College of Sciences

Department of Botany and Microbiology

Industrial microbiology

Industrial microbiology is a branch of microbiology that focuses on the practical applications of microorganisms in various fields such as agriculture, industry, environmental management, and biotechnology. It involves the utilization of microorganisms to achieve specific beneficial outcomes or solve practical problems. Here is an introduction to Industrial microbiology:

1. **Agriculture:**

Industrial microbiology plays a crucial role in agriculture by harnessing the power of beneficial microorganisms to improve soil fertility, enhance plant growth, and combat plant diseases. For example, the use of rhizobacteria and mycorrhizal fungi can promote plant health and increase crop yields through mechanisms like nitrogen fixation and nutrient uptake.

2. **Industrial Microbiology:**

Microorganisms are extensively used in various industrial processes such as fermentation, biofuel production, enzyme production, and bioremediation. Industrial microbiologists work to optimize these processes by selecting or engineering microorganisms with desirable traits for industrial applications.

3. **Environmental Microbiology:**

In environmental management, Industrial microbiology focuses on using microorganisms to degrade pollutants, clean up contaminated sites, and maintain ecological balance. Bioremediation techniques employ microorganisms to break down hazardous substances in soil, water, and air.

4. **Biotechnology:**

Industrial microbiology is at the forefront of biotechnological advancements by utilizing microorganisms for the production of valuable compounds like antibiotics, enzymes, and biofuels. Genetic engineering techniques are often employed to modify microorganisms for specific industrial or medical purposes.

5. **Food Microbiology:**

Microorganisms play a critical role in food production, preservation, and safety. Industrial microbiologists work to develop probiotics, food additives, and biocontrol agents to improve food quality, shelf-life, and safety. They also study foodborne pathogens and develop strategies to prevent food contamination.

6. Pharmaceutical Microbiology:

In the pharmaceutical industry, Industrial microbiology is essential for the production of antibiotics, vaccines, and other pharmaceutical products derived from microorganisms. Microbial fermentation processes are used to produce a wide range of therapeutic compounds.

7. Clinical Microbiology:

Industrial microbiology in clinical settings involves the diagnosis, treatment, and prevention of infectious diseases caused by pathogenic microorganisms. Microbiologists study microbial pathogens, develop diagnostic tests, and monitor antimicrobial resistance to ensure effective patient care.

8. Research and Development:

Industrial microbiology drives innovation through research and development efforts aimed at discovering new microbial strains, improving fermentation processes, and developing novel biotechnological applications. This research contributes to advancements in medicine, agriculture, industry, and environmental science.

In conclusion, Industrial microbiology bridges the gap between theoretical knowledge of microorganisms and their practical applications in diverse sectors. It plays a vital role in addressing global challenges related to food security, environmental sustainability, healthcare, and industrial development by harnessing the potential of microorganisms for beneficial outcomes.

Experiment no.: 1 and 2
Exp. title: Isolation of microorganisms

Microorganisms are found everywhere in the environment: in the air and water; on the surface of our clothes, walls, furniture; in soil and dust; and on and in our own bodies (skins, hair, and mucous membranes). Many of the soil organisms for example, play important roles in processing vital elements such as phosphorus and nitrogen, making them available to other living organisms. On the other hand, there are organisms that are harmful. To study any microorganism it should firstly be isolated from its own environment. There are many isolation methods for recovering fungi and bacteria and the results may be differed according to used method and isolation media.

Isolation of fungi from soil:-

Objective:-

Learning how to isolate soil fungi.

Materials :

Soil sample.

Acidified Czapek–Dox + 0.5 % yeast extract agar medium.

Sterilized Petri plates.

Sterilized distilled water in test tubes (9cm water/each).

Golf shaped like glass road.

Procedures:-

Simple plating technique (Direct isolation):

The direct inoculation method may be best for isolating various and general soil fungi simply, readily, and economically.

1- Transfer a small amount (0.005–0.015 gm) of soil to a sterilized Petri dish.
2- Added 8–10 ml. of semi-cooled (45°C) nutrient medium and shake the plate to let the soil particles dispersed throughout the thin layer of agar medium before it solidify.

3- If the soil is very dry, or contains a high proportion of clay, it is preferable to mix the particles with a drop of sterile water in the plate, before adding the medium.

4- Incubate treated plates at 20-30°C, investigate the colonies appearance after 48 and record the results.

Handling note:

- The amount of soil used in the preparation of a soil plate varies with the soil investigated, and is determined by trial.
- Acidified Czapek–Dox + 0.5 % yeast extract agar, is a very good isolation medium for many soil fungi as well as it stimulate the growth and sporulation.
- No need for antibiotic (i.e. streptomycin) addition to the isolation medium to avoid bacterial contamination.

