) Where appropriate, it must adhere to the microbiological restrictions listed in Table 2.3.

Table 2.3:

KEBS Recommended Microbiological Restrictions for Pathogens

Microorganisms	Allowable Level
E. coli	1000 cfu/g
Salmonella	Nil
Faecal streptococci	<500 cfu/g
Infective parasites	Nil

Source: (Kenya Bureau of Standards [KEBS],2023)

Compared to faeces, urine contains fewer pathogens and is thus easier to purify and reduce. Lactic acid is effective for removing odour in urine. This is because lactic acid suppresses pathogens in FS and organic material preservation by reducing the bulk pH of the surrounding medium, hence influencing the activity of membrane-bound enzymes and exoenzymes (Odey *et al*., 2018). This happens when lactic acid penetrates the cytoplasmic membrane of microorganisms in concomitant form, resulting in the decline of the intracellular pH of pathogens and bacteria, suppressing them (Anderson *et al*., 2015).

Abdullahi *et al*., 2020, in an experiment carried out to determine the impact of co-fermentation of food waste and municipal sludge on lactic acid production noted that lactic acid displayed strong antimicrobic activity against pathogenic organisms, including *Pseudomonas aeruginosa, Enterobacter aerogenes* and *Escherichia coli (E. coli)*. Byakika *et al*., 2019, also carried out an experiment to determine the antagonistic properties of lactic acid against *E. coli* present in contaminated fermented food called Obushera (fermented porridge), and found out that lactic acid can be used in food

processing to constrain pathogens (Byakika *et al*., 2019). LA can therefore be relied on for pathogen removal properties in urine, making it safe for reuse as nitrogen fertiliser.

2.10 Analytical Methods

Analytical methods are the basis of modern industrial operations, offering vital instruments for exact measurement and material characterisation. They give useful insights into the composition and quality of products, and enhance industrial operations through precision and dependability (Koel & Kaljurand, 2019).

2.10.1 Lactic acid detection

Analytical procedures for detecting the existence of lactic acid use chemical processes, spectroscopy, and chromatography techniques. LCMS/MS and UV-VIS spectrophotometry techniques were used for the quantitative and qualitative analysis of LA respectively.

UV-VIS spectrophotometry

Spectrophotometry is a technique that measures light intensity as a light beam travel through a sample solution to determine how much a chemical compound absorbs light. The idea is that, within a specific wavelength range, every substance either transmits or absorbs light (De Caro & Claudia, 2025). Using this technique, the amount of a known chemical substance can also be measured. A UV-VIS Spectrophotometer utilises light in the visible (400–700 nm) and ultraviolet (185–400 nm) regions of the electromagnetic energy spectrum and can quantitatively be used to determine the quantity of Lactic acid in a sample. The analysis techniques used in spectrophotometry are very sensitive and selective and thus, the quantities of lactic acid in various samples can be determined precisely and specifically (Gupta, 2022).

The study used the UV-VIS spectrophotometer to quantify LA production from the fermentation process. Iron (III) chloride was employed as a blank solution in the

spectrophotometric analysis of lactic acid because it produces a coloured complex that can be detected at a certain wavelength. In an aqueous solution, iron (III) chloride reacts with lactate ions to produce yellowish-green iron (III) lactate which has optical characteristics. Iron (III) chloride is also a sensitive and selective reagent for lactic acid, enabling for accurate and exact readings at low concentration. Absorption spectra for pure lactic acid solution were obtained by measuring the absorbance of iron (III) lactate (Ngouénam *et al*., 2021).

Liquid chromatography mass spectrometry (LCMS/MS)

Liquid Chromatography-Mass Spectrometry (LCMS/MS) is an analytical chemistry technique that combines the mass separation capabilities of the LC and Mass Spectrometry (MS) with physical separation abilities (Bhole *et al*., 2020)**.** Mass Spectrometry offers high molecular specificity and detection sensitivity for identifying the structure of individual components based on their molecular mass and charge ratio, while liquid chromatography is used to separate mixtures with multiple components by passing them through the chromatographic column (Wong *et al*., 2018). Liquid chromatography involves transferring a sample between a polar mobile phase and a non-polar stationary phase. The analytes are separated from the LC unit, ionised, and then directed to the MS/MS unit, where the molecules are broken down based on their mass/charge ratio for further determination (Vishwakarma, 2021).

2.10.2 Nitrogen detection

Nitrogen retention in urine is analysed by measuring the nitrogen content in the urine sample. This is typically done by quantifying the amount of urea nitrogen present, as urea is urine's primary nitrogenous waste product (Schulz *et al*., 2023). The most used technique for figuring out N in samples taken from animal nutrition research is the Kjeldahl technique (KJ). Nitrogen analysis by KJ does not require the drying of samples

(Morris *et al*., 2019). The samples undergo a three-step process of digestion, distillation, and titration. In the digestion process, the samples are digested with conc. sulphuric acid at high temperatures of up to 400^0C in the presence of catalysts (Hicks *et al.*, 2022).The sulphuric acid breaks down organic nitrogen-containing materials into ammonium ions (NH_4^+). The reaction for the digestion is as below.

H_2SO_4 + Organic N ⟶ $(NH_4)_2SO_4$ + CO_2 + H_2O (2.6)

The distillation process involves titrating with sodium hydroxide to convert the ammonium salt into ammonia.

$(NH4)_2 SO_4 + 2NaOH \longleftrightarrow 2NH_3 (gas) + Na_2 SO_4 + 2H_2 O$ (2.7)

Boric acid acts as an absorbing solution, trapping the ammonium vapours by forming an ammonium borate complex with the ammonia.

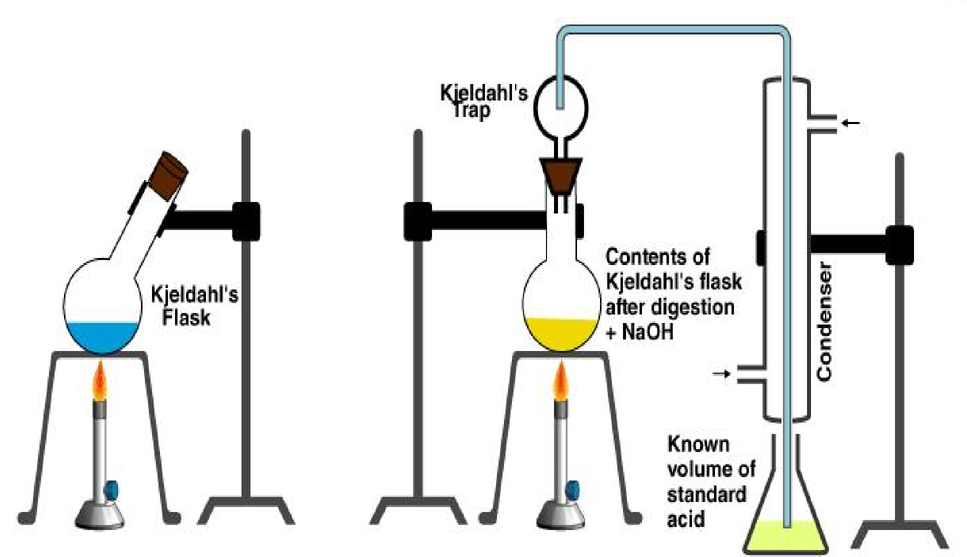
$B(OH)_3 + NH_3 + H_2O \longleftrightarrow NH_4^{\ +} + B(OH)_4^{\ -}$ (2.8)

The ammonia in the absorbing solution is subsequently quantified and determined by titration using an acid and a mixture of indicators (Hicks *et al.*, 2022).The study employed the use of Tashiro indicator (a mix of Methylene blue /methyl red indicators) in the titration for its ability to distinctly change the colour from light green to pink, indicating a complete reaction with hydrochloric acid.

$B(OH)_4 - + HCl \longleftrightarrow Cl^- + B(OH)_3 + H_2 O$ (2.9)

Figure 2.4:

Kjeldahl Technique



Source: (Sáez-Plaza et al., 2013)

2.10.3 Test for pathogens

One indicator organism can be used for pathogen analysis to roughly represent the degree of pathogen inactivation attained during treatment, as assessing many pathogens is typically time-consuming and expensive (Zerihun *et al*., 2021). The various methods of pathogen analysis that have been used in various research include:

Flow cytometry

By immediately identifying bacteria and other cells in various biological fluids, flow cytometry reduces the time it takes to make a microbiological diagnosis. It analyses and quantifies microbial cells in urine samples, measuring individual cells' physical and chemical properties as they pass through a laser beam, allowing for differentiation and quantification of different cell types, including pathogens (Rubio *et al*., 2019).

Molecular methods

Traditional microbiological quantification techniques rely on the culture of the pathogens on specialised media and their isolation, followed by biochemical identification, during which time the pathogens spread further from the source of contamination (Zhang *et al*., 2022). Molecular methods offer a more sensitive and rapid approach to detecting and quantifying pathogen. The polymerase chain reaction (PCR) is one of the molecular methods for accomplishing this. It enables a more thorough genetic sequence analysis by rapidly increasing copies of a DNA sequence specific to a given pathogen The PCR requires prior knowledge of target pathogens and their specific DNA sequences (Zhang *et al*., 2022). *E. coli* can also be quantified using Compact Dry ECO plates (Zerihun *et al*., 2021).

Microbiological culture-based detection methods

While many novel pathogen detection techniques have emerged, classic microbiological culture-based methods still play a key role in pathogen identification. Culture-based approaches are the earliest methods for detecting microorganisms, including harmful strains. Traditional culture entails cultivating microorganisms on or in specialised media to isolate and identify them based on their growth characteristics, morphology, biochemical activity, and the bacteria's ability to grow, reproduce, and form visible colonies on laboratory media. They give qualitative or quantitative data on the amount and kind of live microorganisms present in samples (Foddai & Grant, 2020). This approach provides a confirmed result for the presence of a specific pathogen. These cultures take between 18 and 24 hours to grow. One of the culture-based approaches is the use of Sorbitol MacConkey agar (SMAC), which is based on the principle of sorbitol fermentation and is used to identify *E. coli* O157:H7 (Priyanka *et al*., 2016).

The MacConkey agar contains bile salts and crystal violet, which prevent the development of Gram-positive bacteria while allowing Gram-negative bacteria,

particularly enteric bacteria, to proliferate. It may also identify different types of microorganisms based on their biological properties. MacConkey agar contains lactose and a pH indicator (neutral red) (Jung & Hoilat, 2022). Gram-negative bacteria that can ferment lactose generate acid, which lowers the pH and changes the colour of the medium around the colonies to pink or red. non-lactose fermenters create colourless or pale colonies. MacConkey agar is commonly used to identify and distinguish members of the Enterobacteriaceae family, which includes several intestinal pathogens like *Escherichia coli* and *Salmonella spp*. *E. coli* often ferments lactose, resulting in pink colonies (Jacob *et al*., 2020).

In this study, the efficacy of lactic acid for bacterial inactivation in human urine was determined, by using MacConkey agar to identify E. coli, which is a gram-negative bacterium and will therefore grow on the Agar.

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

The steps and methodology that were used to conduct the research project are described in detail in this chapter. The sample collection and preparation methods as well as physiochemical analytical methods are also outlined.

3.2 Study Design

The study was conducted in Meru University of Science and Technology. The research design involved was a laboratory experiment. The experiments were carried out in the Biological Sciences, Food Science and Sanitation Research Institute (SRI) laboratories. The parameters of interest were closely regulated and monitored.

3.3 Selection Criteria

The volunteers were initially selected using convenience sampling, based on their availability and consent to participate in the provision of urine samples. The vegetable and fruit peels samples were also chosen based on availability and consent of the vendors to provide their waste. Exclusion criteria was further employed to ensure the participants were not sick or under any form of medication. Vendors who were not willing to provide the fruit and vegetable waste were also excluded.

3.4 Materials

The materials, equipment and reagents used in the experiment were as below.

