OPTIMIZING SWEET POTATO AS A SUBSTRATE FOR INDIGENOUS MICROORGANISMS (IMO) GROWTH

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ABSTRACT

There is a potential of using sweet potato as suitable substrate for trapping, culturing and multiplying IMOs for improved crop and livestock production. However, there are limited studies on optimal concentration of sweet potato substrates for IMO growth and multiplication. This study focused on optimizing sweet potato as a substrate for indigenous microorganisms (IMO) growth. A randomized complete design (RCD) with three replicates was employed to test the five- sweet potato substrate concentration (20%, 40%, 60%, 80%, 100%). Data was collected on shape, color, texture, colony forming units (CFU) and colony size of bacteria and fungi in different concentrations of sweet potato substrates. This study showed that there was a significance differences (P-Value **<.001)** on CFU and colony sizes. **Rice substrate concentration of 100% showed highest (**7266667^{d}**) CFU of bacteria, while 100% concentration of sweet potato showed the lowest (**4333333^{a}**). The largest colony size (**3.333^{d}**) shown in 100% of rice whereas 100% concentration of sweet potato had the smallest size (**1.167 ± 0.0000^{a} **) and all concentrations had only Gram positive bacteria. Sweet potato concentration of 60% had the highest number (6677778^{c}) of CFU while 100% concentration of sweet potato showed the number (4100000^{a}) Largest colony size (10.667^{c}) of fungi was shown in 100% concentration of sweet potato whereas 100% of rice showed the lowest size (4.000^{a}).** This implies that the concentration having 20% sweet potato substrate supports good bacterial growth and 60% of sweet potato substrate supports fungal growth. Using sweet potato substrate as a supplement to rice can be used for growth and multiplication Indigenous micro-organisms for improved animal and crop production.

DEDICATION

I dedicate this work to my parents Mr. Owor Anthony and Mrs. Peres Owor, whose unwavering support, sacrifice and encouragement have been my foundation. Your influence is deeply appreciated.

DECLARATION

I, OTHIENO BRIAN GABRIEL, declare that this Research Dissertation is submitted to the Faculty of Agricultural Sciences of Uganda Christian University, Mukono is my original work. Therefore, it has never been submitted and documented in any way to any academic institution in exchange for a degree or other educational credentials.

SIGNATURE:.....

DATE: 16th / April / 2025

APPROVAL

This is to confirm that OTHIENO BRIAN GABRIEL has finished his year four research project under my supervision and the report has been submitted with the approval of the academic supervisor.

Signature: ..... **Date:** 16/April/2025

MISS. WINNIE NAMUTOSI

ACKNOWLEDGEMENT

I sincerely thank Almighty God for the grace, wisdom and knowledge to complete this research and my bachelors' degree.

My deepest gratitude goes to my supervisor, Madam Winnie Namutosi, for her invaluable guidance.

I also appreciate my lectures, family, and friend for their unwavering support and encouragements given to me.

Appreciation goes to Madam Linet, for guidance, providing necessary resources during my study.

God bless you abundantly!

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ACRONOMES

IMO: Indigenous microorganisms

E.coli: Escherichia coli

CFU: Colony Forming Unit

MAAIF: Ministry of Agriculture, Animal Industry and Fisheries

UBOS: Uganda Bureau of Statistics

RCD: A randomized complete design

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Indigenous microorganisms (IMO) are naturally occurring microbial communities found in local environments, such as soil, water and plant surfaces. These microorganisms include bacteria, fungi, actinomycetes and yeasts which play very important role in nutrient cycling, organic matter decomposition and plant growth promotion **(Smith et al., 2019; Bayu, 2024; Teruo Higa, 1994).** In sustainable agriculture, IMO is also increasingly utilized as bio-fertilizer, bio-control agent and soil conditioner due to their ability to enhance soil fertility, suppress pathogens and improve crop resilience **(Abu-Bakar & Ibrahim, 2013; Adal, 2024; Carandang, 2003; Kumar & Gopal, 2015).** A key factor in cultivating IMO's is the choice of substrate which serves a nutrient source for microbial proliferation **(Kumar & Gopal, 2015)**. Common substrates include rice, rice bran, and wheat bran, which provide carbohydrate, proteins and minerals necessary for microbial growth **(Kumar & Gopal, 2015; Khan et al., 2019)**. However, there is a growing need to explore alternative, cost effective and locally available substrates to improve accessibility and efficiency of IMO-based farming practices **(Kumar et al., 2015; Smith et al., 2019).**

In 2019, production of rice on global scale, about 756 million metric tons over about 164 million hectare, hence contributing to its third position among agricultural production after sugarcane and maize. In Africa, rice production was predicted to be around 37.9 million metric tons from about 17.2 million hectares **(FAO, 2021).**

Uganda is the is third in rice production and experience deficit in rice production which necessities reliance on imports **(FAO, 2021)**. According to FAO (2023), Uganda spends about $60 million annually on rice imports. Sweet potatoes (*Ipomoea batatas*) is therefore a promising candidate due to its high carbohydrate content (starch, sucrose, glucose and fructose), rich nutrient profile and widespread availability in tropical and subtropical region **(Woolfe, 1992)**. This makes sweet potatoes a suitable carbohydrate source for trapping, culturing, and multiplying indigenous microorganisms. Traditional substrates like rice and wheat brand have been widely studied yet there is a growing imperative to explore alternative substrates that are both eco-friendly and locally available **(Navarro et al., 2020; Zhao et al., 2022).** This study focused on optimizing sweet potatoes as a substrate for indigenous microorganisms (IMO) growth.

1.2 PROBLEM STATEMENT

Fouls smell in pig sty is one of the major problems in animal production **(Ndyomugyenyi et al., 2020)**. Indigenous Micro-organisms is increasingly being utilized as bio fertilizer, bio-control agent and soil conditioner due to their ability to enhance soil fertility, suppress pathogens and improve crop resilience **(Abu-Bakar & Ibrahim, 2013; Adal, 2024; Carandang, 2003; Kumar & Gopal, 2015)**. Rice and sweet potato are widely used as substrates in the production of indigenous microorganisms **(Kobusinguzi, 2024; Park1 et al., 2010; Kumar & Gopal, 2015)**; however a comparative study on efficiency of rice and sweet potato as IMO substrate is needed. Research pointed out that rice supports bacterial growth however, they did not look at specific microorganisms found in it **(Anyanwu et al., 2015; Kumar &**

Gopal, 2015), Kobusinguzi (2024), also reported that there is a potential of using sweet potato as suitable substrate for trapping, culturing and multiplying IMOS; which was evidenced by the formation of white mold within 7 days **(Kobusinguzi, 2024)**. However, there are limited studies on optimal concentration of sweet potato substrates for IMO growth and multiplication. Therefore, this study optimized sweet potato as a substrate for indigenous microorganisms (IMO) growth for improved animal and crop production.

1.3 OBJECTIVES

Main objective

To assess the potential of sweet potato as a substrate for cultivating indigenous microorganisms (IMO)

Specific objectives

1. To isolate and identify the IMO strains grown on media.
2. To determine the optimal level of sweet potato substrate for IMO growth.

