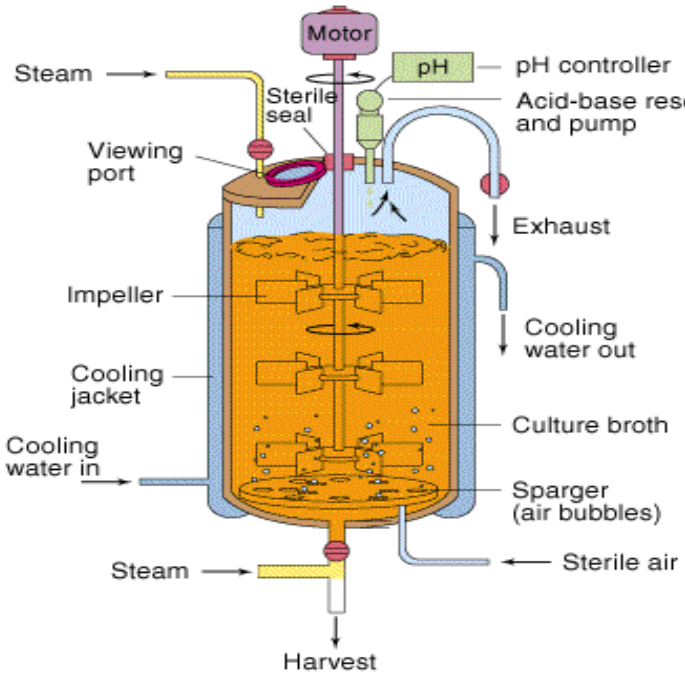
ANNAMALAI UNIVERSITY

FACULTY OF AGRICULTURE

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

III. B. Sc (Ag.) **VI SEMESTER**

PRACTICAL MANUAL CUM RECORD
OPC – AGM 326
ADVANCED MICROBIAL BIOTECHNOLOGY (1 + 1)



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CERTIFICATE

III. B. Sc., (Ag.) 2019 -2020 VI – Semester

*This is to Certify that Selvan / Selvi*_____*of III.* B.Sc., (Ag.) *Class bearing Reg No*_____*has duly completed the* practicals *for the course OPC – AGM 426 Advanced Microbial Biotechnology (1 +1) conducted under my Supervision during the Sixth Semester of 2019 -2020.*

Signature of the course teacher

Examiners

1.

Annamalainagar 2.

Date…….

EXERCISE NUMBER	DATE	TITLE OF THE EXERCISE	PAGE NUMBER	SIGNATURE
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EX.NO:1	FERMENTOR DESIGN AND OPERATION
DATE:	

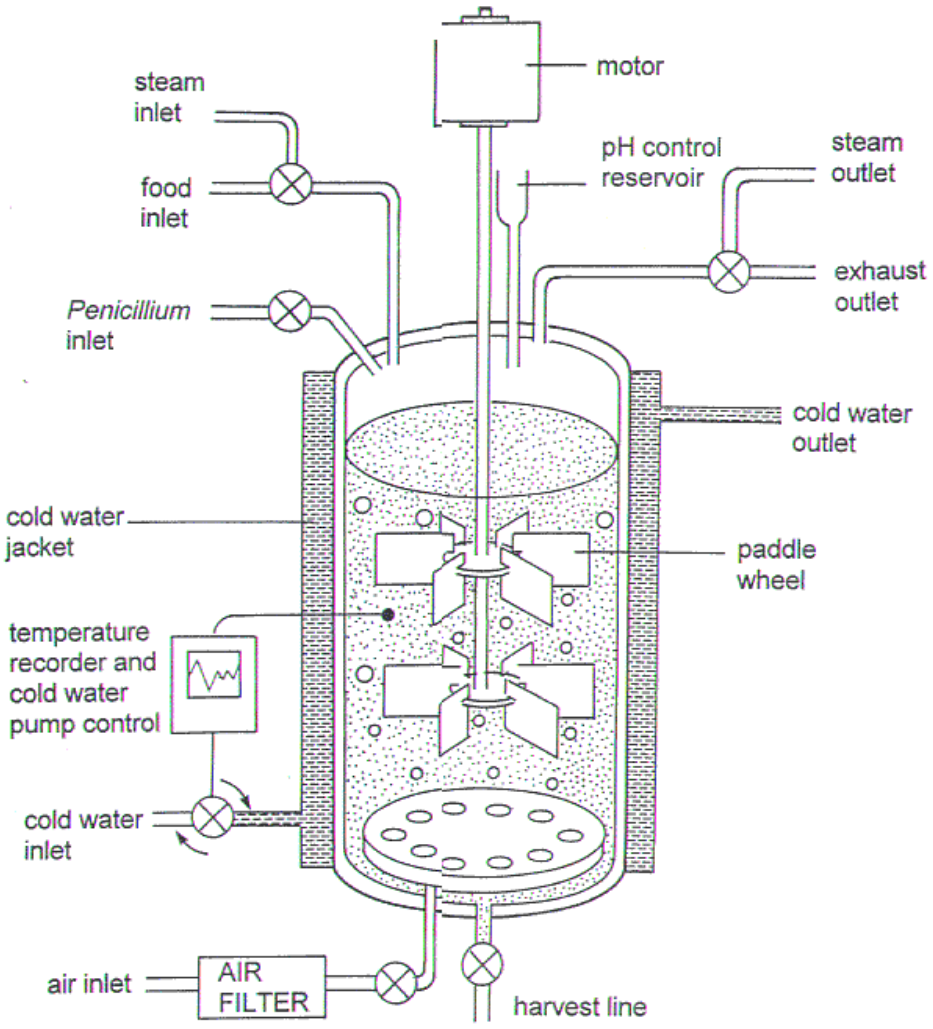
The main function of a fermentor or bioreactor is to provide a controlled environment for growth of a microorganisms or a defined mixture of microorganism to obtain a desired product. Their size may vary from mini scale to large (<1000 liters capacity) to meet out the industrial needs. Fermentors are usually made up of stainless and glasses made vessels are employed when photobionts are used as inoculants.

