

PRODUCTION OF BIO-FERTILIZER FROM AGRO WASTES AND ITS EFFECTS ON PLANT GROWTH

Submitted in partial fulfillment of requirements for the award of
Bachelor of Technology degree in Chemical Engineering

BY

MOHAMED IBRAHIM.S (39190018)

NAVDEEP.K.M (39190020)



DEPARTMENT OF CHEMICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

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JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI – 600119

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of Mohamed Ibrahim.S (39190018) & Navdeep.K.M (39190020) who Carried out the Project entitled "**Production of Bio-Fertilizer from Agro Wastes and its Effects on Plant Growth**" Under my Supervision from July 2022 to March 2023.

Internal Guide

Dr.D.Venkatesan M.Tech., Ph.D.,

(Associate Professor)

Dr.S.Sathish M.E., Ph.D.,

(Associate Professor, Head of the Department)

HEAD OF THE DEPARTMENT
DEPARTMENT OF CHEMICAL ENGINEERING
SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Jeppiaar Nagar, Rajiv Gandhi Salai
Chennai - 600 119

Submitted for Viva voce Examination held on

Internal Examiner

External Examiner

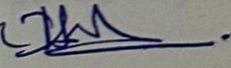
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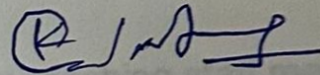
DECLARATION

We **MOHAMED IBRAHIM S (39190018) AND NAVDEEP K M (39190020)** hereby declare that the Project Report entitled "**Production of Bio-Fertilizer from Agro Wastes and its Effects on Plant growth**" done by us under the Guidance of **Dr.D.VENKATESAN M.Tech., Ph.D., (Internal)** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Chemical Engineering.

DATE : 19-04-2023

PLACE: CHENNAI

1. 

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SIGNATURE OF THE CANDIDATES

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ABSTRACT

The biofertilizer with significant potential and the capacity to stimulate crop development. The chief difference among bio – fertilizer as well as other fertilizers, such as inorganic fertilizers, is the fact that it doesn't alter the soil's quality. The feedstock material utilized is Fruit Wastes.

The objective is to convert Fruit wastes into a beneficial bio - fertilizers that are also friendly to the environment. Fertilizers are in great demand as a result of poor soil condition and a rising need for food production.

Anaerobic digestion is being considered as a substitute for the integration of alternative energy. A purposeful involvement by the government via biofertilizer policy, finance, the creation of effective quality assurance systems, and marketplace approaches is required to promote expanded firm output and appropriate deployment of Feed among farmers. More specifically, major commercialization and adoption of biofertilizer will be facilitated by both the public and private sectors involvement in consultation & raising money.

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CHAPTER – 1

INTRODUCTION

Our team's ultimate focus in this Review was to preserve the environment. Currently, proper waste conversion into valuable products is essential. The utilization of bio-fertilizers is Vital for preserving the environment. They Add Nutrients needed for plant growth. Anaerobic digestion is a process that disintegrates organic waste with anaerobic microorganisms in an airtight container in a reactor called a bioreactor or biodigester. The final products are chiefly biogas, which is mainly made up of methane, CO_2 , and digestate bio-fertilizer used as soil conditioners.

The nitrogen in the soil is fixed to the plants by biofertilizers. However, it also converts the insoluble phosphate forms, including modified form, iron, as well as aluminium phosphates, into usable forms. Additionally, this was found that bio-fertilizers generate hormonal antagonists and metabolites that enhance root development and that they take soil layers' phosphates. They also reported that they break down organic substances, which aids in the surface mineralization. Bio-fertilizers boost yield and nutrient availability by 20% when used on seeds or soil, neither affecting environment nor the soil.

Biofertilizer is a term that refers to fertilizers that contains living microorganisms. It's anticipated that these organisms' operations will alter and the soil environment provide more food to plants. Recent research and studies have concentrated on the development and advertising biofertilizer based on agro - waste.

1.1 MYCORRHIZA

Mycorrhiza inoculums are the most commonly used type of biofertilizer.

In Malaysia, where commercial use of microbial inoculants dates back to the late 1940s. Ali and other biofertilizer producers like IBG Manufacturing Sdn. Bhd had proposed conducting MINT-MAH collaborative research between 2002 through 2004. The application of stably occurring fermentation in the creation of numerous bio-products and operations has gained a great deal of attention over the years. A process known as SSF is one that uses solid substrates or supports to take place in the absence of free water.

The many types of bioreactors used to produce bio-fertilizers are reviewed in this paper, as well as the feedstocks — which primarily consist of meat processing waste, fruit peels, animal manure, human excrement, and other agricultural residues. Additionally, the numerous bio-fertilizer processes are examined, such as anaerobic digestion and kinematic models of microbial development.

1.2 SOLID STATE FERMENTATION

Solid State Fermentation (SSF) is the development and/or cultivation of microorganisms under controlled circumstances in the absence of free water for the creation of desired products of interest, in contrasting to Submerged (liquid state) Fermentation. Industrial enzymes, fuels, and nutrient-rich animal feeds are a few examples of Solid State Fermentation products. The use of contemporary biotechnological understanding and control systems technologies can greatly boost production from this antiquated technique.

Water serves as a solvent to deliver nutrients, scavenge metabolic wastes, and sustain biological structures organized at the molecular and cellular levels in SSF systems, where water content typically ranges from 30% to 85%. SSF is known to frequently offer a number of benefits over submerged (or liquid) fermentation (SmF), despite being less widely used in industry. These benefits include higher yields and productivities, extended product stability, lower production costs, lower protein breakdown (which is crucial if an enzyme is the target product), lower contamination risk, lower energy requirements, lower energy costs for sterilization, lower fermenter volume, and lower (or absent) Catabolite levels.

1.3 NITROGEN FIXATION

The nitrogen in the soil is fixed to the plants by biofertilizers. However, it also converts the insoluble phosphate forms, including modified form, iron, as well as aluminium phosphates, into usable forms. Additionally, this was found that bio-fertilizers generate hormonal antagonists and metabolites that enhance root development and that they take soil layers' phosphates. They also reported that they break down organic substances, which aids in the surface mineralization. Bio-fertilizers boost yield and nutrient availability by 20% when used on seeds or soil, neither affecting environment nor the soil.

1.4 LITERATURE REVIEW

Assessment of Bio-fertilizer Quality of Anaerobic Digestion of Watermelon Peels and Cow Dung.

D. U. Hassan and S. Abdulsalam, (2017)

Journal of Chemical and Bio molecular Engineering, 2 (3) 135–141.

In this study, waste products from fruits and veggies including papaya, melon, bananas, papayas, and orange were used. The supplies came from the neighborhood fresh market. The size of the agricultural wastes was decreased. Agro-waste was fermented solid-state employing the approach. The years of effort to ensure food safety have culminated in a significant decision to outlaw the use of uncooked foods as feed. A thorough and stringent veterinary rule (EC) 1774/2002 went into effect since May 2003 and therefore is presently undergoing revisions. Aiming to prevent not just to TSE but also other representatives that may cause illness in humans or animals, Regulation 1774 classifies animal by-products, defines required processing procedures, and specifies acceptable final uses of the by-products. These very specific health rules apply to the collection, processing, as well as disposal practices or use of species by-products.

500 gram melon water trash was placed inside a 2.5 L plastic bottle. 500 ml water was poured from the container. Then the bottle was fixed in a 298 K tank until soluble material was produced. This soluble material was passed through the constructed filter (not shown here). The fermented solution is the one that has been filtered, often known as the first solution.

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Use of animal byproducts from slaughterhouses and falling stock as feed had long been widespread and seen as economically advantageous. According to the survey, the majority of pig farms (82%) in Malaysia and Seremban not just to cause eutrophication and parasitic and bacterial pollution of the water, but also place a significant demand on oxygen. The European Commission became aware of animal by-products as a result of the recognition that hemorrhagic fever encephalopathies (TSE) may well be transmitted by food and feed.

With few adjustments, the determination of indole acetic acid synthesis followed the methodology outlined by Bric, Bostock, & Silverstone (1991). LB broth laced with L-Tryptophan was used to culture the isolates for 24 hours. The cells were then 10 minutes of centrifuging at 4°C using 10,000 Revolutions per minute. Salkowski reagent combined with one milliliter of the supernatant and 30 minutes of room temperature incubation in dark. Indole synthesis was detected by a yellow to pink colour change in the reagent.

The amount of agricultural waste produced by agro-based industries has risen by more than a third, including factories that process wood, rubber, and palm oil. 65.7% of all detected pollution sources in the industry and agricultural sectors are located in Selangor, Perak, and Johor. Livestock manure is a substantial source of pollution and a major factor in BOD.

CHAPTER – 2

AIM AND SCOPE

1. To Convert Agro Wastes into Useful Items (Bio-Fertilizers)
2. To Perform Process Synthesis to the Agro Wastes.
(i.e.) Anaerobic Fermentation (30 Days) for Batch – 1 and
(20 Days) for Batch – 2 then, Filtrate the Liquid Fertilizer.
3. To Perform Characterization of Liquid Fertilizer.
 - ✓ pH Analysis
 - ✓ Electrical Conductivity
 - ✓ Elemental Composition Analysis (N P K)
4. To Examine the Application of Bio-Fertilizer

The biofertilizer has a lot of potential and can encourage crop development. The main distinction between biofertilizer and other fertilisers, such as inorganic fertilisers, is that it doesn't change the quality of the soil. Due to the deplorable state of the soil and the growing demand for food production, fertilisers are in high demand. The infusion of alternative energy is being replaced with anaerobic digestion. More precisely, participation in consultation and funding from both the government and the private sectors will promote widespread commercialization and use of biofertilizer.

CHAPTER – 3: EXPERIMENTAL OR MATERIALS AND METHODS, ALGORITHMS USED

3.1 MATERIALS ANALYSIS

3.1.1 AGRO WASTES

In this Project, waste products from fruits and veggies including papaya, melon, mangoes, bananas, papayas, and orange were used. The supplies came from the neighborhood fresh market. The size of the agricultural wastes was decreased. Agro-waste was fermented solid-state employing the approach.

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Animal dung and other agriculture waste should not be used in ways that open up new paths for the spread of pathogens and diseases to humans, animals, and the environment. Bacteria, virus, intestinal parasites, and most recently TSE prions, can all be major pollutants.

Using an atomic absorption spectrophotometer, the Agro Waste samples' macro- and micro-mineral contents were examined for magnesium, calcium, potassium, iron, zinc, and manganese. Nitrogen, copper, and phosphorus were identified.

3.1.2 SOIL FERTILITY ANALYSIS

The capacity of the soil to support plant growth and maximize crop yield is known as soil fertility. By adding organic and inorganic fertilisers to the soil, this can be improved. Data from nuclear methods improves crop output and soil fertility while having a little negative environmental impact.

A scientific method for quickly characterizing the fertility level and forecasting the nutrient requirements for crop development, soil testing relates to the physicochemical equations to describe. The goal of land analysis is to determine whether there are enough nutrients available for plant life and to monitor any changes brought. The natural environment is distorted by mining, especially the arable land and the forest area region.

Calculations in the soil pH, calcium, electrical conductivity, mg, phosphorus, chloride, and sulfate, overall Iron, zinc, copper, sodium, potassium, organic carbon, nitrogen were made as part of the analysis of the soil's fertility.

Physicochemical changes are influenced by anthropogenic activity. Transportation, waste management, industrialization, societal, and agricultural practices have a negative impact on ecosystem health and environmental degradation.

Adding water to a soil sample and chemically separating the N, P, and K as nitrate, phosphate, and potassium are the fundamental steps in evaluating soil fertility. The solution is compared to a colour chart to identify the levels of N, P, and K in the sample.

3.1.3 BIOCHEMICAL CHARACTERISATION

Tests including the Citrate Utilization, Urease, Oxidase, Indole, Methyl Red, Triple Sugar Iron, Oxidase, and Catalase were run as part of the biochemical screening process.

3.1.4 SCREENING AND COMPOSITION OF BIO – FERTILIZER

The appropriate locations were used to gather vegetable and agricultural waste. The wastes — 50% agricultural wastes & 50% vegetable wastes were suitably crushed into granules with a mixer-grinder that were around 40 mm or smaller in diameter.

The powder form of the waste was then sterilized in an air-heated oven at 161°C for two to three hours to remove contaminants.

3.1.5 PH ANALYSIS OF THE COMPOST

By using long forceps to collect samples from three distinct locations of the composting mixture, the pH of the manure was assessed every two days. Its pH of the solution was measured using a pH meter. Effluent after the specimen was shaken for half an hour on a rotary shaker while immersed in water (w/v, 1:10).

Many nutrients' availability and the activity of soil microbes are both impacted by pH changes in the soil. Fertilizer Affect the pH of Soil. The primary nutrient that can modify soil pH is nitrogen. Depending on the nitrogen fertiliser used, soils may become more acidic.

3.2 TESTS DURING PREPARATION OF BIO – FERTILIZER

Different temperature, moisture, and inoculum size conditions were used to create bacterially inoculated biofertilizer. Table 3.1 contains information about temperature variation. Microbes broke down during the creation of the biofertilizer. The heat produced by fermentation and released the organic materials. During composting, the biofertilizers temperature rose quickly for up to 21 days. After that it gradually dropped off as the biofertilizer matured. The patterns of temperature were comparable to the preparation and commercial composting procedure.