1.4 HYPOTHESIS

1. All the bacteria in IMO will be Gram positive
2. The composition and diversity of isolated indigenous microorganisms (IMO) will vary with different concentrations of sweet potato and the concentration having 40% and 60% will enhance growth of bacteria and fungi respectively

1.5 JUSTIFICATION

Bad odor in pigsty is a major constraint to the growth of pig farming in East Africa, leading to community complaints, regulatory penalties and reduction in pig production **(Mamawi et al., 2020; Schiffman et al., 2001).** Unlike commercial farmers that use the commercial IMO to control smell in the pig sty, smallholder farmers who dominate this sector in Uganda cannot afford a sustainable solution to manage smell and this is evident by most farmers not having shelter for their pig and do tethering **(UBOS, 2024)**. This study therefore, seeks to bridge this gap by developing eco-friendly locally adaptable IMO using sweet potato, an underutilized yet abandoned crop in some regions of the country **(Kobusinguzi, 2024)**.

1.6 SIGNIFICANCE

This study holds critical importance for Uganda's rapidly growing big farming sector, which contribute to livelihoods but faces persistent odor related challenges, due to poor waste management. It will provide benefit, smallholder farmers who dominate Uganda's pig industry, most especially those that are from regions where sweet potato production is high **(Kobusinguzi, 2024)** and are struggling with expensive commercial IMO. This study will greatly contribute to Uganda's vision of 2040, delivering employment to the youth and women, reducing future greenhouse gas emission **(Mugyenyi et al., 2020)** as well as contributing to developing sustainable agricultural practices that addresses the pressing need for effective waste management solution in Uganda's pig farming sector to reduce emission of foul smell**(Arum & Wahyudi, 2022; Manzoni et al., 2023).**

1.7 CONCEPTUAL FRAMEWORK

Independent variable

The concentration of sweet potato substrate (carbon or nutrient source) tested at varying levels **(**20%,40%,60,80% and 100%), to determine the optimal level for IMO growth. Sweet potato is rich in carbohydrate rich, hence have influence on microbial metabolic activity. Microbial growth increase with increase in nutrient availability but but decrease when nutrient availability is limited and when subjected to osmotic stress

Dependent variable

Colony forming units, colony sizes and morphological traits like color, shape, and appearance which collectively measure microbial growth responses to substrate concentration. CFU counts, help in quantification of the viable microbial population serving as a direct indicator of growth efficiency across the different sweet potato levels**.** Colonic size reflect the metabolic activity and health of the microorganisms with larger colonies suggesting more favorable growth condition. Morphological traits such as shape, color and texture provide insight into species diversity and potential adaptation to varying substrate condition. Together, these variables offer a comprehensive assessment of how sweet potato concentration influences IMO development.

Environmental conditions

To ensure reliability of the results, key environmental conditions were standardized throughout the experiment. Temp pressure is maintained between 30^0C degrees an optimal range for mesophillic microorganisms commonly found in soil. Humidity is

controlled at 60 to 70% Relative humidity to prevent, media dehydration, or excessive moisture, which could lead to contamination **(Anyanwu, 2015; Seema et al., 2019).** Aerobic conditions are preserved through the proper ventilation as most IMOS require oxygen for growth. Additionally, the media composition were kept constant and through controlling these variables, the study ensures that observed differences in microbial growth are solely attributable to the changes in substrate concentration(Anyanwu, 2015).

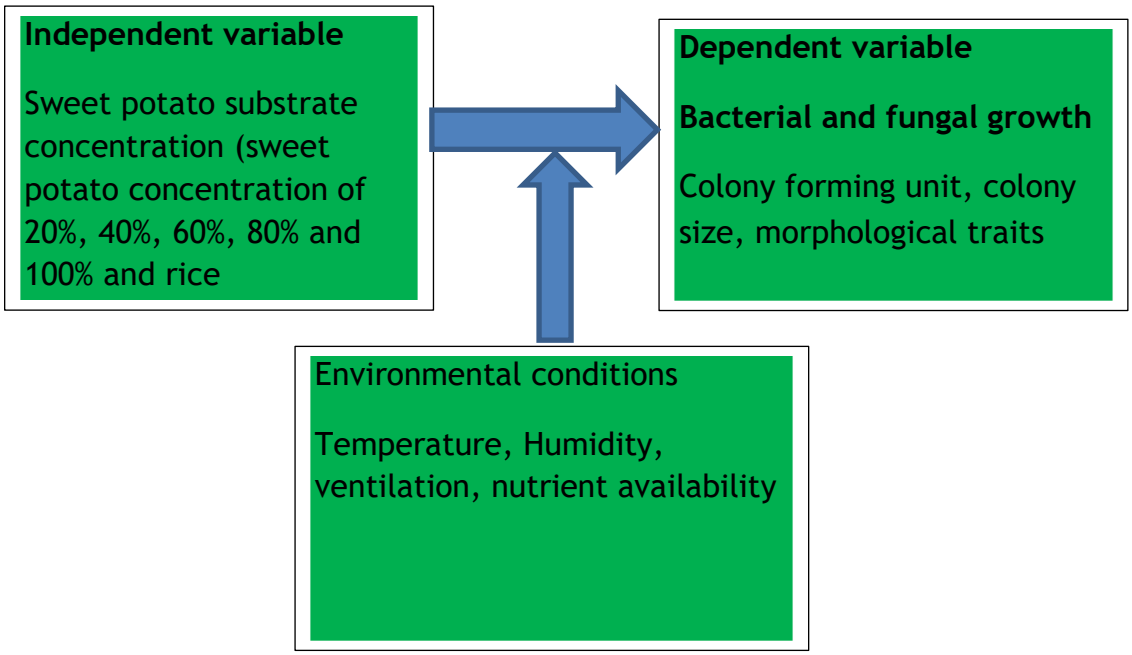

Figure 1: Conceptual framework

1.8 SCOPE OF THE STUDY

The scope of this investigation encompasses the isolation of microbial strain from sweet potato at different concentrations and the mapping of these strains' growth performance under controlled laboratory settings. This research focuses explicitly on the use of local resourced sweet potato, addressing current limitations and challenges in conventional organic farming practices. The study will contribute to the agricultural resources available to smallholder farmers, particularly in Uganda, where reliance on expensive commercial IMOs can hinder productivity. Additionally, this research aligns with Uganda's vision 2040 for sustainable agriculture, showcasing how harnessing locally available resources can lead to environmental and economic benefit, ultimately improving public health and farmer welfare.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance of Indigenous microorganisms

Indigenous microorganisms play crucial roles in sustainable agriculture, particularly enhancing organic waste decomposition and soil health (Abu-Bakar et al., 2013). Their application in composting systems demonstrate significant benefits for nutrient recycling within the farm for as seen in chicken litter system where the waste is naturally converted in to fertilizer and microbial ecology as evidence by studies from their production and utilization (Carandang, 2003)

IMO enhance microbial diversity by introduce beneficial fungi bacteria and actinomycetes to agricultural system which are essential for broking down complex organic matter. These microorganisms dominate different composting phases with mesophilic bacteria initiating decomposition followed by thermophilic bacteria and finally actinomycetes stabilizing manure compost**(Abu-Bakar et al., 2013)**. The thermophilic phase during composting, supported by IMO activity, helps in suppression of pathogens through heat generation. Addition of IMOs to composting mixture accelerates organic matter decomposition for example according to Abu-Backer et. al,(2013), treatment IMOs had higher microbial counts, particularly actinomycetes, which indicate compost maturity **(Abu-Bakar et al., 2013).**

