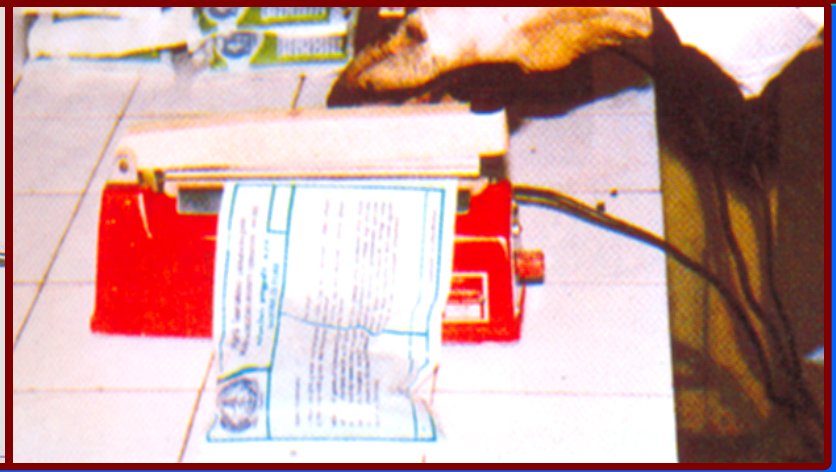
INTRODUCTION

The procedure involves isolation, mass culture production and preparation of inoculants along with inoculant quality control. The individual organism can be mass multiplied using specific media either as small scale or as large-scale commercial production procedure using fermenters. The desired growth of organisms is then mixed with carrier materials and sealed in culture packets. The entire procedure is carried out under aseptic condition to avoid contamination from other undesired organisms. The quality of inoculant is regularly checked prior to distribution of individual biofertilizer culture.

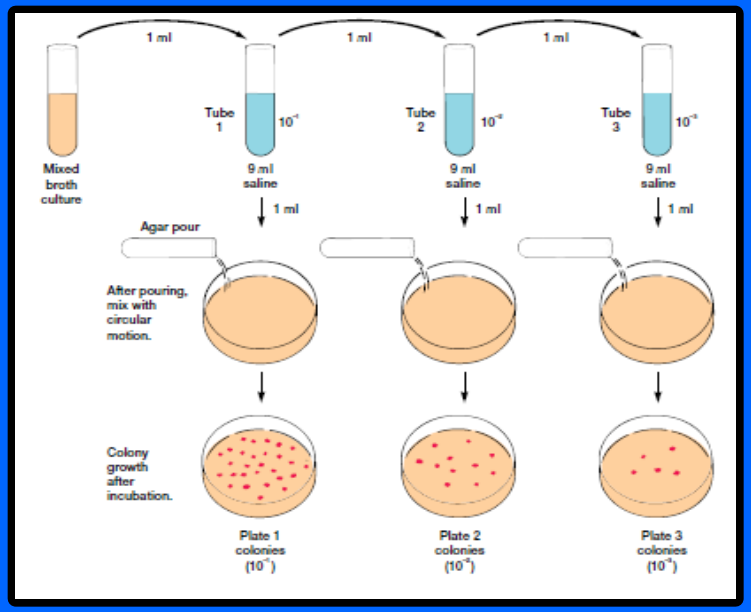
MASS MULTIPLICATION OF BIOFERTILIZERS

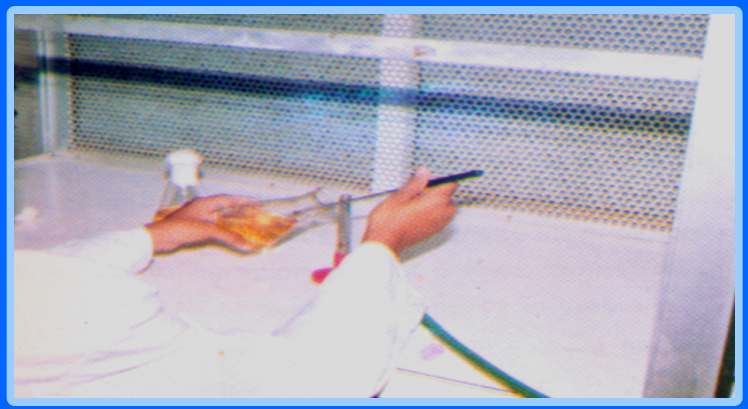


BACTERIAL INOCULANT PRODUCTION

Isolation Procedure

ISOLATION OF BACTERIAL CULTURE PERFORMED UNDER INOCULATION CHAMBER USING SERIAL DILUTION METHOD TO GET PURE COLONIES OF DESIRED MICROBE