During biofertilizer preparation, microbial inoculants need supplementation of bulk materials for their survival. They utilized the organic matter present in the waste as a source of their nutrient. The data for variation in pH have been represented. Initially the pH decreased with incubation period up to 14 and 21 days due to production of organic acid by the process of fermentation but increased Thereafter. Due to the acidic atmosphere, microbial populations tended to drop sharply since day 0 to day 14 before balancing during the preparation of the biofertilizer. Rhizobium biofertilizer quality tests can be separated into three categories.

- ✓ The Pre - culture test
- ✓ The Broth test and
- ✓ The Peat test

The following factors should be examined in the pre-culture before manufacturing Rhizobium biofertilizer:

- ✓ Progress
- ✓ Purification
- ✓ Gram staining
- ✓ Broth structure

✓ pH

Over time, composts' pH progressively rose to a neutral pH after 21 days of composting because the components that contain nitrogen deteriorate the development of NH_4^+ ions and the hydrolysis-induced release of OH^- . The neutral or slightly pH of biofertilizer is alkaline and it is beneficial because in agriculture of its contribution towards neutralization of the acidic agricultural soil.

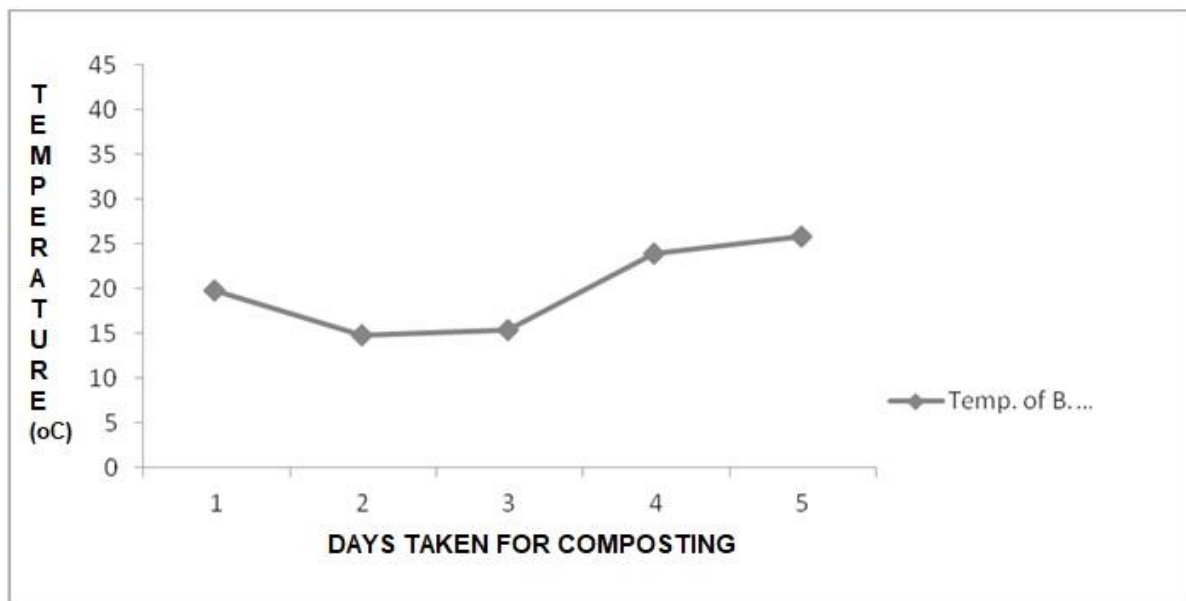


Fig 3.1 (SHOWS TEMPERATURE VARIATIONS OF AGRO-WASTES)

3.3 ANAEROBIC FERMENTATION:

- ✓ From the fruit market, agro-wastes (rotten fruits) were gathered. Watermelon and Mangoes were the fruits used in this investigation. Fruits were broken up into tiny pieces and muddled. For solid-state fermentation, they were employed (SSF).



Fig 3.2 (FRUIT WASTES COLLECTED FROM MARKET)

Two batch of fermentation process were carried out - BATCH – I & II.

3.3.1 MATERIALS REQUIRED

1. Polyethene bottle
2. Fruit wastes (rotten)
3. Distilled water

3.3.2 BATCH – I

- A polythene bottle with a 2.5 L capacity was filled with 500 grammes of water melon waste.
- It then received 100 milliliters of water. The soluble substance was created after the bottle was left alone for 30 Days Until the Soluble Product was formed.
- This soluble product underwent filtration using a manufactured filter. The first batch of water melon biofertilizer is the fermented solution.



Fig 3.3 (FRUIT WASTES CUTTED AND MUDDLED FOR BATCH-I)



Fig 3.4 (BATCH – I LIQUID BIO-FERTILIZER)

3.3.3 BATCH – II

- ✓ The purified solution was used as an inoculum precursor for the following SSF process in a volume of 100 milliliters. Wastes from a fresh Mango weighing 500 g were put in a polythene bottle.
- ✓ The precursor shortens the SSF process's length and speeds up fermentation. Until the soluble product was created, the bottle was left at room temperature unopened for 20 days.
- ✓ This soluble product underwent filtration using a manufactured filter. The second batch Watermelon – Mango biofertilizer is the name given to this filtered mixture.



FIG 3.5 (FRUIT WASTES CUTTED AND MUDDLED FOR BATCH - II)

3.4 MOISTURE CONTENT DETERMINATION OF THE COMPOSTING

Wet or dry weight calculations were used to calculate the moisture content, which was expressed as wet weight. It provides the proportion of the sample's initial wet weight that was made up of water. As a result, the compost's original weight was determined, placed in the oven, and dried for three hours at 105°C. After constant drying, the ultimate mass of the compost was also calculated; as a result, the moisture content was computed, indicating the compost's initial moisture content by utilizing Equation (1).

The anaerobic fermentation process can be more sustainably carried out if the digestate material is used as a nutrients source for plants. However, when applied, the digestate might contain pathogens and toxic substances that could harm people and reduce crop output. In contrast to the treated substrate at this epoch utilized as supply for AD, animal manure, this study attempted to assess the digestate's viability as a soil organic amendment.

3.5 BIO-CHEMICAL ANALYSIS

For the counting of bacteria, nutrient agar was employed, and for the counting of fungi, potato dextrose agar. The bio fertilizers sample was weighed at 10 g and then aseptically transferred into a beaker containing sterile saline to create a stock solution. The sample was then transferred into a second tube containing 9 ml of the same normal saline sterile and serial dilution until 10⁵ dilute was obtained. The diluted was placed septically into a dish of nutrition and potato glucose agar, respectively, using a sterile pipette size 00 (m). The pure culture [Nutrient Agar (NA) & Potato Dextrose Protein (PDA)] were then covered with the eye.

After the inoculation, the Nutrient agar plates were incubated for 24 hours at 37 °C (for bacteria) and plates of agar combined potato dextrose (for fungi) were incubated at room temperature (Drawer) for about 5–7 days and following the incubation phase, the bacteria and fungi colonies grown on the culture media (NA/PDA) were tallied, with results were used to describe a colony-forming unit per gram (Cfu/g) using Equation (2).

$$\text{Cfu/ g} = \frac{\text{Total colonel count X Concentration}}{\text{Volume}}$$

The outflow line was opened after 30 days, and the fertilisers were gathered through it. The one chosen for use had the markings N: P: K 20:10:10. To compare the liquid obtained from the biodigester with the very fine white particles that made up the chemical fertilizer, it was necessary to place both of these in the same physical container.

In order to accomplish this, 50 grammes of chemical fertilizer were weighed and then mixed with 500 ml of water to create the fertilizer solution. The properties of this combination were also sent to the lab for evaluation. Three nurseries were seeded with maize to perform this test after test pairs were enhanced with chemical fertilizer.

To get rid of rocks and other pollutants, tile next. Then 1.2kg of dirt was poured into 3 translucent containers, labeled cradles 1, 2, and 3 correspondingly.

The nurseries were constructed from 5 liter transparent buckets. Nursery one received 250 ml of chemical fertilizer, nursery two received 250 ml of liquid from the biodigester, and nursery three received 250 ml of chemical fertilizer.

The study of the concentration of organic material, ash content, and moisture content employed the ASTM D2974-07 (ASTM, 2007).

Sobiecka et al. used Kjeldahl's technique to assess the nitrogen content (2007). Using an Atomic Absorbance spectroscope (AAS), the levels of phosphorus and potassium were examined. Using a pH-meter 340I/SET (Texcare Equipment, India's New Delhi 0.01/59.16 mV/pH @25°C precision/sensitivity), pH was determined throughout the filter solution. For determining the organic carbon content, a method for determining organic cellulose (TOC) from sediments and ground has been utilized.

3.6 OTHER METHODS OF PRODUCTION

3.6.1 POTASSIUM SOLUBILIZATION

The methods through which KSB can dilute K-bearing elements and release K to improve plant development and yield are currently poorly understood. The prevailing consensus is that microbes play myriad different roles in the liberation of K⁺ in K-bearing rocks. Due to the sluggish releases of easily available exchangeable K, emitted H⁺ can immediately dissolve the mineral K. The main method of K mineral solubilization, similar to P solubilization, involves the both organic & mineral acid production as well as manufacturing of particles (acidolysis mechanism). These processes are able to change insoluble K (found in mica, muscovite, and quartzite feldspar) into soluble variations of K that are effectively absorbed by plants.

Potassium is indeed the third vital element as growing plants. In addition, it contributes to the breakdown of sugars and photosynthesis. The soil contains inorganic k, non-exchangeable potassium, & exchangeable k as types of this macronutrient. K makes up 0.04% to 3% of the soil's overall content. The majority of the potassium in soil, however, is mineral K. It is hence completely inaccessible to plants.

Because they produce a variety of organic acids, many types of microorganisms, including bacteria and fungi, boost the availability of potassium. The mechanisms of complex K solubilization, according to references, involve *Bacillus mucilaginous* & *Bacillus edaphicus* converting scarce K into available form.

In Thailand, organic fertiliser was applied to the soil to boost its nutrient content. When organic fertiliser was added, it did not the amount of sandy loam organic matter, but also the quantity of phosphate, potassium, calcium, and magnesium. How organic composts affect growth and crop and vegetable yield increases do not due to component variations, uniform, and unstable microorganisms at work.

Adding some biofertilizer can aid in enhancing the impact of having access to compost's nutrient content. In the soil, K can be found in mineral, non-exchangeable, exchangeable, and solution forms. Figure 1 illustrates how several soil K forms interact with one another. 90 of between 98% of soils K are mineral K, and the majority of this K is not available for plant absorption depending on the kind of soil. Minerals include mica and feldspar (orthoclase and microcline) that contain K. (biotite and muscovite). About 1–10% of soil K is made up of a non-exchangeable form of potassium, which is entrapped among the sheets or layers of precise types of mineral clay. Solutions K are the K form that is immediately and easily absorbed by soil-dwelling plants and microorganisms. Rhizotrophic bacteria also have significant abilities to solubilize potassium. Between potassium six strains of solubilizing bacteria were chosen from 41 isolates, KT7/2 displayed the greatest potassium levels. Activity that dissolves under soil incubation, mobilizes potassium is converted into minerals to a usable form.

Azospirillum sp., a variety of rhizotrophic bacteria, can create auxins such indol-3-acetic acid, which promote plant development. We also looked into bacteria with high auxin production. KJB 9/2 had the most activity.

Potassium insufficiency has been noted in most crop plants as a result of the majority of Potassium in the ground being in a set format (not accessible to plants directly), unequally distributed fertiliser use, significant increases the harvest production (eroding soil nutrient Potassium), and the exhaustion in the topsoil is potassium due to of farmers not adding crop residue to the soil. It is essential to discover an alternate indigenous supply of Potassium and preserve Potassium levels in soils for a sustainable crop production because Potassium -fertilizer costs are rising year (the price of potash has been \$470 per tonne since 2011).

Compared to the 24 microbial isolates belonging to the species *Azospirillum*, KJB 9/2's auxin production was not very high (IAA/l, 29-761 mg). KJB 9/2, on the other hand, produced more auxin than 11 *Pseudomonas* isolates (23.4-53.2 mg/l) and 10 isolates from *Azotobacter* (7.3-32.8 mg/l), which were chosen from rhizospheric lands in India.

It has been demonstrated that the microbial soil community can affect soil fertility by influencing soil processes such mineralization, decomposition, and the storage and release of nutrients. Some helpful soil microorganisms, including a variety of saprophytic microbes, fungal strains, and actinomycetes, have been reported to be able to convert insoluble potassium into soluble potassium through a variety of mechanisms, including the manufacturing organic and inorganic components enzymes, acidolysis, oligosaccharides, chelate, complexolysis, carbohydrates, and consequences of interaction.

By using a variety of action mechanisms, KSB can quicken the weathering processes of K minerals, particularly during the time when located in close proximity to mineral coatings. There have been attempts to increase plant nutrition by using K mobilizing bacteria to solubilize K from various K containing minerals. Although KSB may be a feasible alternative method for converting solid K towards soluble form, there are currently a number of obstacles preventing their use in agricultural practice.

For instance, (i) farmers' lack of knowledge about bio-fertilizers; (ii) the K bio-slow fertilizer's effect on crop yield; (iii) the scientific community's lack of interest in the research of K bio-fertilizer innovations; and (iv) the lack of culture collection banks for KSB .

3.6.2 NITROGEN FIXING BIO – FERTILIZER

The most crucial component for controlling plant nutrition is nitrogen. Nitrates (NO_3) and ammonium are the two forms of nitrogen that plants can receive from the soil (NH_4).

