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Biofertilizers

Mass Multiplication

Mass multiplication

- Isolation
- Mass culture production
- Preparation of inoculant along with quality control.
- Individual organism has to be multiplied using specific media – either as small scale – laboratory condition/ large scale – using fermentors.

- Allow to grow
- After desired growth – it is mixed with carrier materials
- Sealed in packets
- Entire procedure is carried out in aseptic condition to avoid contamination of undesired organism.



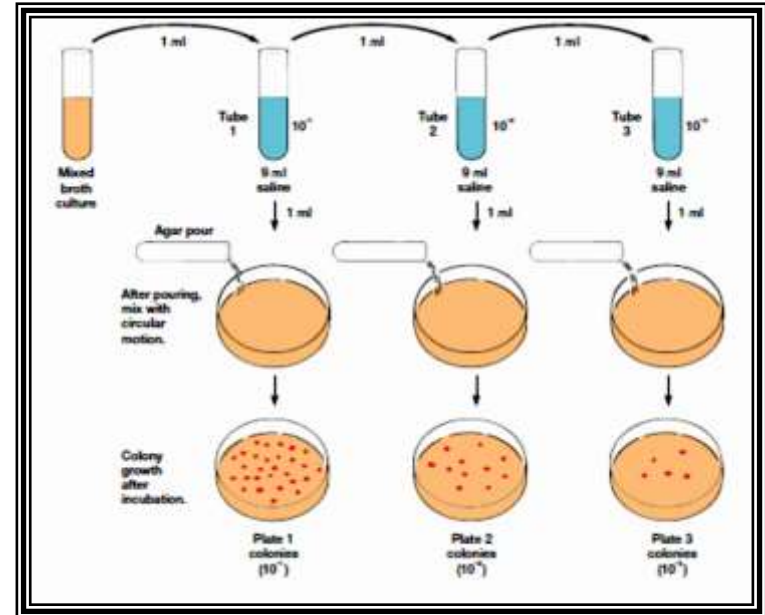
MASS MULTIPLICATION OF BIOFERTILIZERS



BACTERIAL INOCULANT PRODUCTION

Isolation Procedure

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



- 100 ml broth – flask and autoclave
- Inoculate pure culture in to flask and allow to grow in a shaker at 28-30oC for 3-6 days – submerged culture.
- Harvest the culture

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.