According to Abu-Bakar et al.,(2013)IMO contribute to soil ecology by increasing microbial population and maintains the balance during composting ensuring sequential dominance of mesophillic bacteria , thermophiles and actinomycetes, hence enhance

nutrient availability and organic matter turnover , indirectly promoting plant growth **(Abu-Bakar et al., 2013)**. The locally sourced microbes, lacto bacilli and lactic acid bacteria have been reorganized for their potential to enhance soil fertility, improve plant and animal health and reduce dependence on synthetic inputs. The bacteria lacto bacilli plays a great role in arresting foul odors, associated with anaerobic decomposition whereas lactic acid bacteria thrive and feed on ammonia released during decomposition hence it is vital in reducing foul odor in pig sties **(Carandang, 2003).** IMO based composting reduces the need for chemical additives, aligning with eco-friendly practices which is vital in sustainability. Also the production of IMOs using low cost materials like cooked rice, cooked sweet potatoes further supports accessible, small-scale agriculture**(Abu-Bakar et al., 2013; Carandang, 2003)**. The indigenous microorganisms are also important in harnessing natural resources

2.2 Application of Indigenous microorganisms

IMO is vital in waste management through efficient composting where it enables recycling of agricultural residues like mixture of farm waste, rice straw into nutrient-rich compost, reducing reliance on synthetic fertilizers important in composting organic matter **(Serafin et al., 2015; Le Thi Xa et al., 2020)**.

It is vital in soil structure improvement through soil aggregation, water retention and aeration which creates favorable condition for earthworms and symbiotic microbes**(Kumar & D.V.R., 2015)**

Disease suppression through competition with harmful microbes for resources and production of microbial compounds that inhibit the growth of pathogens like *Salmonella, Shigella, E. coli and Caliform*

IMOs are also applied in eco-friendly recycling of wastes through the break down of complex organic matter , hence close nutrient loops, supporting circular econmy practices **(Serafin et al, 2015)**

2.3 Substrates used for production of Indigenous microorganisms

Different substrates have been used to grow IMOs for crop and animal production. Rice , maize and wheat have been commonly used. According to FAO (2023), Uganda spends about $60 million annually on rice imports.

Rice's starch undergoes complete gelatinization, at 65^0C to 75 ^{0}C, yielding more readily fermentable carbohydrates than sweet potatoes which contain resistant starch structure **(Xu et al., 2023)**.

Rice also has neutral pH and lacks natural antimicrobial compound unlike Sweet Potato, which contains growth-inhibiting entities like phenolic compounds that negatively affect microbial growth**(Alam et al., 2016)**.

2.4 Characterization of Indigenous microorganisms

Isolation and characterization of microorganisms are important steps in microbes identification and usage. Basic isolation procedures for indigenous microorganism have been suggested by(Anyanwu, 2015). While isolating IMOs, key parameters like colony section and purification, color, shape , size, texture, elevation, appearance and optical properties are important for bacteria, whereas for fungi, cultural

characteristics like color, colony shape, size, texture and elevation are important **(Seema Yadav et al., 2019)**

2.5 Optimization of Indigenous microorganisms

Sweet potato substrate have dual benefits, addressing food, security challenges while simultaneously supporting the production of indigenous microorganisms that can contribute to sustainable farming practices. Many smallholder famers in the region where conventional carbohydrate sources (rice) are less accessible may find so potato particularly valuable as they work to promote sustainable systems that reduce resilience and synthetic chemicals thereby commercial environmental conservation hence incorporating sweet potatoes into agricultural practices for production of IMOs provides a promising avenue for enhancing food security and sustainability. The effectiveness of sweet potato as carbon source for trapping and culturing indigenous microorganisms highlights their potential to become a staple in eco-friendly farming system **(Kobusinguzi, 2024).** There is no literature about the effect of different concentrations in the growth of IMOs necessitating the relevance of optimization of sweet potato

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study location

The research was conducted at Uganda Christian University, Mukono. The area is located in Uganda's central region, about 27km, East of Kampala. Its elevation range from 1158m to 1219m above sea level. Mukono receives two wet seasons with an annual rainfall ranging between 1100mm and 1400mm. the temperature varies between 21 and 29^0C with coordinates $00^0$20'N 32^0 45'E **(Nakanwagi, Mildred Julian, Godfrey Sseremba & Kizito, 2017).** The experiment was conducted both in the field and laboratory between January, 2025 to March 2025.

Materials

Sweet potatoes, and rice, Petri dishes, inoculation loops, growth media(tryptone glucose yeast extract agar, potato dextrose Agar), fluorescent microscope, laminar flow hood, an oven, Stains(crystal violet, iodine solution) Acetone, neutral red solution **(Bartholomew et al., 1952; University of AL-Mustansiriyah, 2022)**

3.2 Collection of microbes from the field

Substrates that were purchased from Mukono Market were used in this study.

3.3 Experimental design

A randomized complete design (RCD) with three replicates was employed. Five sweet potato substrate concentration mixed with rice; (20% of sweet potato and 80% rice), (40% of sweet potato and 60% of rice), (60% of sweet potato and 40% of rice), (80% of sweet potato and 20% of rice) (100% of sweet potato), and (100% of rice) were arranged with each having three petri dishes per replicate.

TREATMENTS

T1: 100% rice

T2: 100% of sweet potato

T3: 20% of sweet potato and 80% rice

T4: 40% of sweet potato and 60% rice

T5: 60% of sweet potato and 40% rice

T6: 80% of sweet potato and 20% rice

Table 1. Treatments

Replicate 1	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
Replicate2	**T3**	**T2**	**T6**	**T1**	**T4**	**T5**
Rplicate3	**T5**	**T3**	**T4**	**T1**	**T6**	**T2**

3.4 Preparation of Sweet potato Substrate

The substrate concentration was prepared by boiling the rice and sweet potato separately, mashed (sweet potato) , allowed to cool to room temperature (Park1 et al., 2010) and then mixed based on the percentages like 100% rice(control), 20% , 40%, 60%,80% and 100% sweet potatoes by mixing rice and sweet potatoes in the ratio of 800g: 200g, 600g:400g, 400:600g, 200g:800g and 1000g of sweet potatoes sweets

Equal quantity (1kg) of the mixed substrates were put in plastic containers, wrapped in a paper, secured with a string and buried at a depth of 10cm. The boxes were covered with leaf litter and little soil to mimic the natural environmental condition, protected from rain using a polythene paper and left undisturbed for 7 days **(Anyanwu et al., 2015; Kumar et al., 2015; Park1 et al., 2010).** Physical changes were recorded (color, texture and odor) as qualitative indicator of microbial growth and microbial growth quantified by preparing serial dilution of substrate samples

3.5 Media preparation

For bacteria 17.5g of Tryptone Glucose Yeast Agar was put in a conical flask and suspended in 1 litre of distilled water and dissolved by boiling and sterilized by autoclaving at 121^0C for 15 minutes and the media allowed to cool to a 40^0C temperature and poured in the petri dishes and allowed to gel. For Fungi, 39g of Potato Dextrose Agar was used and the procedure was repeated as in Tryptone Glucose Yeast Extract Agar.