Thus, to allow for the atmosphere's nitrogen will available to plants, it might first undergo a microbiological process known as nitrogen fixation, which is accompanied by a process called as nitrification. Due to a lack of the soil's organic matter or nitrogen being washed away by the wind, there are too many azotobacters in cultivated lands and not enough of them in plant rhizosphere. Applying nitrogen-fixing biofertilizer will therefore increase the presence of these bacteria. A biofertilizer, on the other side, made of better microorganisms, can take over some of the roles played by organic fertiliser. According to Zadi and Khan⁶, adding nitrogen-fixing bacteria, potassium-solubilizing bacteria, certain fungi, and Rhizotrophic microorganisms improved wheat's growth and yield as well as its ability to absorb nutrients.

Micro - organisms like Rhizobium, Actinobacteria, Azotobacter, & Azospirillum are present in the nitrogen-fixing biofertilizers. They facilitate the conversion of nitrate into organic molecules. One technique for transforming basic nitrogen into a form the plants can use is biological nitrogen fixation. It involves turning nitrogen (N_2) into ammonia (NH_3). As a result, there is a growing demand for sustainable alternative sources, like nitrogen-fixing biofertilizers. This is due to growing awareness of water contamination and nitrate emissions.

Rhizobium is one of the microorganisms that are most frequently employed because of how well it fixes nitrogen. A symbiotic nitrogen-fixing bacterium called Rhizobium combines air nitrogen with plant roots nodules. This bacteria's pH ranges from 6.8 to 7.5 and it doesn't produce foam or have an offensive odour.

A variety of microorganisms are used in this effort to with a large quantity for N_2 fixation, as well as phosphate & potassium solubilization, was performed using the soil of agricultural areas in Thailand in order to build a new biofertilizer. Additionally, bacteria that produce a lot of auxins were chosen. Also looked at were the effects of growth promotion on a crop (corn) and a vegetable (Chinese kale).

For nitrogen reduction, nitrogenase needs a source of energy and electron donors. Mg-ATPs are created when Mg^{++} ions interact with ATPs generated through the breakdown of carbohydrates, proteins, and lipids. A Fe protein binds to the Mg^{++} of Mg-ATP to create an active compound. In order to produce energy, this Mg-ATP is converted to Mg-ADP & inorganic phosphate (ip).

1 molecule of N_2 must be converted into NH_3 using around 12–15 Mg-ATPs. Bacteroids oxidise the sugars to produce ATP, proton (H^+), and electron donors such as $NADPH_2$ and ferredoxin. Mo-Fe protein receives electrons from reduced ferredoxin. The Mg-ATPs are hydrolyzed to provide energy to the enzyme nitrogenase complex. Using the electrons, the Mo-Fe protein converts N_2 into NH_3 .

One N_2 molecule must be reduced by a minimum of six electrons into two NH_3 molecules. Then, the Fe protein and the Mo-Fe protein split apart. When there are numerous free H^+ ions present, the nitrogenase converts 2 H^+ ions into 1 H_2 molecule simultaneously delivering the Fe protein's electrons to the Mo-Fe protein.

The *ntrA*, *ntrB*, *ntrC*, and *gln A* genes, which are found elsewhere on the chromosomal DNA, control the *Nif* genes of *Azotobacter*. The *ntr A* product binds to the *Nif* gene cluster's promoter and triggers transcription. It is yet unknown how *ntr B* & *ntr C* regulate processes. Choosing bacteria: Following Forster's approach, maize, millet, rice, sugar beets, soybean, and grassland rhizospheric soil samples were gathered from 12 Thai provinces: Lopburi, Kanjanaburi, Phetchaboon, Nakon Sawan, Tak, Chiang Rai, Utharadit and Prachuab Khiri Khan.

The bacterium *Klebsiella pneumonia* is diazotrophic & free-living. Its chromosomal DNA contains *Nif* genes. The *Nif* clade has a length of 24×10^3 base pairs and 18×10^6 daltons in terms of molecular weight. It is made up of 17 *Nif* genes spread across 7 operons and is positioned between the histidine (*his*) and shikimic acid uptake (*shi*) genes. *Nif-QB*, Gene encoding, *Nif-F*, *Nif-MVSU*, *Nif-XNE*, *Nif-YKDH*, and *Nif-J* are their names.

Other genes apart from the Nif genes chromosomal DNA in *Klebsiella pneumoniae* potentially determines the expression the Nif genetics. The ntrC gene's product binds to Nif-A and activates the Nif gene cluster when NH₄ and glutamate are in short supply. The product of the ntr B gene binds to the product of the ntrC gene on Nif-A and suppresses transcription if their quantity in the cell is high. If O₂ or NH₄ levels are high, the Nif-L product binds to the Nif-A product and inhibits transcription.

Eubacteria & methanogenic archaea are both broadly yet randomly distributed in nitrogen fixation. The present knowledge of variety of nitrogenases is largely premised on evolutionary investigations of the structural genes for nitrogenases, nifH and nifD. NifH, NifD, NifK, NifE, and NifN proteins are among those encoded by the nif genes that make up nitrogenase, and a genomic analysis of these genes identified five groups: There are five types of Mo-Fe nitrogenases: (1) typical Mo-Fe nitrogenases, which are primarily made up of members of the phylas of cyanobacteria and proteobacteria; (2) absence of oxygen Mo-Fe nitrogenases, which are made by Anaerobic microorganisms predominate and a few methanogens; (3) additional nitrogenases, which include Anf & Vnf proteins, which are not dependent on Mo.

Applying a biofertilizer (containing of three different types of microorganisms, including *Azotobacter* and *Bacillus*) improved the development, output, and essential oil content of fragrant marjoram more than applying compost alone did.

3.6.3 PHOSPHORUS SOLUBILIZING BIO-FERTILIZER:

Phosphorus is among the vital macronutrient it is crucial for the plant's growth and development. It is found in the soil between 400–and 1200 mg/kg. It is present in both inorganic and organic forms in soil. Reference stated that (0.05%) Both organic and inorganic phosphorus are present in large quantities in soil. Still, hardly 0.1% is available.

The primary cause of the non-availability of soil phosphorus is it present in the soil as insoluble salts? Which is around 95%–99% of the total phosphorus in the soil? Plants can only use phosphate as H_2PO_4^- or HPO_4^- . Microorganisms such as rhizo bacteria can dissolve the complicated insoluble inorganic phosphate groups. To accessible the phosphate ions. Procedures are being performed by low-molecular-weight organic acids produced as a result of bacterial sugar metabolism. They can operate as chelating agents of divalent cations, causing insoluble phosphates to become soluble.

Bacteria that dissolve phosphorus serve as a bio fertiliser that can separate soluble molecules from insoluble inorganic phosphorus. One of the most significant characteristics linked to plant phosphorus nutrition is the ability of rhizosphere bacteria to solubilize nutrients. Phosphorus Biofertilizer, or PSB, has been presented to the agricultural sector. For plants, one of the crucial macronutrients, phosphorus is added to the soil as phosphorus fertilisers. The amount of the soil's phosphorus is around 0.05%. It is generally acknowledged that the secretion of acidic phosphates and low-molecular-weight organic acids including succinic, fumaric, acetic, formic, lactic, acetic, and lactic acids.

The same as phytase produced by soil are involved in the method of petroleum phosphorus solvation by phosphorus emulsifying bacteria strains.

Phosphorus solubilizing bacteria in the soil like *Pseudomonas* and The fungus and bacteria such as *Penicillium* and *Aspergillus* dissolve phosphate and fixed phosphorus to make it accessible for plants. 20–25% phosphorus requirement of plants is content by PSB. Due to its immobile nature, phosphorus is deficient in tropical and subtropical soils. It helps in converting food energy into chemical energy during photosynthesis and into chemical energy to be removed during respiration.

Phosphorus solubilizing bio-fertilizer application is used to aid remedy and make phosphorus more bio available and bio-accessible for the development and growth of plants. For instance, reference stated that phospho-bacterin aids in solubilizing phosphate, increasing its availability to plants.

In the literature, a variety of techniques are employed to yield bio-fertilizer, including anaerobic digestion, aerobic co-composting/re-composting procedures, closed system cultivation using sunlight, and vermicomposting (a procedure involves different genera of pests, especially red wigglers, white worms, etc.). Additionally, according to reports, the key components of a vermicomposting system include flasks, crushers, liquid, solid waste, *Eudriluseugeniae* (an African worm), a thermometer, a pH scale, and a sieve. The feedstocks also consist of banana, orange, pineapple, and watermelon peels, with a total result of 17.21% N, 10.24% P, and 48.32% K that does not fit any suitable NPK ratio.

Report indicates the bio-fertilizer produced by the fermentation method utilizing pineapple and watermelon peel has a pH value of 5.15 and low potassium content. Agar-based Pikovskaya medium but also fructose minimum medium with salts is used to select phosphorus solubilizing microorganisms (PSB) and potassium solubilizing bacteria (KSB), respectively. In Pikovskaya's medium, calcium phosphate was swapped out for 10 g/l of ores (RP, 100 mesh) as the only provider of phosphorous and 10 g/l of potassium-containing tourmaline (K-fel, 100 micron) as the sole potassium source for liquid cultivation.

Wheat, rice, chickpeas, lentils, peas, sorghum, rice, millets, oil seeds, pulses, and vegetables are some examples of common staple foods.

Dosage: Rice, finger millet, oil palm, pulses, and vegetables are all crops. May be treated with PSB. The following techniques are advised for use:

Seed Procedure: For 1 kg of seed, combine 10 ml/10 gm liquid formulation/PSB in enough water to completely cover the seeds. Let the seeds dry under cover before sowing. Spread the mixture across a 1-acre area close to the root zone after thoroughly mixing it. Phosphorus Solubilizing Bacteria help the plant access Rock Phosphorus, which is a major source of Phosphorus.

The negative impacts of phosphorus fertilisers on crop and soil health are reduced by using Phosphorus Solubilizing Bacteria, or PSB Biofertilizers.

These were introduced to the medium containing sucrose minimum salts. Approximately 10⁸ CFU/ml of each separate PSB and KSB were added, and the cells were then grown for 3 and 6 days, respectively. In the dirt quality, 0.1% weight-per-weight of RP and K-fel were added, the moisture level was regulated at field capacity, and PSB was incubated for 6 days at 37 C.

Tricalcium, iron, & aluminium phosphorus, among other insoluble forms of phosphorus, are solubilizing into soluble forms.

- ✓ They plunder the soil's phosphorus strands.
- ✓ They create hormonal antagonists and metabolites that encourage the growth of roots.
- ✓ They aid in the mineralization of soil and the breakdown of organic materials.
- ✓ When added to seeds or soil, bio fertilisers increase nutrient availability and boost yields by 10% to 25% without having a negative impact on the land or the environment.

3.7 ANAEROBIC DIGESTION

The biochemical process of anaerobic digesting involves the bio-metabolism phases of hydrolyzed, acetogenesis, acetogenesis, and methanogenesis. Larger molecular weight chemical matter, such as lipids, carbohydrates, and proteins, reportedly breaks down into smaller liquid organic matter, such as fatty, sugar, and amino acids, during the first step of hydrolysis when exo-enzymes are present.

The outputs of the hydrolyzing process are either activated by the acid created during in the second stage, known as acidogenesis, or they are transformed directly into volatile fatty acids. In the third stage, called acetogenesis, bacteria are reduced to produce the esters CHCOO^- and H_2 . Methanogens, the last stage, are essential for bacterial methane production into the atmosphere (methanogens). Applied the anaerobic digesting procedure in the lab using reactors, 20 mm aluminium crimp seals, and Amber boron glass serum vials.

They employed the effluent from Brewer's wasted grain and palm oil mills as their feedstocks, and the results showed that adding manure improved the bio-fertilizing qualities of the effluent.

The anaerobic fermenter Method is compared to what goes on inside a cow's gut, where microorganisms break down foodstuffs into biofuel, which is a combination of both carbon dioxide and methane and semi-solid waste (Fruit Wastes) or fertiliser.

The presence of microbial species, which consume and digest biomass and also produce biogas and biofertilizer, is necessary for anaerobic degradation of plant or animal material, according to. The bacterium is naturally present in soils, water bodies like lakes and wastewater from treatment plants, as well as in the digestive tracts of people and animals.

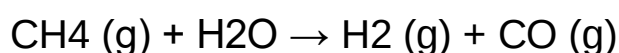
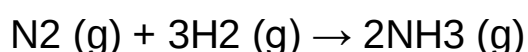
Microbial populations that are susceptible to pH changes carry out the AD process. Anaerobic microbes preferred a somewhere around 6.5 and 7.5 pH range for the best methane generation. The pH did not change between both the AD procedure and prior ($p > 0.05$). As has been demonstrated in earlier research the quality and nutrient composition of the digestate are influenced by the substrate type utilized in biogas production.

According to the study, biofertilizer production from cow dung and watermelon peel as feedstock can yield up to 83.35%. The metabolic process that leads to the biofertilizer production process is presented in Equation 1.

Substrate $\rightarrow$ CH₄ + CO₂ + H₂ + NH₃ + Bio-fertilizer

3.8 HABER BOSCH PROCESS:

One of the most effective and successful industrial processes used to produce ammonia. In order to perform the reaction on a lab scale, German researchers Fritz Haber and his helper created high-pressure equipment and catalysts in the 20th century. Carl Bosch later used the invention to produce an industrial-level machine in the year 1910. Indeed, this was a significant advancement in the scientific community.