-Dilution (Plate) Method:-

- Take a proper amount of airy dried soil sample after saving it to remove any undesirable materials (plant duperies and beg granuls).
- Prepare cereal dilution (i.e. 1:10, 1:100, 1:1000…….etc) from the soil sample.
-Transfer one drop from each of the last two dilution samples to plated isolation media using a sterile pipette.
- Use golf shaped like glass road to spread the droplets onto the agar surface.
- Incubate treated plates at 20-30°C, investigate the colonies appearance after 48 and record the results.

There are many selective media that could be used such as:-

- Pepton-pentachloronitrobenzene (PCNB) agar and V-8 juice-dextrose-yeast extract agar (VDYA)-PCNB, for the selective isolation of *Fusarium* spp.
- Pimaricin-vancomycin-PCNB (P 10 VP) and hymexazol (3-hydroxy-5-methylisoxazole, HMI) containing P 10 VP or PDA at concentrations of 25 to 50 µ m/ml, together with various antibiotics for isolation of *Phytophthora*

Experiment no.: 3
Exp. title: Alcohol fermentation

Alcohol fermentation is a biochemical process in which microorganisms such as yeasts convert sugars into alcohol and carbon dioxide. This process is commonly used in the production of alcoholic beverages like beer, wine, and spirits, as well as in industrial ethanol production. Here is an overview of the alcohol fermentation production process:

Aim of the experiment:
The aim of an experiment focused on alcohol fermentation production typically revolves around studying and optimizing the various factors that influence the process of converting sugars into alcohol by yeast. Here are some common objectives and aims of experiments in alcohol fermentation production:

Optimizing Fermentation Conditions:

- Experimenting with different parameters such as temperature, pH, nutrient availability, and oxygen levels to optimize the fermentation process and maximize alcohol yield.

Yeast Strain Selection and Comparison:

- Evaluating different yeast strains for their fermentation efficiency, alcohol tolerance, flavor profiles, and other characteristics to determine the most suitable strain for a specific type of alcohol production.

Substrate Evaluation:

- Investigating the impact of different substrates (e.g., various sugars, grains, fruits) on the fermentation process to determine the most efficient and cost-effective raw material for alcohol production.

Materials, Methods & Equipment:

 Raw Materials:

- Malted barley, corn, wheat, grapes, or other fermentable sources containing sugars.

- **Yeast**:
  - Saccharomyces cerevisiae or other selected yeast strains for fermentation.
- **Water**:
  - Clean and preferably filtered water for preparing the fermentation medium.
- **Nutrients**:
  - Yeast nutrients such as nitrogen sources (e.g., diammonium phosphate), vitamins, and minerals.

Procedures:

1- Prepare Malt broth medium and dispense into 6 flasks.

2- Sterilize the medium using autoclave at 121C for 20 min

3- Inoculate the flasks with *Saccharomyces service* and incubate 2 flasks at 30 c for 3 days

4- The other two flasks will be incubated for 7 days

5- The last two flasks will be incubating for 15 days

6- After incubation periods for each flask (3, 7 and 15 days). The flasks were examined and chemically analyzed for Alcohol, Acetaldehyde and Acetic acid production respectively

Results & Observations

Iodoform test:

1- Take (1 ml) of Alcohol and add (5 ml) of Iodine soln (which prepared by mixing 1gm of I_2 with 2gm of KI ground them together, add (100 ml) of dist water &filter).

1- Add drops of (1 N) NaOH soln (drop by drop), till the color of the mixture become straw yellow (pale yellow).
2- Heat mixture in water bath for (5 min) then allow mixture to cool gradually

3- If a yellow ppt. is formed ----------► Alcohol is Ethanol
If no yellow ppt. is formed ----------► Alcohol is Methanol.

Experiment no.: 4
Exp. title: Antibiotics extraction and their assay
Antibiotics production by Microorganisms (Fungi and Bacteria)

Microorganisms are omnipresent and always exist in a competitive environment. During their metabolism, they produce many type of secondary metabolites which inhibit the growth of the surrounding strains. In such a competitive environment, most microorganisms produce antibiotics to maintain their predominance.

Aim of the experiment:

- **Optimizing Fermentation Conditions**:
  - Experimenting with various parameters such as temperature, pH, oxygen levels, and nutrient availability to maximize the production of antibiotics by the fermenting microorganism.
- **Microbial Strain Selection and Comparison**:
  - Evaluating different microbial strains for their antibiotic production capabilities, potency, and yield to identify the most effective strain for a specific antibiotic compound.
- **Media Formulation**:
  - Developing and optimizing the growth medium composition to support the growth of antibiotic-producing microbes and enhance antibiotic production.
- **Fermentation Kinetics**:

- Studying the kinetics of antibiotic production by monitoring parameters such as microbial growth, antibiotic concentration, substrate utilization, and byproduct formation over time.

 Metabolic Engineering:

- Modifying microbial strains through genetic engineering techniques to enhance antibiotic production pathways and improve yields.

 Purification and Characterization:

- Developing purification methods to isolate and characterize the antibiotic compound produced by the microorganism, ensuring its purity and potency.