3.4.1 Substrates and chemical reagents used

The fruit and vegetables used as substrates in the experiment included: - fresh mangoes, ripe bananas, oranges, pineapple and cabbage wastes.

Analytical grade reagents (AR) and distilled water were used. The chemicals included: concentrated sulphuric acid (H_2SO_4), Potassium sulphate (K_2SO_4), Titanium dioxide (TiO_2), copper (II) sulphate ($CuSO_4$),4% Boric acid (H_3BO_3), 0.1 N Hydrochloric acid

(HCl), Sodium hydroxide (NaOH), Iron (III) chloride ($FeCl_3$), lactic acid standard ($C_3H_6O_3$), RANKEM, CAS No: 50-21-5; 89-92%), Bromocresol green methyl red indicator.

3.4.2 Instrumentation

Various common laboratory apparatus and specialised instruments were used. These included: - thermometer, pH meter, micropipettes, titrators, burettes, conical flasks, measuring cylinders, beakers, analytical balances, spatulas, blender, sieve, stirring rods, test tubes, test tube racks, cold chamber, Ultraviolet-visible (UV-VIS) spectrophotometer (APEL model P-3000V),LCMS/MS (Shimadzu LCMS-8030 Triple Quadruple), Kjeldahl digester (Gerhardt), distillation system (Gerhardt, Vapodest 20s) and tubes, glass cover, refrigerator, centrifuge (HERMLE Z326K model), horizontal laminar flow (BBS-H1800 model) incubator (Bio base model BJPX-2102C), hotplate,10-500 mL and 2- 1 kg plastic tins.

3.5 Sample Collection and Preparation

The various collection processes for each of the samples are mentioned below.

3.5.1 Urine samples collection

About 3 L of fresh urine provided by volunteers was collected from Urine Diverting Dry Toilets (UDDTs) within Meru University of Science and Technology. The urine was collected in tightly capped bottles and taken to the laboratory within ten minutes of collection. Without dilution, they were combined and stored in bottles in the refrigerator. The temperature and pH were determined before the experiment began. Around three litres of urine were collected. A 25 mL portion of the urine was taken and used to quantify *E. Coli* and measure nitrogen content before stabilisation. The urine samples were then sealed in airtight containers and stored in the refrigerator awaiting analysis.

The aim of urine characterisation allows the chemical characteristics of the samples to be investigated before and after stabilisation.

3.5.2 Fruit and vegetable peels samples

The mangoes, oranges, bananas, pineapples and cabbages wastes were obtained from local market vendors in Gakoromone market, in Meru Town. Mango, pineapple, banana, orange and cabbages were chosen because they are among the most consumed fruits and vegetable in Kenya (Korir *et al*., 2015;Standard Media, 2019), in addition to their high fermentable sugar content, organic acid potential, and biodegradability (Abdullahi *et al*., 2020). According to Fenton *et al*., 2021, banana is the most-produced fruit worldwide. Each of them was packaged in different clean bags. An estimate 2 kg of the waste was collected for the research.

These specific fruits and vegetables were chosen based on availability, but also because they are widely used in Meru County and generate significant amounts of waste, making them abundant sources of substrate for LA production. The fruits used were throwaways and not fresh, since the essence of the project was to utilise waste. The age of the fruits was determined through observation of their skin colours, their firmness as well as the strong aroma they exuded, indicating near rottenness. The substrates were washed in tap water then rinsed with distilled water and cut into small pieces using a knife. About 120 g from each of the samples was air dried at 45ºC for 2 days. The remainder of the samples were stored in the refrigerator at 4ºC until use.

Figure 3.1:

Fruit Peel Preparation

Source: (Researcher, 2024)

3.6 Lactic Acid Fermentation of Fruit and Vegetable Peels

Amounts equal to 60 g of each of the samples were measured into a blender totalling to 300 g. The sample mixtures were then blended with water in a ratio of 1:1 and ratio 1:2 (substrate: water). First, 300 g of the homogenised mixture was blended with 300 mL of water. This was then divided equally in three containers. This was done thrice for the three different set ups at 1:1 ratio. The same was done for ratio 1:2 where 300 g of the sample (60 g each) was blended with 600 mL of distilled water. It was then divided equally into three replicates for the three set ups. The peelings and the cabbage leaves were homogenised in order to provide a balanced medium containing all nutrients necessary for LA production (Nandini *et al*., 2014).

The substrates were mixed to mimic the naturally heterogeneous makeup of organic waste found in the environment as well as balance the carbon sources needed for anaerobic fermentation (Liu *et al.,* 2019). Organic waste in real life is rarely uniform, consisting of a variety of fruit and vegetable waste. The study aimed to replicate the

diverse nature of waste-to-resource projects by blending several peels during the fermentation process. This makes the findings more realistic and relevant to large-scale or community-based activities. Their temperatures and pH were recorded and all the containers tightly sealed. They were then fermented organically by closing them up in different anaerobic incubators for 72 hours at 34°C, 37°C and 40°C. The temperatures chosen fall within the mesophilic range of the fermentation temperatures that promoted growth of lactic acid bacteria (LAB)(Tang *et al*., 2016). This was to assess the effect of possible environmental variations on fermentation efficiency. After three days, the mixture was sieved to get rid of biomass residues. It was then centrifuged using the HERMLE Z326K centrifuge to filter the mixture further. The pH and temperature were then recorded.

3.7 Lactic Acid Tests

The lactic acid was qualitatively and quantitatively determined using the procedures below.

3.7.1 Qualitative determination of LA using LCMS/MS method

LA was determined using Shimadzu LCMS-8030 Triple Quadruple LCMS/MS model. Mass analysis was done in Multiple Reaction Monitoring (MRM) mode in negative electrospray ionisation (ESI). The MRM parameters were set as in Table 3.1.

Table 3.1:

MRM Parameters

Molecule	Molecular mass (g/mol)	Precursor ion (M-H)	CE	Daughter ion
Lactic acid	90.03	89.3	7	89.05

Source: (Researcher,2024)

Sample preparation was as below.

About 1 mL of the sample was filtered into two vials. After filtering, 20 µL of each sample was diluted with a 1:1 methanol and water solution at a dilution factor of 50. The samples were then injected into the Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The LCMS Parameters were set as in Table 3.2.

Table 3.2:

LCMS/MS Parameters

Column	Kinetex 2.6 µm XB-C 18 (150mm x 3mm)
Guard column	Phenomenex Security-Guard Ultra cartridge
Mobile phase	A: 0.1 % Formic acid in water B: 0.1 % Formic acid in Methanol
Gradient program (B %)	0 – 1.0 min > 10 %; 1 – 3 min > 10 - 50 %; 3 - 6 min > 50 - 90 %; 6 - 8 min > 90%; 8 -8.01 min > 90 - 10 %; 8.01 – 15 min > 10 %;
Flow rate	0.25 mL/min
Oven temperature	30°C
Injection volume	5 mL
MS interface	ESI
Polarity	Negative
Nitrogen gas flow	Nebulising gas 3 L/min; Drying gas 15 L/min
MS temperature	De solvation line 250 ^{0}C; Heating block 400 ^{0}C

Source: (Researcher, 2024)

The results were then monitored over a period of one hour, with the blanks being run prior to each sample run, and each sample run for 15 minutes. LA presence was then monitored and the results output displayed on the screen.

3.7.2 Quantitative determination of LA using UV-VIS spectrophotometry

The lactic acid produced was determined quantitatively using a UV-VIS spectrophotometer (APEL model PD-3000UV, JAPAN). The cuvettes used were 1 cm in path length. LA used was RANKEN, CAS NO: (50-21-5), with 88-92% purity and a density of 1.2 g/mL. One mL of lactic acid was used to obtain the range of five serial dilutions (test tubes 1 to 5).

Exactly 1 mL of Lactic acid (containing 1.2 g/mL lactic acid) (89-92%) was placed in a 10 mL volumetric flask and diluted with water to the mark. Its concentration was 1324 mg/L. Exactly 5 mL of distilled water was placed in five separate test tubes. The stock solution was used to generate a series of lactic acid solutions by two-fold dilutions, resulting in a stock solution ranging from a concentration of 662 mg/L to 82.75 mg/L. A mass of 0.3 g of anhydrous $FeCl_3$ was placed in a 100 mL flask, diluted to the mark, and swirled to completely dissolve the salt. 100 µL of pure lactic acid from repeated dilutions was added to 4 mL of $FeCl_3$ solution. In separate test tubes, 100 µL from each of the sample replicate from the fermentation stage was added to 4 mL of $FeCl_3$.The absorbance of each solution was measured at 390-450 nm in comparison to the blank solution ($FeCl_3$) to determine the wavelength of maximum absorbance for lactic acid. The reaction and measurements were conducted at room temperature.

A calibration curve for the analysis was established using the prepared concentrations of lactic acid. The absorbances of the standards were measured at 410 nm, which was the wavelength of maximum absorbance. The concentration of lactic acid in the samples was determined using the calibration curve.

3.8 Urine Treatment

The processes of urine stabilisation and physicochemical analyses are described below.

3.8.1 Urine treatment and stabilisation using lactic acid

The lactic acid obtained from the fruit peelings in 3.5 above was added to the urine samples collected in a ratio of 1:2 (25 mL lactic acid extract and 50 mL urine) and sealed in an airtight container and then stored for four, seven and ten days in three replicate measures. In a different container, 50 mL of urine was added and set up in the same way for control. After four, seven and ten days, all the urine samples, treated and untreated were analysed for *E. coli*, nitrogen and pH.

3.8.2 Direct LAF of urine using waste

A volume of 25 ml of the homogenised substrate from the blended mixtures was directly added directly to 50 mL of urine in triplicate set ups. The samples were tightly sealed and stored for four, seven and ten days in similar conditions. This was set up as a comparison of effectiveness between direct LAF of urine and first obtaining lactic acid through fermentation then adding it to urine.

3.9 Physicochemical Analyses

The following parameters were used to determine the physicochemical properties of the treated and untreated stabilised urine samples.

3.9.1 Temperature

A thermometer was used to monitor the temperatures.

3.9.2 pH

The pH was determined using a digital benchtop pH meter (86501 AZ EB) after calibration with pH buffers 4.01 ,7 and 10. Each time after dipping the probe in a sample, it was rinsed with distilled water and the pH values recorded on the monitor.

3.9.3 Total nitrogen content

The nitrogen content of the samples was determined by employing the Kjeldahl method (Sáez-Plaza *et al*., 2013).Total Kjeldahl nitrogen is the total of free-ammonia and organic nitrogen compounds that are transformed to ammonium sulphate $(NH_4)_2$ SO_4.

Exactly 2 mL of each of the sample was put in the Kjeldahl tubes and 0.5 g of a mixture of $CuSO_4$, TiO_2 and K_2SO_4 (ratio 1:1:9) added in as catalysts., then 20 mL of sulphuric acid, H_2SO_4 was added. The solution was digested for 2 hours at 400^oC. Prior to urine treatment, the urine sample was set up in triplicate, with 20 mL sulphuric acid and 0.5 g catalyst used as a blank solution. In the set up with treated urine samples at different conditions, the set ups were set up singly due to the large number of samples to be determined. The residue was then chilled and allowed to cool for around ten minutes. Using NaOH, the acid digestion combination was made strongly alkaline during the distillation step. The Kjeldahl flask was linked to a water condenser and heated to boil off NH_3 from the digest. A solution of 4% boric acid was made by dissolving 20 g of boric acid in 500 mL distilled water. A volume of 50 mL boric acid was added to the sample. Boric acid served as an absorbent solution for ammonia obtained after distillation. After adding two drops of Tashiro's indicator (mixture of methyl red (0.03%) and methylene blue (0.1%), the mixture was titrated against 0.1 N HCl until the colour turned pink. A magnetic stirrer was used to mix the solution during the titration process. The results from the titrations were recorded for nitrogen quantification.

