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# MASS MULTIPLICATION OF BIO-FERTILIZERS



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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.



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# Steps in the mass multiplication of Biofertilizers

Strain Selection ,maintenance of pure culture

Preparation of broth culture.

Preparation of broth culture at commercial scale, inoculant quality control

Mixing broth culture with carrier (blending) and curing Packing

All steps to be undertaken under aseptic conditions.

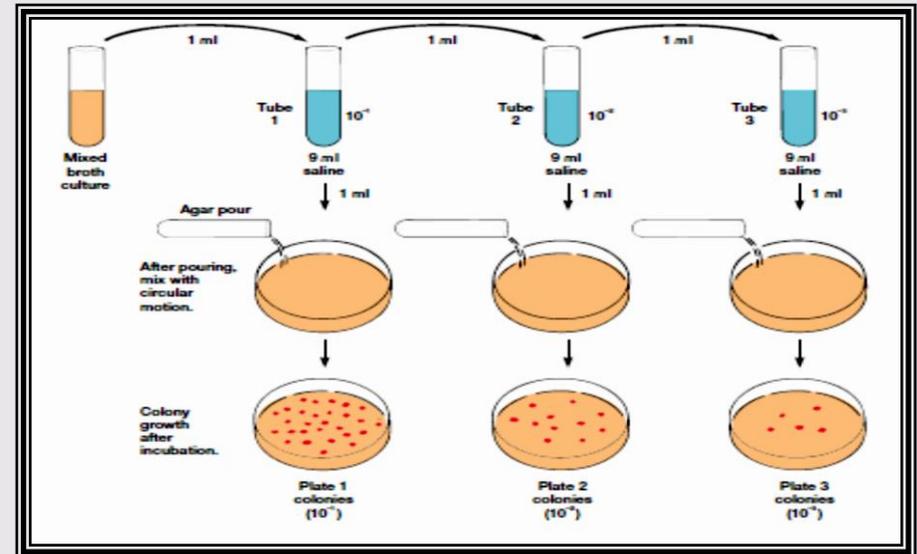


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## Isolation Procedure

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



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## Laboratory scale production

### Preparation of the Inoculum (broth) 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 desired pure culture of bacteria aseptically into the flasks with the help of inoculation needle.

Incubate flasks at  $30 \pm 2^\circ\text{C}$  on rotary shaker for 2-6 days depending on the organism.



## Preparation of broth culture

Submerged culture technique employed for growing bacteria on mass scale.

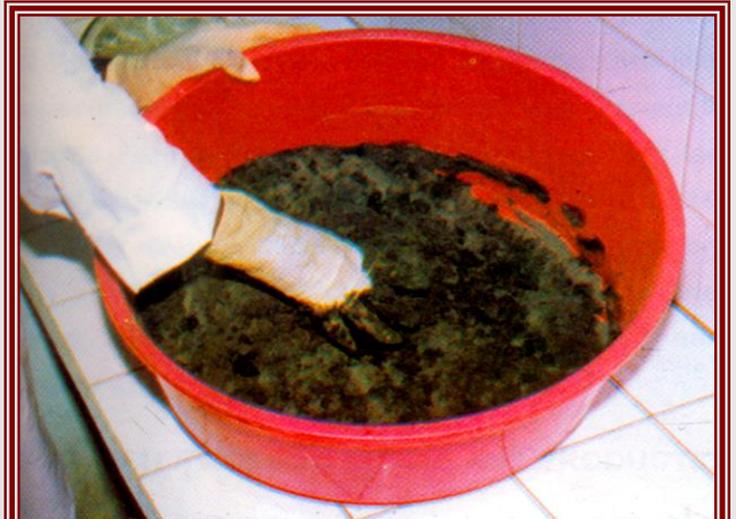
Sterilize battery of conical flasks each containing 400 ml broth medium in an autoclave.