An ideal fermentor should fulfill the following criteria.

a. Long term operation in aseptic condition.
b. Adequate aeration and agitation.
c. pH – control system.
d. Sampling facility.
e. Minimum labour in operation, harvesting, cleaning and maintenance.
f. Temperature controlling system.
g. Minimum evaporation losses for fermentors.
h. Suitable for a variety of process.

For mass production of any organism first the liquid medium is prepared and poured into the fermentor vessel. Steam from generator is passed followed by circulating cooling water in around the inner vessel to have optimum temperature range to avoid time consumption for cooling. The medium is inoculated with microorganism's starter cultures either by manually or by using peristatic pump. Then the fermentor is provided with favorable environments like temperature, pH, oxygen agitation etc. with various accessories.

Now a day all this parameter can be automatically controlled by microprocessors. During fermentation process the antifoaming agents are added to a medium as on requirement. Periodical sampling can help to access the maturity of culture. Then the fermented medium is harvested by downstream processing like filtration and centrifugation, the cells are separated, and the end product is recovered.



Work done:

EX. NO: 2	ISOLATION OF LACTIC ACID BACTERIA FROM DAHI (CURD)
DATE:	

CURD (Dahi) is prepared by souring milk with a mixed culture of lactic acid bacteria, the starter. The milk is boiled and cooled. The starter culture is added and incubated for 12 hrs at room temperature. When the culture develops 0.80 to 0.90 % lactic acid, the fermentation is stopped by cooling. The curd is broken by agitation to enhance the flavour and odour of cultured butter milk. The starter culture for curd fermentation generally contain *Streptococcus lactis, S. cremoris, Leuconostoc citrovorum*, and *L. dextranicum*

Materials required: curd, water blanks, Perti plates, pipettes and Yeast extract lactose agar

Composition:

Peptone	1.0 g
Yeast extract	0.5 g
K_2HPO_4	0.2 g
Lactose	0.5 g
Bromocresol purple	0.2 ml (1.6 g in 100 ml)
Distilled water	100 ml

PROCEDURE: The curd sample is serially diluted up to 10^{-5} dilution. Plated one ml each from dilutions 10^{-4} and 10^{-5}. The plates are incubated at room temperature for two days. After the incubation period the lactic colonies are counted and population of lactic acid bacteria per ml of curd is estimated.

RESULT:

EX. NO:3	BACTERIAL BIOFERTILIZERS – MASS PRODUCTION, QUALITY CONTROL AND METHOD OF APPLICATION
DATE:	

Introduction

The term Biofertilizer can be defined as preparation containing live (or) latent cells of efficient strains of N-fixing, phosphate solubilizing or mobilizing microorganisms used for the application of seed, Soil with the objective of increasing the number of such organisms to augment the availability of nutrients in a form which can be easily assimilated by plants.

I. MASS PRODUCTION OF BACTERIAL BIOFERTILIZERS

Mass production of biofertilizers involved the following steps.

i. Culturing of microorganisms
ii. Preparation of carrier materials
iii. Preparation of carrier-based inoculants

i. Culturing of microorganisms

Pure culture of efficient strains of *Rhizobium*/ *Azospirillum*/ *Azotobacter*/ phosphobacteria maintained in slants as mother culture are transferred to the respective broth (100 ml quantities) in 250 ml Erlenmeyer flask and allowed to grow under aseptic condition at 30±2°C as submerged culture. The culture is incubated until maximum cell production of 10^{10} to 10^{11}cfu ml^{-1} is produced. Under optimum conditions, the population level could be within 15 days for *Rhizobium*, 5 – 7 days for *Azospirillum*; and 2 to 3 days of phosphobacteria. This will serve as starter culture for mass production. Starter culture in small containers are transferred to bigger size flask (1 – 2 lit capacity) or the seed tank fermentor at the rate of 1.5% and grow till the required population reaches to get the primary inoculum. Then, primary inoculum is used to mass multiply the culture in the production fermentor at the rate of 1-5% v/v of the broth in the fermentor until it attains the maximum cell count of about 10^9/ml. Batch fermentation is often followed in inoculum production.

The incubation period in the fermentor can be reduced by increasing the inoculum level upto 5%. Aeration and agitation of cultures hasten the growth.