Le Chatelier's principle states that low temperature and high pressure are favorable conditions for ammonia synthesis. The Haber procedure is typically used at temperatures of 500°C and pressures of 200 to 400 atmospheres. As NH_3 is created, it is continuously eliminated in the commercial manufacturing of ammonia. Le Chatelier's principle states that more hydrogen and nitrogen mix when the products are removed. Reversibility characterizes the reaction. The constitution of the addition reaction, the rate of the reaction, and the economics of the entire process are all impacted by variations in temperature, pressure, and the catalyst used.

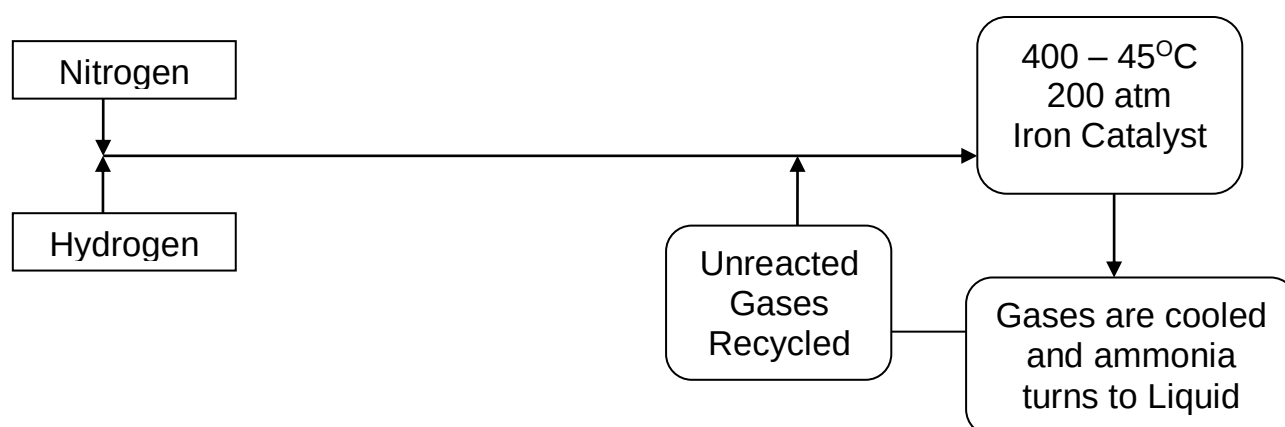


FIG 3.6 (HABER BOSCH PROCESS)

3.9 ABSORPTION OF PHOSPHORUS BY MYCORRHIZAL FUNGI

Tropical soils are deficient in phosphorus. Further most of the microorganisms solubilize P and thus make it available for plant growth. It is estimated that in most tropical soils, 75% super phosphate applied is fixed and only 25% is available for plant growth.

There are some fungi such as *Aspergillus awamori*, *Penicillium digitatum*, etc. and bacteria like *Bacillus polymyxa*, *Pseudomonas striata*, etc. that solubilize unavailable form of P to available form. India has 250 mt of rock phosphate deposits. The cheaper source of rock phosphate like Mussoorie rock phosphate and Udaipur rock phosphate available in our country can be used along with phosphate solubilizing microorganisms.

Many crop plants' roots have vesicular-arbuscular mycorrhizal (VAM) fungus colonising them. They are zygomycetous fungi from the genus *Glomus*, as well as those from the *Gigaspora*, *Acaulospora*, *Sclerocystis*, and other families.

These must be maintained as obligatory symbionts and can't be grown on artificial media. Through enhanced phosphorus nutrition, they promote plant growth, and they also shield the roots from diseases. Inoculation with effective VAM fungus can save roughly 25–30% of phosphate fertiliser.

3.10 PRODUCTION OF MYCORRHIZAL BIO-FERTILIZER

Mycorrhizal fungi are produced through a variety of inoculation techniques, depending on whether they are Ectomycorrhizal or endomycorrhizal in nature.

3.10.1 ECTOMYCORRHIZAL FUNGI

As an inoculum, you may utilise basidiospores, minced sporocarps, spore, pure mycelial cultures, broken-up mycorrhizal roots, or soil from the mycorrhizosphere region. After combining the inoculum with nursery soil, seeds are sowed.

A mycelial spore of *Pisolithus tinctorius* in a fungal growth montmorillonite moss substrate with the brand name "MycoRhiz" has been created by the Institute for Mycorrhizal Development and Research in the USA and Abbot Laboratories in the USA.

3.10.2 CYANOBACTERIA METHOD

In order to address the issues of soil quality, soil production, and environmental quality, biological nitrogen fertilisers are essential. Cyanobacterium *Anabaena azollae* coexists symbiotically with the free-floating water fern *Azolla*. The symbiotic system *Azolla*-*Anabaena* complex is known to contribute 40-60 kg N ha⁻¹ per rice crop. *Anabaena azollae* can grow photo auto-trophically and fixes atmospheric nitrogen. The nitrogen fixing cyanobacteria such as *A. azollae* and *variabilis* when immobilized in polyurethane foam and sugar cane waste have significantly increased the nitrogen fixing activity and ammonia secretion.

By secreting ammonia into flood water, the injection of phytoplankton in rice crop dramatically influenced the development of rice crop. Neem cake use as well as the inoculation of Forages substantially improved rice crop nitrogen utilization efficiency.

In addition to *Anabaena*, additional nitrogen-fixing cyanobacteria have been blamed for the natural fertility of the tropical rice fields, including *Aulosira*, *Calothrix*, *Hapalosiphon*, *Scytonema*, *Tolypothrix*, and *Westiellopsis*.

In addition to adding N, cyanobacteria also improve the physical and chemical composition of the soil by adding organic matter and growth-promoting compounds like auxins and vitamins. It has been demonstrated that alkalization can improve saline-alkaline soils, aid in the development of soil aggregates, lessen soil compaction, and reduce the C: N ratio.

These organisms allow the crop to use more of the provided nutrients, increasing the crop's ability to use fertiliser. The majority of cyanobacteria supplement nitrogen fertiliser, providing up to 30 kg N ha⁻¹ season⁻¹. The increase in crop output ranges from 5 to 25%.

3.11 MASS PRODUCTION OF CYANOBACTERIAL BIOFERTILIZERS

For outdoor cultivation of cyanobacterial biofertilizers, the regional specific strain should be used. In such practices, a mixture of 5 or 6 regionally acclimatized strains of cyanobacteria e.g. species of *Anabaena*, *Aulosira*, *Cylindrospermum*, *Gloeotrichia*, *Nostoc*, *Plectonema*, *Tolypothrix* etc. are generally used as starter inoculum.

3.12 MASS CULTIVATION OF AZOLLA

In addition to adding N, cyanobacteria also improve the physical and chemical composition of the soil by adding organic matter and growth-promoting compounds like auxins and vitamins. It has been demonstrated that algalization can improve saline-alkaline soils, aid in the development of soil aggregates, lessen soil compaction, and reduce the C: N ratio.

These organisms allow the crop to use more of the provided nutrients, increasing the crop's ability to use fertiliser. The majority of cyanobacteria supplement nitrogen fertiliser, providing up to 30 kg N ha⁻¹ season⁻¹. The increase in crop output ranges from 5 to 25%.

3.13 MACRONUTRIENTS AND MICRONUTRIENTS IN PLANTS

The elements that plants require in the greatest amounts are known as macronutrients, as the name suggests. These include carbon, oxygen, hydrogen, potassium, calcium, phosphorus, sulphur, and phosphorus. Carbon, hydrogen, and oxygen are obtained from both water and air whereas the others are derived from soil.

Micronutrients are the micronutrients that plants only incredibly little amount of. They include things like iron, zinc, and boron. Higher plants also require other minerals as silicon, cobalt, selenium, and salt. The macronutrients & micronutrients can be further divided into the following groups:

Elements make up bio-molecules, which in turn create the structure of the cell. Like oxygen, nitrogen, and carbon.

The elements that plants require in the greatest amounts are known as macronutrients.

Substances that are found in plants and are a component of chemical compounds that deal with energy, like the phosphorus in ATP.

Substances that help to inhibit or deactivate enzymes. Zinc is an illustration of a mineral that helps with alcohol dehydrogenates activation. Elements that alter the osmotic potential of the cell. Let's now look at the roles played by these macro- and micronutrients.

3.13.1 FUNCTIONS OF MACRONUTRIENTS AND MICRONUTRIENTS IN PLANTS:

- 1. Calcium:** Both cell division and the normal operations of the cellular membranes depend on this mineral. Additionally, this mineral controls metabolic functions.
- 2. Potassium:** It is an element that is essential for the development of plants. Stomata must open and close in order for life to exist. It helps keep the cell stiff and helps in the functioning of enzymes.
- 3. Magnesium:** It aids in the production of DNA and RNA and is essential in the activating of enzymes in photosynthesis and respiration. Additionally a crucial component of ribosome's, it aids in ribosome structural maintenance.
- 4. Sulfur:** a significant component of coenzymes, vitamins, and amino acids.
- 5. Iron:** Iron must be consumed in larger concentrations than that of other micronutrients. It is an essential part of protein and assists in stimulating enzymes.
- 6. Manganese:** Manganese stimulates a variety of enzymes, including those responsible for respiration, photosynthetic, and other activities. It facilitates the splitting of water during photosynthesis.
- 7. Zinc and Copper:** Several enzymes are activated by zinc and copper as well. Interstitial fluid is essential for the membrane's function as well as pollen emergence, cell expansion, germination, and other processes. Molybdenum is a component of numerous enzymes, such as nitrogenase.
- 8. Chlorine:** Analyzing the anion-cation equilibrium and cellular solute levels with chlorine is helpful. Just like humans, plants have basic needs that must be met.

Plants require constant access to both macro and micronutrients. These two groups divide macronutrients into primary and secondary types. The three

fundamental macronutrients, potassium (K), phosphorus (P), and nitrogen (N), are the most important ones (K). The truth is that more of these three basic nutrients are needed than there are of all the other macronutrients put together.

Auxiliary nitrogen and phosphorus are also required for plant health, albeit in smaller quantities than core macronutrients. The secondary macronutrients are calcium, magnesium, and sulphur (S) (Mg). In contrast to their macronutrient cousins, micronutrients are only required in trace levels for plant development and growth.

CHAPTER – 4

RESULTS AND DISCUSSION, PERFORMANCE ANALYSIS

4.1 PH ANALYSIS

By using long forceps to collect samples from three distinct locations of the composting mixture, the pH of the manure was assessed every 2 days. Its pH of the solution was measured using a pH meter. Effluent after the specimen was shaken for half an hour on a rotary shaker while immersed in water.



Fig 4.1 (PH ANALYSIS OF LIQUID BIO-FERTILIZER)

4.2 ELECTRICAL CONDUCTIVITY OF LIQUID BIO-FERTILIZER

Biofertilizers enhance plant growth and soil quality. They prevent the spread of diseases. They are economical and environmentally favorable. As natural fertilisers, biofertilizers shield the environment from contaminants.



Fig 4.2 (ELECTRICAL CONDUCTIVITY OF LIQUID BIO-FERTILIZER)

4.3 ELEMENTAL ANALYSIS OF LIQUID BIO-FERTILIZER



CIN: U93000TN2000PTC043869

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TEST REPORT

Test Report No & Date	CTL/CH/N-19705/2022-23 & 27.03.2023
Sample Number	N-19705/22-23
Name of the Customer	Mr. Mohamed Ibrahim.S.,
Address	No.12, Ambujammal Street, Alwarpet, Chennai - 600 018.
Sample Drawn by	Customer
Sample Name	Bio Fertilizer
Sample Description	Bio Fertilizer
Sampling Location	NA
Sample Drawn on	NA
Sample Collected by	NA
Sampling Plan & Procedure	NA
Sample Quantity	500 ml
Sample Condition	Good & Received in Plastic Container
Environmental Conditions	NA
Equipment used for Sampling	NA
Sample Received on	21.03.2023
Analysis Started on	22.03.2023
Analysis Completed on	24.03.2023

Test Results:

The above sample tested as received, and results are as follows:

S.No	PARAMETERS	TEST METHOD	UNITS	RESULTS
1	Nitrogen as N	Schedule - IV, Part D(6) of FCO, 1985	%	1.3
2	Phosphorous as P	Schedule - IV, Part D(8) of FCO, 1985	%	0.37
3	Potassium as K	Schedule - IV, Part D(9) of FCO, 1985	%	1.17

END OF REPORT

Verified by

For Chennai Testing Laboratory Pvt Ltd

Authorised Signatory

A. RAJKUMAR
Technical Manager
(CHEMICAL)

Page 1 of 1

The Report shall not be used to assign, defend and for any malicious purpose.
The Report is meant only for sole use of the addressee to promote his/her own business.

A - Super 19 | T.V.K. Industrial Estate | Guindy | E-mail : chennaitestinglab@gmail.com
Chennai - 600 032 | Tamil Nadu | India | Telefax : +91-44-2250 1757

4.4 EFFECT ON PLANT NUTRITION

The cultivation of the nutrition of the world's expanding population depends on crops and healthful food. A vital element of sustainable agriculture is plant nutrition. Natural stocks of plant nutrients exist in soils, however only a little quantity is produced annually via biological or chemical processes, and plants are unable to access these reserves. This flow is too sluggish to both meet crop needs and make up for nitrogen losses brought on by agricultural output. The quantities of the 16 essential plant minerals (Fe, Zn, Cu, Mo, Manganese, B, and Cl; N, P, K, Calcium, Mg, and S; Micronutrients) needed to produce crops with the highest yield are well-established.