 Antibiotic Stability Studies:

- Investigating the stability of the antibiotic compound under various storage conditions to determine its shelf-life and optimal preservation methods.

 Antibiotic Efficacy Testing:

- Evaluating the antimicrobial activity of the produced antibiotic against target pathogens through in vitro and in vivo experiments to assess its effectiveness.

Materials, Methods & Equipment:

a. Bacterial production medium (for *B. subtitles*)

Bacterial Production Medium is composed from:

Glucose	(3gm)
$NaNO_3$	(0.6gm)
KH_2PO_4	(0.1gm)
KCl	(0.5gm)
$MgSO_4$	(0.02gm)
$FeSO_4$	(0.01gm)
Peptone	(5gm)
Beef Extract	(3gm)
H_2O (100 ml)	

b- **Dox broth medium** (for antibiotics production from *Penicillium* A, B) Flasks of 150 ml

Procedure:

1- bacterial broth medium is prepared, dispensed in flasks and sterilized by autoclave for 15 min.

2- Dox broth medium for (fungal strains) is prepared, dispensed in flasks and sterilized by autoclave for 15 min.

3- The identified bacterial strain of *B. subtitles* and two strains of *Penicillium* A & B is taken carefully with an inoculating needle and inoculated on the bacterial broth and Dox broth media.

4- The Flasks of bacterial strain are incubated at 37^{0}c for 48 hrs while flasks of fungal strains are incubated in Shaker incubator at 25 ^{0}c for 5 days.

Extraction (Principles): After two days, the maximum amount of antibiotic will have been produced as the secondary metabolites are released into the medium during their stationary phase and the extraction can be done. Depending on the specific antibiotic produced, the fermentation broth is processed by various purification methods. For example, for antibiotic compounds that are water soluble (as streptomycin and neomycin), an ion-exchange method may be used for purification. The compound is first separated from the waste organic materials in the broth and then sent through equipment, which separates the other water-soluble compounds from the desired one. To isolate an oil-soluble antibiotic such as penicillin and tetracycline, a solvent extraction method is used. In this method, the broth is treated with organic solvents such as chloroform, which can specifically dissolve the antibiotic. The dissolved antibiotic is then recovered using various organic chemical means. It can be further refined into different product types.

Extraction of Water soluble antibiotics:

1- Acidify the fermentative medium to pH: 2.7 using sulfuric acid, them filtrate
2- Resultant filtrate is adjusted at pH; 8 using sod hydroxide soln of Conc. 50%
3- Activated Charcoal is added and the suspension is agitated for 1 hrs then filter
4- Antibiotics will be absorbed on Charcoal and then eluted with 10 % acetone of pH,2
5- Add pure acetone which will precipitate water sol. Antibiotics
6- This Water sol. antibiotic was redissolved again in water and adjust pH; at 6.2

Preparation of Crude Extract:

5ml of the inoculated production broth is taken and is subjected to centrifugation at 6000 rpm for 10 minutes. Bacterial cells were pelleted out. The cells pellet is discarded and supernatant is stored at 4 deg C in a refrigerator this supernatant is the crude extract which is tested for the antibiotic activity.

Results & Observations

Antibiotic Assay of the Crude Extract:

Various methods are available for testing the sensitivity of organisms to antibiotics.

The most widely used method for routine use is the disc diffusion method on Mueller-Hinton agar.

The accepted methodology was standardized by Kirby, Bauer, and Sherris. Automated technologies (Autobac, Vitek, etc.) use turbidimetric responses to determine antimicrobial susceptibility, and are becoming increasingly utilized in large clinical laboratories. In sensitivity testing, there is a high degree of standardization to ensure reproducible results. This standardization includes the

size of inoculum, the ionic strength of the medium and type of medium used, the amount of antibiotic tested and in the case of Kirby-Bauer (a plate qualitative disc diffusion method), the thickness of the agar. Zone size recommendations for interpretation of the Kirby-Bauer (resistant, intermediate, sensitive) are supplied by the manufacture.

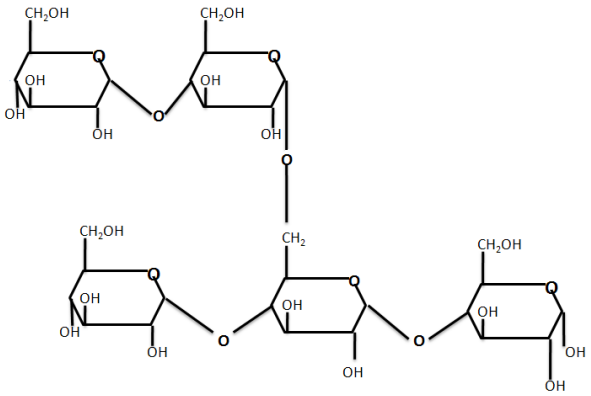
Experiment no.: 5
Exp. title: Production of Enzyme

Carbohydrates account for the major storage form of energy in plants and in animals. Starch is a homopolysaccharide which is the most important storage polysaccharides in plant cells. It is composed of two types of glucose polymer, amylose and amylopectin. The amylose consists of long, unbranched chains of D-glucose residues joined by (α-1,4) linkages. The successive glucose residues in amylopectin are joined by α-1,4 glycosidic linkages and α-1,6 linkages for branch points.