Laboratory scale production

Preparation of the Inoculum culture

- Dispense 100 ml aliquots of the specified broth into flasks, and plug with non-absorbent cotton
- Autoclave flasks at 121°C for 20 minutes
- Transfer 5-6 days old non contaminant cultures of bacteria into the flasks with inoculation needle
- Incubate flasks at 30: t 2°C on shaker for 2-6 days

Preparation of broth culture

- Submerged culture technique employed for growing bacteria on mass scale
- Sterilize battery of conical flasks each containing 400 ml broth medium in an autoclave
- Add 5 to 25 ml of inoculums culture of desired organism aseptically into each flask and incubate on rotary shaker at 30± 1°C for 2-6 days

Commercial scale production

For large-scale production, fermenter are used for growing bacteria. pH is adjusted to 6.5 - 7. 0. Inoculum should be added @ 5%. Continuous aeration is done by forcing sterile air through sparger. Incubate culture till the bacterial population reaches 108 cells/ml, and added to carrier.

Standards

The organism count in final broth cultures shall not be less than 108 to 109 cells / ml. Otherwise, the broth should be rejected

Schematic diagram for mass scale production of bacterial Biofertilizers

Carriers for Bacterial inoculants

Carrier is the medium in which organisms are allowed to multiply . Different carrier materials viz., peat lignite , compost, leaf manures, cellulose powder, charcoal powder , coconut shell powder, rice husk powder, press mud etc are extensively used carrier for inoculum preparation.

A : Press mud ; B : Lignite : C: Charcoal : D: Coconut Shell : E: Rice Husk : F: Cellulose Powder : G: Leaf Manure : H: Peat

Preparation of carrier material

Drying and grinding of the carrier

- Carrier is sun-dried upto a moisture level of 5 %.
- The carrier is ground to pass through a 100-200 mesh sieves.
- Particles coarser than this cause 'balling up' when wetted
- The survival of rhizobia is also poor in coarser carrier materials.

Preparation of carrier material

Pretreatment of carrier

- The carriers are mixed with calcium carbonate to neutralize pH
- Carrier is mixed with 10 % water before sterilization

Sterilization of carrier

- The pretreated carrier is filled up to 2/3 of the capacity of the Containers
- Carriers are sterilized at 121°C for 3-4 hrs. for three successive days.

Mixing Broth With Carrier (Curing)

- Grow culture in fermenter till population reaches to 106 cells /ml
- Blend inoculum broth with the finely powdered and sterilized carrier.
- Add broth @ 1/3 of the water holding capacity of the carrier.
- Thoroughly mix the broth culture with sterilized carrier aseptically
- Keep blended carrier for 24 hrs for curing

PACKING AND STORAGE

- After curing, the inoculant is ready to be packed
- Select 50-75 micron polythene bags (6 x 10 in.)
- Dispense 200 g of inoculant in each bag
- Seal the polythene bags leaving 2/3 vacant spaces
- Pin bags on few places for aeration
- Keep inoculants for a week at room temperature
- Store in a cold room and despatch

QUALITY STANDARD OF INOCULANT MICROORGANISMS

Inoculant quality refers to the number of specific effective organisms in the inoculant

QUALITY STANDARD

- The inoculant shall be a carrier-based one
- The inoculant shall contain 108 viable cells within 15 days of manufacture
- The inoculant shall contain 107 viable cells within 15 days before expiry
- The inoculant shall have a maximum expiry period of 6 months
- The inoculant shall not have any contamination
- The pH of the inoculant shall be between 6.0-7.5
- The inoculant should be infective and effective when tested on crop
- The carrier material shall be in powder form
- The manufacturers shall control the quality of broth and maintain records
- The inoculant be packed in 50-75 micron polyethylene packets
- The inoculant shall be stored cool place preferably at 15°C - 30°C ± 2°C.

Each packet shall be marked with information like: product name, specific crop, manufacturer's name, batch no, ISI mark, date of manufacture, date of expiry, net quantity and storage instructions

LET US SUM UP

- Mass multiplication of biofertilizers involves small scale and large scale production system.
- The detailed procedure includes isolation, maintenance, characterization and mass culture production.
- A well furnished laboratory having specific equipments and complete aseptic condition is required for mass multiplication of biofertilizers.

contd…

contd…

- The organism once isolated, purified is mixed with carrier materials as solid support and packaged into low density polybags prior to use.

- Periodic quality assessment of individual culture is a very basic for quality produce.

REFERENCES

i) Soil Microbiology – N. S. Subba Rao

ii) Advances in Microbiology – N. S. Subba Rao

iii) Bio-fertilizer and Organic Farming – NIIR Board

iv) Biofertilizers-

Potentialities and Problems – S. P. Sen and P. Palit