3.10 Pathogen Assessment

E. coli was studied for pathogen assessment of urine. This was done using MacConkey agar plates. A mass of 51.53 g of MacConkey agar was dissolved in 1000 mL of distilled water and heated in a hot plate to allow complete dissolution of the agar. The agar was sterilised by autoclaving at 15 lbs. pressure (121°C) for 15 minutes. It was then cooled to

45-50°C. The solution was mixed well before pouring into sterile Petri plates. The plates were warmed to room temperature. The agar was poured into the sterilised petri plates and the agar surface allowed to dry. The plates were inoculated with 100 µL of the samples in triplicates at a dilution sufficient to generate distinct colonies. It was incubated aerobically at 37ºC for 24 hours.

3.11 Quality Control

All reagents for the investigation were analytical grade quality. Standards of known concentration were used for instrument calibration. Three urine samples were treated and stabilised with lactic acid, and all physicochemical and biological characteristics were determined in triplicates. Prior to stabilisation, the nitrogen concentration of the urine samples was measured to determine nitrogen retention. All the samples were either analysed immediately or stored at -4°C in the refrigerator if the analysis was not completed in time. For instance, the urine was analysed and used within thirty minutes of its collection. After treatment, *E. coli* tests were performed on all samples.

3.12 Statistical Analysis

The experimental data was statistically processed using the SPSS software. The mean difference between the different parameters and conditions was examined using one-way ANOVA, and pair-wise significant differences were found using the Tukey multiple comparison test. A paired samples *t*-test was used to compare the pH of urine and direct substrate set up because only the means of the two pH values was determined. The pH for LA produced from fermentation at different temperatures, absorbance of the different fermentation products, urine pH before and after treatment and nitrogen content in all the set ups were compared for significance differences at a 95% confidence level at probability value $p = 0.05$. Results were judged statistically significant at $p < 0.05$.

3.13 Ethical Approval

Prior to data collection introductory letter was obtained from School Engineering and Architecture (SEA), department of civil and environmental engineering (Appendix C). Ethical approval was obtained from the Meru University of Science and Technology Institutional Research Ethics Review Committee (MIRERC) as shown as in Appendix E. A study permit was also obtained from the National Commission for Science Technology and Innovation (NACOSTI) (Appendix D). Before sample collection, consent was sought from the volunteers, with complete prior information on the nature of the experiment (Appendix B). The information was given to enable the volunteers choose whether or not to engage willingly in sample provision.

CHAPTER FOUR: RESEARCH RESULTS

The chapter provides a detailed presentation of the data obtained, followed by an analysis of the results. Each section focuses on unique research problems and theories presented in previous chapters.

4.1 Lactic Acid Extraction from Fruit and Vegetable Substrates through Anaerobic Fermentation

The study sought to extract LA from fruit and vegetable substrates through anaerobic fermentation using water medium.

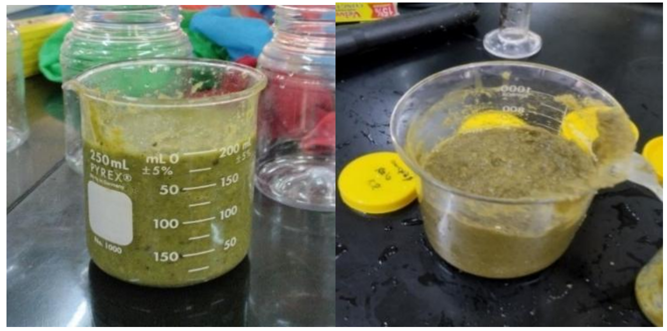
Two different parameters were compared in this procedure: the effect of two substrate to water ratios, and the effect of three different temperature between 30°C and 40°C on LA production and subsequent treatments. This was done to determine optimal conditions for LA production, and to assess whether changing the conditions affected its production. The fermentation process was carried out at three distinct temperatures (34°C, 37°C, and 40°C) and two different substrate-to-water ratios (1:1 and 1:2).

4.1.1 Substrate to water ratio

The fermentation setups were carried out with two substrate-to-water ratios, 1:1 and 1:2, achieved by blending 300 g of waste substrate with 300 mL of distilled water and 300 g of substrate with 600 mL of distilled water, respectively, for each temperature condition. The paste colour was green as in Figure 4.1. The paste for ratio 1:1 had higher viscosity than the set up at 1:2 ratios. Effervescence was observed in the beaker with the 1:2 ratio.

Figure 4.1:

Plates Showing Paste After Blending Sample with Water



Source:(Researcher,2024)

The jars were then set up anaerobically at 34ºC, 37ºC and 40ºC for three days. At all temperatures, after three days, the 1:1 ratio consistently had higher absorbance and concentration values than the 1:2 ratio.

4.1.2 Effect of temperature on LA production

The samples were set up at 34°C,37°C and 40°C for three days under anaerobic conditions (Bio base incubator model BJPX-2102C).

Figure 4.2:

Plates Showing Lactic Acid Extract Set Ups After Three-Day Fermentation Process

Source: (Researcher,2024)

The results after three days revealed that fermentation temperature had a considerable effect on lactic acid generation. The maximum lactic acid production was recorded at 37°C (concentration of 1304.7 mg/L) while the lowest was at 34°C (concentration of 316.9 mg/L).

4.1.3 pH

The pH for each set was measured before and after fermentation. The pH of the lactic acid before and after fermentation are shown in Table 4.1. For each triplicate, the means were obtained.

Table 4.1:

pH of LA Producing Samples Before and After Fermentation at Different Temperatures (n=3).

	34°C		37°C		40°C	
Substrate-water ratio	**Initial pH**	**Final pH**	**Initial pH**	**Final pH**	**Initial pH**	**Final pH**
1:1 (Mean± RSD)	4.70± 0.00	3.43± 4.45	4.30± 0.00	3.60± 0.00	4.70± 0.00	3.40± 2.94
1:2 (Mean± RSD)	4.70± 0.00	3.30± 3.03	4.40± 0.00	3.60± 0.00	4.70± 0.00	3.43± 3.36

Source: (Researcher, 2024)

Across all set ups, there is a consistent drop in pH indicating successful fermentation. The final pH ranged averagely between 3.3 and 3.6 from a range of 4.3-4.7 across the temperatures.

4.2 Lactic Acid Tests

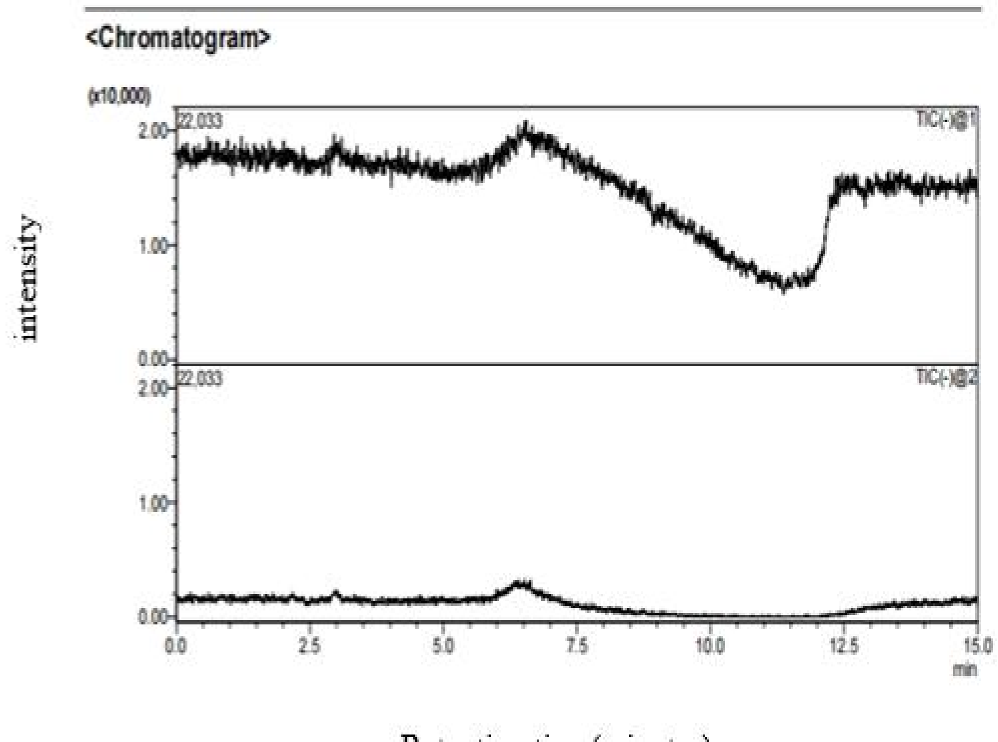
The LA obtained from fermentation was analysed both quantitatively and qualitatively. Its presence in the samples was first assessed through liquid chromatography/mass spectrometry (LCMS/MS), whose identification was marked through distinct chromatographic peaks and retention times, and then it was quantified through UV-VIS spectrophotometry.

4.2.1 Qualitative determination of lactic acid

LA was identified in the sample using LCMS/MS. The results were as shown in Figures 4.3, 4.4 and 4.5. The blank used was methanol (MeOH) and water in a 1:1 ratio.

Figure 4.3:

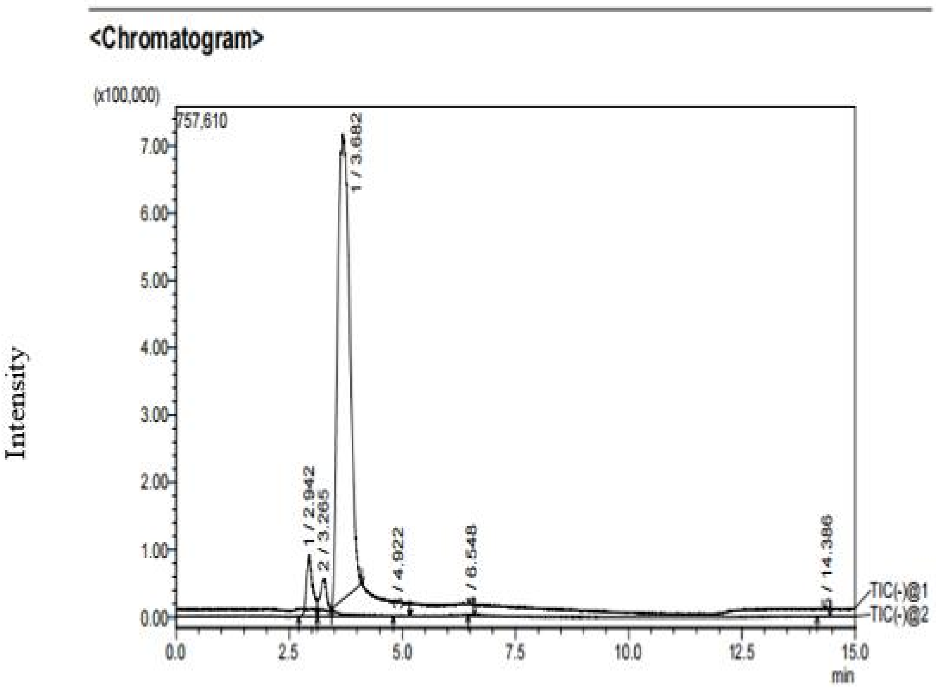
Chromatogram of Blank Sample (MOH: H2O, 1:1)



Source: (Researcher,2024)

Figure 4.4:

Chromatogram of Sample 1:1 showing Lactic Acid at 3.682 Minutes.