Amylases are enzymes that hydrolyze starch. The enzyme β-amylase catalyses the hydrolysis of α-1,4 glycosidic linkages from the non-reducing end of the polysaccharides (starch –amylose, amylopectin), to yield maltose units. The glucose residues at the nonreducing ends of the outer branches are removed enzymatically to facilitate the mobilization of starch for energy production. Thus it is also known as 1-4-α-D-Glucan maltohydrolase. β-Amylase is specific for amylose chains of six glucose units.

Starch

Amylose



Amylopectin

The aim of an experiment focused on the production of enzymes typically involves studying and optimizing the process of synthesizing specific enzymes from microbial or other sources. Here are some common objectives and aims of experiments in enzyme production:

1. **Optimizing Production Conditions**:
    - Experimenting with various parameters such as temperature, pH, substrate concentration, and oxygen levels to maximize the production of the target enzyme by the producing organism.
2. **Strain Selection and Comparison**:
    - Evaluating different microbial strains or genetically modified organisms for their ability to produce the target enzyme efficiently and at high yields.
3. **Media Optimization**:
    - Developing and optimizing the composition of the growth medium to provide essential nutrients and inducers required for enzyme production.
4. **Induction Studies**:
    - Investigating the effect of different inducers (such as specific substrates or chemicals) on enzyme synthesis to enhance production levels.
5. **Fermentation Kinetics**:
    - Monitoring the growth of the producing organism, enzyme production, substrate consumption, and byproduct formation over time to understand the dynamics of the fermentation process.
6. **Enzyme Purification**:
    - Developing purification strategies to isolate and concentrate the enzyme from the fermentation broth, ensuring high purity and activity.

Materials Required:

Sweet potato.

1. Knife/peeler.
2. Mortar and Pestle.
3. A Blender.
4. Blue capped tubes.
5. 20mM sodium Phosphate buffer at pH 7.
6. Vortexer.

Procedure:

Take a clean sweet potato and peel the skin off.

1. Weigh the peeled sweet potato and note the weight.
2. The sweet potato is cut into small pieces and transferred into a mortar and pestle.
3. The pieces are crushed and then transferred into a blender.
4. Add 40 ml of cold 20mM sodium phosphate buffer saline. Blend it until it forms a paste.
5. Gently transfer the potato slurry into a blue capped tube.
6. Allow the enzyme to extract over a 1 hour period at room temperature, with frequent vigorous stirring on a vortex mixer.
7. Then the extract is filtered using a GF A glass fibre filter and the filtrate is collected in a new blue capped tube.
8. Centrifuge the filtrate at 12000rpm for 20 minutes at 4 degree Celsius.
9. After the centrifugation, take out the blue capped tube.
10. Carefully transfer the supernatant into a new blue capped tube and is stored at 4 degree Celsius in refrigerator and discard the pellet.

Experiment no.: 6

Exp. title: Production of Yogurt

Yogurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. All yogurt must contain at least 8.25% solids not fat. Full fat yogurt must contain not less than 3.25% milk fat, low fat yogurt not more than 2% milk fat, and nonfat yogurt less than 0.5% milk. The two styles of yogurt commonly found in the grocery store are set type yogurt and swiss style yogurt. Set type yogurt is when the yogurt is packaged with the fruit on the bottom of the cup and the yogurt on top. Swiss style yogurt is when the fruit is blended into the yogurt prior to packaging.

The aim of an experiment

The aim of the production of yogurt typically involves studying the fermentation process of milk by specific bacterial strains to produce yogurt. Here are some common objectives and aims of experiments in yogurt production:

1. **Optimizing Fermentation Conditions**:
    - Experimenting with different temperatures and incubation times to determine the optimal conditions for the growth of yogurt bacteria and production of lactic acid.
2. **Bacterial Strain Selection**:

    - Evaluating different strains of lactic acid bacteria, such as Lactobacillus bulgaricus and Streptococcus thermophilus, for their ability to ferment milk and produce high-quality yogurt.
3. **Milk Composition**:
    - Investigating the impact of different types of milk (whole milk, skim milk, etc.) on the texture, flavor, and nutritional content of the yogurt.
4. **Starter Culture Optimization**:
    - Determining the best combination and ratio of bacterial strains in the starter culture to achieve desired yogurt characteristics.
5. **Acidification Kinetics**:
    - Monitoring the pH levels during fermentation to understand the acidification process and its effect on the development of yogurt texture and flavor.
6. **Texture and Viscosity Studies**:
    - Assessing the impact of fermentation parameters on the texture, consistency, and mouthfeel of the yogurt, including factors like syneresis and gel formation.