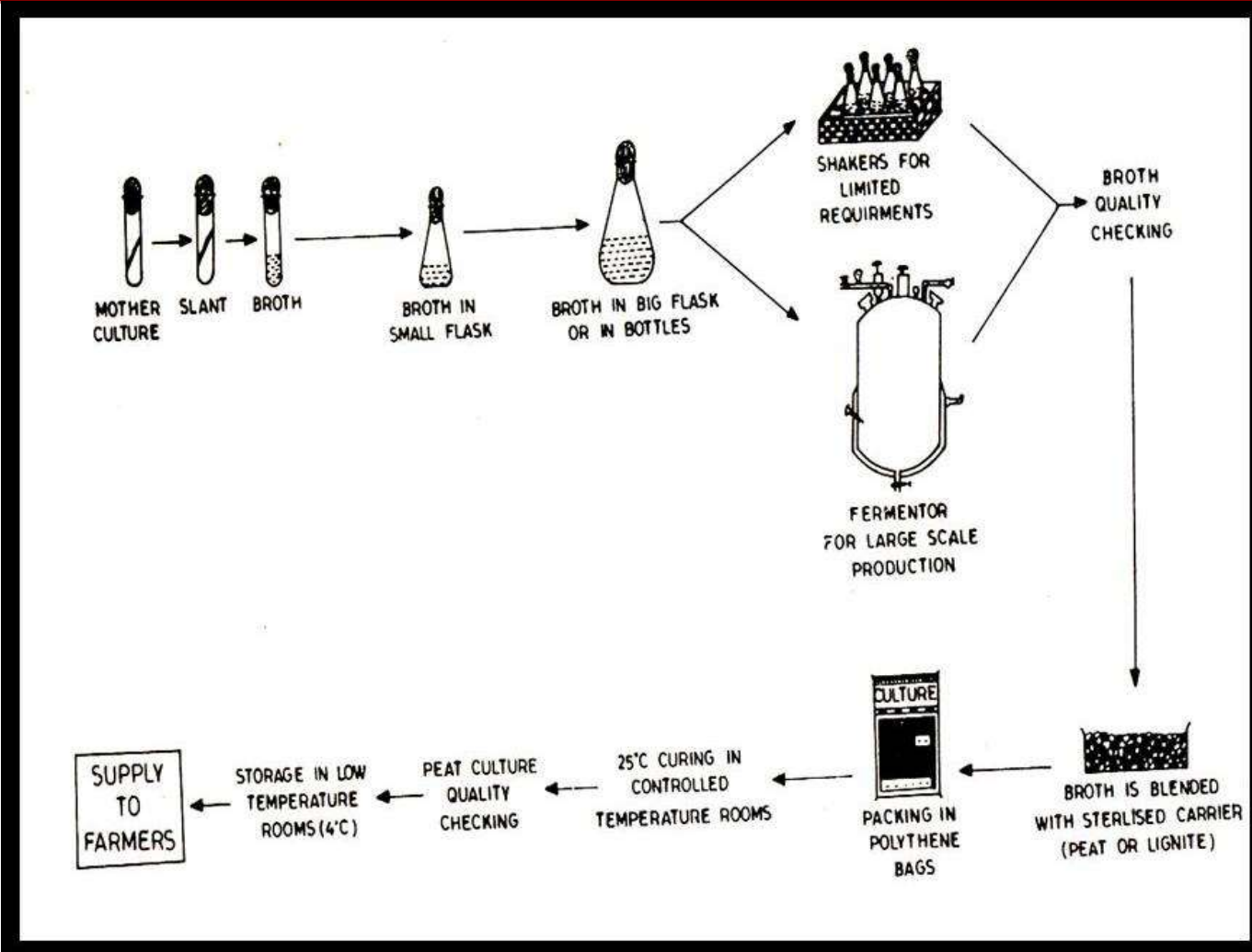


Standards

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

Mass Multiplication of Biofertilizers

Schematic diagram for mass scale production of bacterial Biofertilizers



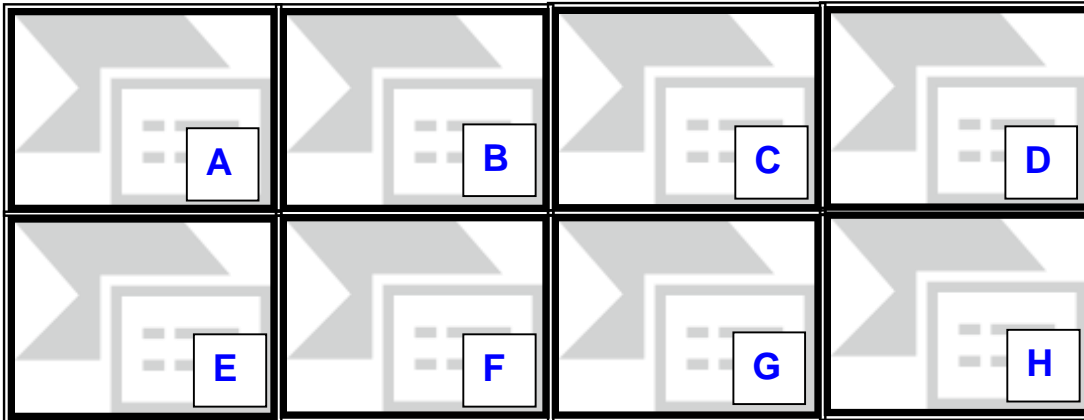
Grow

Process

Test

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

Mixing Broth With Carrier (Curing)

- **Grow culture in fermenter till population reaches to 10^6 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 10^8 viable cells within 15 days of manufacture
 - The inoculant shall contain 10^7 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 $15^{\circ}\text{C} - 30^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

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

MASS MULTIPLICATION OF AZOLLA

HOMESTEAD METHOD



- ❁ Prepare pit 2m length, 1m width & 20cm depth
- ❁ Spread polythene sheet (2.6mx1.6m) over the pit
- ❁ Add SSP (10g), MOP (10g), dry cow dung (100g) & Azolla 300 g
- ❁ Mud plastering the surroundings & pour water level (10 cm)
- ❁ Multiply for 15 days. Harvest and repeat the above procedure