The bacterial strain, initial inoculum level, aeration, temperature and incubation period influences the output of cells. Attaining high population in minimum time is the prime objective. The optimum temperature is maintained by passing water through outer jacket. It is not advisable to store, the broth for more than 24 hours after fermentation period, even at 4°C since it may affect the viability of cells.

ii. Preparation of carrier materials

The inoculant packets are prepared by mixing the broth with a solid material, known as carrier, for easy handling, transportation and application. The carrier offers increased protection to the cells when they are in contact with chemical fertilizers. Selection of an ideal carrier material is necessary for biofertilizer preparation. The good quality carrier material has the following characteristics.

- Costly wise cheaper
- Easily available nearby in plenty
- Should not have toxic chemicals
- The organic matter content should be around 40%.
- Should have the water holding capacity more than 50%
- Amenable for adjusting the pH to neutral
- Easy access for mixing broth

The common carriers used are peat soil, lignite, vermiculite, charcoal and press mud. The Indian peat soil is having the organic matter content of 41.6% and WHC is 53% lignite is having the organic matter content of 75% and WHC of 198%.

Before using the carrier material, it should be ground to a fine powder and the pH should be neutralized using $CaCO_3$. The neutralized carrier material is sterilized at 15 lb for 1 hours to eliminate the contaminants.

The neutralized peat soil/ lignite is found to be the best carrier materials for inoculum production.

iii. Preparation of carrier-based inoculants

Appropriately grown bacterial cultures suspension is mixed with the neutralized carrier material to the moisture content of 35 to 40% on wet weight basis.

Broth having excess cell population can be suitably diluted before mixing. The carrier and broth are mixed either manually or mechanically and left in trays for 2 to 5 days for curing. Curing improved the cell count to 10^9 – 10^{10} cells/g. After curing, it is then packed in low density polythene bags. The inoculants may be allowed for curing even after packaging in polythene bags for 3 – 4 days at room temperature.

After filling the inoculants, the bags are thoroughly sealed using a electric sealer/ automatic sealing machine.

Storage of inoculants packets

Storage is one of the important factors in biofertilizer production.

1. It should be stored in cool place away from heat or direct sunlight.
2. At normal room temperature, the inoculants can be stored up to 3 months.

II. QUALITY CONTROL OF BACTERIAL BIOFERTILIZERS

The Biofertilizer quality can be tested by using Serial dilution method.

Materials required:

1. Bio-fertilizer
2. Medium
3. Sterile water blanks
4. Sterile Petri plates and pipettes

Procedure:

10 gm of Bio-fertilizer is taken, and it is added to the 100ml sterile water blank. Mixed thoroughly, this dilution was 10^{-1}. By using 10^{-1} dilution serially diluted up to 10^{-7}. 1ml suspension was pipette out from 10^{-7} dilution added to Petri plates and were poured with appropriate medium and incubated at room temperature. After the incubation period, the colonies were counted by using colony counter.

Microbial population can be calculated by using this formula

Microbial population=average no of colonies × dilution factor

The microbial population should attain 10^8 cells/gm of carrier.

Many private companies are producing biofertilizer in the country and they have been found vary in their quality. Hence Bureau of Indian standards has

prescribed certain specification for the inoculants to maintain good quality. They are as follows.

- The inoculants should be a carrier based one.
- The inoculants should contain a minimum of 10^8 cells/g of carrier with 15 days of manufacture and 10^7 cells/gm within 15 days of expiry.
- The inoculants should have a maximum expiry period of 6 months from the date of manufacture.
- The inoculants should not have any contamination with other microorganisms.
- The pH of the inoculants should be between 6.0 – 7.5.
- The carrier material should be in the form of powder passing through 17-106 µ sieve that is peat, lignite, peat soil and humus materials neutralized with $CaCO_3$ and sterilized.
- The manufacture should control the quality of the broth and maintain records of test.
- The inoculants should be packed in 50-75µ low-density polythene bags.
- Each pocket should give the following information's.
    a) Name of product, specific name
    b) For which crop it is intended
    c) Name and address of the manufacturer
    d) Type of carrier
    e) Batch and code number
    f) Date of manufacture
    g) Date of expiry
    h) Net quantity
    i) Storage instructions words under 'Store in a cool place away from direct sun and heat'.
- The above items should be printed on coloured ink background
- The pocket may be marked with ISI certification mark.

III. METHOD OF APPLICATION OF BACTERIAL BIOFERTILIZERS

1) Seed treatment (*Rhizobium*)

1 pocket/acre

One pocket contains 250 gms. Mix pocket inoculants with 250ml cooled rice gruel. After mixing the recommended seeds for 1 acre is mixed and shade dried for half-an-hour and sow the seeds.

2) **Dipping of roots**:

2 pockets/acre.

Prepare one plot and allow the water to stand 5cm height. Mix 3 pockets inoculants in to the water. Dip the roots of seedlings for 10min and then transplanted.

3) **Direct broad casting**:

4 pockets/acre

Four pockets inoculants is mixed with 25kg of soil or FYM and broadcasted in the field.

RESULTS

EX. NO: 4	MASS PRODUCTION OF ALGAL BIOFERTILIZERS
DATE:	

MASS PRODUCTION OF BLUE GREEN ALGAE

BGA can be mass produced as soil – based composite culture (algal flasks) by different methods.