All of the A productive soil should contain both macro- and micronutrients since these components assist plant nutrient uptake. The application of fertilisers and organic manure is a crucial graining activity since good fertility is essential for effective plant growth. Keeping soil's nutrient levels appropriate is important. Crucial for the establishment of healthy plants all the minerals are not provided by chemical fertilisers alone in the proper balance that plants require the organic matter content of the soil decreases as a result, which has an effect on the biological systems and soil's physical attributes. The use of biological manures has sparked interest as a result of all these general considerations.

In favor of the use of organic manure the activity of the management of organic manure includes other factors as well. Crop yields, but is also essential for demonstrating influences the nutrition in a direct and indirect manner. Increasing soil porosity through improved physical, chemical enhances the physiological, biological, and Chemical, Utilization and efficacy of Fertilizers Applied.

CHAPTER – 5

SUMMARY AND CONCLUSION

5.1 SUMMARY

According to the results of research on the liquid byproduct of Agro Wastes anaerobic decomposition Crop residues like Fruit Wastes can be utilized in a biodigester to create additional useful products like methane and fertilisers that functions as a replacement energy source as well as another waste management technique. And it informed us that the residue of the anaerobic Agro waste decay or other types of biological waste can provide excellent manure for the typical farmer. The characteristics of the crop production that sprouted from the 3 different soil types used implied that the chemical fertiliser tends to increase rate of growth of crops but is dangerous to the land and the plants been developed on it - this results in the expansion of leaves, flowers, and fruit and vegetables that tend to diverge from the standard and in some terrible case scenarios, it may cause the death of a plant. It should also be noted that tests have shown liquid biofertilizer, which is the product of the anaerobic decomposition of Fruit Wastes, to be excellent for nourishing plants and soil.

5.2 CONCLUSION

The Anaerobic Digestion process is made environmentally friendly and economically viable by using its by-products, particularly the digestate. Compared to using the digestate as bio-fertilizer, the transformation of biogas to power is more well-established. As a result, the current study's main objective is to ascertain the nutrients that make up the feed and digestate. It was also done to identify and characterize the microbial communities' capacity to promote plant growth.

CHAPTER – 6

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PRODUCTION OF BIO-FERTILIZER FROM AGRO
WASTES AND ITS EFFECTS ON PLANT GROWTH

⁴
Submitted in partial fulfillment of requirements for the award of
Bachelor of Technology degree in Chemical Engineering

BY

MOHAMED IBRAHIM.S (39190018)

NAVDEEP.K.M (39190020)

²
DEPARTMENT OF CHEMICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of Mohamed

Ibrahim.S (39190018) & Navdeep.K.M (39190020) ⁴ who Carried out the

Project entitled "Production of Bio-Fertilizer from Agro Wastes and its

Effects on Plant Growth" ² Under my Supervision from July 2022 to March

2023.

Internal Guide

Dr.D.Venkatesan

(Assistant Professor, M.E., Ph.D.,)

²
Dr.S.Sathish M.E., Ph.D.,

(Associate Professor, Head of the Department)

Submitted for Viva voce Examination held on

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iii

DECLARATION

I MOHAMED IBRAHIM S (39190018) ² hereby declare that the Project Report entitled "Production of Bio-Fertilizer from Agro Wastes and its Effects on Plant growth" ¹⁴ done by me under the Guidance of MR.D.VENKATESAN (Internal) is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Chemical Engineering.

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iv

DECLARATION

I NAVDEEP K M (39190020) ² hereby declare that the Project Report entitled "Production of Bio-Fertilizer from Agro Wastes and its Effects on Plant growth" ⁴ done by me under the Guidance of

MR.D.VENKATESAN (Internal) ⁴ is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Chemical Engineering.

DATE :

PLACE : SIGNATURE OF THE CANDIDATE

v

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I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Chemical Engineering and Electronics Engineering who were helpful in many ways for the completion of the Project.

vi

ABSTRACT

The biofertilizer with significant potential and the capacity to stimulate crop development. The chief difference among bio – fertilizer as well as other fertilizers, such as inorganic fertilizers, is the fact that it doesn't alter the soil's quality. The feedstock material utilized is Fruit Wastes. The objective is to convert Fruit wastes into a beneficial bio - fertilizers that are also friendly to the environment. Fertilizers are in great demand as a result of poor soil condition and a rising need for food production. Anaerobic digestion is being considered as a substitute for the integration of alternative energy. A purposeful involvement by the government via biofertilizer policy, finance, the creation of effective quality assurance systems, and marketplace approaches is required to promote expanded firm output and appropriate deployment of Feed among farmers. More specifically, major commercialization and adoption of biofertilizer will be facilitated by both the public and private sectors involvement in consultation & raising money.

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CHAPTER – 1

INTRODUCTION

Our team's ultimate focus in this Review was to preserve the environment.

Currently, proper waste conversion into valuable products is essential. The utilization of bio-fertilizers is Vital for preserving the environment. They Add

Nutrients needed for plant growth. Anaerobic digestion is a process that disintegrates organic waste with anaerobic microorganisms in an airtight container in a reactor called a bioreactor or biodigester. The final products are chiefly biogas, which is mainly made up of methane, CO_2 , and digestate biofertilizer used as soil conditioners.

The nitrogen in the soil is fixed to the plants by biofertilizers. However, it also converts the insoluble phosphate forms, including modified form, iron, as well as aluminium phosphates, into usable forms. Additionally, this was found that bio-fertilizers generate hormonal antagonists and metabolites that enhance root development and that they take soil layers' phosphates. They also reported that they break down organic substances, which aids in the surface mineralization. Bio-fertilizers boost yield and nutrient availability by 20% when used on seeds or soil, neither affecting environment nor the soil.

Biofertilizer is a term that refers to fertilizers that contains living microorganisms. It's anticipated that these organisms' operations will alter and the soil environment provide more food to plants. Recent research and studies have concentrated on the development and advertising biofertilizer based on agro - waste.

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1.1 MYCORRHIZA

Mycorrhiza inoculums are the most commonly used type of biofertilizer.

In Malaysia, where commercial use of microbial inoculants dates back to the late 1940s. Ali and other biofertilizer producers like IBG Manufacturing Sdn.

Bhd had proposed conducting MINT-MAH collaborative research between 2002 through 2004. The application of stably occurring fermentation in the creation of numerous bio-products and operations has gained a great deal of attention over the years. A process known as SSF is one that uses solid substrates or supports to take place in the absence of free water.

The many types of bioreactors used to produce bio-fertilizers are reviewed in this paper, as well as the feedstocks — which primarily consist of meat processing waste, fruit peels, animal manure, human excrement, and other agricultural residues. Additionally, the numerous bio-fertilizer processes are examined, such as anaerobic digestion and kinematic models of microbial development.

1.2 SOLID STATE FERMENTATION

¹⁵ Solid State Fermentation (SSF) is the development and/or cultivation of microorganisms under controlled circumstances in the absence of free water for the creation of desired products of interest, in contrasting to Submerged ²⁷ (liquid state) Fermentation. Industrial enzymes, fuels, and nutrient-rich animal feeds are a few examples of Solid State Fermentation products. The use of contemporary biotechnological understanding and control systems technologies can greatly boost production from this antiquated technique.

3

Water serves ⁸ as a solvent to deliver nutrients, scavenge metabolic wastes, and sustain biological structures organized at the molecular and cellular levels in SSF systems, where water content typically ranges from 30% to 85%. ⁸ SSF

is known to frequently offer a number of benefits over submerged (or liquid) fermentation (SmF), despite being less widely used in industry. These benefits include higher yields and productivities, extended product stability, ⁸ lower production costs, lower protein breakdown (which is crucial if an enzyme is the target product), lower contamination risk, lower energy requirements, lower energy costs for sterilization, lower fermenter volume, and lower (or absent) Catabolite levels.

1.3 NITROGEN FIXATION

The nitrogen in the soil is fixed to the plants by biofertilizers. However, it also converts the insoluble phosphate forms, including modified form, iron, as well as aluminium phosphates, into usable forms. Additionally, this was found that bio-fertilizers generate hormonal antagonists and metabolites that enhance root development and that they take soil layers' phosphates. They also reported that they break down organic substances, which aids in the surface mineralization. Bio-fertilizers boost yield and nutrient availability by 20% when used on seeds or soil, neither affecting environment nor the soil.

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1.4 LITERATURE REVIEW

¹⁹ Assessment of Bio-fertilizer Quality of Anaerobic Digestion of Watermelon Peels and Cow Dung.

D. U. Hassan and S. Abdulsalam, (2017)

Journal of Chemical and Bio molecular Engineering, 2 (3) 135–141.

In this study, waste products from fruits and veggies including papaya, melon,

bananas, papayas, and orange were used. The supplies came from the neighborhood fresh market. The size of the agricultural wastes was decreased. Agro-waste was fermented solid-state employing the approach. The years of effort to ensure food safety have culminated in a significant decision to outlaw the use of uncooked foods as feed. A thorough and stringent veterinary rule (EC) 1774/2002 went into effect since May 2003 and therefore is presently undergoing revisions. Aiming to prevent not just to TSE but also other representatives that may cause illness in humans or animals, Regulation 1774 classifies animal by-products, defines required processing procedures, and specifies acceptable final uses of the by-products. These very specific health rules apply to the collection, processing, as well as disposal practices or use of species by-products.

500 gram melon water trash was placed inside a 2.5 L plastic bottle. 500 ml water was poured from the container. Then the bottle was fixed in a 298 K tank until soluble material was produced. This soluble material was passed through the constructed filter (not shown here). The fermented solution is the one that has been filtered, often known as the first solution.

5

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Use of animal byproducts from slaughterhouses and falling stock as feed had long been widespread and seen as economically advantageous. According to the survey, the majority of pig farms (82%) in Malaysia and Seremban not just to cause eutrophication and parasitic and bacterial pollution of the water, but also place a significant demand on oxygen. The European Commission became aware of animal by-products as a result of the recognition that hemorrhagic fever encephalopathies (TSE) may well be transmitted by food and feed.

With few adjustments, the determination of indole acetic acid synthesis followed the methodology outlined by Bric, Bostock, & Silverstone (1991). LB broth laced with L-Tryptophan was used to culture the isolates for 24 hours.

The cells were then 10 minutes of centrifuging at 4°C using 10,000 Revolutions per minute. Salkowski reagent combined with one milliliter of the supernatant and 30 minutes of room temperature incubation in dark. Indole synthesis was detected by a yellow to pink colour change in the reagent.

The amount of agricultural waste produced by agro-based industries has risen by more than a third, including factories that process wood, rubber, and palm oil. 65.7% of all detected pollution sources in the industry and agricultural sectors are located in Selangor, Perak, and Johor. Livestock manure is a substantial source of pollution and a major factor in BOD.

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CHAPTER – 2

AIM AND SCOPE

1. To Convert Agro Wastes into Useful Items (Bio-Fertilizers)

2. To Perform Process Synthesis to the Agro Wastes.

(i.e.) Anaerobic Fermentation (30 Days) for Batch – 1 and

(20 Days) for Batch – 2 then, Filtrate the Liquid Fertilizer.

3. To Perform Characterization of Liquid Fertilizer.

☐ pH Analysis

☐ Electrical Conductivity

☐ Elemental Composition Analysis (N P K)

4. To Examine the Application of Bio-Fertilizer

The biofertilizer has a lot of potential and can encourage crop development.

The main distinction between biofertilizer and other fertilisers, such as

inorganic fertilisers, is that it doesn't change the ³¹quality of the soil. Due to the

deplorable state of the soil and the growing demand for food production,

fertilisers are in high demand. The infusion of alternative energy is being

replaced with anaerobic digestion. More precisely, participation in consultation

and funding from both the government and the private sectors will promote

widespread commercialization and use of biofertilizer.

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CHAPTER – 3: EXPERIMENTAL OR MATERIALS AND METHODS,

ALGORITHMS USED

3.1 MATERIALS ANALYSIS

3.1.1 AGRO WASTES

In this Project, waste products from fruits and veggies including papaya,

melon, mangoes, bananas, papayas, and orange were used. The supplies came from the neighborhood fresh market. The size of the agricultural wastes was decreased. Agro-waste was fermented solid-state employing the approach.

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broth laced with L-Tryptophan was used to culture the isolates for 24 hours.

The cells were then 10 minutes of centrifuging at 4°C using 10,000

Revolutions per minute. Salkowski reagent combined with one milliliter of the supernatant and 30 minutes of room temperature incubation in dark. Indole synthesis was detected by a yellow to pink colour change in the reagent.

The amount of agricultural waste produced by agro-based industries has risen by more than a third, including factories that process wood, rubber, and palm oil. 65.7% of all detected pollution sources in the industry and agricultural sectors are located in Selangor, Perak, and Johor. Livestock manure is a substantial source of pollution and a major factor in BOD. According to a recent study from the Veterinary Services Department, there were roughly 717 standing pigs per farm in the WCPM. Large amounts of manure are produced by pig farms, while some of these farmlands release their effluents untreated directly into marshes and tidal rivers.

Animal dung and other agriculture waste should not be used in ways that open up new paths for the spread of pathogens and diseases to humans, animals, and the environment. Bacteria, virus, intestinal parasites, and most recently TSE prions, can all be major pollutants.