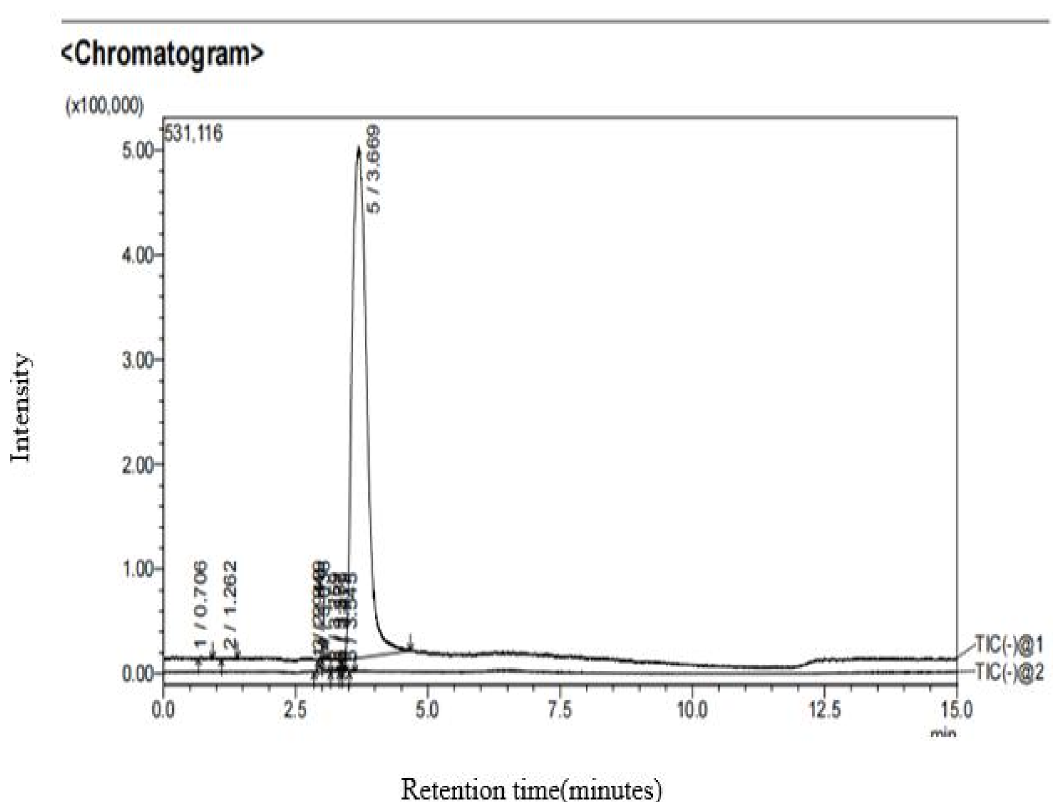
Retention time(minutes)

Source: (Researcher, 2024)

The peaks at 2.265–2.842 minutes represented acetic acid. The other minor peaks at 4.922, 6.548, and 14.396 minutes were likely due to the presence of other fermentation products or coeluting substances.

Figure 4.5:

Chromatogram of Sample 1:2 showing Lactic Acid at 3.669 Minutes



Source: (Researcher, 2024)

The peaks at 1.076-1.282minutes represented acetic acid. Multiple peaks observed between 3.0 and 3.5 minutes was likely a mix of co-eluting materials or ion related signals associated with the main analyte.

4.2.2 Quantitative determination of LA

Lactic acid was determined spectrophotometrically using a UV-VIS spectrophotometer (APEL's JAPAN model PD-3000UV).

The concentration in the LA standards (test tubes 1–5) was first passed through several wavelengths ranging from 350–430 nm to determine the wavelength of maximum absorbance. Table 4.2 shows the absorbance for the run across different wavelengths. It is also represented graphically, with the peak at 410 nm.

Table 4.2:

Absorbance of Iron III Lactate at Different Wavelengths

		Wavelength (nm)						
Serial dilution	**Conc. (mg/L)**	**390**	**400**	**410**	**420**	**430**	**440**	**450**
1	1324	0.179	0.434	1.188	0.848	0.647	0.474	0.291
2	662	0.109	0.291	0.510	0.346	0.419	0.189	0.060
3	331	0.160	0.226	0.410	0.262	0.171	0.028	0.013
4	165	0.100	0.032	0.175	0.098	0.055	0.030	0.047
5	83	0.026	0.06	0.090	0.036	0.018	0.051	0.011

Source: (Researcher,2024)

The maximum absorption of iron (III) lactate absorption was seen at 410 nm. The maximum absorbance was 1.188, indicating the highest standard concentration of LA in test tube 1, while the minimum absorbance was 0.090, indicating the lowest concentration of LA in test tube 5. The LA standards were diluted two-fold sequentially from test tube 1 (1324 mg/L) to 5 (83 mg/L) prior to the measurement.

The absorbance of LA standards at 410 nm maximum absorption wavelength is shown in Table 4.3 for the different dilutions.

Table 4.3:

Concentration and Absorbance of Lactic Acid Calibration Standards at 410 nm

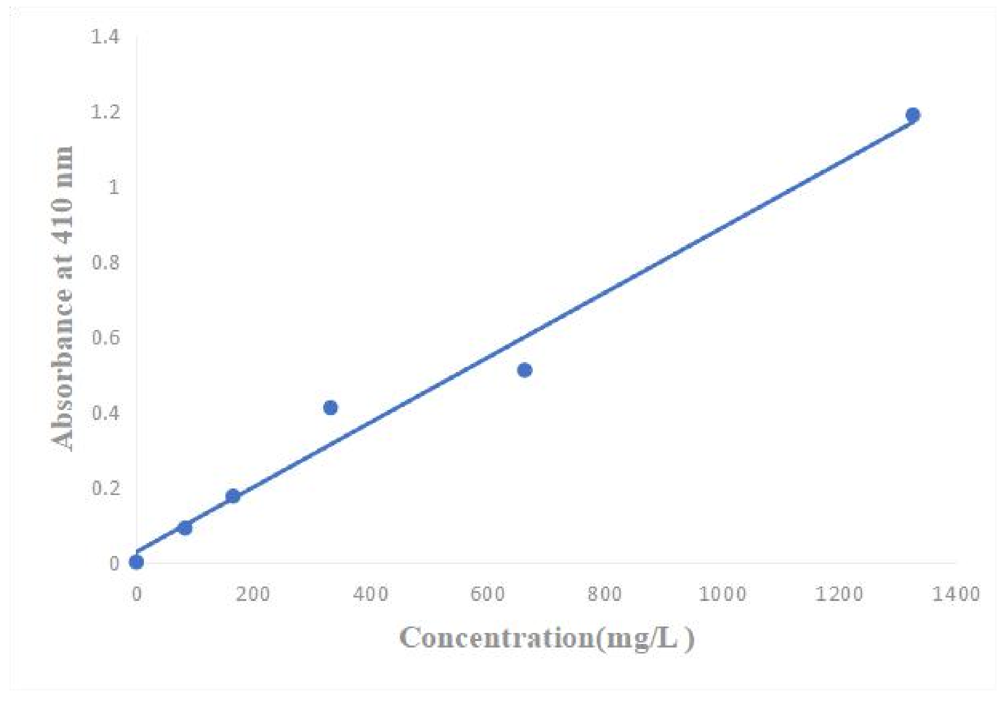
Serial dilutions	Concentration (mg/L)	Absorbance at 410 nm
1	1324	1.188
2	662	0.510
3	331	0.410
4	165	0.175
5	83	0.090
Blank solution	0.000	0.000

Source: (Researcher,2024)

The calibration curve was then plotted. Figure 4.6 shows the calibration curve of the lactic acid obtained.

Figure 4.6:

Calibration Curve for LA Standards



Source: (Researcher, 2024)

From the calibration curve, the equation obtained was $y = 0.0009x + 0.0268$, and $R^2 = 0.9804$, showing good linearity.

The absorbance of samples obtained from the fermentations at different temperatures were also measured at 410 nm. The regression equation for the calibration curve was used to obtain analyte (LA) concentration in the samples obtained from the fermentation. The results are shown in Table 4.4 and 4.5.

Table 4.4:

Absorbance (Abs) and Concentration (Conc.) for the Samples of Ratio 1:1

	34°C		**37°C**		**40°C**	
Samples	**Abs**	**Conc. (mg/L)**	**Abs**	**Conc. (mg/L)**	**Abs**	**Conc. (mg/L)**
1	0.711	760.2	0.835	898.0	0.511	538.0
2	0.800	859.1	0.895	964.7	0.650	692.4
3	0.654	696.9	1.201	1304.7	0.584	619.1
Mean	0.722	772.1	0.977	1055.8	0.582	616.5
SD	0.060	66.75	0.160	178.09	0.057	63.06
RSD	10.20	10.59	20.09	20.66	11.95	12.53

Source: (Researcher, 2024)

Table 4.5:

Absorbance (Abs) and Concentration (Conc.) for the 1:2 Ratio Samples

	34°C		**37°C**		**40°C**	
Samples	**Abs**	**Conc. (mg/L)**	**Abs**	**Conc. (mg/L)**	**Abs**	**Conc. (mg/L)**
1	0.312	316.9	0.472	494.7	0.420	436.9
2	0.450	470.2	0.558	590.2	0.366	376.9
3	0.465	486.9	0.369	380.2	0.346	354.7
Mean	0.409	424.7	0.466	488.4	0.377	389.5
SD	0.084	93.701	0.095	105.143	0.038	42.524
RSD	20.620	22.060	20.290	21.530	10.140	10.920

Source: (Researcher, 2024)

The sample produced the highest yield at 37°C, compared to 34°C and 40°C in both ratios.

A multiple comparisons Tukey's test on the absorbance of samples at the different temperatures showed significant difference, (mean difference = -0.24217, p = 0.005), especially for the absorbance at 37°C and 40°C, confirming that higher temperatures significantly impact absorbance values.

Table 4.6:

Multiple Comparison of Absorbance Tukey's HSD Test

(I) Temperature (°C)	(J) Temperature (°C)	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
34	37	-.15633	.060697	.059	-.31826	.00560
	40	.08583	.060697	.365	-.07610	.24776
37	34	.15633	.060697	.059	-.00560	.31826
	40	.24217*	.060697	.005	.08024	.40410
40	34	-.08583	.060697	.365	-.24776	.07610
	37	-.24217*	.060697	.005	-.40410	-.08024

*Based on observed means. The error term is Mean Square (Error) = .011. *. The mean difference is significant at the 0.05 level; Source: (Researcher,2024)*

At 34°C, there was no significant difference in the absorbance measured (mean difference = -0.15633, p = 0.059).

4.2 Stabilisation of Fresh Urine using the Extracted Lactic Acid

Lactic acid (LA) obtained from fermented fruit and vegetable substrate was added to urine samples collected at a 1:2 ratios (25 mL LA and 50 mL urine), which were confined in airtight containers (Figure 4.7). Analysis was carried out after four, seven and ten days for each sample set up.

Figure 4.7:

Plates Representing Sample Images after Treatment

Source: (Researcher,2024)

4.3 Determination and Characterisation of Urea Hydrolysis Inhibition

Urea inhibition was assessed by determining various parameters, which directly relate to the hydrolysis process. pH and nitrogen concentration were measured in the samples after treatment.

4.3.1 Urine pH

The pH before and after the treatment set ups was noted and recorded in Table 4.7. This was done to assess the efficacy of the fermentation efficiency of the lactic acid treatment.

Table 4.7:

pH of LA+ Treated Urine and Untreated Urine Samples after 4-, 7- and 10-days Period (n=3).