I. Multiplication in Tank

- Cement tank (9'×6'× ¾') or big metallic trays can be used for small scale production.
- Take 10 kg of paddy field soil, powder well and spread in the tank
- Fill water in the tank to a depth of 3"
- Add 150 g of super phosphate and 30 g of lime and mix well with the soil in the tank.
- Add 25 g carbofuran to control the insects
- Maintain water level in tank
- After 10 to 15 days, the blooms of BGA will start floating on the water sources.
- At this stage, stop the irrigation and allow the soil to dry completely.
- Collect the dry soil-based inoculum as flakes
- Store in a dry place. By this method, 5 to 7 kg of soil-based inoculum can be obtained.

II. Multiplication under field condition

- Select an area of 40 m^2 (20 × 2 m) near a water source which is directly exposed to sunlight.
- Make a bund all around the plot to a height of 15 cm and give it a coating with mud to prevent loss of water due to percolation.
- Plot is well prepared and leveled uniformly and water is allowed to a depth of 5 -7.5 cm and left to settle for 12 hrs.
- Apply 2 kg of superphosphate and 200 g lime to each plot uniformly over the area

- The soil based composite starter culture of BGA containing 8 – 10 genera is powdered well and broadcasted at 5 kg/ plot.
- Carbofuran at 200 g per plot is also applied to control soil insect occurring in BGA.
- Water is let in at periodic intervals so that the height of water level is always maintained at 5 cm.
- The growth of BGA is rapid and in about 10 – 15 days, a thick mat is formed which floats on the surface of the water.
- After 15 days of inoculation, the plot are allowed to dry up in the sun and the algal flakes collected and stored.

Observation

The floating algal flakes are green or blue green in colour. From each harvest, 30 to 40 kg of dry algal flakes are obtained from 1 cent plot.

METHOD OF INOCULATION OF BGA IN RICE FIELD

Blue green algae may be applied as soil – based inoculum to the rice field following the method described below.

- ❖ Powder the soil – based algal flakes very well.
- ❖ Mix it with 10 kg of soil or sand (10 kg of powdered algal flakes with 10 kg of soil/sand).
- ❖ BGA is to be inoculated on 7 – 10 days after rice transplanting.
- ❖ Water level at 3 – 4" is to be maintained at the time of BGA inoculation and then for a month so as to have maximum BGA development.

MASS PRODUCTION OF *Azolla* BIOMASS

Azolla is a floating water fern and it is ubiquitous in distribution. There are six species of ***Azolla.***

A. carolinana
A. nilotica
A. filiculoides
A. mexicana
A. microphylla
A. pinnata

The common species of the fern occurring is ***A. pinnata.*** It grows in stagnant water along with other water weeds such as lemna. The plant has a floating branched stem, deeply bilobed leaves, and true roots which penetrate the body of water. Each leaf has dossal and a ventral lobe. The dorsal fleshy lobe is exposed to air and contains chlorophyll. It has an algal symbiont, ***Anabaena azollae*** with in a control cavity. The ventral lobe is thin, is partly submerged in water, and loss chlorophyll. The alga fixes atmospheric nitrogen and is present at all stages of growth and development of the present.

Mass multiplication

- ***Azolla*** is mass multiplied under field conditions in one cent plots as follows.
- Select a wet land field and prepare thoroughly and level uniformly.
- Mark the field into one cent plots (20 x 2 m) by providing suitable bunds and irrigation channels.
- Maintain water level to a height of 10 cm and Mix 10 kg of cattle dung in 20 litres of water and sprinkle in one cent plot.
- Apply l00 g superphosphate per plot as basal dose.
- Inoculate fresh ***Azolla*** at 8 kg to each plot.
- Apply super phosphate at 100 g as to top dressing fertilizer on 4^{th} and 8^{th} day after ***Azolla*** inoculation to each plot.
- Apply carbofuran (Furadan) granules at 100 g/plot on 7^{th} day after ***Azolla*** inoculation to each plot.
- Maintain the water levels at 10 cm height throughout the growth period of two or three weeks.

Observations

Note the ***Azolla*** mat floating on the plot. Harvest the ***Azolla,*** drain the water and record the biomass.

RESULTS

EX.NO: 5	MASS PRODUCTION OF AM FUNGI
DATE:	

VAM fungi can be mass produced only in the presence of living root due to the inability to grow separately without plant host. The inoculum production of VAM fungi requires a host plant and a growth medium. There are wide ranges of host plants to reproduce VAM fungi. Host plants such as maize, sorghum, andropogon, cenchrus, sudan and guinea grasses can be used. Host plants should be selected on consideration of climatic and edaphic adaptation to the location. The host plants that harbor pathogenic organisms determine to the crops should not be selected.

Soil is a commonly used medium as it provides optimum conditions for growth and reproduction of fungus. Inert materials like vermiculite, perlite and sand are used.

Mass production technology for VAM inoculation

The procedure adopted by TNAU, Coimbatore for mass production of VAM fungi is as follows.

- 1 m^2 trench is formed to a depth of 30 cm. the trench is lined with black polythene sheet on the surface and made as per plant growth tub.
- 50 kg of vermiculite and 50 kg of sterilized soil are mixed and packed in the trench up to 20 cm height.
- 2 kg of VAM inoculum (mother culture) is applied uniformly 2 – 5 cm below the surface of vermiculite.
- Maize seeds surface sterilized with 5% Sodium hypochlorite for 2 minutes are used.
- 2 g of urea, 2 g super phosphate and 1 g of Muriate of potash are applied for each trench at the time of sowing seeds. Further, 1 g of urea is applied twice on 30 and 45 days after sowing for each trench (1 m^2 area).
- Quality tests on VAM colonization in root samples is recorded on 30^{th} and 45^{th} day.
- Stock plants are grown for 60 days (8 weeks). The inoculum is obtained by cutting all the roots of stock plants. The inoculum so produced consists of a mixture of vermiculite, spores, pieces of hyphae and infected root pieces.