Procedure:

1. Adjust Milk Composition & Blend Ingredients

Milk composition may be adjusted to achieve the desired fat and solids content. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. Ingredients such as stabilizers are added at this time.

2. Pasteurize Milk

The milk mixture is pasteurized at 185°F (85°C) for 30 minutes or at 203°F (95°C) for 10 minutes. A high heat treatment is used to denature the whey (serum) proteins. This allows the proteins to form a more stable gel, which prevents separation of the water during storage. The high heat treatment also further reduces the number of spoilage organisms in the milk to provide a better environment for the starter cultures to grow. Yogurt is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yogurt after fermentation to act as probiotics; if the yogurt is pasteurized after fermentation the cultures will be inactivated.

3. Homogenize

The blend is homogenized (2000 to 2500 psi) to mix all ingredients thoroughly and improve yogurt consistency.

4. Cool Milk

The milk is cooled to 108°F (42°C) to bring the yogurt to the ideal growth temperature for the starter culture.

5. Inoculate with Starter Cultures

The starter cultures are mixed into the cooled milk.

6. Hold

The milk is held at 108°F (42°C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavor of yogurt. This process can take several hours.

7. Cool

The yogurt is cooled to 7°C to stop the fermentation process.

8. Add Fruit & Flavors

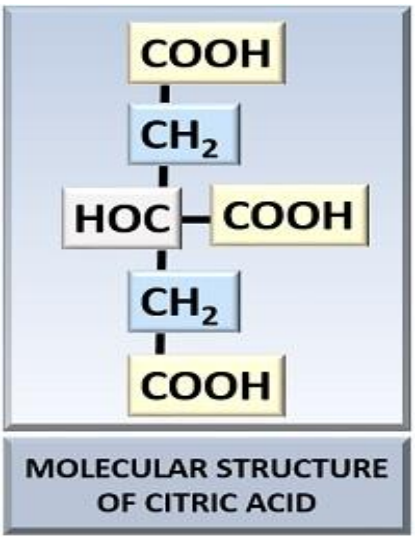
Fruit and flavors are added at different steps depending on the type of yogurt. For set style yogurt the fruit is added in the bottom of the cup and then the inoculated yogurt is poured on top and the yogurt is fermented in the cup. For swiss style yogurt the fruit is blended with the fermented, cooled yogurt prior to packaging.

9. Package

The yogurt is pumped from the fermentation vat and packaged as desired.

Experiment no.: 7
Exp. title: Citric acid production

Citric acid can define as the most common **weak organic acid** which was derived from the Latin word "**Citrus**", which includes all the species of genus Citrus. Lemon, orange etc. are the best examples of having citric acid as the major constituent.



Important points about Citric Acid

- A Production of citric acid is an industrial process, which makes the use of raw materials like **substrates**, citric acid growth promoting **microorganisms** and enzymes etc. for the commercial production of citric acid.
- Generally, the commercial production of citric acid works out best by employing the method of **fermentation**.
- Globally, there is around 7, 36,000 tonnes/year production of citric acid.
- It is having high demand in food, pharmaceuticals and some other industries like cosmetics, toiletries etc.
- In the year **1826**, the commercial production of citric acid was first achieved by the **John** and **Edmund Sturage Company**, UK.

The production of citric acid can define as the process in which a citric acid can be derived by two methods like:

Natural process: Produce **naturally** from the citrus plants like lemon, orange etc.

Synthetic processes: Includes **chemical synthesis** of citric acid by the enzymes and **biological fermentation** by the microorganisms.

The aim of an experiment

The aim of an experiment or industrial process focused on citric acid production typically involves optimizing the fermentation process by certain strains of mold, usually Aspergillus niger, to produce citric acid. Here are some common objectives and aims of experiments in citric acid production:

1. **Strain Selection and Optimization**:
    - Evaluating and selecting the most efficient strains of Aspergillus niger for citric acid production.
    - Optimizing culture conditions, such as pH, temperature, aeration, and nutrient supply, to enhance citric acid yield.
2. **Substrate Utilization**:
    - Studying the utilization of different carbon sources (such as molasses, glucose, or other carbohydrates) for citric acid production.
    - Investigating the impact of nitrogen sources and other nutrients on citric acid yield.
3. **Fermentation Process Optimization**:
    - Developing and optimizing fermentation strategies to maximize citric acid production rates and yields.
    - Monitoring parameters like dissolved oxygen levels, pH, and temperature during the fermentation process.
4. **Metabolic Engineering**:
    - Implementing genetic and metabolic engineering techniques to enhance the productivity of the citric acid-producing strain.
    - Modifying the strain to increase its citric acid synthesis capabilities.
5. **Product Purification**:
    - Developing purification processes to isolate and recover citric acid from the fermentation broth effectively.
    - Implementing techniques such as filtration, precipitation, and chromatography for citric acid purification.