3.6.1 For Objective 1. To isolate and identify the IMO strains present in the different concentrations of sweet potatoes.

3.6.1.1 Serial dilution

Microbial growth was quantified through the assessment of colony-forming units (CFUs) across various treatment conditions. Serial dilation and plating o were employed for accurate measurement, while other morphological analyses were used in the study of the isolated microorganisms. Stock solution was made by weighing one gram of fermented substrate and transferred into a sterile test tube containing 9ml of

distilled water. The tube was shaken thoroughly to form a uniform solution of 1:10 (substrate: distilled water). Ten folds serial dilution was done using a pipette by transferring 1ml of the stock solution to a test tube containing 9ml of distilled water. 1mL of this dilution was added to the next tube (10^{-2}) and mixed thoroughly and this procedure was repeated for the next tubes (10^{-3}, 10^{-4}, 10^{-5}). Pipettes were changed for each dilution to avoid cross contamination **(Reynolds, 2005)**.

3.6.1.2 Plating

1 mL of the diluted solution was planted on the petri dishes containing the prepared media that is Tryptone Glucose Yeast Extract Agar for bacteria and Potato Dextrose Agar for and transferred to an oven where incubation is done at 30^0C for 24 hours and 7 days for bacteria and fungi respectively. The colony forming unites are then counted, color, and texture are recorded **(Meenakshi, 2024).**

3.6.1.3 Morphological analysis

Observation of bacterial colonies and fungi colonies, taking note of the color, shape measuring the size, and counting the number of colonies

3.6.1.4 Microscopic examination

Gram Staining

Procedure

A colony was picked using inoculation loop and spread/ smeared on the glass slide and fixed to the slide by gently passing it over the heat source

The heat fixed smear of cells were flooded with crystal violent (primary stain) for 1 minute **(Nishant, 2023; O'Toole, 2016; Paray et al., 2023; Bartholomew et al., 1950).** The slide was washed in a gentle and indirect stream of tap water for 2 seconds. The slide was then flooded with iodine solution (a mordant) and washed after I minute. Washing of the slide with tap water was done for 2 seconds. A decolorizer (acetone) was added and immediately washed to avoid over decolorization. Safranin counterstain, was then added and left to stay for 1 minute before washing. The slide is then washed until no color appears in the effluent and then left to dry.

The cells on the slides were observed under oil immersion and ×40 objective was initially used in the examination to evaluate the smear distribution, and then ×100 oil immersion objective was used.

This technique was used to identify bacteria based on the Gram-reactions where Gram positive bacteria appear blue or purple whereas gram negative bacteria appear pink or red due to the retention of the crystal violent-iodine complex **(Paray et al., 2023)**

Data collection

Data was collected on shape, color, for bacteria and fungi in texture, different concentrations of sweet potato substrates

3.6.1.5 Data analysis

Data was entered in Microsoft excel and imported to GenStat 12 ed, for analysis, where analysis of variance (One way ANOVA) was done, and the Least Significant Difference (LSD) was used to separate means at 5% significance level

3.6.2 For objective two: To determine the optimal level of sweet potato substrate for indigenous microorganisms growth

3.6.2.1 Colony forming unit

Procedure for colony forming unit count

It is a technique used to establish the number of viable microorganisms in a sample by culturing them on an appropriate medium. After incubation, the plates with 30-300 colonies were selected as this range provided reliable results and the number of colonies on each plate was counted. Calculation of the colony forming units per gram (CFU/g) of the sample using the formula below was done.

$$\text{CFU/g} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{Volume Plated (mL)}}$$

CFU for each dilution and replicate was recorded and the mean CFU value for each sample was used for analysis

Data collection

Data was collected on shape, color, elevation, appearance, colony size for bacteria and fungi in color, texture, and size of colony in different treatments.

3.6.2.3 Data analysis

Data was entered in Microsoft excel and imported to GenStat 12 ed, for analysis, where analysis of variance (One way ANOVA) was done, and the Least Significant Difference (LSD) was used to separate means at 5% significance level.

CHAPTER FOUR

4.0 RESULTS

4.1 For Objective 1: To isolate and identify the IMO strains present in the different concentrations of sweet potatoes.

The result showed that IMO from all the concentrations had bacteria and fungi. All the concentrations had only gram positive bacteria (Staphylococcus aureus, Streptococcus peneumoniae, streptococcus pyogenes and bacillus)

Characterisitcs

Streptococcus peneumoniae; grayish to whitish mycelia, small translucent , mucoid colnies, diplococci

Staphylococcus aureus; smooth, creamy, circular colonies with a glossary surface, with rapid robust growth. Cocci are in grape-like clusters. Streptococcus pyogenes; small translucent , slitely glossy colonies, whitish-gray with moderate growth. Cocci are in chains. Bacillus; rod shaped, large irregular, spreading colonies with a dry wrinkled texture, whitish to cream and rapid growth **(Anyanwu, 2015).**

The fungi included Rhizopus, Penicillium, aspergilus and mucor. Rhizopus; rapily growing, fluffy, cotton-like colonies. Initially white,turning gray-black as sporangia mature, aseptate hyphae with rhizoids. Penicillium; powdwery texture with radial growth, white colonies, septate hyphae, chains of round,spores**(Anyanwu, 2015)**