Add 5 to 25 ml of inoculum culture of desired organism aseptically into each flask and incubate on rotary shaker at  $30 \pm 1^\circ\text{C}$  for 2-6 days.



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## 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  $10^8$  cells/ml, and added to carrier.**



### Standards

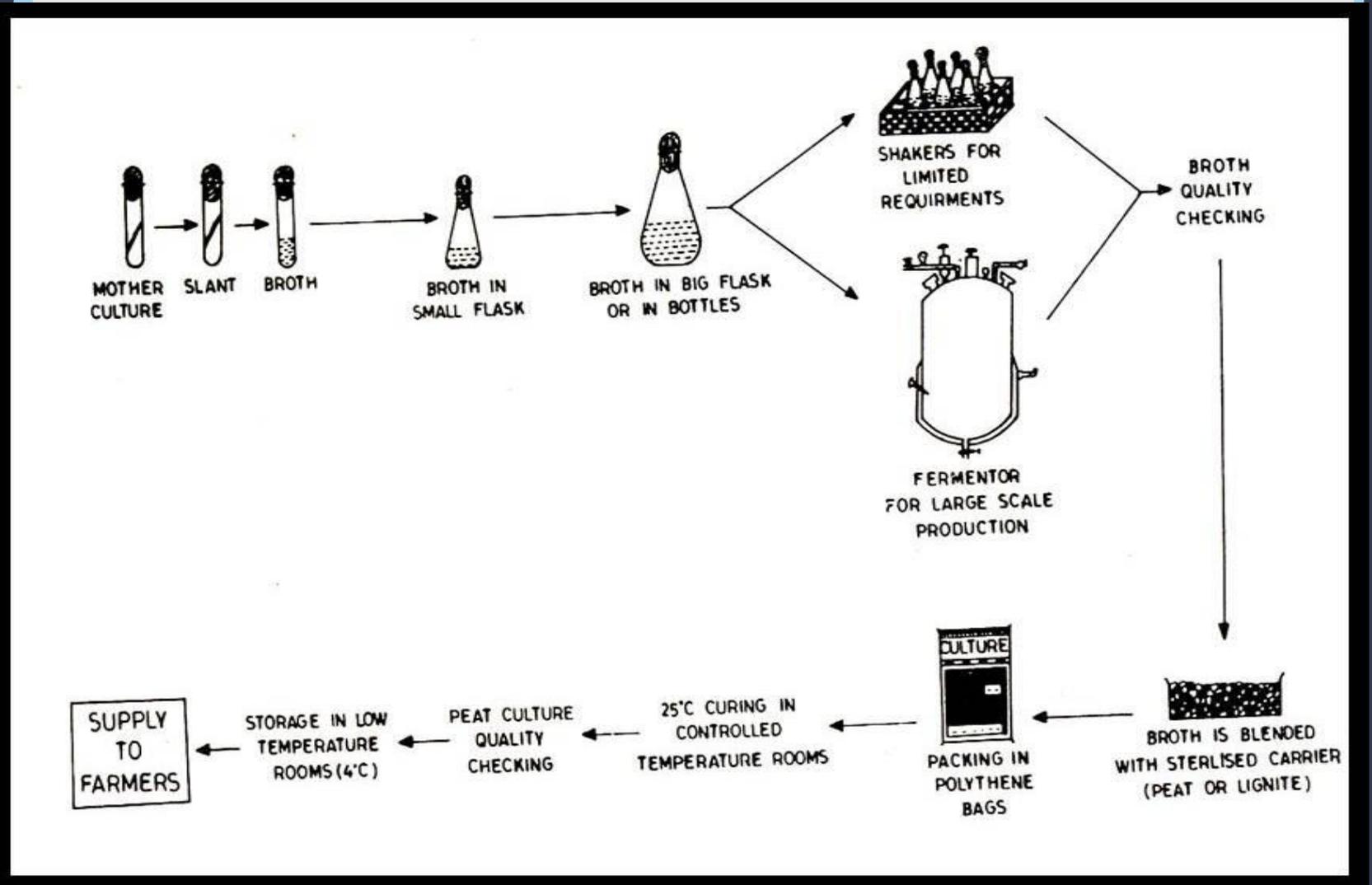
**The organism count in final broth cultures shall not be less than  $10^8$  to  $10^9$  cells / ml. Otherwise, the broth should be rejected**