Using an atomic absorption spectrophotometer, the Agro Waste samples' macro- and micro-mineral contents were examined for magnesium, calcium, potassium, iron, zinc, and manganese. Nitrogen, copper, and phosphorus were identified.

3.1.2 SOIL FERTILITY ANALYSIS

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The capacity of the soil to support plant growth and maximize crop yield is known as soil fertility. By adding organic and inorganic fertilisers to the soil, this can be improved. Data from nuclear methods improves crop output and soil fertility while having a little negative environmental impact.

A scientific method for quickly characterizing the fertility level and forecasting the nutrient requirements for crop development, soil testing relates to the physicochemical equations to describe. ²⁰ The goal of land analysis is to determine whether there are enough nutrients available for plant life and to monitor any changes brought. The natural environment is distorted by mining, especially the arable land and the forest area region.

Calculations in the soil pH, calcium, electrical conductivity, mg, phosphorus, chloride, and sulfate, overall Iron, zinc, copper, sodium, potassium, organic carbon, nitrogen were made as part of the analysis of the soil's fertility.

Physicochemical changes are influenced by anthropogenic activity.

Transportation, waste management, industrialization, societal, and agricultural practices have a negative impact on ecosystem health and environmental degradation.

Adding water to a soil sample and chemically separating the N, P, and K as nitrate, phosphate, and potassium are the fundamental steps in evaluating soil fertility. The solution is compared to a colour chart to identify the levels of N, P, and K in the sample.

3.1.3 BIOCHEMICAL CHARACTERISATION

Tests including the Citrate Utilization, Urease, Oxidase, Indole, Methyl Red, Triple Sugar Iron, Oxidase, and Catalase were run as part of the biochemical screening process.

3.1.4 SCREENING AND COMPOSITION OF BIO – FERTILIZER

The appropriate locations were used to gather vegetable and agricultural waste. ³ The wastes — 50% agricultural wastes & 50% vegetable wastes were suitably crushed into granules with a mixer-grinder that were around 40 mm or smaller in diameter.

³ The powder form of the waste was then sterilized in an air-heated oven at 161°C for two to three hours to remove contaminants.

3.1.5 PH ANALYSIS OF THE COMPOST

By using long forceps to collect samples from three distinct locations of the composting mixture, the pH of the manure was assessed every two days. Its ¹² pH of the solution was measured using a pH meter. Effluent after the specimen was shaken for half an hour on a rotary shaker while immersed in water (w/v, 1:10).

Many nutrients' availability and the activity of soil microbes are both impacted by pH changes in the soil. Fertilizer Affect the pH of Soil. The primary nutrient that can modify soil pH is nitrogen. Depending on the nitrogen fertiliser used, soils may become more acidic.

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3.2 TESTS DURING PREPARATION OF BIO – FERTILIZER

Different temperature, moisture, and inoculum size conditions were used to create bacterially inoculated biofertilizer. Table 3.1 contains information about temperature variation. Microbes broke down during the creation of the biofertilizer. The heat produced by fermentation and released the organic materials. During composting, the biofertilizers temperature rose quickly for ³ up to 21 days. After that it gradually dropped off as the biofertilizer matured.

The patterns of temperature were comparable to the preparation and commercial composting procedure.

³ During biofertilizer preparation, microbial inoculants need supplementation of bulk materials for their survival. They utilized the organic matter present in the waste as a source of their nutrient. The data for variation in pH have been represented. ³ Initially the pH decreased with incubation period up to 14 and 21 days due to production of organic acid by the process of fermentation but increased Thereafter. Due to the acidic atmosphere, microbial populations tended to drop sharply since day 0 to day 14 before balancing during the preparation of the biofertilizer. Rhizobium biofertilizer quality tests can be separated into three categories.

- ☐ The Pre - culture test
- ☐ The Broth test and
- ☐ The Peat test

The following factors should be examined in the pre-culture before manufacturing Rhizobium biofertilizer:

- ☐ Progress

- Purification
- Gram staining
- Broth structure

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- pH

Over time, composts' pH progressively rose ³ to a neutral pH after 21 days of composting because the components that contain nitrogen deteriorate the development of NH_4^+ ions and the hydrolysis-induced release of OH^- . The neutral or slightly pH of biofertilizer is ³ alkaline and it is beneficial because in agriculture of its contribution towards neutralization of the acidic agricultural soil.

Fig 3.1 (SHOWS TEMPERATURE VARIATIONS OF AGRO-WASTES)

3.3 ANAEROBIC FERMENTATION:

- From the fruit market, agro-wastes (rotten fruits) were gathered.

Watermelon and Mangoes were the fruits used in this investigation. Fruits were broken up into tiny pieces and muddled. For solid-state fermentation, they were employed (SSF).

13

Fig 3.2 (FRUIT WASTES COLLECTED FROM MARKET)

¹⁰ Two batch of fermentation process were carried out - BATCH – I & II.

3.3.1 MATERIALS REQUIRED

1. Polyethene bottle

2. Fruit wastes (rotten)

3. Distilled water

3.3.2 BATCH – I

□ A polythene bottle with a 2.5 L capacity was filled with 500 grammes of water melon waste.

□ It then received 100 milliliters of water. The soluble substance was created after ¹⁰the bottle was left alone for 30 Days Until the Soluble Product was formed.

□ This soluble product underwent filtration using a manufactured filter.

The first batch of water melon biofertilizer is the fermented solution.

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Fig 3.3 (FRUIT WASTES CUTTED AND MUDDLED FOR BATCH-I)

Fig 3.4 (BATCH – I LIQUID BIO-FERTILIZER)

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3.3.3 BATCH – II

□ The purified ¹⁰solution was used as an inoculum precursor for the following SSF process in a volume of 100 milliliters. Wastes from a fresh Mango weighing 500 g were put in a polythene bottle.

□ The precursor shortens the SSF process's length and speeds up fermentation. Until the soluble product was created, the bottle was left at room temperature unopened for 20 days.

□ This soluble product underwent filtration using a manufactured filter.

The second batch Watermelon – Mango biofertilizer is the name given to this filtered mixture.

FIG 3.5 (FRUIT WASTES CUTTED AND MUDDLED FOR BATCH - II)

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3.4 MOISTURE CONTENT DETERMINATION OF THE COMPOSTING

Wet or dry weight calculations were used to calculate the moisture content, which was expressed as wet weight. It provides the proportion of the sample's initial wet weight that was made up of water. As a result, the compost's original weight was determined, placed in the oven, and dried for three hours at 105°C. After constant drying, the ultimate mass of the compost was also calculated; as a result, the moisture content was computed, indicating the compost's initial moisture content by utilizing Equation (1).

The anaerobic fermentation process can be more sustainably carried out if the digestate material is used as a nutrients source for plants. However, when applied, the digestate might contain pathogens and toxic substances that could harm people and reduce crop output. In contrast to the treated substrate at this epoch utilized as supply for AD, animal manure, this study attempted to assess the digestate's viability as a soil organic amendment.

3.5 BIO-CHEMICAL ANALYSIS

For the counting of bacteria, nutrient agar was employed, and for the counting of fungi, potato dextrose agar. The bio fertilizers sample was weighed at 10 g and then aseptically transferred into a beaker containing sterile saline to

create a stock solution. The sample was then transferred into a second tube containing 9 ml of the same normal saline sterile and serial dilution until 10⁵ dilute was obtained. The diluted was placed septically into a dish of nutrition and potato glucose agar, respectively, using a sterile pipette size 00 (m). The pure culture [Nutrient Agar (NA) & Potato Dextrose Protein (PDA)] were then covered with the eye.

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After the inoculation, ²³ the Nutrient agar plates were incubated for 24 hours at 37 °C (for bacteria) and plates of agar combined potato dextrose (for fungi) were incubated at room temperature (Drawer) for about 5–7 days and following the incubation phase, the bacteria and fungi colonies grown on the culture media (NA/PDA) were tallied, with results were used to describe a colony-forming unit per gram (Cfu/g) using Equation (2).

$$\text{Cfu/ g} = \text{Total colonel count} \times \text{Concentration}$$

Volume

The outflow line was opened after 30 days, and the fertilisers were gathered through it. The one chosen for use had the markings N: P: K 20:10:10. To compare the liquid obtained from the biodigester with the very fine white particles that made up the chemical fertilizer, it was necessary to place both of these in the same physical container.

In order to accomplish this, 50 grammes of chemical fertilizer were weighed and then mixed with 500 ml of water to create the fertilizer solution. The

properties of this combination were also sent to the lab for evaluation. Three nurseries were seeded with maize to perform this test after test pairs were enhanced with chemical fertilizer.

To get rid of rocks and other pollutants, tile next. Then 1.2kg of dirt was poured into 3 translucent containers, labeled cradles ¹¹ 1, 2, and 3 correspondingly.

The nurseries were constructed from 5 liter transparent buckets. Nursery one received 250 ml of chemical fertilizer, nursery two received 250 ml of liquid from the biodigester, and nursery three received 250 ml of chemical fertilizer. The study of the concentration ¹⁷ of organic material, ash content, and moisture content employed the ASTM D2974-07 (ASTM, 2007).

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Sobiecka et al. used Kjeldahl's technique to assess the nitrogen content (2007). Using an Atomic Absorbance spectroscope (AAS), the levels of phosphorus and potassium were examined. Using a ¹⁷ pH-meter 340I/SET (Texcare Equipment, India's ¹⁷ New Delhi 0.01/59.16 mV/pH @25°C precision/sensitivity), pH was determined throughout the filter solution. For determining the organic carbon content, a method for determining organic cellulose (TOC) from sediments and ground has been utilized.

3.6 OTHER METHODS OF PRODUCTION

3.6.1 POTASSIUM SOLUBILIZATION

The methods through which KSB can dilute K-bearing elements and release K to improve plant development and yield are currently poorly understood.

The prevailing consensus is that microbes play myriad different roles in the liberation of K^+ in K-bearing rocks. Due to the sluggish releases of easily available exchangeable K, emitted H^+ can immediately dissolve the mineral K. The main method of K mineral solubilization, similar to P solubilization, involves the both organic & mineral acid production as well as manufacturing of particles (acidolysis mechanism). These processes ¹⁶ are able to change insoluble K (found in mica, muscovite, and quartzite feldspar) into soluble variations of K that are effectively absorbed by plants.

Potassium is indeed the third vital element as growing plants. In addition, it contributes to the breakdown of sugars and photosynthesis. The soil contains inorganic k, non-exchangeable potassium, & exchangeable k as types of this macronutrient. K makes up 0.04% to 3% of the soil's overall content. ³⁴ The majority of the potassium in soil, however, is mineral K. It is hence completely inaccessible to plants.

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Because they produce a variety of organic acids, many types of microorganisms, including bacteria and fungi, boost the availability of potassium. The mechanisms of complex K solubilization, according to references, involve *Bacillus mucilaginous* & *Bacillus edaphicus* converting scarce K into available form.

In Thailand, organic fertiliser was applied to the soil to boost its nutrient content. When organic fertiliser was added, it did not the amount of sandy loam organic matter, but also the quantity of phosphate, potassium, calcium,

and magnesium. How organic composts affect growth and crop and vegetable yield increases do not due to component variations, uniform, and unstable microorganisms at work.

Adding some biofertilizer can aid in enhancing the impact of having access to compost's nutrient content. In the soil, K can be found in mineral, nonexchangeable, exchangeable, and solution forms. Figure 1 illustrates how

several soil K forms interact with one another. 90 of between ¹⁶98% of soils K are mineral K, and the majority of this K is not available for plant absorption depending on the kind of soil. Minerals include mica and feldspar (orthoclase and microcline) that contain K. (biotite and muscovite). About 1–10% ²¹of soil K is made up of a non-exchangeable form of potassium, which is entrapped among the sheets or layers of precise types of mineral clay. Solutions K are the K form that is immediately and easily absorbed by soil-dwelling plants and microorganisms. Rhizotrophic bacteria also have significant abilities to solubilize potassium. Between potassium six strains of solubilizing bacteria were chosen from 41 isolates, KT7/2 displayed the greatest potassium levels. Activity that dissolves under soil incubation, mobilizes potassium is converted into minerals to a usable form.

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Azospirillum sp., a variety of rhizotrophic bacteria, can create auxins such indol-3-acetic acid, which promote plant development. We also looked into bacteria with high auxin production. KJB 9/2 had the most activity.

Potassium insufficiency has been noted in most crop plants as a result of the

majority of Potassium in the ground being in a set format (not accessible to plants directly), unequally distributed fertiliser use, significant increases the harvest production (eroding soil nutrient Potassium), and the exhaustion in the topsoil is potassium due to of farmers not adding crop residue to the soil. It is essential to discover an alternate indigenous supply of Potassium and preserve Potassium levels in soils for a sustainable crop production because Potassium -fertilizer costs are rising year (the price of potash has been \$470 per tonne since 2011).

Compared to the 24 microbial isolates belonging to the species *Azospirillum*, KJB 9/2's auxin production was not very high (IAA/I, 29-761 mg). KJB 9/2, on the other hand, produced more auxin than 11 *Pseudomonas* isolates (23.4-53.2 mg/l) and 10 isolates from *Azotobacter* (7.3-32.8 mg/l), which were chosen from rhizospheric lands in India.