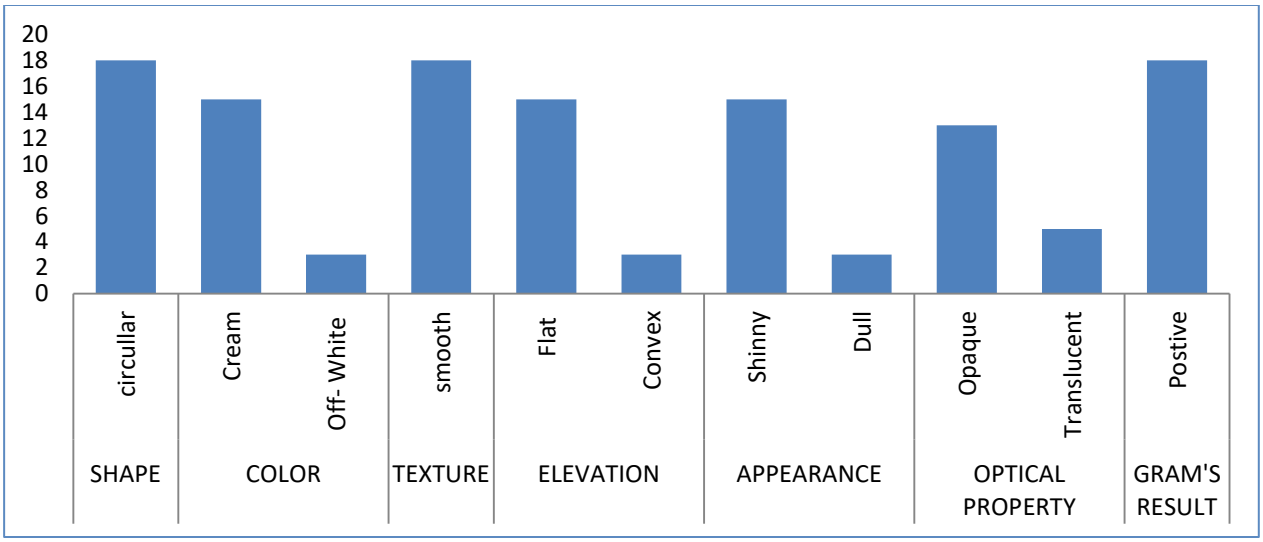

Figure 2: Frequency of bacteria cultural characteristic

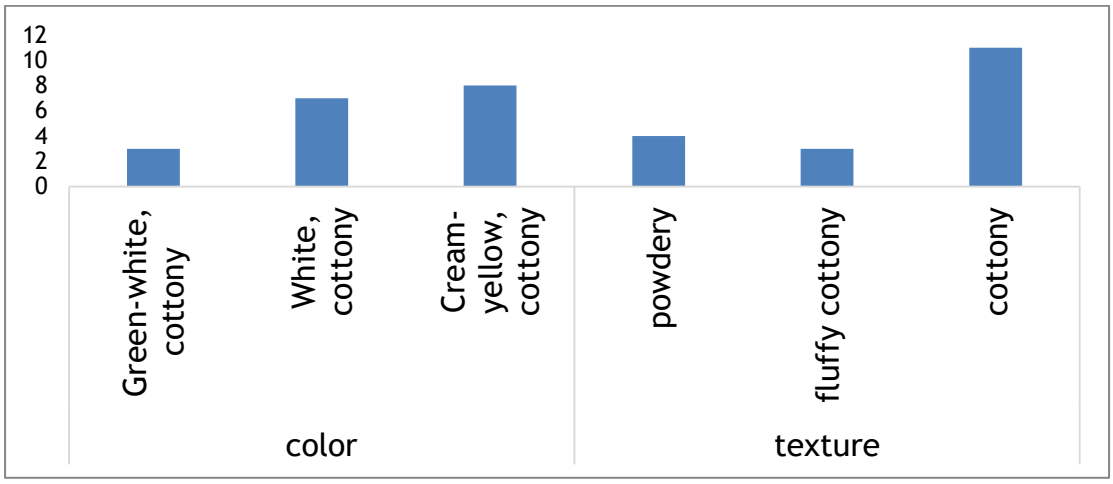

Figure 3. Frequency of fungi cultural characteristic

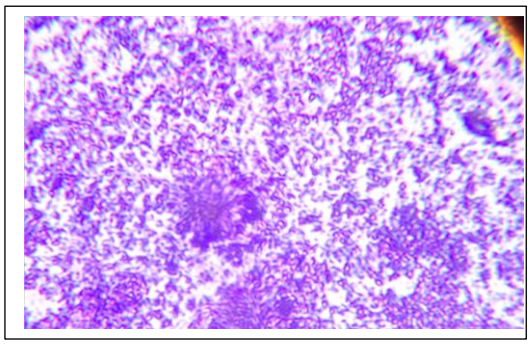

Figure 5. Staphylococcus aureus

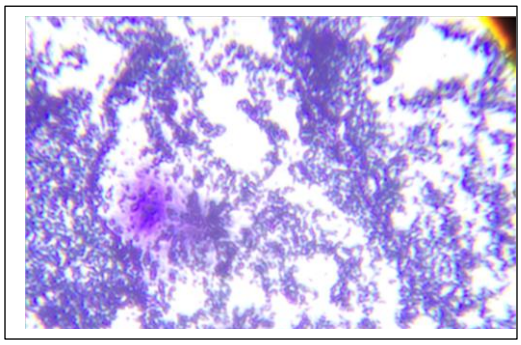

Figure 4. Streptococcus peneumoniae

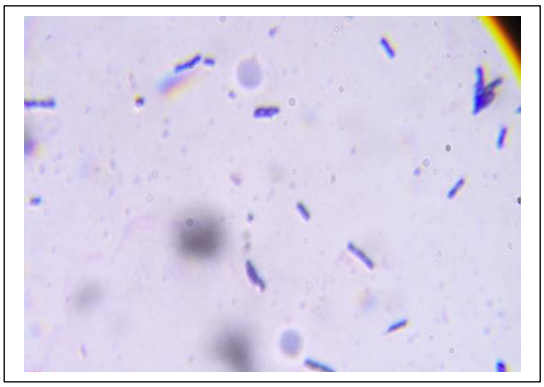

Figure 6. Bacillus

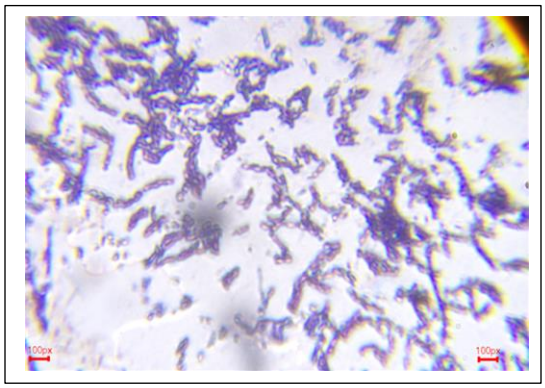

Figure 6. streptococcus pyogenes

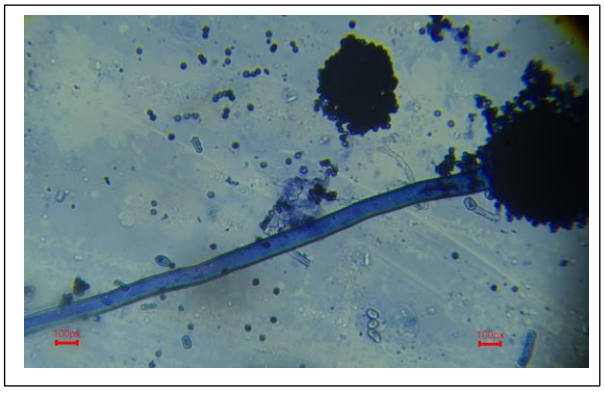

Figure 7. Mucor

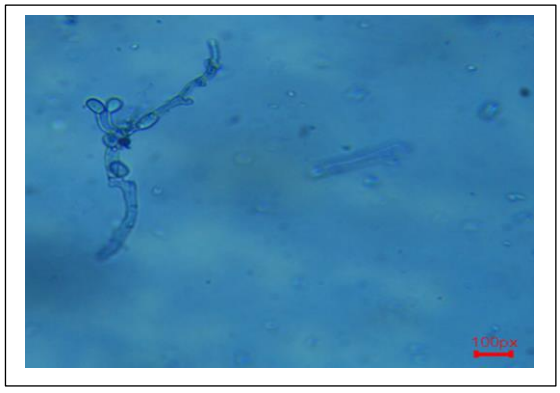

Figure 8. Penicillium

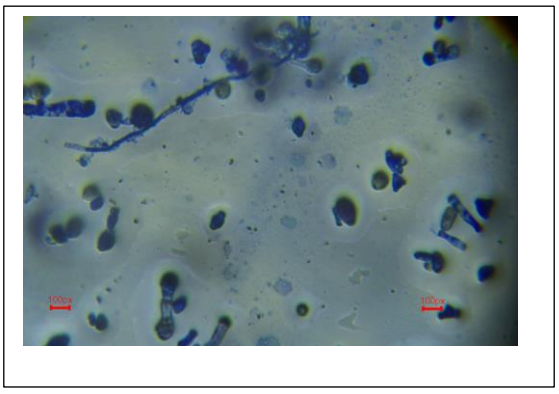

Figure 9. Aspergillus

4.2 For objective two: To determine the optimal level of sweet potato substrate for indigenous microorganism growth

4.2.1 The Mean Colony Forming Unit and Colony Size for fungi

The highest fungal colony forming unit was observed at 60% sweet potato (6677778±134715**),** which was even higher than the 100% rice (4966667±404145). This supports the hypothesis that sweet potato can favor fungal growth at different concentrations. Fungal colony forming units peaks at 60% sweet potato and declines at higher concentrations (80% and 100%), suggesting an optimal range for fungal growth