		Treatment Period								
		4 days			7 days			10 days		
LA extract +urine	**Initial pH**	**Final pH**			**Final pH**			**Final pH**		
Temperature °C		**34**	**37**	**40**	**34**	**37**	**40**	**34**	**37**	**40**
i)Substrate: water (1:1) + Urine Mean ± RSD	6.1± 0.95	3.8± 6.96	3.9± 2.56	3.8± 2.63	3.9± 5.29	4.0± 2.50	3.7± 8.33	4.0± 2.50	3.6± 5.84	3.8± 5.53
ii)Substrate: water (1:2) + Urine Mean± RSD	6.1± 1.64	4.2± 2.38	4.0± 8.70	4.1± 2.84	3.9± 5.13	3.8± 6.96	4.1 ± 6.93	4.0± 1.43	3.9± 1.49	4.0± 1.46
Untreated urine Mean± RSD	6.1± 0.00	7.5± 2.00	7.5± 2.00	7.5± 2.00	8.0± 0.00	8.0± 0.00	8.0± 0.00	8.6± 0.00	8.6± 0.00	8.6± 0.00
Direct LAF of urine Mean± RSD	6.1± 0.00	6.5± 0.00	6.5± 0.00	6.5± 0.00	5.8± 0.00	5.8± 0.00	5.8± 0.00	5.4± 0.00	5.4± 0.00	5.4± 0.00

Source: (Researcher, 2024)

All samples exhibited significant pH drop, showing hydrolysis inhibition. The average pH ranged from 3.6 to 4.2. The initial pH for all samples was 6.1, which was slightly acidic. The lowest pH was shown after ten days for the sample set up that had been fermented at 37°C (3.6), while the highest pH set up was the set up fermented at 34°C (4.2) after four days. Compared to the sample set up at ratio 1:1, the sample at 1:2 showed relatively higher pH, ranging from 3.8 (37°C) to 4.2 (34°C), with most of the samples recording pH>4. The pH change was very low in the set up that involved direct

addition of urine to the sample substrate, without initial fermentation. For the direct lactic acid fermentation (LAF) of urine, the pH ranged from 5.4-6.5 from 6.1. The set ups were stored at room temperature for the days of treatment.

A paired t-test analysis done to analyse the initial and final pH of the urine reacted directly with the unfermented substrate showed that the *P*-Value = 0.467 > 0.05, alluding that there was no significant difference in the pH values before and after the treatment (Table 4.8). A paired samples Test was used because only the means of the two pH values was determined. This implies that LA had not been sufficiently produced to stabilise the urine effectively.

Table 4.8:

Paired T-Test for Urine+ Directly Added Substrate Sample

Paired Samples Test

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	**Std. Deviation**	**Std. Error Mean**	**Lower**	**Upper**	**t**	**df**	**Sig.(2-tailed)**
Pair 1	Initial pH Final pH	.2793	.5436	.31384	-1.0710	1.6297	.8900	2	.4670

Source: (Researcher,2024)

4.3.2. Nitrogen determination using the Kjeldahl method

The Nitrogen (N) content in the samples before and after the treatment was measured using the Kjeldahl method. The Kjeldahl technique is used in determining the content of nitrogen in organic and inorganic substances. Before being set up for treatment, some of the fresh urine was tested for nitrogen using the Kjeldahl. The mean nitrogen concentration for the pure urine was 2643.33 mg/L. The nitrogen concentrations were calculated using the formulae (4.1):

$$\text{Nitrogen (mg/L)} = \frac{(V_f - V_b) \times N \times 14.007 \times 1000}{\text{(Sample volume (mL)}} \quad (4.1)$$

Where V_f =volume of titrant

V_b =volume of blank

N= normality of HCl (0.1 N in this case).

Sample volume=2 mL

Given the variables are the titrant volumes, the formula is simplified to:

Nitrogen (mg/L) = 700 (V_f - V_b)

The volume of the blank solution was deducted from the sample volumes to account for any potential background interferences and errors.

Results for the urine samples treated with LA are shown in Table 4.9.

Table 4.9:

Mean Kjeldahl Nitrogen Concentration (mg/L) After Four, Seven- and Ten-Days

		Treatment Period								
		4 days			7 days			10 days		
LA Extract	Initial TKN	Final TKN (mg/L)								
Temperature ^{o}C		34	37	40	34	37	40	34	37	40
Substrate : water (1:1) Mean± RSD	2643 ± 0.00	490 ± 14.29	2123 ± 13.73	1703 ± 10.34	1353 ± 7.90	2450 ± 7.56	1523 ± 11.68	1400 ± 8.66	2403 ± 13.76	2053 ± 13.78
Substrate : water (1:2) Mean± RSD	2643 ± 0.00	560 ± 12.50	1470 ± 9.52	793 ± 13.48	443 ± 18.23	1657 ± 8.80	1167 ± 9.17	887 ± 12.05	2100 ± 12.02	1703 ± 6.28

Treatment for Different Sample Ratios (n= 3)

Source: (Researcher,2024)

The concentrations ranged from averagely from 490 to 2450 mg/L for the different LA stabilised samples across treatment days. The sample treated with LA that had been produced at 34°C consistently showed the lowest concentration across the three days. Conversely, the samples obtained from fermentation at 37°C had the highest concentrations. Samples treated with LA produced from 1:1 substrate to ratio set ups showed higher concentration compared to those at 1:2 ratio, with the highest being for sample set at 37°C, after seven days treatment (2450 mg/L). The average concentration of the urine determined immediately after collection was 2643.33 mg/L. The highest concentration from the treated sample retained 92.69% of the nitrogen quantified in the

fresh urine. The lowest concentration, which was at four days, with the sample treated with LA obtained at 34°C retained only 18.54% of the nitrogen in our sample urine. For the set up where the blended substrates had been directly treated with urine, there was a consistent increase, with the highest volume being recorded after ten days as in Table 4.10.

Table 4.10 :

Titration Results for Urine and Direct Substrate Addition in Urine (n=3).

		Treatment Days								
		Four days			**Seven days**			**Ten days**		
LA extract	**Initial TKN (mg/L)**	**Final TKN (mg/L)**								
Temperature °C		**34**	**37**	**40**	**34**	**37**	**40**	**34**	**37**	**40**
Direct LAF of urine Mean± RSD	2643 ± 0.00	910 ± 0.00	910 ± 0.00	910 ± 0.00	1120 ± 8.83	1120 ± 8.83	1120 ± 8.83	2240 ± 13.26	2240 ± 13.26	2240 ± 13.26
Untreated urine (control) Mean± RSD	2643 ± 0.00	350 ± 0.00	350 ± 0.00	350 ± 0.00	840 ± 8.83	840 ± 8.83	840 ± 8.83	630 ± 11.10	630 ± 11.10	630 ± 11.10

Source: (Researcher,2024)

A Tukey's HSD test was conducted to compare the mean nitrogen concentrations across different treatment days and temperature (4, 7, and 10 days) as in table 4.11 and 4.12.

Table 4.11:

Comparison Between Days of Tukey's HSD Test

Multiple Comparisons

Dependent Variable: Concentration

Tukey HSD

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
4	7	-277.220	149.621	.167	-642.940	88.500
	10	-602.780*	149.621	.001	-968.500	-237.060
7	4	277.220	149.621	.167	-88.500	642.940
	10	-325.560	149.621	.089	-691.270	40.160
10	4	602.780*	149.621	.001	237.060	968.500
	7	325.560	149.621	.089	-40.160	691.270

Based on observed means. The error term is Mean Square (Error) = 201477.778.

**. The mean difference is significant at the 0.05 level.*

Source: (Researcher, 2024)

Table 4.12:

Comparison Between Temperatures of Tukey's HSD Test for Samples

Multiple Comparisons

Dependent Variable: Concentration

Tukey HSD

(I) Temp	(J) Temp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
q34	37	-1174.440*	149.621	.000	-1540.160	-808.730
	40	-592.220*	149.621	.001	-957.940	-226.500
37	34	1174.440*	149.621	.000	808.730	1540.160
	40	582.220*	149.621	.001	216.500	947.940
40	34	592.220*	149.621	.001	226.500	957.940
	37	-582.220*	149.621	.001	-947.940	-216.500

Based on observed means: The error term is Mean Square (Error) = 201477.778.

**. The mean difference is significant at the 0.05 level.*

Source: (Researcher,2024)

Across all set ups, the Tukey's test indicated significant differences in concentration over the number of days, especially between 4 and 10 days ($p=0.001<0.05$). For each of the temperature settings, there was significant difference in the concentration of nitrogen($p=0.000,0.001,0.002<0.05$). Nitrogen concentration significantly increased from 34°C to 37°C, with 37°C ($p = 0.000$) having higher concentrations, and from 34°C and 40°C, with 40°C ($p = 0.001$) showing higher nitrogen concentrations.

4.4 Pathogen Analysis of the Stabilised Urine by Testing for *E. coli*

Pathogen determination on the samples was done using MacConkey agar. The aim was to evaluate the effectiveness of lactic acid obtained from fermented fruit and vegetable substrate in inhibiting *E. coli* growth in urine samples. The MacConkey agar was first prepared and autoclaved before the samples were added to each plate within the horizontal laminar flow (BBS-H1800 model) to prevent the plates from picking other microorganisms which might cause contamination. After all the samples had been spread, they were carried to the bio base incubator and stored for 24 hours at 37°C. After 24 hours, the plates were studied to identify *E. coli* contamination.

Both the pure, fresh urine and the stabilised urine were assessed for *E. coli* presence. *E. coli* quantification was not done since the main aim was to simply assess and compare *E. coli* presence before and after stabilisation. Assessment was based on growth or no growth of *E. coli* on the plates. From the results, most of the samples treated with lactic acid did not show any growth of *E. coli* on the MacConkey agar plates. The untreated urine had great growth in *E. coli*.

The temperature at which LA had been fermented during its production had an influence on its inactivation capacity. Samples that had been fermented at 34°C showed some growth of *E. coli* on the plates. At 37°C, most of the samples had no growth on the plates. Samples that had been treated at 40°C showed growth, though it was most rampant at samples that had initially been set at substrate to water ratio of 1:2. The samples at 37°C had the least growth of *E. coli*, since this was the optimal temperature for LA production. The higher LA production meant more optimal action on inhibition of microbial activity.

The initial substrate to water ratio employed in LA production also influenced the *E. coli* inhibition. The most growth was observed in the substrate that had been directly added to urine without initial fermentation, especially the one assessed after four days of treatment.

The sample ratios at 1:2 had more growth than 1:1 ratio, especially those that had been conditioned to 34°C, and treated for four days.

CHAPTER FIVE: DISCUSSION

The chapter gives detailed analysis of the findings within the context of existing literature and theoretical frameworks. In addition, any unexpected findings are highlighted, and their possible impact on the study's conclusions are assessed.

5.1 Lactic Acid Extraction from Fruit and Vegetable Substrate through Anaerobic Fermentation

The choice of selected fruits and vegetable waste to use was based on their wide consumption in the region as well as their high nutritional value and carbon content (Wongkaew *et al*., 2021). It has been demonstrated that the readily fermentable sugars and starches in banana and mango peels encourage the growth of LAB and increase acidity (Zahid *et al*., 2021). Orange and pineapple peels also have carbohydrates and antibacterial compounds, such as citric acid and flavonoids which enable LAB growth (Gunwantrao *et al*., 2016). The cabbage waste was used because of its rapid ability to reduce the pH and sustain it at that low level (Zerihun *et al*., 2021).

The fermentation of the substrates was carried out in anaerobic settings because LAB grow in anaerobic, carbohydrate-rich environments with low pH and various anabolic compounds, such as fermented milk, meat, and vegetables. These conditions improve the efficiency of lactic acid synthesis, prevent contamination, and are necessary for the metabolic pathways involved (Rombouts *et al*., 2020). In these environments, the LAB is able to use different carbohydrate sources, fermenting them and converting them into lactic acid.

The fermentation was done for three days to enable maximum LA production, because after this time, if the substrates are used up in acid formation, the LA produced can be broken down and used as substrate to produce other organic acids, which are not under study (Abedi & Hashemi, 2020). Abdullahi *et al*., 2020, found this to be the maximum

LA yield after carrying out batch fermentation for 72hrs. The fruit and vegetable substrate were blended at different substrate to water ratio to determine the effect of substrate concentration on LA production. After fermentation, the samples were sieved into beakers and centrifuged to ensure they are free of particulates that could interfere with the spectrophotometric analysis. The resulting solution was analysed for different parameters.

5.1.1 Substrate-to-water ratio

Assessment across all temperature set ups showed that the 1:1 ratio consistently produced higher absorbance and concentration values than the 1:2 ratio. This is because having a denser substrate ratio, it offered a greater concentration of fermentable carbohydrates and nutrients per unit volume. A research by Daharbha *et al*., 2015 to optimise vegetable waste for lactic acid production also found that the 1:1 ratio of vegetable waste slurry to water produced the highest lactic acid (4.948 g). This aligns with the optimal ratio obtained in the study. Thus, for practical application, the 1:1 substrate-to-water ratio is more efficient in producing relatively more LA.