Thus, within 60 days we can produce 55 kg of VAM inoculum from 1 sq meter area. The inoculum will be enough to treat 550 m^2 nursery area of 11,000 seedlings in the nursery.

MODE OF APPLICATION

1) Nursery application

100 g of bulk inoculum is enough for 1 m2. The inoculum should be applied at 2 – 3 cm below the soil at the time of sowing. The seeds/cuttings should be sown/planted above the VAM inoculum to cause infection.

2)For polythene bag – raised crops

5 – 10 g of bulk inoculum is enough for each packet. Mix 10 kg of inoculum with 1000 kg of sand potting mixture and pack the potting mixture and pack the potting mixture in a polythene bag before sowing.

3) For out planting

20 g of VAM inoculum is required per seedling. Apply the inoculum at the time of planting.

4) For existing trees

200 g of VAM inoculum is required for inoculating one tree. Apply the inoculum near the root surface at the time of fertilizer application.

RESULTS

EX. NO. 6	VERMICOMPOSTING
DATE:	

Introduction

The agricultural wastes contain important plant nutrients, which would greatly improve the physical, chemical and biological properties of the soil. The concept of waste utilization and recovery of nutrients through vermiculture will go long way in sustaining soil fertility.

Vermicomposting is a method of environment friendly waste recycling system using earth worms. Vermicompost contains all the essential plant nutrients including micronutrients. It is a balanced organic manure and supplied the various major and minor nutrients to the growing plants. It acts as food material for soil microorganisms, increasing their population and activity in soil.

Earthworms – surface dwellers (Epigeics)

1. *Eudrilus eugeniae*
2. *Eisenia foetida*
3. *Lampito mauritii*

Solid wastes such as crop residues, weeds, farm waste, animal droppings, household residue are used in vermicomposting. It can be done in wooden boxes or on floors with side lining or pits of suitable sizes, depending on the volume of organic waste. The vermicompost pits are lined with granite slabs to avoid the rodents.

METHOD OF VERMICOMPOSTING

1. Selection of earthworm: Earthworm that is native to the local soil may be used.
2. Size of pit: Any convenient dimension such as 2m x 1m x 1m may be prepared.
3. Preparation of vermibed: A layer 15-20 cm thick of good loamy soil above a thin layer of (5 cms) broken bricks and sand should be made.

4. Inoculation of earthworms: About one hundred earthworms are introduced as an optimum inoculating density into a compost pit of about 2m x 1m x 1m, provided with vermibed.

5. Organic layering: Chop cellulosic biomass waste materials in to small bits and mix with farm yard manure. It is done on the vermibed with fresh cattle dung. The compost pit is then layered to about 5 cm with dry leaves or hay or organic wastes. Moisture content of the pit is maintained by the addition of water.

6. Wet organic layering: It is done after four weeks with moist green organic waste, which can be spread over it to a thickness of 5 cm. This practice can be repeated every 4 days. Mixing of wastes periodically without disturbing the vermibed ensures proper vermicomposting. Wet layering with organic wastes can be repeated till the compost pit is nearly full. During this primary degradation 3-4 turns of the mix are to be given and maintain 50% moisture, for initial decomposition and temperature stabilization. After passing through the alimentary canal of earthworms, the food emerges as a compact, granular mass termed 'casting'.

7. Harvesting of compost: At maturation (after 90-120 days), the moisture content is brought down, by stopping the addition of water. At this stage, the worms start aggregating at the base of the pits. Collect entire material from the pit and make small pyramidal heaps on the ground. The worms move to the base of the heap. Slowly brush aside the heap to collect the worms in the bottom. The mature compost, a fine loose granular mass (about 1500 Kg), is removed from the pit, sieved, dried and packed. Matured vermicompost is rich in nutrient and recommended @ 1-10 t ha^{-1}.

OBSERVATION:

EX.NO: 7	PRODUCTION OF SINGLE CELL PROTEIN (SCP)
DATE:	

Introduction

Single cell protein is defined as the dried cells of microorganisms such as algae bacteria, actinomycetes and fungi, that are grown in large scale culture systems and used as food or feed. The large-scale production of microorganisms as a direct source of microbial protein has been well realized. SCP can be used to replace totally or partially the valuable amount of conventional vegetable and animal protein feed. Using microorganisms as SCP has many advantageous. A variety of substrates are used for SCP production. The materials which contain sugars / starch/ lignocelluloses can be used as substrates for SCP production. Production of yeast biomass as SCP is to be done in this experiment.

Materials required

Good strain of yeast; Tapioca waste / Sago industry waste, culture medium, container, centrifuge.

Procedure

- Grow a good strain of yeast in MYGP broth in a conical flask for about 3-4 days and use it as starter culture.
- Prepare the substrate in liquid form by mixing the sago industry waste with water and collect in a container.
- Inoculate yeast cell suspension @ 10 ml/l of substrate to the container and incubate it till the density of yeast cells reaches to 1.0 g/ml with intermittent shaking at 30-35°C.
- Harvest the cells by decantation, centrifugation and drying treatment methods.