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## Schematic diagram for mass scale production of bacterial Biofertilizers



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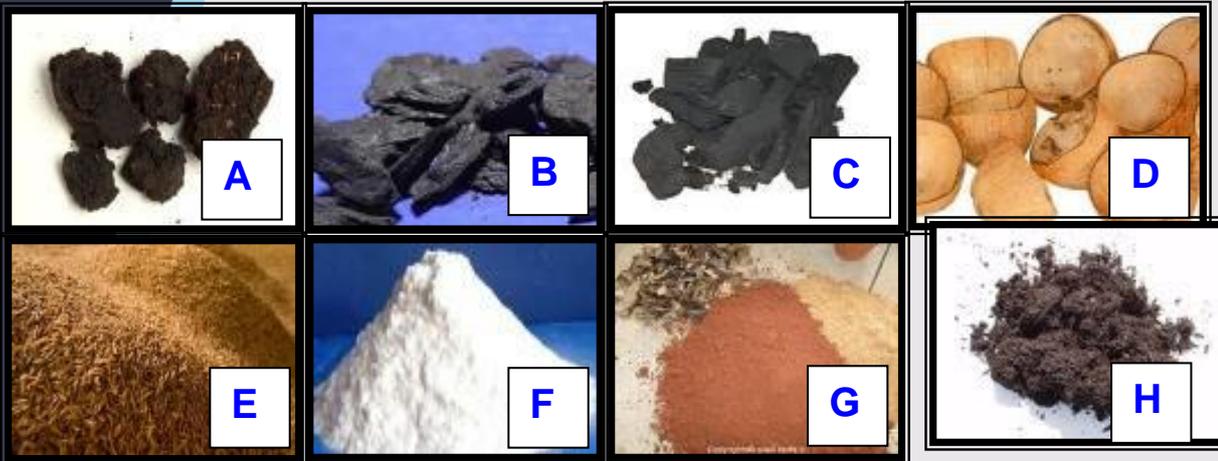
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# Carriers for Bacterial inoculants

- 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.





# 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 and adhere poorly to the seed coat at the time of inoculation .**
- **The survival of rhizobia is also poor in coarser carrier materials.**



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## Preparation of carrier material

- The carriers are mixed with calcium carbonate to neutralize pH(6.5-7.0).
- 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 (steel trays, 2x1-0.5 x0.5feet).
- Carriers are sterilized at 121°C for 3-4 hrs.continuously or 1hr each for three successive days.



## Mixing Broth With Carrier (blending) and Curing

- **Grow culture in fermenter till population reaches to  $10^9$  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 in trays aseptically.**
- **Keep blended carrier for 24 hrs for curing to ensure acclimatization of bacteria with the carrier .**



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# PACKING AND STORAGE

- After curing, the inoculant is ready to be packed.
- Select 50-75 micron polythene bags (6 x 10 inches).
- Dispense 200 g of inoculant in each bag manually or with automatic dispenser.
- Seal the polythene bags leaving 2/3 vacant spaces.
- Pin bags on few places for aeration. Keep inoculants for a week at room temperature (25-30°C).
- Store in a cold room(4°C) and despatch.



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## QUALITY STANDARD OF INOCULANT MICROORGANISMS

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

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## QUALITY STANDARD

**The inoculant shall be a carrier-based one.**

**The inoculant shall contain  $10^8$  viable cells within 15 days of manufacture and  $10^7$  viable cells within 15 days before expiry.**

**When the inoculant is stored at 25-30°C 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.**

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## QUALITY STANDARD

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  $15^{\circ}\text{C} - 30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

**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**