5.1.2 Temperature

The fermentation set ups were incubated anaerobically at the set temperatures for three days. These temperatures were chosen because they fall within the mesophilic range of the fermentation temperatures that promote optimal growth of lactic acid bacteria (LAB) (Souza *et al*., 2017). Maintaining a constant temperature during fermentation is critical for maximum lactic acid production as temperature fluctuations can influence microbial growth and metabolism, resulting in variations in lactic acid production as well as production of other organic acids (Feng *et al*., 2018).

The maximum lactic acid production was recorded at 37°C for 1:1 substrate ratio (concentration of 0.6164), while the lowest was at 34°C (concentration of 0.1498). This

is likely due to the fact that 37°C gives the best mix of microbial activity and process efficiency in producing lactic acid for many LAB. (Silva *et al*., 2018). Xavier *et al*., 2024, discovered that a temperature range of 37°C to 40°C was optimal for lactose conversion by bacterial cells during production of LA. The production was lowest at 34°C.

According to Zhou & Hua, 2025, most LAB preferred higher optimum temperatures to produce lactic acid (35-50°C) compared to lower temperatures (20-35°C). Barroso *et al*., 2022, in an experiment to establish a temperature-based model for LA production found out that the lower the temperature was, the lower the potential lactic acid yielded. At 40°, the high temperature likely exceeded the optimal range for bacterial activity, hence slightly inhibiting their efficiency in the fermentation process by hindering further LA production due to increased energy use (Tang *et al*., 2016). Sarkar, 2019 in an experiment determined that lactic acid production increased significantly with a rise in temperature from 25°C to 37°C, with maximal production recorded at 37°C (43.6 mg/L), but they observed a decline in production at 45°C (34.2 mg/L).

5.1.3 pH

The final pH ranged between 3.3 and 3.6 from an initial range of 4.3-4.7 across the temperatures, which is within the theoretical pH range for lactic acid, showing that LA production occurred (Pau *et al*., 2022). Wu *et al*., 2015, in his experiment to assess LA production from waste discovered that the predominant fermentation product of fruit and vegetable substrate at pH 4.0 was lactic acid with an average amount of 60.4% (based on mass). According to Feng *et al* 2018, fermentation of *Lactobacillus* had dominated the system at a pH of 3.2-4.5. The production of LA is what lowered the pH of the medium. LAB is tolerant to acidic conditions and can produce lactic acid even as the pH drops. The pH drop observed in the study is supported these theoretical findings. Boncz *et al*.,

2016, in an experiment to assess different methods for stabilising urine found that use of organic acids reduced the urine pH from 5.47 to between 3.2-3.90.

The 37°C set up showed the least variance and a moderate pH drop, indicating this temperature as suitable for lactic acid formation. The special pH of LA being between 3.5-4 is what enabled pH to be a key indicator for LA production because of LA's unique chemical properties, drawn from the Intermolecular hydrogen bonding that exists between α-hydroxyl and carboxylate groups (Niaz *et al.*, 2022).

5.2 Lactic Acid Tests

The results from lactic acid tests presented in chapter four (4.2.1 and 4.2.2) are discussed below.

5.2.1 Qualitative LA determination

The LCMS/MS chromatograms showed peaks that corresponded to different components of the sample. The component of assessment was the lactic acid obtained from the fermented fruit and vegetable peelings. Lactic acid was identified by the peak with retention times of 3682and 3669 minutes respectively for both substrate-water ratios. Lactic acid typically elutes at a specific retention time under given chromatographic conditions (Thomas *et al.*, 2022). This consistent retention time across the samples reinforced the identification of LA, showing that it as a major component within the samples. The height peaks for both samples were quite pronounced, with a high intensity of 7.0×10^6 and 5.3×10^6 for 1:1 and 1:2 ratios respectively, indicating a high concentration of the lactic acid at this retention time.

In chromatography, the peak height is proportional to the concentration of the analyte (Zhang, 2024). The sharpness of the peaks indicated that the lactic acid in the samples was relatively pure, with minimal contamination from other components. The intensity in the 1:2 ratio was slightly lower than the 1;1 ratio indicating a slightly lower yield. The

smaller peaks at 2.265-2.842 minutes and 1.076-1.282 minutes for 1:1 and 1:2 ratios were identified to be acetic acid. Other smaller peaks were also noted for both ratios, represented a mix of coeluting compounds or ion related signals associated with the main analyte (Marillier *et al*., 2024). The height peaks for both samples were quite pronounced, indicating a high concentration of the LA at this retention time for both the sample at 1:1 and 1:2. On the width, both peaks were sharp. In chromatographic analysis, sharp peaks typically signify the presence of a singular compound, free from interference or overlap with other substances (Thomas *et al*., 2022). This sharpness, therefore, indicated that the LA in the samples was relatively pure, with minimal contamination from other components. The LCMS/MS thus helped identify singly the presence of LA in the fermented fruit and vegetable peelings.

5.2.2. Quantitative LA determination

Absorption spectra for pure lactic acid solution were obtained by measuring the absorbance of iron (III) lactate (Ngouénam *et al*., 2021). The absorbance of Iron (III) lactate solution was proportional to the lactic acid concentrations determined by serial dilutions. The absorbance measurement had to be performed promptly following sample preparation because after about 15 minutes, the colour of iron (III) lactate showed little decrease in absorbance, most likely due to partial breakdown of the reaction product (De Berg *et al*., 2016). The maximum rate of iron (III) lactate absorption was seen at 410 nm. According to Borshchevskaya *et al*., 2016., the wavelength range of 380-405 nm is ideal for detecting lactic acid because it corresponds to the peak absorption of the coloured complex produced by iron (III) chloride and lactate ions. This complex is responsible for the colorimetric detection of lactic acid using the spectrophotometric approach. Borshchevskaya *et al*., 2016., found their wavelength of maximum absorption to be 390 nm in their spectrophotometric LA determination. The wavelength, which was maximum

at 410 nm, was well within this range, with just a little variance, which might be owing to variable environmental circumstances or experimental settings, such as pH, that might have caused the complex to interfere somewhat with the maximum wavelength.

The sample produced the highest yield at 37°C, at the 1:1 ratio. The 1:1 sample had a higher concentration than the 1:2 ratio for each of the temperature settings. The absorbances at 34°C were slightly higher than those at 40°C. At 34°C, there was no significant difference in the absorbance measured (mean difference = -0.15633, p =0.059). The results showed that temperature had a statistically significant effect on the absorbance of lactic acid, particularly at higher temperatures ($p < 0.05$), confirming the observations made. Such information can be crucial in optimising conditions for lactic acid production in industrial and laboratory settings.

5.3 Stabilisation of Fresh Urine using the Extracted Lactic Acid

The first batch of set ups were analysed after four days treatment. pH measurements were taken alongside *E. coli* presence tests, and nitrogen concentration was assessed using the Kjeldahl method. The remaining samples underwent the same analytical techniques after seven and ten days, allowing for a comparative examination across treatment durations.

5.3.1 Urine pH

The pH for the set ups where the lactic acid initially fermented was added to urine was reduced greatly. The pH range was between 3.6-4.2, which is within the range found by Zerihun *et al.*, 2021 who found the pH to be 4.12 and 4.26 respectively for the 1:1 and 1:2 ratios. This is one of the parameters indicating that stabilisation occurred. There was a consistent trend across all temperatures, where the pH dropped in all setups. The initial pH for all samples was 6.1, which was slightly acidic. The pH values decreased after

treatment with lactic acid, which is consistent with the expected production of lactic acid lowering the pH, making it an effective stabiliser.

Analysis for the 1:1 ratio after the four-day treatment period showed the lowest pH at 34°C and 40°C (3.8). For the 1:2 ratios, the pH values were higher compared to the 1:1 ratio, with the lowest pH again being at 37°C after seven days (3.8). This shows that the dilution effect of the higher water content caused less pronounced acidity, reducing overall efficiency on the treatment. For the seven-day treatment, the pH values were also lower. For the 1:1 ratio, the lowest value was at 40°C (3.7), with some fluctuations from the expected trend, likely due to differing fermentation dynamics and microbial activity. For the 1:2 ratios, the 37°C set up showed the lowest pH (3.8).

pH values dropped after the ten-day treatment duration, with the lowest pH at 37°C, for the 1:1 ratio (3.6) suggesting that extended treatment period allowed for maximum action on urine. Longer fermentation periods (10 days) resulted in the most significant drop, indicating a decrease in the urine's buffering capacity. The 1:1 ratio sample also showed lower pH values compared to the 1:2 ratios, showing higher concentrations of LA. This is due to the higher substrate concentration available for fermentation.

LA has lower pH than other organic acids due to the presence of a hydroxyl group (-OH) adjacent to the carboxyl group, which is highly soluble in water and can establish hydrogen bonds with water molecules. This structural aspect, along with its single dissociation step, gives lactic acid a simple buffering range around its pKa of 3.86. Because of these characteristics, in an aqueous solution LA establishes an equilibrium where a significant portion of the lactic acid molecules donate protons to the urine, resulting in a lower pH (Yadav *et al*., 2022).

The final pH of the untreated urine (control) was between 7.5 and 8.6. This was a consistent increase from 6.1, indicating limited microbial activity, which resulted in

ammonia accumulation and a continuously increasing pH due to urea hydrolysis. Yang *et al.*, 2021, showed that hydrolysis increased urine pH from 6 to between 9-9.3. Andreev *et al.*, 2017 found that for untreated urine, the pH increased rapidly to 8.9 in only one week. This showed further the stabilising action of lactic acid. For the set up where the substrate was directly added to urine, the pH change was very low and ranged from 5.4-6.5. This can be attributed to the fact that the reaction is dependent on natural fermentation and is therefore very slow.

The lactic acid was being produced first before it could act on the urine, hence it would require a longer time compared to the set up where already produced lactic acid was added to urine. An experiment by Andreev *et al.*, 2017, who also carried out a direct LAF of urine found the final pH drop to 4.7 after 36 days of treatment. This suggests that continuous treatment of the LAF urine would have resulted in lower pH than this.

5.3.2. Nitrogen determination using the Kjeldahl method

Nitrogen concentration in the urine samples was determined through the Kjeldahl method because it’s accurate and reliable for nitrogen determination in various materials, including foods, beverages, and pharmaceuticals, and has been tested and standardised for a wide variety of food matrices, indirectly determined by their nitrogen concentration. It is the reference method recognised by many international organisations (Sáez-Plaza *et al.*, 2013). The digestion stage involved the concentrated sulphuric acid oxidising the organic molecule, liberating reduced nitrogen in the form of ammonium sulphate. The digestion process was carried out at 400°C to ensure complete breakdown of the organic compounds by sulphuric acid. This temperature also minimised interference from other substances present in the sample, hence ensuring nitrogen determination is accurate (Hicks *et al.*, 2022).

The results showed that the total Kjeldahl nitrogen content of the urine samples increased with treatment time and LA concentrations obtained at higher temperatures. The sample treated with LA that had been produced at 34°C consistently showed the lowest concentration across the three days. This supports the low LA production at this temperature, insinuating slow stabilisation capacity.

Samples treated with LA produced from 1:1 substrate to ratio set ups showed higher concentration, with the highest being for sample set at 37°C, after seven days' treatment (2450 mg/L). The concentration of the urine determined immediately after collection was 2643.33 mg/L. The theoretical nitrogen for human urine is estimated to reach concentrations of up to 9000 mg/L (Viskari *et al*., 2018) and averagely 3,700-3,830 g N per person per year according to Swedish data (Nagy *et al*., 2019). Alemayehu *et al*., 2020., in their analysis on the effect of urine on cabbage growth found the concentration of the total nitrogen in stored urine to be about 2,862 mg/L. This is within the maximum range obtained in this study, attesting to successful nitrogen retention in the urine through acid stabilisation. The difference in concentration of the fresh urine from the theoretical 9000 mg/L can be attributed to nutritional factors, such as the diet for the volunteers, since they were not on a scheduled and monitored diet system during the time of study.