Colony size varied across treatments, with the largest colonies observed in 100% sweet potato (11.889±0.1925), though CFU was lower at this concentration. This implies that while fewer fungi may grow at 100% sweet potato, those that do thrive may growth larger

Table 2. Mean and SD of Colony Forming Unit and Colony Size of bacteria

Substrate Concentration(%)	CFU/g (Mean ± SD)	Colony Size (Mean ± SD)
Rice 100	7266667±351188[d]	3.333±0.0.1667[d]
Sweet potato 100	4333333±288675[a]	1.167±0.0000[a]
Sweet potato 20	6733333±115470[cd]	2.833±0.1667[cd]
Sweet potato 40	5666667±404145[b]	2.056±0.1925[b]
Sweet potato 60	6133333±305505[bc]	2.556±0.3849[bc]
Sweet potato 80	4533333±404145[a]	1.333±0.0000[a]
P-Value	<.001	<.001
LSD	581016.7	0.3352

Table 3.Colony Forming Unit and Colony Size for fungi

Substrate Concentration (%)	CFU (Mean ± SD)	Colony size (Mean ± SD)
Rice 100	4966667±404145^{ab}	4.056±0.8221^{a}
Sweet potatoes 100	4100000±200000^{a}	11.889±0.1925^{d}
Sweet potato 20	5022222±234126^{ab}	5.889±0.2546^{b}
Sweet potato 40	5800000±529150^{bc}	6.611±0.4194^{b}
Sweet potato 60	6677778±134715^{c}	6.889±0.2546^{bc}
Sweet potatoes 80	4500000±264575^{a}	8.167±0.4410^{c}
P-Value	<.001	<.001
LSD	574762.7	0.800