FIELD METHOD FOR AZOLLA MULTIPLICATION

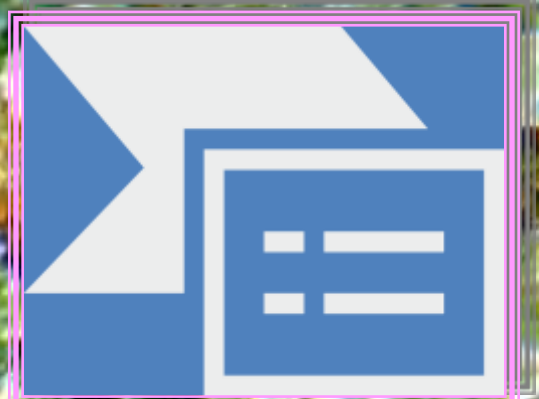
- Prepare and level the field uniformly
- Divide the field into 20x5m providing suitable bunds & irrigation channel
- Maintain 10 cm water depth
- Add 10 kg cowdung+8kg Azolla+100 gm SSP /plot
- Harvest after 15 days



Uses

- Azolla excretes organic nitrogen in water during its growth and also immediately upon trampling.
- Fern fronds are soft and rapidly decomposed.
- It absorbs traces of potassium from irrigation water.
- Azolla provides nitrogen, potassium organic carbon etc.
- It prevents weed growth in rice field water

Azolla as a component of INM



HOMESTEAD AZOLLA CULTIVATION

AZOLLA RICE DUAL CULTURE



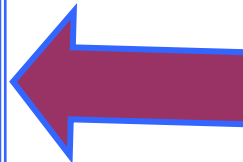
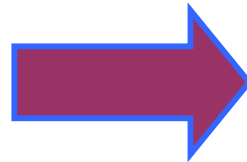
Azolla dual with rice
can save 50 % of
nitrogen
requirement

BLUE GREEN ALGAE

MASS PRODUCTION OF BGA CULTURE

Shallow trays (2m x 1m) of galvanized sheet

Spread 8-10 kg soil plus 200 g SSP



Add water (5-15cm)

Sprinkle algal culture

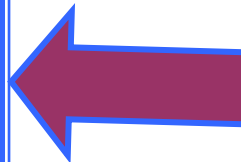
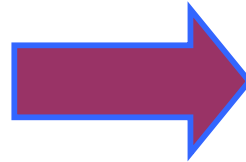
Expose to sunlight

BLUE GREEN ALGAE

MASS PRODUCTION OF BGA CULTURE

**Thick algal mat forms
at 15 days**

**Allow water to
evaporate**



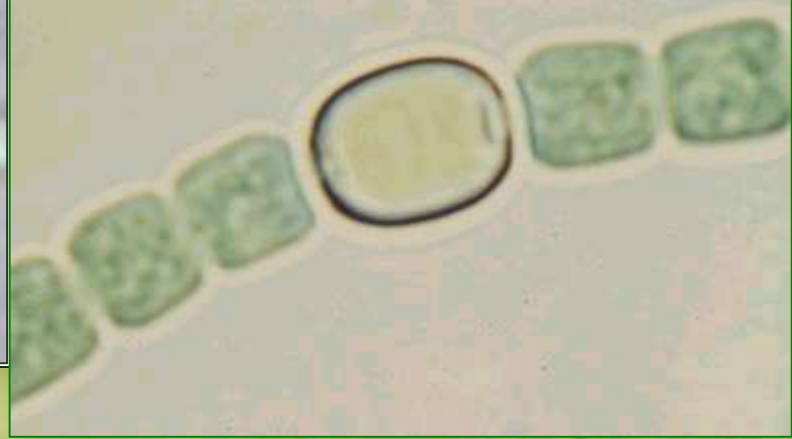
**Collect dry algal
flakes**

**Make into powder and
pack in polybags**

- Cyanobacteria can fix atmospheric nitrogen in aerobic conditions by means of specialized cells called heterocysts. Heterocysts may also form under the appropriate environmental conditions (anoxic) when fixed nitrogen is scarce. Heterocyst-forming species are specialized for nitrogen fixation and are able to fix nitrogen gas into ammonia (NH_3), nitrites (NO_2^-) or nitrates (NO_3^-), which can be absorbed by plants and converted to protein and nucleic acids (atmospheric nitrogen is not bioavailable to plants,