The TKN of untreated urine dropped greatly to between 350-840 mg/L across the days. This was a huge drop from the initial 2643.33 mg/L. This is between 68-86% loss in concentration. This can be attributed to massive nitrogen loss due to ammonia volatilisation, and hydrolysis having occurred (Yang *et al*., 2021). Lv *et al*., 2020 in their experiment to stabilise source separated urine found that more than 70% of urea in unstabilised urine was hydrolysed within one day, showing that the urine samples had a substantial hydrolysis capacity. This percentage loss coincides with our findings.

Samples where the blended substrates had been directly treated with urine had a consistent increase across the days, with the highest concentration being recorded after ten days (2240 mg/L). This could be attributed to the length of time taken for the samples to produce lactic acid with urine as a medium before their stabilising action on urine. As LA was formed it acted on the urine, retaining nitrogen and preventing hydrolysis. Andreev *et al*., 2017, who performed a direct LAF of urine discovered that the ammonium content in LAF urine reduced by around 22-30% from 1.2 to 0.9 g/L, compared to the fresh urine. This supports the hydrolysis inhibition action observed in our study, supporting the LAF potential of urine, as well as the stabilising action of LA in urine.

Across all set ups, the Tukey's test indicated significant differences in concentration over the number of days, especially between 4 and 10 days (p=0.001<0.05). From this analysis, we concluded that there is a significant difference in nitrogen concentration between samples treated from 4 days to 10 days, with the latter showing higher nitrogen levels. This implies that samples taken at ten days had more significant differences in the nitrogen concentration than those at 4 and 7 days. There was no significant difference between 7 days and 10 days. This shows that the results obtained after seven and ten days were statistically similar. The amount of nitrogen determined using the Kjeldahl method did not change significantly between these two intervals.

Each of the temperature settings had a significant difference in the concentration of nitrogen(p=0.000,0.001,0.002<0.05). Nitrogen concentration significantly increased from 34°C to 37°C, with 37°C (p = 0.000) having higher concentrations, and from 34°C and 40°C, with 40°C (p = 0.001) showing higher nitrogen concentrations. This shows that stabilisation occurred at each of the set ups, just at different magnitudes. It supports the results observed, showing that temperature significantly influences nitrogen

concentration, with higher temperatures generally resulting in higher nitrogen levels as measured by the Kjeldahl method.

This supports the fact that LA is suitable for retaining nitrogen in urine, hence stabilising it. This is of key significant as it highlights the probability of introducing LA in the sanitation value chain as a stabiliser, especially for UDDTs. The stabilised urine can then be used as a nitrogen fertiliser, since nitrogen loss is curtailed. The blend of fruit and vegetable peels also introduces a different scent to urine, even as the hydrolysis inhibition serves further to reduce odour in urine.

5.4. E. coli Analysis of the Stabilised Urine

E. coli identification was made using MacConkey agar using a total of 135 agar plates. The MacConkey agar was incubated at 37°C to stimulate physiological conditions conducive for growth of enteric bacteria, including *E. coli.* It's ideal for the development of many harmful bacteria present in excreta, making it appropriate for identifying their presence or absence (Kubizniaková *et al*., 2020).

The consistent incubation conditions across all samples ensured the reliability and comparability of the results obtained. By maintaining standardised incubation parameters, such as temperature and duration, any observed differences in *E. coli* growth can be confidently attributed to the experimental variables under investigation, such as lactic acid treatment (Turhan & Koca, 2024). The LA was tested as a precautionary measure to ensure that it did not become a source of contamination to the urine sample. *E. coli* presence was marked by growth of red or pink colonies on the plates.

113 samples out of the 135 plates did not show any growth of *E. coli* on the MacConkey agar plates. This proves that lactic acid indeed has the potential to inhibit the growth of *E. coli,* by lowering pH levels and excreting inhibitory chemicals, thus supporting its antimicrobial properties as reported in previous studies (Andreev *et al*., 2018;

Borshchevskaya *et al*., 2016). The low pH created by lactic acid action on urine disrupted the cell membrane of gram-negative bacteria such as *E. coli* hence leading to their inhibition and inactivation. MacConkey agar, being selective and differential, allowed for detection and differentiation of *E. coli* colonies based on their ability to ferment lactose and produce acid (Jung & Hoilat, 2022).

Samples treated at 40°C showed growth, especially for samples that had initially been set at substrate to water ratio of 1:2. This is possibly because at this temperature LA production dropped due to bacterial stress caused by the high temperature. The lower temperatures (34°C) may have yielded lower lactic acid productivity therefore reducing its antimicrobial effects on the urine. This is further supported by the fact that at 1:2, the LA concentration was lower, hence less efficient LA production. The sample that had been treated for four days, whose substrate to water ratio was 1:2 and the temperature was 34°C had the most growth after the sample substrate with directly fermented LA in urine. This is because, in addition to the low temperatures slowing production, the lactic acid did not have enough time to act upon the urine. The samples that showed *E. coli* growth suggests incomplete inhibition, possibly due to variations in LA production across different temperatures (Liu *et al*., 2021).

There were some anomalies during the study, that could primarily be attributed to sample handling and fermentation conditions. For instance, the samples that had been treated with directly added urine to the unfermented substrate showed more *E. coli growth* compared to samples treated with LA. This suggests that the fermentation process may have been slower or incomplete hence allowing for microbial growth.

Some plates also showed inconsistent growth of *E. coli* across triplicates, showing possible contamination during sample handling or plate preparation, which might have introduced bacteria or affected the growth patterns of *E. coli* in some samples, causing

disparities which were rather not a true reflection of the microbial activity of the LA. For example, some of the LA prepared at 1:2 at 40°C, and treated for four days, showed growth yet the other two treated to the same conditions had no growth. Generally, the results underscore the potential of LA derived from fruit and vegetable substrate as an effective agent for inhibiting *E. coli* in urine samples intended for use as a nitrogen fertiliser. This validates its potential for safe and sustainable urine management and use in agriculture. This has significant implications for sustainable agriculture, where stabilised urine can be safely used as a fertiliser without the risk of pathogen transmission (Budiati *et al*., 2022).

CHAPTER SIX: CONCLUSION, RECOMMENDATIONS AND PUBLICATION

6.1 Conclusion

This study sought to assess the utilisation of fruit and vegetable substrates (mango, banana, orange, pineapple and cabbage) in producing lactic acid, which was then used in urine treatment to reduce pH and prevent nitrogen from getting lost as a result of hydrolysis. The results showed that fruit and vegetable substrates, specifically mango, banana, orange, pineapple, and cabbage, are effective sources for producing LA through fermentation. The influence of fermentation temperature and substrate-to-water ratio on the effectiveness of LA production was clearly demonstrated, with 37°C and a 1:1 ratio yielding the best results. This supported the theoretical knowledge of 37^oC being the temperature at which LAB, which is found in fruit and vegetable substrates acts optimally to produce LA, signified by the pH reduction (Wang *et al*., 2024).

The urine pH was reduced significantly after treatment with LA from the fermentation process. From the study, all set ups across the different number of days retained some nitrogen, though the concentrations varied widely subject to the conditions they had been subjected to over the treatment period. At seven and ten days, significant increase in nitrogen concentrations through the Kjeldahl technique was observed, especially for the higher temperatures (37^oC and 40^oC). By employing MacConkey Agar for pathogen assessment, *E. coli* was effectively suppressed in LA-treated urine for most plates.

The study deduced that stabilising urine using LA from fruit and vegetable substrates is an effective way of inhibiting urea hydrolysis and retaining Nitrogen, which can potentially be used as fertiliser. The study objectives were successfully achieved: lactic acid was efficiently produced through anaerobic fermentation of selected peels, applied to urine samples, and shown to significantly lower pH, reduce nitrogen loss, and eliminate microbial growth. The results of this investigation were consistent with earlier

studies on urine stabilisation, especially in how effective pH lowering through acidification are to efficient nitrogen preservation and microbial inhibition (Zerihun *et al*., 2021).

Although lactic acid made from fermented fruit and vegetable waste was used in this study as a biological stabilising agent, other techniques have been used with comparable objectives (Yang *et al*., 2021). For example, it has been demonstrated that acidification with strong mineral acids, such as sulfuric, nitric, or phosphoric acid, inhibits urea hydrolysis by quickly bringing the pH below 5.5, which stops ammonia volatilisation (Beltrán-Prieto & Kolomazník, 2021). This method enforced the possibility of reusing the nitrogen as a liquid fertiliser in addition to preserving it in a more stable state. It offered a low-cost, ecologically friendly, and sustainable way to recycle nutrients in agriculture in line with the circular economy's objectives and ecological sanitation principles.

By converting organic waste into a bio-based stabilising agent, this approach not only addressed the environmental burden of both organic and human waste but also contributed to nutrient recycling for agricultural use—aligning with Sustainable Development Goal 6.2 on equitable sanitation and hygiene, and promoting broader ecological sustainability.

6.2 Recommendations

Large scale production of lactic acid using fruit and vegetable peelings especially in food-based industries to enhance circular economy and sustainability. This will also reduce the menace of poor waste disposal, especially in large scale fruit-based industries. Additionally, using organic waste as a fermentation substrate will encourage cyclical resource flows, which helps low-resource environments with waste management and sanitary issues.

Test the stabilised urine's effectiveness in controlled germination of seedlings and comparing it with chemical commercial fertilisers: - This will yield valuable insights regarding the agronomic benefits of stabilised urine in relation to plant absorption, soil compatibility, and nutrient availability. This will allow assessment of aspects like germination rate, seedling vigour, and biomass yield in comparison with crops fed with commercial fertilisers to determine whether lactic acid-stabilised urine is a feasible and cost-effective alternative. The discoveries will especially benefit low-resource communities seeking circular solutions to sanitation and agriculture.

Develop a system to integrate urine stabilised by lactic acid into UDDTs, utilising natural fermentation of food waste- While UDDTs are already in existence, integrating stabilised urine into UDDTs, where food waste is naturally fermented, reduces direct contact with urine while also addressing local sanitation concerns. This strategy has the potential to increase community acceptance and sustainability of UDDT, as well as this stabilisation technique. Further research towards a piping technology that directly transfers the stabilised urine to farms is recommended.

Optimisation of Lactic Acid Production Conditions: - Further explorative research to improve the circumstances for lactic acid production from fruit and vegetable waste, especially on a local basis will greatly enhance waste containment. Temperature, pH, and microbial consortia can all be optimised to increase lactic acid output from urine, thereby setting a standard for stabilisation efficiency. Understanding the appropriate circumstances for lactic acid production ensures consistent urine stabilisation, which promotes sustainable urine usage techniques.

6.3 Publication

Khamala, T. N., Mungai, G. N., & Mwangi, E. K. (2025). Inhibition of Urea Hydrolysis of Human Urine using Lactic Acid from Selected Fruit and Vegetable Waste Fermentation. *African Journal of Science, Technology and Social Sciences,* 4(2), PAS 57–64. https://doi.org/10.58506/ajstss.v4i2.265.

The publication is provided in Appendix A.