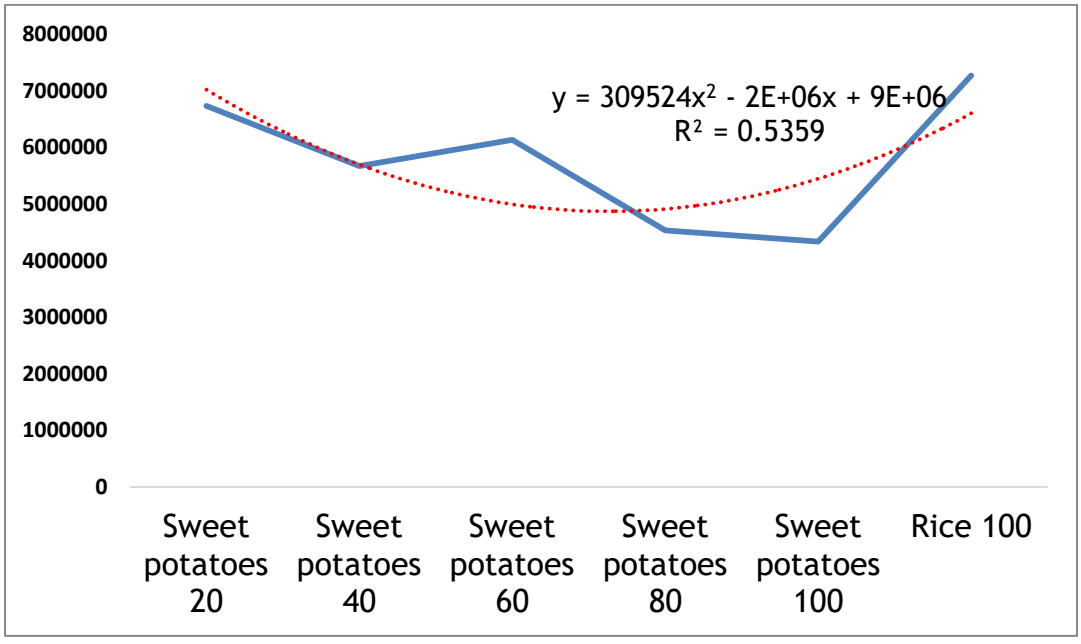

Figure 10. Bacteria colony forming unit

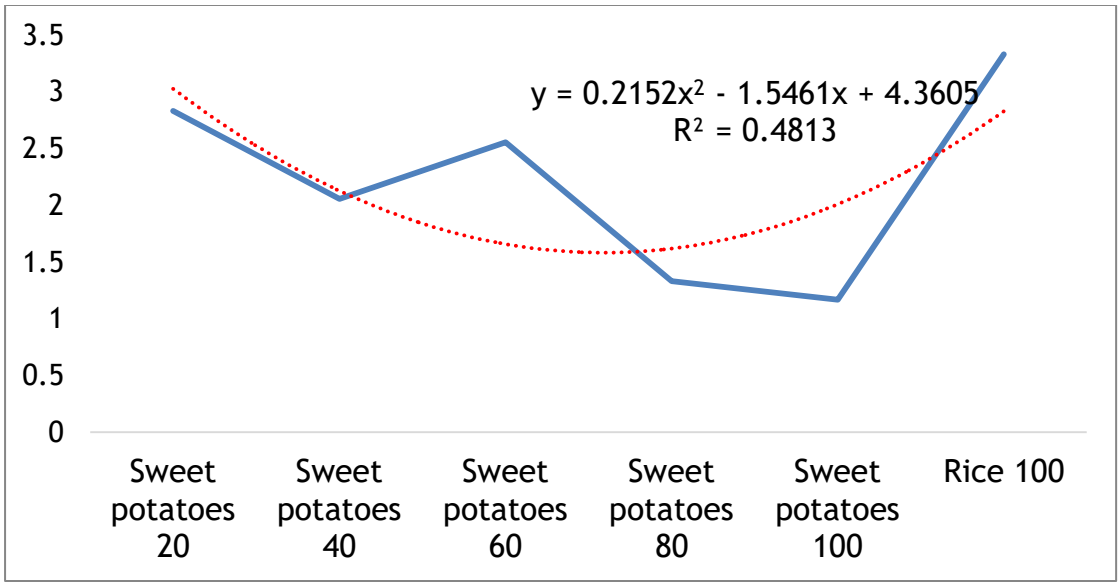

Figure 11. Bacteria colony size

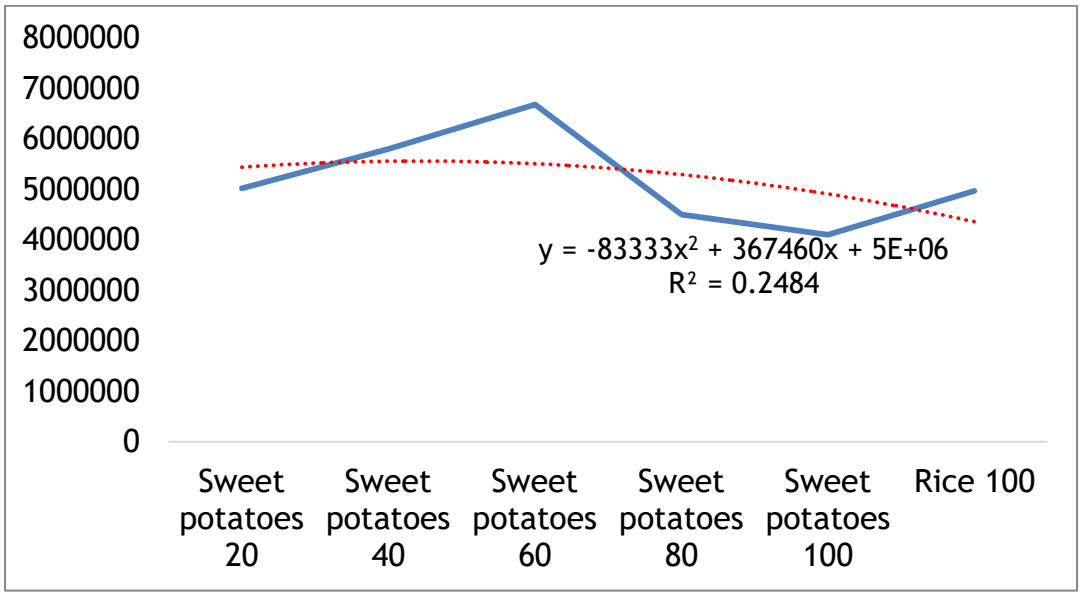

Figure 12. Fungi colony forming unit

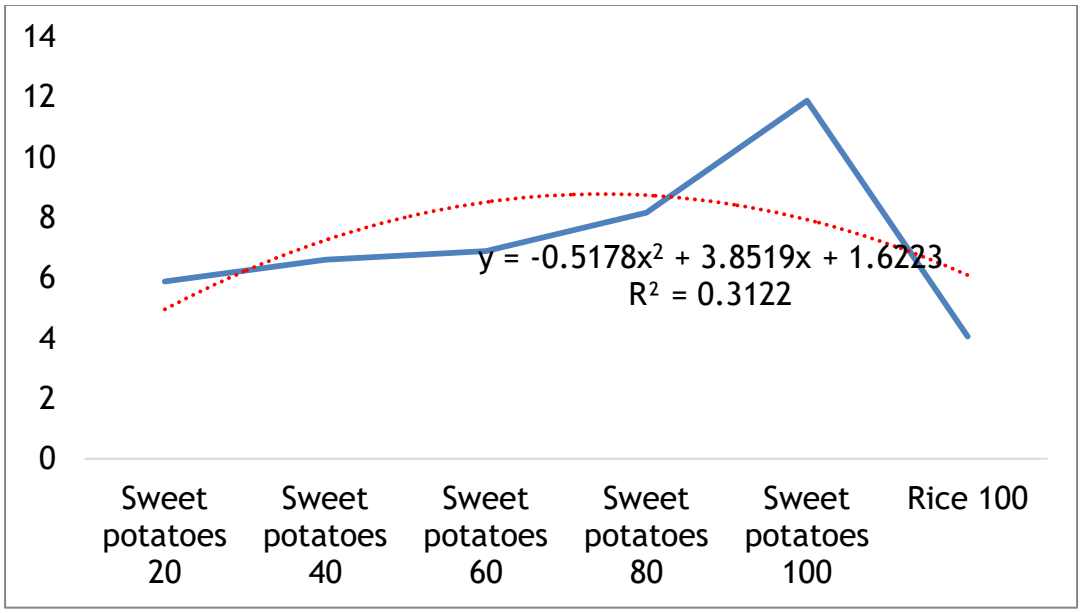

Figure 13. Fungi colony size

CHAPTER FIVE

5.0 DISCUSION

In my study, the media (TGYEA AND PDA) have been noted to efficient in supporting bacteria and fungi growth repectively and this has been reported by Anyanwu **(Anyanwu et al., 2015)**. Bacteria was cultured in TGYEA and had the highest population ranging from 4333333 to 7266667 as compared to fungi (**4100000 to 6677778)**, and the majority of the bacteria were colored cream **(Anyanwu et al., 2015)**. Similar result was reported by Anyanwu et al.,(2015) using the same media for identifying colony characteristics of bacteria and fungi **(Anyanwu et al., 2015).** Also Nguyen et al (2015) had the same results about the CFUs of bacteria being higher than fungi**(Nguyen et al., 2019)**

5.1 Morphological characteristics of microbial isolates

Microorganisms from the 6 different concentrations were characterized (Table 2), and bacteria was the dominate across all treatment with the population ranging from 4333333 to 7266667 and the highest percentage were colored cream and opaque except at 60% sweet potato concentration where the colony were translucent. Similarly Anyanwu et al., (2019) found out that bacteria was the dominate across all treatment their study

5.2 Gram's stain

Gram staining performed on bacterial colonies from the different substrate concentrations revealed the predominance of Gram-positive bacteria across all treatments. Gram-positive microorganisms are essential for suppressing harmful

pathogens like *E. coli* and Salmonella in organic waste , demonstrating their crucial role in mitigation of odors during the decomposition process. This beneficial characteristic underlines the potential of utilizing indigenous microbes from sweet potato and rice substrates in enhancing sustainable agricultural practices and improving soil health**(Seema et al., 2019; Yadav et al., 2020)**. The result also shows that all the different concentrations of substrate contain only gram positive bacteria **(Figure4,5,6 and 7).** These are beneficial microorganisms that suppress the availability and growth of harmful microorganisms like *E.coli and salmonella* in organic waste hence they play a vital role in mitigation of bad smell from decomposing organic waste, therefore contributing to sustainable and eco-friendly practice in agriculture by lowering emissions that are harmful to man and livestock as well as causing air pollution. Similarly, Seema et al (2019) and Anyanwu et al 20215 reported in their study that most of the bacteria were gram positive, implying that most of the IMOs that are useful in decomposition of waste are gram positive **(Seema et al, 2019; Anyanwu et al, 2015;)**

5.4 Growth and performance of the organisms

The result (Table 2 and 3) showed that there was a significant difference between 100% concentration of rice and sweet potato 100%. This could have been due to high water activity in rice compared to the low water activity in sweet potato due to high sugar content. Despite rice, having a lower initial water activity of 0.52 in its dry state compared to sweet potato with. 0.92. It supports significantly greater bacterial growth when hydrated because rice absorbs three to four times its weight in water to achieve an ideal environment for bacterial growth due to the water activity of 0 .97

to 0 .99 **(Adam and Kott, 1989),** while sweet potato's higher soluble sugar content 4-6% lower the water activity to around **0.92-0.95** ,that creates osmotic stress for bacteria hence lowering bacterial activity.