It has been demonstrated that the microbial soil community can affect soil fertility by influencing soil processes such mineralization, decomposition, and the storage and release of nutrients. Some helpful soil microorganisms, including a variety of saprophytic microbes, fungal strains, and actinomycetes, have been reported to be able to convert insoluble potassium into soluble potassium through a variety of mechanisms, including the manufacturing organic and inorganic components enzymes, acidolysis, oligosaccharides, chelate, complexolysis, carbohydrates, and consequences of interaction.

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By using a variety of action mechanisms, KSB can quicken the weathering

processes of K minerals, particularly during the time when located in close proximity to mineral coatings. There have been attempts to increase plant nutrition by using K mobilizing bacteria to solubilize K from various K containing minerals. Although KSB may be a feasible alternative method for converting solid K towards soluble form, there are currently a number of obstacles preventing their use in agricultural practice.

For instance, I farmers' lack of knowledge about bio-fertilizers; (ii) the K bioslow fertilizer's effect on crop yield; (iii) the scientific community's lack of interest in the research of K bio-fertilizer innovations; and (iv) the lack of culture collection banks for KSB .

3.6.2 NITROGEN FIXING BIO – FERTILIZER

The most crucial component for controlling plant nutrition is nitrogen. Nitrates (NO_3) and ammonium are the two forms of nitrogen that plants can receive from the soil (NH_4).

Thus, to allow for the atmosphere's nitrogen will available to plants, it might first undergo a microbiological process known as nitrogen fixation, which is accompanied by a process called as nitrification. Due to a lack of the soil's organic matter or nitrogen being washed away by the wind, there are too many azotobacters in cultivated lands and not enough of them in plant rhizosphere. Applying nitrogen-fixing biofertilizer will therefore increase the presence of these bacteria. A biofertilizer, on the other side, made of better microorganisms, can take over some of the roles played by organic fertiliser.

According to Zadi and Khan⁶, adding nitrogen-fixing bacteria, potassium-solubilizing bacteria, certain fungi, and Rhizotrophic microorganisms improved wheat's growth and yield as well as its ability to absorb nutrients.

22

Micro-organisms like Rhizobium, Actinobacteria, Azotobacter, & Azospirillum are present in the nitrogen-fixing biofertilizers. They facilitate the conversion of nitrate into organic molecules. One technique for transforming basic nitrogen into a form the plants can use is biological nitrogen fixation. It involves turning nitrogen (N_2) into ammonia (NH_3). As a result, there is a growing demand for sustainable alternative sources, like nitrogen-fixing biofertilizers. This is due to growing awareness of water contamination and nitrate emissions.

Rhizobium is one of the microorganisms that are most frequently employed because of how well it fixes nitrogen. A symbiotic nitrogen-fixing bacterium called Rhizobium combines air nitrogen with plant roots nodules. This bacteria's pH ranges from 6.8 to 7.5 and it doesn't produce foam or have an offensive odour.

A variety of microorganisms are used in this effort to with a large quantity for N_2 fixation, as well as phosphate & potassium solubilization, was performed using the soil of agricultural areas in Thailand in order to build a new biofertilizer. Additionally, bacteria that produce a lot of auxins were chosen. Also looked at were the effects of growth promotion on a crop (corn) and a vegetable (Chinese kale).

For nitrogen reduction, nitrogenase needs a source of energy and electron donors. Mg-ATPs are created when Mg^{++} ions interact with ATPs generated through the breakdown of carbohydrates, proteins, and lipids. A Fe protein binds to the Mg^{++} of Mg-ATP to create an active compound. In order to produce energy, this Mg-ATP is converted to Mg-ADP & inorganic phosphate (ip).

23

1 molecule of N_2 must be converted into NH_3 using around 12–15 MgATPs. Bacteroids oxidise the sugars to produce ATP, proton (H^+), and electron donors such as $NADPH_2$ and ferredoxin. Mo-Fe protein receives electrons from reduced ferredoxin. The Mg-ATPs are hydrolyzed to provide energy to the enzyme nitrogenase complex. Using the electrons, the Mo-Fe protein converts N_2 into NH_3 .

One N_2 molecule must be reduced by a minimum of six electrons into two NH_3 molecules. Then, the Fe protein and the Mo-Fe protein split apart. When there are numerous free H^+ ions present, the nitrogenase converts 2 H^+ ions into 1 H_2 molecule simultaneously delivering the Fe protein's electrons to the Mo-Fe protein.

The *ntfA*, *ntfB*, *ntfC*, and *glnA* genes, which are found elsewhere on the chromosomal DNA, control the *Nif* genes of *Azotobacter*. The *ntfA* product binds to the *Nif* gene cluster's promoter and triggers transcription. It is yet unknown how *ntfB* & *ntfC* regulate processes. Choosing bacteria:

Following Forster's approach, maize, millet, rice, sugar beets, soybean, and

grassland rhizospheric soil samples were gathered from 12 Thai provinces:

6

Lopburi, Kanjanaburi, Phetchaboon, Nakon Sawan, Tak, Chiang Rai,

Utaradit and Prachuab Khiri Khan.

The bacterium *Klebsiella pneumonia* is diazotrophic & free-living. Its chromosomal DNA contains Nif genes. The Nif clade has a length of 24 x 10³ base pairs and 18 x 10⁶ daltons in terms of molecular weight. It is made up of 17 Nif genes spread across 7 operons and is positioned between the histidine (his) and shikimic acid uptake (shi) genes. Nif-QB, Gene encoding, Nif-F, Nif-MVSU, Nif-XNE, Nif-YKDH, and Nif-J are their names.

24

Other genes apart from the Nif genes chromosomal DNA in *Klebsiella pneumoniae* potentially determines the expression the Nif genetics. The ntrC gene's product binds to Nif-A and activates the Nif gene cluster when NH₄ and glutamate are in short supply. The product of the ntr B gene binds to the product of the ntrC gene on Nif-A and suppresses transcription if their quantity in the cell is high. If O₂ or NH₄ levels are high, the Nif-L product binds to the Nif-A product and inhibits transcription.

Eubacteria & methanogenic archaea are both broadly yet randomly distributed in nitrogen fixation. The present knowledge of variety of nitrogenases is largely premised on evolutionary investigations of the structural genes for nitrogenases, nifH and nifD. NifH, NifD, NifK, NifE, and NifN proteins are among those encoded by the nif genes that make up nitrogenase, and a genomic analysis of these genes identified five groups:

There are five types of Mo-Fe nitrogenases: (1) typical Mo-Fe nitrogenases, which are primarily made up of members of the phylas of cyanobacteria and proteobacteria; (2) absence of oxygen Mo-Fe nitrogenases, which are made by Anaerobic microorganisms predominate and a few methanogens; (3) additional nitrogenases, which include Anf & Vnf proteins, which are not dependent on Mo.

Applying a biofertilizer (containing of three different types of microorganisms, including Azotobacter and Bacillus) improved the development, output, and essential oil content of fragrant marjoram more than applying compost alone did.

25

3.6.3 PHOSPHORUS SOLUBILIZING BIO-FERTILIZER:

Phosphorus is among the vital macronutrient it is crucial for ¹the plant's growth and development. It is found in the soil between 400–and 1200 mg/kg. It is present in both inorganic and organic forms in soil. Reference stated that (0.05%) Both organic and inorganic phosphorus are present in large quantities in soil. Still, hardly 0.1% is available.

The primary cause of the non-availability ³⁰of soil phosphorus is it presents in the soil as insoluble salts? Which is around 95%–99% of the total phosphorus in the soil? Plants can only use phosphate as $H_2PO_4^-$ or HPO_4^- . Microorganisms such as rhizo bacteria can dissolve the complicated insoluble inorganic phosphate groups. To accessible the phosphate ions. Procedures are being performed by ²²low-molecular-weight organic acids

produced as a result of bacterial sugar metabolism. They can operate as chelating agents of divalent cations, causing insoluble phosphates to become soluble.

Bacteria that dissolve phosphorus serve as a bio fertiliser that can separate soluble molecules from insoluble inorganic phosphorus. One of the most significant characteristics linked to plant phosphorus nutrition is the ability of rhizosphere bacteria to solubilize nutrients. Phosphorus Biofertilizer, or PSB, has been presented to the agricultural sector. For plants, one of the crucial macronutrients, phosphorus is added to the soil as phosphorus fertilisers. The amount of the soil's phosphorus is around 0.05%. It is generally acknowledged that the secretion of acidic phosphates and low-molecularweight organic acids including succinic, fumaric, acetic, formic, lactic, acetic, and lactic acids.

26

The same as phytase produced by soil are involved in the method of petroleum phosphorus solvation by phosphorus emulsifying bacteria strains. Phosphorus solubilizing bacteria in the soil like *Pseudomonas* and The fungus and bacteria such as *Penicillium* and *Aspergillus* dissolve phosphate and fixed phosphorus to make it accessible for plants. 20–25% phosphorus requirement of plants is content by PSB. Due to its immobile nature, phosphorus is deficient in tropical and subtropical soils. It helps in converting food energy into chemical energy during photosynthesis and into chemical energy to be removed during respiration.

Phosphorus solubilizing bio-fertilizer application is used to aid remedy and make phosphorus more bio available and bio-accessible for the development and growth of plants. For instance, reference stated that phospho-bacterin aids in solubilizing phosphate, increasing its availability to plants.

In the literature, a variety of techniques are employed to yield bio-fertilizer, including anaerobic digestion, aerobic co-composting/re-composting procedures, closed system cultivation using sunlight, and vermicomposting (a procedure involves different genera of pests, especially red wigglers, white worms, etc.). Additionally, according to reports, the key components of a vermicomposting system include flasks, crushers, liquid, solid waste, *Eudriluseugeniae* (an African worm), a thermometer, a pH scale, and a sieve. The feedstocks also consist of banana, orange, pineapple, and watermelon peels, with a total result of 17.21% N, 10.24% P, and 48.32% K that does not fit any suitable NPK ratio.

27

Report indicates the bio-fertilizer produced by the fermentation method utilizing pineapple and watermelon peel has a pH value of 5.15 and low potassium content. Agar-based Pikovskaya medium but also fructose minimum medium with salts is used to select phosphorus solubilizing microorganisms (PSB) and potassium solubilizing bacteria (KSB), respectively. In Pikovskaya's medium, calcium phosphate was swapped out for 10 g/l of ores (RP, 100 mesh) as the only provider of phosphorous and 10

g/l of potassium-containing tourmaline (K-fel, 100 micron) as the sole potassium source for liquid cultivation.

Wheat, rice, chickpeas, lentils, peas, sorghum, rice, millets, oil seeds, pulses, and vegetables are some examples of common staple foods.

Dosage: Rice, finger millet, oil palm, pulses, and vegetables are all crops.

May be treated with PSB. The following techniques are advised for use:

Seed Procedure: For 1 kg of seed, combine 10 ml/10 gm liquid formulation/PSB in enough water to completely cover the seeds. Let the seeds dry under cover before sowing. Spread the mixture across a 1-acre area close to the root zone after thoroughly mixing it. Phosphorus Solubilizing Bacteria help the plant access Rock Phosphorus, which is a major source of Phosphorus.

The negative impacts of phosphorus fertilisers on crop and soil health are reduced by using Phosphorus Solubilizing Bacteria, or PSB Biofertilizers.

These were introduced to the medium containing sucrose minimum salts.

Approximately 108 CFU/ml of each separate PSB and KSB were added, and the cells were then grown for 3 and 6 days, respectively. In the dirt quality, 0.1% weight-per-weight of RP and K-fel were added, the moisture level was regulated at field capacity, and PSB was incubated for 6 days at 37 C.

28

Tricalcium, iron, & aluminium phosphorus, among other insoluble forms of phosphorus, are solubilizing into soluble forms.

□ They plunder the soil's phosphorus strands.

- They create hormonal antagonists and metabolites that encourage the growth of roots.
- They aid in the mineralization of soil and the breakdown of organic materials.
- When added to seeds or soil, bio fertilisers increase nutrient availability and boost yields by 10% to 25% without having a negative impact on the land or the environment.

3.7 ANAEROBIC DIGESTION

The biochemical process of anaerobic digesting involves the bio-metabolism phases of hydrolyzed, acetogenesis, acetogenesis, and methanogenesis. Larger molecular weight chemical matter, such as lipids, carbohydrates, and proteins, reportedly breaks down into smaller liquid organic matter, such as fatty, sugar, and amino acids, during the first step of hydrolysis when exoenzymes are present.

The outputs of the hydrolyzing process are either activated by the acid created during in the second stage, known as acidogenesis, or they are transformed directly ¹² into volatile fatty acids. In the third stage, called acetogenesis, bacteria are reduced to produce the esters CHCOO^- and H_2 . Methanogens, the last stage, are essential for bacterial methane production into the atmosphere (methanogens). Applied the anaerobic digesting procedure in the lab using reactors, 20 mm aluminium crimp seals, and Amber boron glass serum vials.

They employed the effluent from Brewer's wasted grain and palm oil mills as their feedstocks, and the results showed that adding manure improved the bio-fertilizing qualities of the effluent.

The anaerobic fermenter Method is compared to what goes on inside a cow's gut, where microorganisms break down foodstuffs into biofuel, which is a combination of both carbon dioxide and methane and semi-solid waste (Fruit Wastes) or fertiliser.

The presence of microbial species, which consume and digest biomass and also produce biogas and biofertilizer, is necessary for anaerobic degradation of plant or animal material, according to. The bacterium is naturally present in soils, water bodies like lakes and wastewater from treatment plants, as well as in the digestive tracts of people and animals.