Procedure:

Koji Process

It also refers to as “Solid-state fermentation”. The koji process was first introduced in **Japan**. It is related to the use of agro-industrial residues for citric acid production. In Koji process, raw materials like apple pomace, sugar cane, beet molasses etc. can be used. These raw materials are utilized by the ***Aspergillus niger***.

The pH and moisture content of the raw material is adjusted to **4-5** and **70% r**espectively. Then the raw material is cooled at 30-60 degrees Celsius and after that, inoculate *A. niger*. After inoculation, the medium is transferred into large **trays** of 3-5cm depth and incubated at 25-30 degrees Celsius for **3-7 days**.

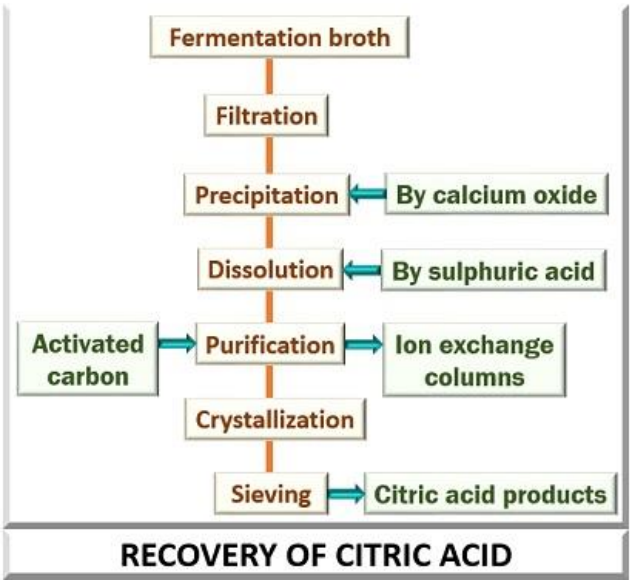
At last, the citric acid is extracted from the fermentation tank. The starch content of the raw material is degraded into citric acid by the amylase enzyme of the *Aspergillus niger*. The koji process does not require the substrate **pre-treatment** because the trace elements do not affect the production of citric acid.

Results and observations

Recovery of Citric Acid

The product formed after fermentation is the fermented liquor looks hazy due to the presence of antifoaming agents, mycelia etc. Therefore, to separate these things, a slurry of calcium hydroxide i.e. **$Ca(OH)_2$** to form a precipitate of calcium citrate.

The precipitate of **calcium citrate** is filtered and washed. After filtration, a filtrate forms which is treated with the **sulphuric acid** for the precipitation of calcium as "Calcium sulphate" **($CaSO_4$)**. Calcium sulphate is then treated with the activated carbon by which it gets demineralized after passing it consecutively from the ion exchange bed.



The solution obtained from this is subjected to the **circulating crystallizers**. The crystals formed as a result of crystallization are then removed by centrifugation. After the completion of these steps, the remaining solvent is dried, sieved and then packed. The remaining mother liquor is again recovered by the same process.

Uses

The production of citric acid is necessary for its versatile uses in the different fields that are given below:

- **Food industries**: Citric acid is used for the production of jams, jellies, candies, frozen fruits etc. In certain foods, citric acid is used as an "Artificial flavouring agent".
- **Beverage industries**: Citric acid is used for the production of soft-drinks and distilled beverages like wine.
- **Hospitals**: Citric acid is used as "Effervescent agent" at the time of blood transfusion.
- **Cosmetic industries**: Citric acid is used in cosmetic products like astringent lotions, hair gels etc.

Experiment no.: 8
Exp. title: Bio pesticide production

The alternatives to the use of chemicals include the following: (a) Predators: Among vertebrates one of the best known is the use of fish especially Gambusia affinis to eat mosquito larvae. Invertebrate predators include other larger insects e.g. wasps, while plant predators include Utricularia (a bladder wort). (b) Genetic manipulations: These include the production (by chemicals or by irradiation) of large numbers of sterile males, whose mating does not result in fertile eggs. (c) The use of hormones or hormone analogs: Pheromones are synthetic compounds which act as sex attractants. The insects attracted are destroyed. (d) The use of pathogens: Pathogens of insects are found among bacteria, fungi, protozoa, viruses and nematodes. The idea of using pathogens to control insects originated from studies of the diseases of the silkworm Bombyx mori. The pioneer work of Bassi was followed by those of Le Conte, Pasteur, Hagen until Metchnikoff actually tested the control of sugar beet pests with the fungus Metarrhizium anisopliae in South Russia.