Most bacteria require high water activity greater than 0.91 for growth, with the optimal level around 0.97-0.99hence the reason for the high colony size in 100% rice concentration **(Colles et al., 2010).**

This is attributed to the fact that the granular structure of rice grains, provide porous architecture that is more suited for biofilm formation, compared to sweet potatoes, dense flesh that limit bacterial colonization, all of which collectively explain why numerous fermentation studies consistently show rice's best substrate yielding higher microbial count than sweet potato**(Alam et al., 2016).**

5.3 Colony size of bacteria

Colony sizes of the bacteria ranged from 1.167 to 3.333 in size, 4 bacteria colonies had flat elevation whereas 2 had convex elevation, a similar report was provided by Anyanwu et al., 2015 who reported that flat elevation was higher in their study. This implies that in IMO, bacteria that have flat elevation always dominate.

The larger colony sizes observed in a rice substrate compared to sweet potato can be attributed to rice's superior nutrient profile and physical structure as it complete starch gentalization during cooking, creating an ideal matrix for bacterial proliferation whereas sweet potatoes' fiber content and complex carbohydrate structure restrict colonial expansion**.** Neutral pH(6-7.0) favored robust cellular division against sweet potatoes, with acidic environment (pH 5.2-5.9) that slows growth rate. The high sugar

concentrations in sweet potato interfere with starch swelling, affecting bacterial activity/ activity by limiting water availability **(Aina et al., 2009).**

Further rice lack of antimicrobial phenolics that would do otherwise restrict cell to cell communication and unlike in sweet potato which ultimately resulted in the characteristics size difference observed between the 100% concentration of rice and 100% concentration sweet potato. This implies that the pH and water activity greatly influence growth on addition to nutrient availability and water activity

5.5 Colony forming unit of fungi

The Fungal Colony forming unit that reveals significant differences in growth depending on substrate concentration. The highest colony forming unit occurred in 60% sweet potato substrate, suggesting that moderate concentration provide optimal condition for proliferation. This implies that a blend between sweet potato and rice provides a favorable environment for fungal growth through balanced nutrients. A study by Maniglia et al.,(2021) confirms that blend substrate (40-60%) enhance fungal growth by providing balanced nutrient while mitigating inhibitory effect of phenolic compounds through dilution effects **(Koranda et al., 2014; Maniglia et al., 2021).** This is likely due to a balanced nutrient profile where a sweet potato's carbohydrate and micronutrients support robust growth while dilation reduces the inhibitory effect of antifungal compound like chlorogenic acid **(Di Lonardo et al., 2020).** In contrast, 100% sweet potato showed lowest CFU which aligns with finding by Cardoso et al. (2013), likely because concentrated sugar lower water activity, creating osmotic stress which reduce fungal proliferation**(Cardoso et al., 2013; Ascough et al., 2010)**. The higher level of level of natural antifungal compounds further restricted growth,

hence contributing to decrease in CFU **(Pangesty et al., 2023)**. The 100% rice substrate supported intermediate fungal count, a similar result was reported by Tian et al., (2023) **(Tian et al., 2023)**

The concentration of 60% sweet potato, provided an optimal level since it created a favorable environment for fungal colonization and growth, promoting a sustainable proliferation rate as fungi are better able to outcompete bacteria **(Murugavelh & Mohanty, 2012).** This result therefore provides a justification that in order to have a desirable microorganism, fungi proper balancing of carbon and nitrogen ratio is vital since they significantly influence microbial community dynamics **(Dijkstra et al., 2015)**

5.6 Colony size of fungi

The size of the fungal colonies exhibited an inverse relationship with the CFU density reflecting nutrient competition, resulting in stress adaptation. The result shows that the in 100% Sweet potato had the largest colonies where low CFU density reduced competition, allowing Fungi to allocate resources to hyphal expansion rather than survival under stress conditions **(Whiteside et al., 2019)**. These substrate stress induced properties like osmotic pressure from sugar may have trigger fungi to elongate hyphae for nutrient scavenging**(McCormack & Iversen, 2019).**

Conversely 100 % rice produced the smallest colonies, despite higher CFU, indicating intense competition for starch derived nutrients, which prioritizes rapid sporulation over hypha growth**(Laarhoven et al., 2016).** Meaning that though s smaller number of fungi grow in 100% concentration of sweet potato, they do not face completion as with 100% concentration hence the larger colony sizes

Intermediate sweet potato concentration (40 to 80%), yielded moderate colony sizes(7.667-8.33mm), showing a trade-off between resource availability and inhibitory effect on growth**(Bai et al., 2023)**. The finding in this research therefore extends to understanding fungal biologically meaningful responses to their environment as shown by the statistical difference of the observed trend (P-Value**<.001),** indicating adaptive effort by fungi towards optimal resource utilization which is determined by the substrate concentration in this case.

This research did not test a broader range of sweet potato concentrations, 10%, 30%,50%, 70% and 80% to identify the threshold at which growth rates significantly increase or decrease.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

For Objective one. To isolate and identify the IMO strains present in the different concentrations of sweet potatoes. I hypothesized that the strains of IMO will be Gram positive and the result revealed that all were gram positive therefore, I accept the hypothesis

For objective two: To determine the optimal level of sweet potato substrate for indigenous microorganisms growth, I hypothesized that the concentration having 40% and 60% sweet potato will enhance growth of bacteria and fungi respectively.

The results revealed that 20% was the optimal concentration of sweet potato for bacteria growth. Therefore **I fail to accept** the first segment of the hypothesis that suggested that 40% concentration of sweet potato was optimal for bacteria of growth and **accept** the second segment that 60% concentration of sweet potato will be the optimal concentration.

I hypothesized that the composition and diversity of isolated indigenous microorganisms (IMO) will vary with different concentrations of sweet potato**.** The result showed that composition and diversity of the microorganisms, vary with different concentrations of sweet potatoes. I therefore, **accept** the hypothesis.

6.2 Recommendations

The study provides valuable insight into the potential application of these findings for sustainable agriculture practices, particularly in the context of odor management in pig farming

Based on the results for this study, in order to enhance the understanding and practical application of sweet potato substrate optimization for cultivating indigenous microorganisms, further research should be conducted to validate and refine the findings regarding optimal sweet potato concentration for both bacteria and fungi. This could involve testing a broader range of concentrations like 10%, 30%,50%, 70% and 80% to identify the threshold at which growth rates significantly increase or decrease. A comprehensive analysis of microbial population dynamics to understand how varying concentrations might influence not only growth but also community diversity

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APPENDICES

ACTIVITIES	MONTHS					
	NOV	DEC	JAN	FEB	MARCH	APRIL
Concept						
Proposal writing						
Setting the experiment						
Data collection and Analysis						
Dissertation writing						
Submitting the dissertation						

Figure 14. Work plan

Figure 19. Mixing substrates

Figure 17. burrying the substrate

Figure 16 Picking substrates with IMOs

Figure 15. collected IMO

Figure 20Media preparation

Figure 22. Plating

Figure 21. Bacteria colonies

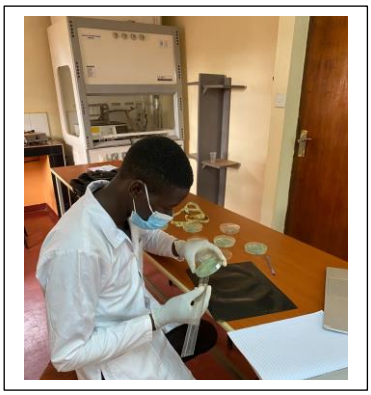

Figure 23. Measuring colony size

Figure 25Gram staining

Figure 24. Serial dilution

Figure 26. Viewing cells