Microbial populations that are susceptible to pH changes carry out the AD process. Anaerobic microbes preferred a somewhere around 6.5 and 7.5 pH range for the best methane generation. The pH did not change between both the AD procedure and prior ($p > 0.05$). As has been demonstrated in earlier research the quality and nutrient composition of the digestate are influenced by the substrate type utilized in biogas production.

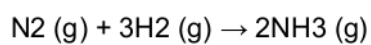
According to the study, biofertilizer production from cow dung and watermelon peel as feedstock can yield up to 83.35%. The metabolic process that leads to the biofertilizer production process is presented in Equation 1.

Substrate $\rightarrow$ CH₄ + CO₂ + H₂ + NH₃ + Bio-fertilizer

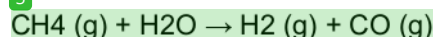
3.8 HABER BOSCH PROCESS:

9

One of the most effective and successful industrial processes used to produce ammonia. In order to perform the reaction on a lab scale, German researchers Fritz Haber and his helper created high-pressure equipment and catalysts in the 20th century. Carl Bosch later used the invention to produce an industrial-level machine in the year 1910. Indeed, this was a significant advancement in the scientific community.



5



Le Chatelier's principle states that low temperature and high pressure are favorable conditions for ammonia synthesis. The Haber procedure is typically used at temperatures of 500°C and pressures of 200 to 400 atmospheres. As NH₃ is created, it is continuously eliminated in the commercial manufacturing of ammonia. Le Chatelier's principle states that more hydrogen and nitrogen mix when the products are removed. Reversibility characterizes the reaction. The constitution of the addition reaction, the rate of the reaction, and the economics of the entire process are all impacted by variations in temperature, pressure, and the catalyst used.

FIG 3.6 (HABER BOSCH PROCESS)

Hydrogen Unreacted

25

Gases

Recycled

Gases are cooled

and ammonia

turns to Liquid

Nitrogen 400 – 450C

200 atm

Iron Catalyst

31

3.9 ABSORPTION OF PHOSPHORUS BY MYCORRHIZAL FUNGI

¹ Tropical soils are deficient in phosphorus. Further most of the microorganisms solubilize P and thus make it available for plant growth. It is estimated that in most tropical soils, 75% super phosphate applied is fixed and only 25% is available for plant growth.

There are some fungi such as *Aspergillus awamori*, *Penicillium digitatum*, etc. and bacteria like *Bacillus polymyxa*, *Pseudomonas striata*, etc. that solubilize unavailable form of P to available form. India has 250 mt of rock phosphate deposits. The cheaper source of rock phosphate like Mussoorie rock phosphate and Udaipur rock phosphate available in our country can be used along with phosphate solubilizing microorganisms.

Many crop plants' roots have vesicular-arbuscular mycorrhizal (VAM) fungus colonising them. ³⁵ They are zygomycetous fungi from the genus *Glomus*, as well as those from the *Gigaspora*, *Acaulospora*, *Sclerocystis*, and other families.

These must be maintained as obligatory symbionts and can't be grown on

artificial media. Through enhanced phosphorus nutrition, they promote plant growth, and they also shield the roots from diseases. Inoculation with effective VAM fungus can save roughly 25–30% of phosphate fertiliser.

3.10 PRODUCTION OF MYCORRHIZAL BIO-FERTILIZER

Mycorrhizal fungi are produced through a variety of inoculation techniques, depending on whether they are Ectomycorrhizal or endomycorrhizal in nature.

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3.10.1 ECTOMYCORRHIZAL FUNGI

As an inoculum, you may utilise basidiospores, minced sporocarps, spore, pure mycelial cultures, broken-up mycorrhizal roots, or soil from the mycorrhizosphere region. After combining the inoculum with nursery soil, seeds are sowed.

¹ A mycelial spore of *Pisolithus tinctorius* in a fungal growth montmorillonite moss substrate with the brand name "MycoRhiz" has been created by the Institute for Mycorrhizal Development and Research in the USA and Abbot Laboratories in the USA.

3.10.2 CYANOBACTERIA METHOD

In order to address the issues of soil quality, soil production, and environmental quality, biological nitrogen fertilisers are essential.

Cyanobacterium *Anabaena azollae* coexists symbiotically with the freefloating ¹ water fern *Azolla*. The symbiotic system *Azolla*-*Anabaena* complex is known to contribute 40-60 kg N ha⁻¹ per rice crop. *Anabaena azollae* can grow photo auto-trophically and fixes atmospheric nitrogen. The nitrogen fixing

cyanobacteria such as *A. azollae* and *variabilis* when immobilized in polyurethane foam and sugar cane waste have significantly increased the nitrogen fixing activity and ammonia secretion.

By secreting ammonia into flood water, the injection of phytoplankton ¹ in neem crop dramatically influenced the development of rice crop. Neem cake use as well as the inoculation of Forages substantially improved rice crop nitrogen utilization efficiency.

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In addition to *Anabaena*, additional nitrogen-fixing cyanobacteria have been blamed for the natural fertility of the tropical rice fields, including *Aulosira*, *Calothrix*, *Hapalosiphon*, *Scytonema*, *Tolypothrix*, and *Westiellopsis*.

In addition to adding N, cyanobacteria also improve ¹² the physical and chemical composition of the soil by adding organic matter and growth-promoting compounds like auxins and vitamins. It has been demonstrated that alkalization can improve saline-alkaline soils, aid ¹ in the development of soil aggregates, lessen soil compaction, and reduce the C: N ratio.

These organisms allow the crop to use more of the provided nutrients, increasing the crop's ability to use fertiliser. The majority of cyanobacteria supplement nitrogen fertiliser, providing ¹ up to 30 kg N ha⁻¹ season⁻¹. The increase in crop output ranges from 5 to 25%.

¹ 3.11 MASS PRODUCTION OF CYANOBACTERIAL BIOFERTILIZERS

For outdoor cultivation of cyanobacterial biofertilizers, the regional specific strain should be used. In such practices, a mixture of 5 or 6 regionally

acclimatized strains of cyanobacteria e.g. species of *Anabaena*, *Aulosira*, *Cylindrospermum*, *Gloeotrichia*, *Nostoc*, *Plectonema*, *Tolypothrix* etc. are generally used as starter inoculum.

3.12 MASS CULTIVATION OF AZOLLA

In addition to adding N, cyanobacteria also improve ¹² the physical and chemical composition of the soil by adding organic matter and growth-promoting compounds like auxins and vitamins. It has been demonstrated that algalization can improve saline-alkaline soils, aid ¹ in the development of soil aggregates, lessen soil compaction, and reduce the C: N ratio.

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These organisms allow the crop to use more of the provided nutrients, increasing the crop's ability to use fertiliser. The majority of cyanobacteria supplement nitrogen fertiliser, providing ¹ up to 30 kg N ha⁻¹ season⁻¹. The increase in crop output ranges from 5 to 25%.

3.13 MACRONUTRIENTS AND MICRONUTRIENTS IN PLANTS

The elements that plants require in the greatest amounts are known as macronutrients, as the name suggests. These include carbon, oxygen, hydrogen, potassium, calcium, phosphorus, sulphur, and phosphorus. Carbon, hydrogen, and oxygen are obtained from both water and air whereas the others are derived from soil.

Micronutrients are the micronutrients that plants only need in a very little amount of. They include things like iron, zinc, and boron. Higher plants also require other minerals as silicon, cobalt, selenium, and salt. The macronutrients &

micronutrients can be further divided into the following groups:

Elements make up bio-molecules, which in turn create the structure of the cell. Like oxygen, nitrogen, and carbon.

The elements that plants require in the greatest amounts are known as macronutrients.

Substances that are found in plants and are a component of chemical compounds that deal with energy, like the phosphorus in ATP.

Substances that help to inhibit or deactivate enzymes. Zinc is an illustration of a mineral that helps with alcohol dehydrogenates activation. Elements that alter the osmotic potential of the cell. Let's now look at the roles played by these macro- and micronutrients.

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3.13.1 FUNCTIONS OF MACRONUTRIENTS AND MICRONUTRIENTS IN PLANTS:

1. Calcium: Both cell division and the normal operations of the cellular membranes depend on this mineral. Additionally, this mineral controls metabolic functions.
2. Potassium: It is an element that is essential for the development of plants. Stomata must open and close in order for life to exist. It helps keep the cell stiff and helps in the functioning of enzymes.
3. Magnesium: It aids in the production of DNA and RNA and is essential in the activating of enzymes in photosynthesis and respiration. Additionally a crucial component of ribosome's, it aids in ribosome structural maintenance.

4. Sulfur: a significant component of coenzymes, vitamins, and amino acids.
5. Iron: Iron must be consumed in larger concentrations than that of other micronutrients. It is an essential part of protein and assists in stimulating enzymes.
6. Manganese: Manganese stimulates a variety of enzymes, including those responsible for respiration, photosynthetic, and other activities. It facilitates the splitting of water during photosynthesis.
7. Zinc and Copper: Several enzymes are activated by zinc and copper as well. Interstitial fluid is essential for the membrane's function as well as pollen emergence, cell expansion, germination, and other processes. Molybdenum is a component of numerous enzymes, such as nitrogenase.
8. Chlorine: Analyzing the anion-cation equilibrium and cellular solute levels with chlorine is helpful. Just like humans, plants have basic needs that must be met.

Plants require constant access to both macro and micronutrients. These two groups divide macronutrients into primary and secondary types. The three

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fundamental macronutrients, potassium (K), phosphorus (P), and nitrogen (N), are the most important ones (K). The truth is that more of these three basic nutrients are needed than there are of all the other macronutrients put together.

Auxiliary nitrogen and phosphorus are also required for plant health, albeit in smaller quantities than core ²¹ macronutrients. The secondary macronutrients

are calcium, magnesium, and sulphur (S) (Mg). In contrast to their macronutrient cousins, micronutrients are only required in trace levels for plant development and growth.

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CHAPTER – 4

RESULTS AND DISCUSSION, PERFORMANCE ANALYSIS

4.1 PH ANALYSIS

By using long forceps to collect samples from three distinct locations of the composting mixture, the pH of the manure was assessed every 2 days. Its pH of the solution was measured using a pH meter. Effluent after the specimen was shaken for half an hour on a rotary shaker while immersed in water.

Fig 4.1 (PH ANALYSIS OF LIQUID BIO-FERTILIZER)

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4.2 ELECTRICAL CONDUCTIVITY OF LIQUID BIO-FERTILIZER

Biofertilizers enhance plant growth and soil quality. They prevent the spread of diseases. They are economical and environmentally favorable. As natural fertilisers, biofertilizers shield the environment from contaminants.

Fig 4.2 (ELECTRICAL CONDUCTIVITY OF LIQUID BIO-FERTILIZER)

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4.3 ELEMENTAL ANALYSIS OF LIQUID BIO-FERTILIZER

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4.4 EFFECT ON PLANT NUTRITION

The cultivation of the nutrition of the world's expanding population depends on

crops and healthful food. A vital element of sustainable agriculture is plant nutrition. Natural stocks of plant nutrients exist in soils, however only a little quantity is produced annually via biological or chemical processes, and plants are unable to access these reserves. This flow is too sluggish to both meet crop needs and make up for nitrogen losses brought on by agricultural output. The quantities of the 16 essential plant minerals (Fe, Zn, Cu, Mo, Manganese, B, and Cl; ³² N, P, K, Calcium, Mg, and S; Micronutrients) needed to produce crops with the highest yield are well-established.

All of the A productive soil should contain both macro- and micronutrients since these components assist plant nutrient uptake. The application of fertilisers and organic manure is a crucial graining activity since good fertility is essential for effective plant growth. Keeping soil's nutrient levels appropriate is important. Crucial for the establishment of healthy plants all the minerals are not provided by chemical fertilisers alone in the proper balance that plants require the organic matter content of the soil decreases as a result, which has an effect on the biological systems and soil's physical attributes. The use of biological manures has sparked interest as a result of all these general considerations.

In favor of the use of organic manure the activity of the management of organic manure includes other factors as well. Crop yields, but is also essential for demonstrating influences the nutrition in a direct and indirect manner. Increasing soil porosity through improved physical, chemical enhances the physiological, biological, and Chemical, Utilization and efficacy

of Fertilizers Applied.

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CHAPTER – 5

SUMMARY AND CONCLUSION

5.1 SUMMARY

According to the results of research on the liquid byproduct of Agro Wastes anaerobic decomposition Crop residues like Fruit Wastes can be utilized in a biodigester to create additional useful products like methane and fertilisers that functions as a replacement energy source as well as another waste management technique. And it informed us that the residue of the anaerobic Agro waste decay or other types of biological waste can provide excellent manure for the typical farmer. The characteristics of the crop production that sprouted from the 3 different soil types used implied that the chemical fertiliser tends to increase rate of growth of crops but is dangerous to the land and the plants been developed on it - this results in the expansion of leaves, flowers, and fruit and vegetables that tend to diverge from the standard and in some terrible case scenarios, it may cause the death of a plant. It should also be noted that tests have shown liquid biofertilizer, which is the product of the anaerobic decomposition of Fruit Wastes, to be excellent for nourishing plants and soil.

5.2 CONCLUSION

The Anaerobic Digestion process is made environmentally friendly and economically viable by using its by-products, particularly the digestate.

Compared to using the digestate as bio-fertilizer, the transformation of biogas to power is more well-established. ³³ As a result, the current study's main objective is to ascertain the nutrients that make up the feed and digestate. It was also done to identify and characterize the microbial communities' capacity to promote plant growth.

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CHAPTER – 6

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