MASS PRODUCTION OF SPIRULINA

Spirulina is a blue green alga used as a potential source of SCP. It grows in waste waters like rice mill effluent and produces SCP photosynthetically as well. Generally, circular cement tanks with shallow depth (25 cm) are constructed in required size according to convenience and yield needed. Open tanks are suitable for tropical and subtropical regions.

The tanks are designed as race way ponds with length/ width ratio of 40. Spirulina requires effective pond management for outdoor production. Agitation of culture by paddles, rotators or pipe pumps provides aeration and efficient mixing of nutrients. Supply of CO_2 during day time improves the quality and yield. Maintenance of optimum levels of nutrients is mandatory, culture medium contains $NaHCO_3$, NPK, $MgSO_4$, at the ratio of 8: 1: 0.1. The depth of culture medium is maintained at 20 cm. Spirulina grows well between pH 9 and 11 and there is a least chance of contamination at this pH. The optimum pH of nutrient medium is shifted from 8.4 to 9.5 during mass cultivation due to consumption of $NaHCO_3$. Atmospheric temperature of 30-35°C is ideally suited for getting higher yield of spirulina. The optimum light intensity for growth is between 20-30 kilo lux. The alga is harvested by filtration. The culture liquid is pumped and filtered through nylon cloth in a rotary machine. The yield is about 15 g / m^2/day (20 tonnes / ha/year). The collected algal suspension is dried (sun dried or spray dried) and used as single cell protein.

OBSERVATION:

EX.NO. 8	DETERMINATION OF RATE OF MICROBIAL GROWTH BY TURBIDOMETRIC TECHNIQUE
DATE:	

Estimation of the growth rate of a bacterial culture can be successfully done with the help of a Photo colorimeter. A bacterial culture in broth acts as a colloidal suspension which can block or reflect light passing through the culture. The light blocked or reflected by a culture of bacteria is proportional to the concentrations of the cells in the suspension. Thus, by measuring the reflection of light rays (nephelometry) or the percentage of light obstructed (turbidimetry) by bacterial cells in a suspension you can calculate the density of bacterial cells in a culture and there by you can estimate the growth rate of the bacteria. Most estimation are done by photo colorimeter to determine turbidity.

In turbidometry, the percentage of light transmitted through a bacterial suspension is inversely proportional to the cell concentration. You can however express turbidity as optical density (O.D.) also which is directly proportional to the cell concentration in a bacterial suspension and can be expressed as:

O.D. = log 100 – log of galvanometer reading. Convert the galvanometer reading into O.D.

Materials required**:** Bacterial culture Nutrient broth Turbidometer Distilled water, Tissue paper

Protocol:

1) Make 5 ml suspension of bacteria (e.g. E. *coli*) in sterile distilled water and transfer it to a flask containing 200ml of sterile nutrient broth. Mix well and allow incubating at 37°C. Note the time of inoculation.

2) Turn the colorimeter on and allow a few minutes to warm up if needed. Select a wavelength of 600 nm and bring the needle to zero on the percentage transmission scale.

3) Transfer 5ml of the sterile nutrient broth from another flask to a clean colorimeter tube. See that all liquid and finger prints are completely wiped off with the help of cleansing tissue. Close the cover of the sample holder. Rotate the light control until the meter reads 100 on the percentage transmission scale.

4) Shake the inoculated flask and transfer 5ml portion of it into another colorimeter tube and insert the unknown tube in the place of the standard and read the percentage of transmittance. Note the result obtained at zero hour. Discard the sample. Wash the tube with disinfectants followed by several changes of sterile water.

5) Incubate the inoculated flask at 37 0C.

6) Then on every 2,4,6,8,16 and 24 hours remove 5ml of the sample with sterile 5ml pipettes and determine the O.D.

7) Plot a graph showing O.D. against time in hour. The curve obtained is called a growth curve for that specific organism.

OBSERVATIONS:

EX.NO: 9	WINE MAKING
DATE:	

Aim:

Wine is a product of alcoholic fermentation of fruit juice that are rich in fermentable sugars. During fermentation the yeast digest sugar found in fruit juice, producing alcohol and carbon dioxide in the process. Most wines are made from grapes only. Wine naturally contains 85-89 % water, 10-14 % alcohol, < 1% fruit acids and hundreds of flavour and aroma compounds in very small amounts. There are many types of wine and three basic groups of wines are most easily distinguishable for the consumer: table wines, sparkling wines, and fortified wines. **Table wines**, also known as still or natural wines, are produced in many different styles and make up the majority of wines on the market. Traditionally consumed as part of a meal, table wines contain between 10 and 14 percent alcohol and are further classified by their color, sugar content, and the variety and origin of the grapes that were used. Depending on the grape variety and wine-making technique, wines can be white, red, or pink in colour. The **red wines** are prepared by fermentation of grape juice along with the grape skins. Red pigments called anthocyanins and other compounds in the grape skins are extracted during the fermentation process to impart the characteristic red color of the wine as well as other features. **White wine,** which is actually straw to golden-yellow in color, is produced from white grapes, by taking the grape juice only. White grapes are crushed, and the juice separated from the skins prior to fermentation. A blush or rosé wine is light pink in color and is produced from red grapes not fermented with the skins. A little pigment is released when the red grapes are crushed, but not to the same extent as during fermentation.