Desirable Properties in Organisms to be Used for Biological Control
The following are desirable in microorganisms to be used in the biological control of insects:
(a) The agent should be highly virulent for the target insect, but should kill no other insects.
(b) The killing should be done quickly so that in the case of crops, damage is kept as low as possible, and in the case of vectors of disease before extensive transmission of the disease occurs.
(c) The killing ability should be predictable.
(d) The agent should not be harmful to man, animals or crops; in other words it should be safe to use.
(e) It should be technically amenable to cheap industrial production.
(f) When produced, it should be stable under the conditions of use such as under the high temperature and ultra violet light of ordinary sunlight.

(g) It should be viable over reasonably long periods to permit storage and transportation as necessary.
(h) It should ideally persist or recycle and/or be able to search for its host.

Candidates Which have been Considered as Biological Control Agents
(i) *Bacteria*
(a) *Bacillus thuringiensis*:
(b) *Bacillus moritai*:
(c) *Bacillus popilliae*:
(d) *Bacillus thuringinensis var. israelensis*
(e) *Bacillus sphericus*
(ii) *Viruses*:
(iii) *Fungi*:
(iv) *Protozoa*:

The aim of an experiment

The aim of an experiment or project focused on biopesticide production typically involves developing effective and environmentally friendly pest control solutions using naturally occurring organisms or compounds. Here are some common objectives and aims of experiments in biopesticide production:

1. **Strain Selection and Optimization**:
    - Evaluating and selecting effective biocontrol agents, such as bacteria, fungi, viruses, or botanical extracts, for pest management.
    - Optimizing culture conditions and growth media to enhance the biopesticide yield and efficacy.
2. **Bioactive Compound Identification**:
    - Identifying and characterizing bioactive compounds produced by the selected biocontrol agents that exhibit insecticidal, fungicidal, or herbicidal properties.
    - Studying the modes of action of these bioactive compounds against target pests.
3. **Formulation Development**:
    - Developing formulations that enhance the stability, shelf-life, and application efficiency of the biopesticides.
    - Exploring different carrier materials, adjuvants, and delivery systems to optimize biopesticide efficacy.
4. **Target Pest Specificity**:
    - Assessing the specificity of the biopesticides towards target pests to minimize non-target effects and environmental impact.
    - Conducting bioassays and toxicity studies to evaluate the efficacy of biopesticides against specific pest species.

5. **Field Efficacy Studies**:
    - Conducting field trials to evaluate the effectiveness of the biopesticides under real-world conditions.
    - Monitoring pest populations, crop damage levels, and yield outcomes to assess the biopesticide's impact on pest control and crop protection.
6. **Safety and Environmental Impact Assessment**:
    - Evaluating the safety of biopesticides for humans, non-target organisms, and the environment.
    - Conducting ecotoxicity studies and risk assessments to ensure the sustainable use of biopesticides.
7. **Integration with Integrated Pest Management (IPM)**:
    - Integrating biopesticides into IPM strategies to enhance pest control efficacy while reducing reliance on synthetic chemical pesticides.
    - Developing biopesticide-based IPM programs for sustainable pest management in agriculture.
8. **Regulatory Compliance**:
    - Addressing regulatory requirements and obtaining necessary approvals for biopesticide registration and commercialization.
    - Ensuring compliance with quality standards and regulations governing biopesticide production and use.

Procedure:

he production of biopesticides from Trichoderma viride involves several steps to cultivate and extract the beneficial compounds produced by this fungus for pest control purposes. Here is a general procedure for producing biopesticides from Trichoderma viride:

1. **Isolation and Characterization of Trichoderma viride**:
    - Obtain a pure culture of Trichoderma viride from a reliable source or isolate it from environmental samples.
    - Characterize the strain through molecular and morphological techniques to ensure its identity.
2. **Inoculum Preparation**:
    - Start by growing Trichoderma viride on a suitable growth medium such as potato dextrose agar (PDA) or malt extract agar (MEA).
    - Incubate the culture at an optimal temperature (usually around 25-30°C) for spore production.
3. **Liquid Culture Preparation**:
    - Inoculate a liquid medium (e.g., broth medium like potato dextrose broth) with spores from the solid culture to scale up the culture volume.

    - Incubate the liquid culture on a shaker at the appropriate temperature and agitation speed for optimal growth.
4. **Fermentation**:
    - Transfer the actively growing culture to a larger fermenter for bulk production.
    - Control fermentation conditions such as pH, temperature, aeration, and agitation to promote biomass and metabolite production.
5. **Bioactive Compound Extraction**:
    - Harvest the fermented broth containing bioactive metabolites produced by Trichoderma viride.
    - Extract the biopesticidal compounds using appropriate extraction techniques like solvent extraction or filtration.
6. **Formulation Development**:
    - Formulate the extracted biopesticide compounds into a suitable formulation for application.
    - Incorporate stabilizers, adjuvants, and carriers to enhance the stability and efficacy of the biopesticide.
7. **Quality Control and Testing**:
    - Conduct quality control tests to ensure the potency, purity, and safety of the biopesticide product.
    - Perform bioassays to evaluate the efficacy of the biopesticide against target pests and assess its environmental impact.