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APPENDICES

Appendix A: Journal Article Publication

African Journal of Science, Technology and Social Sciences. 4 (2) 2025, pgs 57-64

AFRICAN JOURNAL OF SCIENCE, TECHNOLOGY AND SOCIAL SCIENCES

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Inhibition of urea Hydrolysis of human urine using lactic acid from selected fruit and vegetable waste fermentation

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ABSTRACT

In Kenya, enormous amounts of fruit and vegetable waste are improperly disposed of, contributing to environmental pollution and odour. Meanwhile, urine contains nitrogen that can be used as a fertiliser, but its utility is impeded by urea hydrolysis, which causes a rise in pH, nitrogen loss, and ammonia formation. This study investigated the potential of lactic acid fermentation utilising fruit and vegetable waste to prevent urea hydrolysis, enabling nitrogen recovery. Anaerobic fermentation of selected fruit and vegetable waste was carried out in an incubator at a regulated optimum temperature of 37°C for 72 hours. The lactic acid formed was then utilised to treat urine samples for 4, 7, and 10 days. To assess urea hydrolysis inhibition, total nitrogen content was measured using the Kjeldahl method, and pH monitored with a pH meter. The results showed a considerable decrease in the stabilised urine's pH, ranging from 6.1 to between 3.6 and 3.9. The pH for the untreated urine rose to between 7.5 and 8.5 across the days. Statistical analysis using the one-way ANOVA indicated significant difference in the pH across the days ($P = 0.047$). The highest total nitrogen concentration for the stabilised urine was 2450 mg/L after seven days of treatment, demonstrating urine stability and nitrogen preservation. The total nitrogen concentration for untreated urine was approximately 607 mg/L across the days, indicating clear nitrogen loss from the original 2643 mg/L obtained in fresh urine. One-way ANOVA test demonstrated a statistically significant fluctuation in TKN concentrations over treatment durations ($P = 0.021$). The findings showed that lactic acid significantly suppressed urease activity, making it a cheap, ecologically friendly alternative for urine stabilisation. The results showed the effectiveness of lactic acid obtained from fruit and vegetable waste in inhibiting urea hydrolysis in urine, hence, enhancing the recovery of nitrogen nutrient.

Introduction

On-site sanitation systems supply 65% - 100% sanitation services in Sub-Saharan Africa. Of these, Urine-Diverting Dry Toilets (UDDTs) are relatively low-cost, minimise urea hydrolysis and allow for reuse of nutrients from excreta, hence low nutrient loss to the environment (*Mungai et al.*, 2019). Collection and treatment of excreta play a crucial role in attaining Sustainable Development Goals (SDGs) regarding clean water, sanitation, sustainable cities and communities (*Xu et al.*, 2022).

Human excreta constitute both urine and faeces. Most nutrients are expelled through urine. Human urine is an ultra potent blend of chemicals, including

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Appendix B: Consent Form

Research topic: Urine stabilisation and treatment using lactic acid from fruit and vegetable peel fermentation: a potential source of nitrogen fertiliser

Researcher: Tabitha Khamala

Institution: Meru University of Science and Technology

P.o. box, 972-60200 Meru, Kenya.

sri@must.ac.ke.

+254 712524293

Supervisors: Dr George Mungai, Meru University of Science and Technology

: Dr Erastus Mwangi, Meru University of Science and Technology

Informed Consent for: Volunteers

1. Introduction

My name is Tabitha Khamala, a post graduate student in Meru University of Science and Technology pursuing a Masters in Sanitation. I am carrying out a study on the possibility of obtaining nitrogen fertiliser from human urine through stabilisation using lactic acid from fruit and vegetable waste.

The results from the study will play a big role in validating direct usage of urine in agriculture after stabilisation.

Who can participate?

Anyone healthy, who is not sick, under any medication, or undergoing their menses can participate.

Procedures

The study will need you to provide urine samples in a container you will be provided with. You will be guided on how to aseptically collect the urine and deliver it should you consent to the study. Taking part in this research study is fully voluntary and you are free to decline.

Risks

There are no risks involved in the study, it only involves collecting your urine.

Confidentiality

Your name and identity will not be exposed anywhere. The urine will be labelled numerically, so nothing can point back to you as the source. The study takes one month, but your interaction is required only during urine collection. This will take at most ten minutes of your time.

Contact Information

Should you have any further inquiries on the study, you can email the ethical review secretariat at mirerc@must.ac.ke.

2. Consent of participant

I've read and understood the research study's description. After discussing the study with me, the investigator satisfactorily addressed all of my concerns. I've been informed about the study's potential disadvantages as well as its prospective advantages. I have not been forced to participate in this study; I have given my consent voluntarily. I am aware that there are no implications if I decide to discontinue participating in this study at any point throughout the study.

____________________	______________________	___________________
Name of Participant	Signature/thumbprint of subject	Date and Time

_____________________________	________________________________
Name of Representative/Witness	Relationship to Subject

_____________________	______________________	___________
Name of person Obtaining Consent	Signature of person	Date

_______________________	___________________________	___________
Name of Investigator	Signature of Investigator	Date

Appendix C: Introductory Letter

MERU UNIVERSITY OF SCIENCE & TECHNOLOGY

P.O Box, 972-60200 Meru-Kenya
Phone: +254 (0) 712 524 293, +254 (0)799 529 958, +254 (0)799 529 959
Email: engineering@must.ac.ke *Website:* www.must.ac.ke

SCHOOL OF ENGINEERING AND ARCHITECTURE

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

TO: Whom It may concern **DATE:** 1st July, 2024

Dear Sir/Madam,

RE: INTRODUCTORY LETTER FOR TABITHA NEEKESA KHAMALA, REG NO. EG407/201598/21

The above-named, is a student in the Department of Civil and Environmental Engineering at Meru University of Science and Technology, pursuing a Master's degree in Sanitation. She has been approved to conduct research on **"Urine stabilization and treatment using lactic acid from fruit and vegetable peels: A potential source of nitrogen fertilizer**" aimed at completing her studies. This is therefore, to request that you grant her any assistance needed to enable her meet the program requirements for her graduation.

Kindly contact us for any further enquiries.

Thank you

Mirara simon w.
Chair of Department Civil and Environmental Engineering
Meru University of Science and Technology
Email: CODcivilengineeing@must.ac.ke
Smirara@must.ac.ke

Appendix D: NACOSTI Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: 548549

Date of Issue: 24/July/2024

RESEARCH LICENSE

This is to Certify that Miss.. Tabitha Nekesa Khamala of Meru University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Meru on the topic: URINE STABILISATION AND TREATMENT USING LACTIC ACID FROM FRUIT AND VEGETABLE PEEL FERMENTATION: A POTENTIAL SOURCE OF NITROGEN FERTILISER for the period ending : 24/July/2025.

License No: NACOSTI/P/24/37777

548549

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

Appendix E: MIRERC Approval Permit

MERU UNIVERSITY INSTITUTIONAL RESEARCH & ETHICS REVIEW COMMITTEE (MIRERC)

Email: mirerc@must.ac.ke **Website:** https://research.must.ac.ke/research-ethics/

Ref: MU/1/39/28 Vol.3 (156) Date: 7th March 2025

To: Ms. Tabitha Nekesa Khamala (MSc. Sanitation)

Dear Sir/madam

RE: Urine Stabilization and Treatment Using Lactic Acid from Fruit and Vegetable Peels: A Potential Source of Nitrogen Fertilizer.

This is to inform you that *MIRERC* has reviewed and approved your above research proposal. Your application approval number is *MIRERC 009/2025*. The approval period is *7th March 2025 – 7th March 2026*.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *MIRERC*
iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *MIRERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to *MIRERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *MIRERC*.

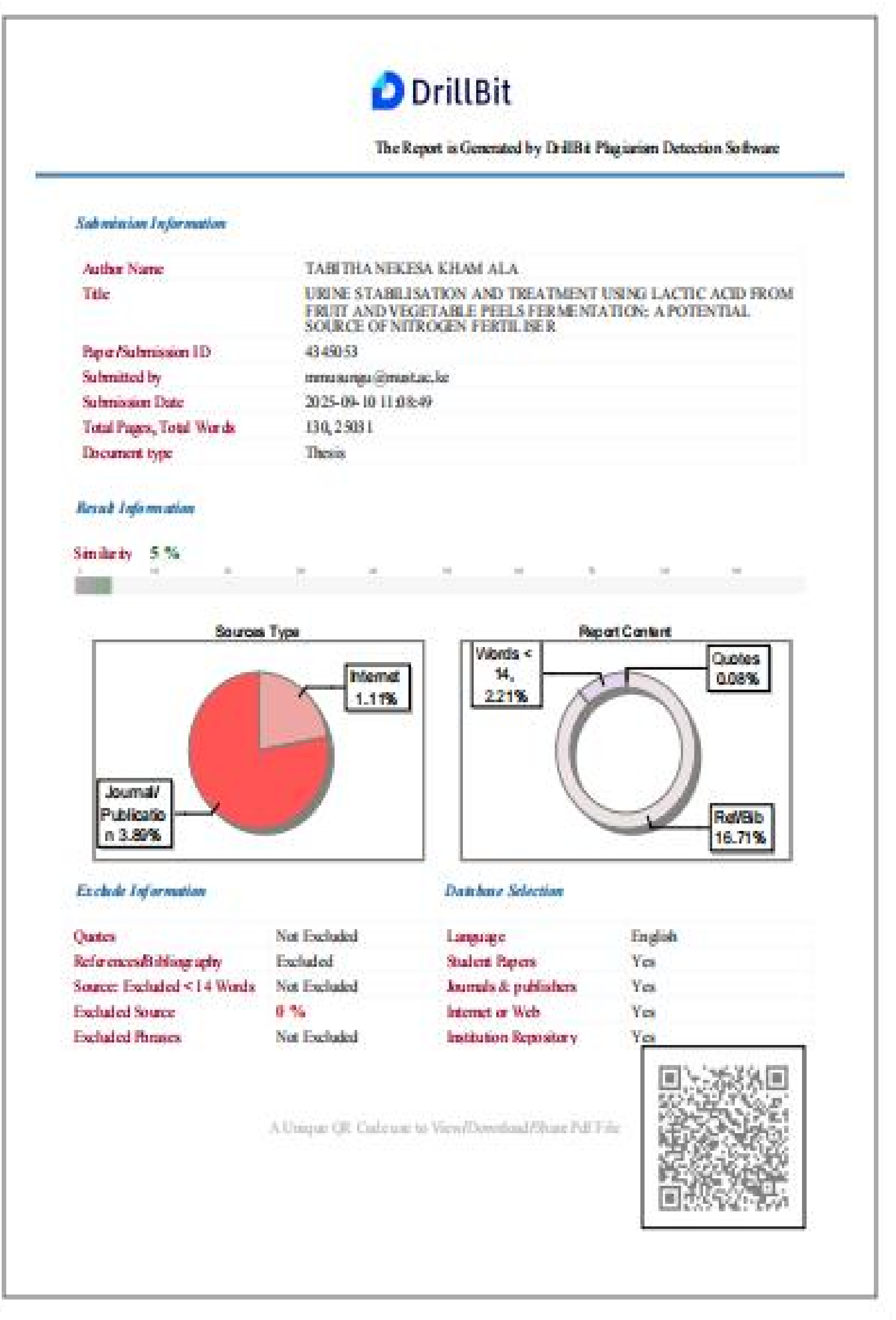
You may also be required to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI), visit: https://research-portal.nacosti.go.ke and also obtain other clearances that your study may require.

Yours sincerely

Prof. Peter Masinde, Ph.D.
Chairperson, MIRERC

MUST IS ISO 9001:2015 and ISO/IEC 27001:2013 CERTIFIED

Appendix F: Plagiarism Report



DrillBit

The Report is Generated by DrillBit Plagiarism Detection Software

Submission Information

Author Name	TABITHA NEKESA KHAMALA
Title	URINE STABILISATION AND TREATMENT USING LACTIC ACID FROM FRUIT AND VEGETABLE PEELS FERMENTATION: A POTENTIAL SOURCE OF NITROGEN FERTILISER
Paper/Submission ID	4345053
Submitted by	mmusunga@must.ac.ke
Submission Date	2025-09-10 11:08:49
Total Pages, Total Words	130, 25031
Document type	Thesis

Result Information

Similarity 5 %

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Quotes	Not Excluded
References/Bibliography	Excluded
Source: Excluded < 14 Words	Not Excluded
Excluded Source	0 %
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Database Selection

Language	English
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Institution Repository	Yes

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