- Still wine (without carbonation)
- Sparkling wine (with carbonation)

Material required:

1) Grapes
2) Yeast culture
3) Potassium meta-bi-sulphate
4) Sterilized water
5) Juice maker
6) Conical flask

Procedure:

- Fully ripened grapes with sweet taste and optimum flavour are selected
- the stem and stalks of the berries are removed as they contain tannin
- Grapes are washed with clean water and crushed to yield grape juice.
- The crushed juice called Must has a pH of 3.0. If the sweetness of Must is low (Brix value < 20), sugar is added.
- SO_2 is added to remove wild yeast contaminants and potassium meta bisulphite is added at the rate of 1.0 mg per kg of grapes as a preservative.
- After six hours of adding SO_2 the starter culture is added. The Must is inoculated with 3% yeast starter culture, *Saccharomyces cerevisiae* var *ellipsoideus*.
- Nutrients like, ammonium sulphate is added as nitrogen source. Anaerobic condition, a temperature of 21 – 27 °C and pH at 3.0 – 3.6 are maintained during fermentation.
- Partially fermented wine is decanted and again allowed to ferment for 7 – 10 days.
- After fermentation is completed the wine is decanted from sediment of yeast and particles of grape tissues.
- Fermented Must is filtered and fining agents such as casein, gelatin, egg white, tannin may be added for clarification.
- Clearing of wine and development of flavour take place during aging of at least 8 – 10 months. The aged wine is again filtered, pasteurized and bottled before consumption.

FLOW CHART - WINE MAKING

GRAPES PICKING

⇩

REMOVAL OF stem, stalks etc.

⇩

WASHING IN CLEAN WATER

⇩

WEIGHING, CRUSHING

⇩

MUST –Brix value < 20 ° need addition of sugar

⇩

SO_2 ADDED to destroy wild yeast

⇩

YEAST INOCULUM at 3 %

Saccharomyces cerevisiae var *ellipsoideus*

⇩

FERMENTATION

⇩

DECANTING

Fermented material passed through a press

Skin and seeds removed

⇩

CLARIFICATION

Fining material added

⇩

AGING OF WINE - 8 – 10 months

⇩

FILTRATION, PASTEURIZATION

⇩

BOTTLED/ PACKED

Work done:

EX.NO: 10	PRODUCTION OF EXTRA CELLULAR ENZYME AMYLASE
DATE:	

Aim:

To demonstrate the production of extra cellular enzyme amylase by Bacteria.

Introduction:

Microorganism is in general utilize complete organic compounds as nutrients. Some of these compounds are insoluble. If the microbe has to use them as substrate for growth and every, they have to necessarily breakdown to simple substrates which are soluble. Such hydrolytic reactions are carried out by the extra cellular enzymes secreted by some microorganisms that are producing surrounding. Those organisms that are producing these specific enzymes can utilize certain complex organic substances (starch, cellulose, casein). As substrates for their growth.

Materials required:

1) Starch agar medium, Gelatin agar medium.
2) Sterilized petridishes, pipettes.
3) Bacterial culture of *Eschericha coli, Pseudomonas aeruginosa, Bacillus subtilis, Streptococcus faecalis and Azotobacter chroococcum,*
4) *Iodine solution.*

Procedure:

1. Melt one tube of starch agar cool it to 45°C and pour it aseptically into a sterile Petri dish and it to solidify.
2. Divide the agar plate into 4 sectors by labeling at the bottom as 1, 2, 3, and 4 with glass marking pen.
3. Inoculate one spot in sector 1 with loopful of *B. subtilis* sector 2 with *A. chroococcum* and sector 3 with *S. faecalis*. Have sector 4 in inoculated.

4. Incubate the inoculated starch agar plate in inverted position at 30°C for two days.
5. At the end of the incubation period, flood the agar surface with gram's iodine solution keep it for a minute and then pour off.
6. Observe the plate carefully and declare the results. Iodine reacts with starch and forms a blue colour.

Starch- Casein Agar Medium:

Starch	–	10.0 g
Casein (vitamin free)	–	2.0 g
KNO_3	–	2.0 g
NaCl	–	2.0 g
K_2HPO_4	–	2.0 g
$MgSO_4$	–	0.50 g
$CaCO_3$	–	0.02 g
$FeSO_4$	–	0.01 g
Agar	–	18.0 g
Distilled water	–	7.0- 7.2

(Nystatin and actidione – 50 mg/ ml each to be added to cooled medium before pouring)

Work done:

EX.NO: 11	PRODUCTION OF ENZYMES USING IMMOBILIZATION TECHNIQUE
DATE:	

Aim:

To demonstrate continuous enzyme production using immobilized yeast cells.

Introduction:

Immobilization is a technique where in enzymes or cells involved in the fermentation processes are immobilized. This method is highly cost effect and the effect of native enzymes subjected to inactivation by chemical, physical and biological factor are minimized. The bonding between beads (carrier) and the enzymes (or) cells should be such that there should not be any alteration in the structure of the enzyme (or) cell it's sensitively and specificity. This way the reactions can be carried out even in small qualities of enzymes (or) cells without any wastage.