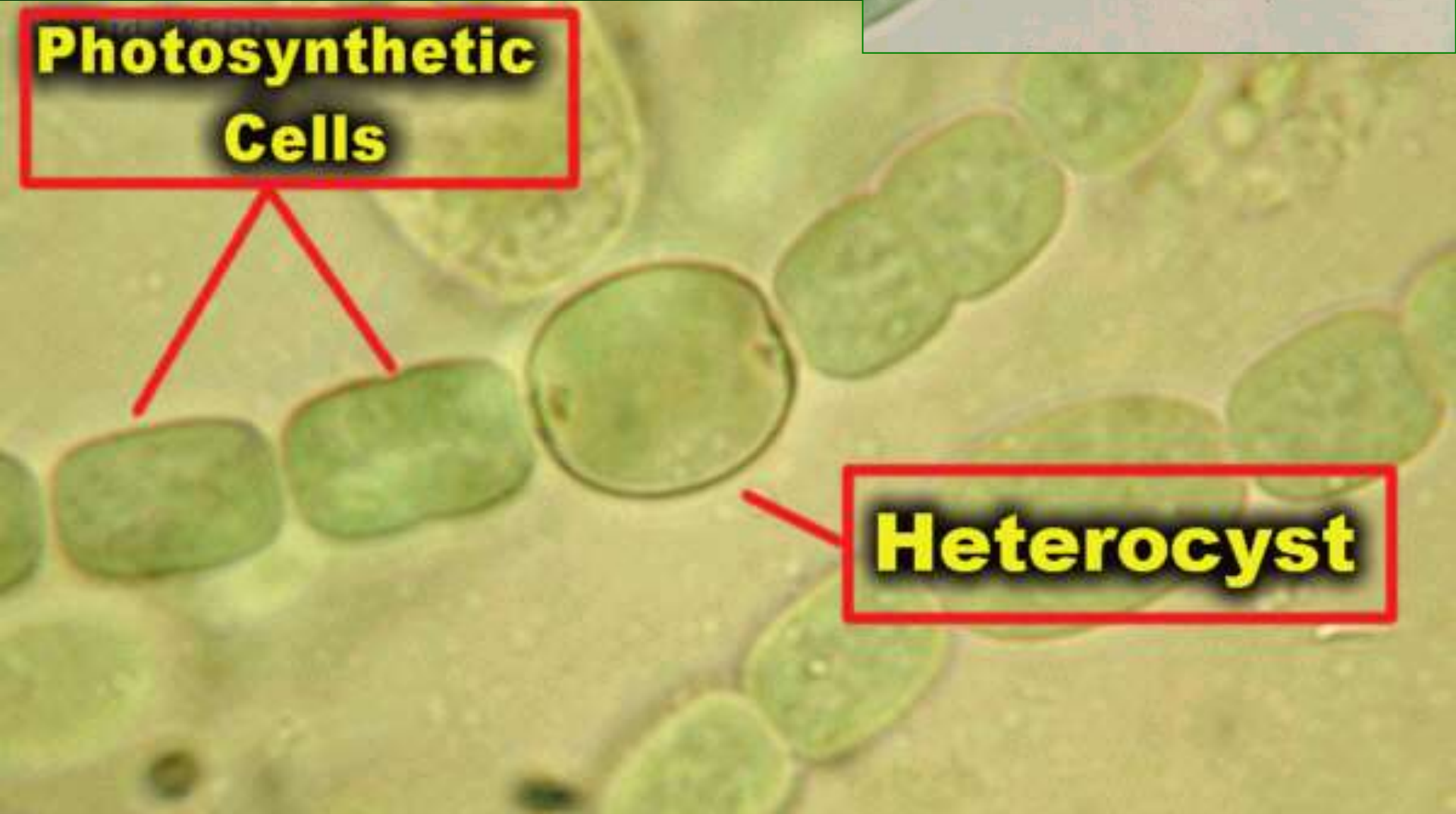
- ***Anabaena*** is a genus of filamentous cyanobacteria that exists as plankton. It is known for its nitrogen fixing abilities, and they form symbiotic relationships with certain plants

- Under nitrogen-limiting conditions, vegetative cells differentiate into heterocysts at semi-regular intervals along the filaments. Heterocysts are cells that are terminally specialized for nitrogen fixation.
- nitrogen fixed in heterocysts moves into the vegetative cells, at least in part in the form of amino acids.



**Photosynthetic
Cells**

Heterocyst



Thank You