Experiment no.: 9
Exp. title: Microbial inoculant production

Nitrogen is a key element in the nutrition of living things because of its importance in nucleic acids. Gaseous nitrogen is present in abundance in the Earth's atmosphere, unfortunately, most living organisms cannot utilize gaseous nitrogen but require it in a fixed form; that is, when it forms a compound with other elements.

Nitrogen can be fixed both chemically and biologically. Chemical fixation is employed in the production of nitrogenous chemical fertilizers, which are used to replace nitrogen removed from the soil by plants. The ability to carry out biological fixation is found only in the bacteria and blue-green algae. Some of these organisms fix nitrogen in the free-living state and thereby contribute to the improvement of the nitrogen status of the soil. Others do so closely associated (in symbiosis) with higher plants. In some others the microorganism penetrates the

roots and forms outgrowths known as nodules within which the nitrogen is fixed. Of the nodule-forming nitrogen fixing associations between plant and micro-organisms, the most important are the legume bacteria associations.

The bacteria which form nitrogen-fixing nodules with legumes are members of the genus *Rhizobium.* Today there are thriving industries producing rhizobia inoculants in most parts of the world. The need for legume inoculation has become more urgent in recent years because of the rise in the cost of chemical fertilizers, the inefficient use of chemical fertilizers by agricultural crops and the short-term and long-term environmental consequences of unused nitrate fertilizers which find their way into, and cause the pollution of, drinking water.

The aim of an experiment

The aim of Rhizobium production and formulation typically revolves around optimizing the growth of Rhizobium strains, which are beneficial nitrogen-fixing bacteria that form symbiotic relationships with leguminous plants. The ultimate goal is to produce effective inoculants that enhance plant growth and soil fertility. Here are some common objectives and aims related to Rhizobium production and formulation:

1. **Strain Selection and Optimization**:
    - Selecting effective Rhizobium strains that establish symbiotic relationships with specific legume hosts and exhibit high nitrogen-fixing efficiency.
    - Optimizing culture conditions such as temperature, pH, carbon and nitrogen sources, and growth media to enhance Rhizobium biomass production.
2. **Mass Multiplication of Rhizobium**:
    - Scaling up Rhizobium cultures to achieve high cell densities for inoculant production.
    - Implementing fermentation processes in bioreactors to produce large quantities of viable Rhizobium cells.
3. **Formulation Development**:
    - Developing carrier-based formulations for Rhizobium inoculants to improve their shelf-life, viability, and efficacy.
    - Incorporating protective agents, nutrients, and adjuvants in the formulation to enhance Rhizobium survival and nodulation efficiency.
4. **Quality Control and Viability Testing**:
    - Conducting quality control tests to assess the viability, purity, and effectiveness of Rhizobium inoculants.

- Performing viability tests, like plate counts or molecular methods, to ensure the presence of viable Rhizobium cells in the formulated product.

5. **Compatibility with Seed Coating**:
   - Ensuring compatibility of Rhizobium inoculants with seed coating processes for convenient application to legume seeds.
   - Developing seed coating formulations that promote adhesion and colonization of Rhizobium on seed surfaces.
6. **Field Performance Evaluation**:
   - Conducting field trials to evaluate the efficacy of Rhizobium inoculants in promoting plant growth, nitrogen fixation, and crop yield.
   - Monitoring nodulation rates, nitrogen content in plants, and overall crop performance in treated fields.

Procedure

The production and formulation of Rhizobium inoculants involve several key steps to ensure the viability and efficacy of these nitrogen-fixing bacteria for enhancing plant growth. Here is a general procedure for Rhizobium production and formulation:

1. **Isolation and Identification**:
   - Isolate Rhizobium strains from nodules of leguminous plants or obtain them from a culture collection.
   - Characterize the isolated strains based on their species, nitrogen-fixing efficiency, and host plant specificity.
2. **Inoculum Preparation**:
   - Start by growing Rhizobium cultures on suitable growth media such as YEMA (Yeast Extract Mannitol Agar) to obtain pure cultures.
   - Incubate the cultures at the optimal temperature (around 28-30°C) for optimal growth and development.
3. **Liquid Culture Establishment**:
   - Inoculate a liquid medium (e.g., YEM broth) with a small number of Rhizobium cells from the solid culture to initiate liquid culture growth.
   - Incubate the liquid culture on a shaker at appropriate conditions to promote biomass production.
4. **Fermentation**:
   - Transfer the actively growing liquid culture to a larger fermentation vessel to scale up Rhizobium production.
   - Control fermentation parameters such as pH, temperature, aeration, and agitation for optimal biomass accumulation.

5. **Harvesting and Concentration**:
    - Harvest the Rhizobium biomass from the fermentation broth by centrifugation, filtration, or other separation techniques.
    - Concentrate the harvested cells to achieve high cell densities for formulation.