Materials required:

1) Sodium alginate
2) $CaCl_2$
3) Starch solution
4) Distilled water
5) Yeast cells (or) paste
6) Calorimeter

Procedure:

1. 3.5 g of sodium alginate dissolved in 100 ml distilled water (avoid any air bubbles)
2. Then, pour two yeast tablets mix it gently and thoroughly using vortex.
3. Prepare 1% calcium chloride solution in which the slurry is dropped using syringe, which forms beads.

4. The beads are collected and washed in distilled water.
5. At least 2-3 beads are added to 5 ml of the different concentrations of starch (1-10%) solution.
6. After an hour, add iodine solution (a drop to each test solution).
7. Estimate the enzyme activity by obtaining optical density at 540 nm.
8. Draw the graph substrate (starch concentration) vs., their optical density at one hour.

Work done:

EX.NO:12	PRODUCTION OF PENICILLIN
DATE:	

Aim:

To study the production of *Penicillin.*

Introduction:

Penicillin was also called as green mould *Penicillium notatum* antibiotic activity was studied by Alexander fleming in 1928 in the plates of *Staphylococcus aureus*. The product penicillin was obtained from *Penicillium notatum* in the earlier days later efficient strains of *Penicillium chrysogenum* is used for penicillin production. It acts as chemotherapeutic agents; About 38% of the penicillin's produced commercially are used in human medicine, 12% in veterinary medicine and 43% are used as starting materials to produce semi synthetic Penicillium.

Materials Required:

1) Rose Bengal Agar medium
2) Penicillin producing strain of *Penicillium chrysogenum*
3) Sterile flasks
4) Inoculating needle/ Cork borer

Procedure:

1. The Rose Bengal broth is prepared in a 250 ml flask and sterilizes it.
2. With help of a cork borer take a disc from the *Penicillium* plate and transfer to the Rose Bengal broth aseptically.
3. The conical flask is incubated at 25°C for 7-11 days.
4. The flask is observed periodically to produce *Penicillin.*

5. The development of golden yellow colour is the medium (or) the droplets in the mycelium will indicate the production of *Penicillin.*

Work done:

EX.NO: 13	ANTIBIOTIC ASSAY – STREPTOMYCIN AND PENICILLIN
DATE:	

Aim:

To determine the efficacy of Streptomycin and Penicillin antibiotic sensitivity against *Bacillus subtilis* culture.

Introduction:

The term "antibiotic" has been defined by "Selman. A. Waksman" as being an organic compound, produced by one micro organism that, at great dilution, inhibits the growth (or) kills the other microorganisms. In this method the filter paper discs are used to the study inhibition effect of Streptomycin and Penicillin against the test organism *Bacillus subtilis.* The effectiveness of the antibiotics in this test based on the size of the inhibition zone. The resulting value is called the minimal inhibitory concentration (MIC) is determined by measuring the diameter of growth inhibition (clear) zone surrounding the antibiotic disc.

Material required:

1. 24 hours old culture of *Bacillus subtilis* (or) *E. coli*
2. Solution of Streptomycin and Penicillin antibiotics of known concentration (250ppm, 500ppm, 1000ppm)
3. Sterile water blank
4. Sterile Petri plate
5. Filter paper disc
6. Nutrient Agar medium / Special Medium

Medium composition:

Nutrient Agar Medium:

Peptone	-	5.0 g
Beef extract	–	3.0 g
NaCl	-	5.0 g
Agar	–	15.0 g
Distilled water	–	1000 ml
pH	–	7.0

Special Medium:

Peptone	–	6.0 g
Beef extract	-	1.5 g
Yeast extract	–	3.0 g
Agar	–	15.0 g
Distilled water	-	1000 ml
pH	–	7.0

Procedure:

1. Streptomycin solution is prepared at different concentration *i.e.*250ppm, 500ppm, and 1000ppm.
2. Filter paper disc are dipped in different concentration of streptomycin solution.
3. The seeded special medium / nutrient agar medium is poured in the sterile Petri plates and allowed to solidification.
4. After the solidification, the paper discs are placed aseptically over the seeded medium in a quadrangular manner and mark the concentration on the Petri plate and filter paper disc dipped in sterile water act as control.
5. The plates are incubated for 24 hours.
6. The inhibition zone is measured, and its area is calculated.
7. The area of inhibition zone is calculated by using the formula

   Area = $\pi(R+r)\,(R-r)$

Where

π = 3.14

R – Radius of inhibition zone + filter paper disc

r- Radius of the Filter paper disc

PENICILLIN

Procedure:

1. The nutrient agar or special medium is prepared and sterilized.
2. To the medium add the culture at low temperature which is as called seeded medium.
3. The seeded medium is poured into sterile Petri-plates and Different concentration of penicillin solution i.e. 250ppm, 500ppm, 1000ppm is prepared using sterile water.
4. Sterile filter paper discs are dipped in different concentration of *Penicillin* solution.
5. The paper disc is placed aseptically over the seeded medium in a quadrangular manner.
6. The plates are incubated at 24 hours and the inhibition zone is measured and its area is calculated. The filter paper dipped in sterile water act as control.

Work done:

The area of the inhibition zone was calculated as.

1. 250ppm –
2. 500ppm –
3. 1000ppm –

Work done: