B. Sc. (Hon's) Fifth Semester examination, 2013-12-11
Botany
Paper: LBC-506
Biofertitlizer Technology

Answer 1.

(i) ***Microcystis*** is not use in algalization. Option "c" is correct.
(ii) Siratro (*Macroptilum artopurpurem*) is a **Forage legume.** Option "d" is correct.
(iii) Nitrogenase was first described in ***Clostridium pasteurinum.*** Option "c" is correct.
(iv) Hydrogenase is sensitive to **oxygen.** Option "a" is correct.
(v) **Chelation of nutrients from rock** is the main function of Lichenic acid. Option "c" is correct.
(vi) ***Sinorhizobium*** is not associative bacteria. Option "d" is correct.
(vii) *Heliobacteria* is **Purple non sulphur.** Option "c" is correct.
(viii) **75%** of phycocyanin found in cyanobacteria.
(ix) Rhizia is mainly associated with **Fabaceae.** Option "a" is correct.
(x) ***Oscillatoria*** lost nitrogen fixation ability. Option "d" is correct.

Answer 2.

(i). The leguminosae is a family of flowering plants found in both temperate and tropical zones. The family comprises three major subfamilies, the papilionoidae (the largest group0, the mimosoidae and caeselpinoidae.Between 80 and 90% of the species in the papilionoidae form nodule, but only about a quarter of mimosoidae and relatively few of the caesalpinoidae.

Papilionoidae
Pisum sativum.....Garden pea
Vicia faba.....Broad bean
Phaseolus vulgaris.....Kidney bean
Trifolium repens.....White clover
Medicago sativa.....Lucerne
Ulex.....Gorse
Lupinus polyphyllus.....Garden lupin
Lotus corniculatus.....Bird's foot trefoil
Melitotus officinalis.....Melilot
Glycine max.....Soya bean
Arachis hypogaea.....Peanut
Cicer arientinum.....Chick pea

Mimosoidae
Accacia.....Babool
Xilia dolabriformis.....Ironwood
Mimosa pudica.....Sensitive mimos
Caesalpinoidae
Cassia fistula.....Senna

Tamarindus indica.....Tamarind
Ceris siliquastrum.....Juelas tree

Molecular basis of *Rhizobia*-Host relation

Molecular basis of nodule forming bacterium-legume plant interaction is known. The bacterium conatins nodulation (nod or nol) genes. Most of these gene are interactive in the absence of the plants. However, the root exudates compounds such as flavonoids and nucleotides activate the positive regulatory protein, nod D. This results in the transcription of iducible nod gene which are required in the biosynthesis of series of compound such as nod factor or chitin oligosaccharides (LCOS). These host specific LCOS include the formation of nodule primodia. Other gene such as nif or fix genes present in the bacterium are also involved in the nitrogen fixation process. Host plant plays important role in the synthesis of nodulins which are nodule specific proteins. The synthesis of some of these nodulins can also induced by purified LCOS. The specific LCOS are major determinants of the host range nodulation.

In contrast, usually flavonoids do not act as host range determinant. During evolution the nod D gene seems to have fine-tuned its interaction with the flavonoid inducers or its host plant. Another important factor which is crucial in the host range is plant lectin.

(ii). A simple rural-oriented method that has been developed at the Indian Agricultural Research Institute at New Delhi. The procedure, starting in the laboratory and ending in the field. The algae used in this system is a mixture of species of *Tolypothrix, Aulosira, Anabaena, Nostoc* and *Plectonema.*

Cyanobacteria used in field

1. Maintain stock culture of different nitrogen-fixing blue-green algae on 1 to 1.5 % agar slants.
2. Maintain the same cultures also in soil extract medium (1g of soil+10 cm^3 of Fogg's medium (or cyanobacterial medium).
3. Grow the algae in 250 cm^3 flask containing 100 cm^3 of Fogg's medium in the light.
4. Scale up the cultures in aspirator bottles or carboys or crooks bottle.
5. Transfer the algae to Troughs to prepare soil

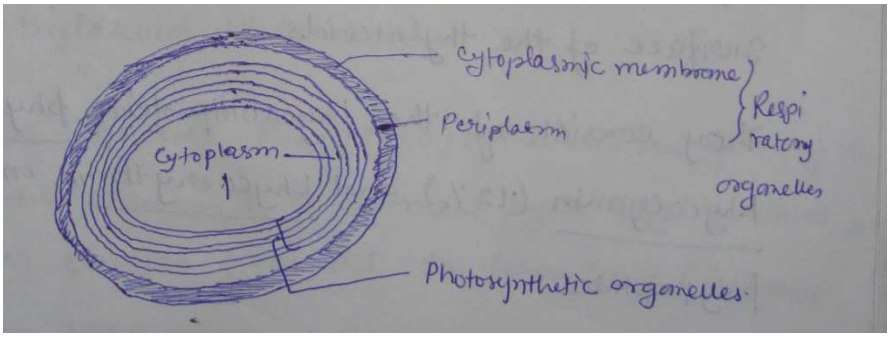
Trough method

1. Prepare shallow trays (2m x 23 cm) of galvanized iron sheet or permanent tanks. The size can be increased if more material is to be produced.
2. Introduce 8-10 kg of soil and mix well with 200 g of super phosphate.
3. Place from 5 cm to 15 cm of water in the tray depending on the local conditions and rate of evaporation. The reaction of the soil should be about neutral; if acidic corrected by adding lime.
4. To prevent insects, add carbofuron (3% granules) at the rate of 25 g per tray or BHC or other suitable insecticide.
5. After the soil has settled, sprinkle the alagl culture on the surface of the standing water. Keep the unit in the open air and completely exposed to the sun.
6. In hot summer months, the growth of the algae will be rapid and in about seven to ten days they form a thick mat.
7. Allow the water to evaporate completely in the sun, the dry algae cracks into flakes.
8. Collect the dry algal flakes from the trays and store in bags for use in field.

Pit method

This method does not differ from the Trough method except in magnitude. Instead of trough or tanks, shallow pits are dug in the ground and layered with a thick polythene sheet to hold the water. Other procedure are the same as in the trough method. This method is easy and less expensive to operate by small farmers.

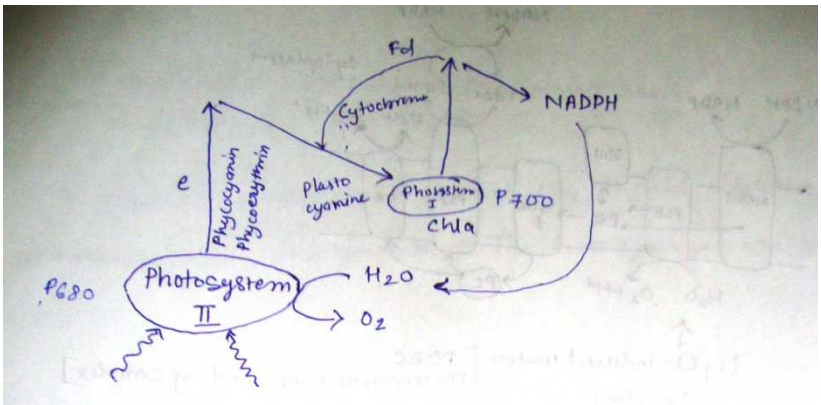
(iii). Cyanobacteria are thought to be among the evolutionary oldest organism. They are among the very few groups that can perform oxygenic photosynthesis and respiration simultaneously in the same compartment, and may cyanobacterial series are able to fix nitrogen. Photosynthesis and respiration require electron transport path ways that to a large extent are catalysed by protein complex in membranes.



Compartmentalization of cyanobacterial cell.

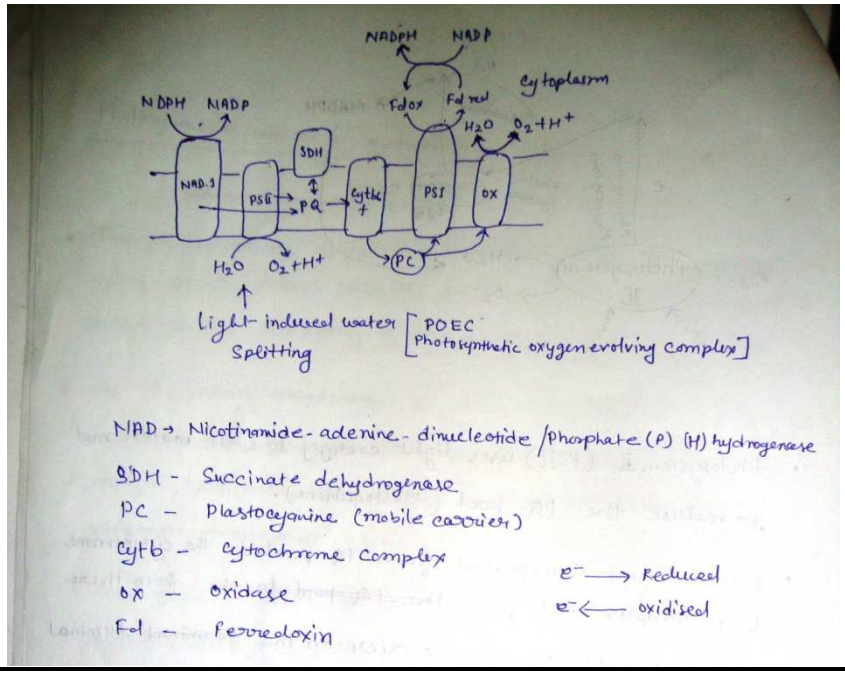
Photosynthesis process in cyanobacteria

- The photosynthetic apparatus is present in the form of thylakoid, which occur either parallel to the cytoplasmic membrane or coiled at the periphery of the photosynthetic space.
- The thylakoid membrane contains Chlorophyll a, β-carotene, and oxo-carotenoids like myxoxanthophyll, chinemon, and zeaxanthin, as well as components of the photosynthetic electron transport system.
- The special feature of the thylakoid of cyanobacteria are the phycobilisomes, disclike structre attached to the outer surface of the thylakoids.
- They consist of the phycobiliproteins phycocyanin (75%), allophycocyanin (12%) and phycoerythrin and some colourless polypeptides.



- Phycocyanobilin, phycoerythrin and allophycocyanin act as “pigment antennae” and deliver the energy that absorb mainly to photosystem II.
- Chlorophyll a serve as photosystem I.

- Photosystem II (PS II) uses light energy to split water and to reduce the PQ pool (plastoquinone).
- Electron are transported from PQ pool to the cytochrome b_6f complex and from there to a soluble electron carrier on the luminal side of the thylakoid membrane.
- Electron move towards PSI (P 700). The oxidised form of the reaction centre Chlorophyll is formed by a light-induced transfer of an electron from PS I to ferredoxin (Fd) and eventually to NADP. Reduced NADP can be used for CO_2 fixation.



(iv) (a). Salient feature of biofertilizer

There are two types of supplies for agriculture, specially fertilizer and pesticide. It can be said the fertilizer is food and pesticide is medicine for plants in conventiaonal agriculture.Biofertilizers are low cost renewable sources of plant nutrients which supplement chemical fertilizers. These are nothing but selected strains of beneficial soil microorganisms cultured in the laboratory and packed in a suitable carrier. They can be used either for seed treatment or soil application. Biofertilizers generate plant nutrient like nitrogen and phosphorus through their activities in the soil or rhizosphere and make available to plants in a gradual manner. Howwere biofertilizer is most commonly referred to the use of soil microorganism to increase the availability and uptake of mineral nutrients for plant.Numerous species of soil bacteria which flourish in the rhizosphere of plants, but which may grow in on or around plant tissue stimulate plant growth. These bacteria collectively known as plant growth promoting rhizobacteria (PGPR).Mode of PGPR include fixing N_2 increasing the availability of nutrients in the rhizoshpere, positively influencing root growth and morphology and promoting other beneficial plant microbe symbiosis.

Types of biofertilizers

- Nitrogen fixing biofertilizers e.g. *Rhizobium, Bradyrhizobium, Azospirillum* and *Azotobacter.*
- Phosphorous solubilising biofertilizers (PSB) e.g. *Bacillus, Pseudomonas* and *Aspergillus.*

- Phosphate mobilizing biofertilizer e.g. Mycorrhiza.
- Plant growth promoting biofertilizers e.g. *Pseudomonas* sp.

(b). Biofertilizer is still an unclear term. It can be easily found that biofertilizers are identified as plant extract composted urban wastes and various microbial mixtures with unidentified sonstituents and chemical fertilizer formulations supplemented with organic compounds.

How Biofertilizers work?

- Biofertilizers fix atmospheric nitrogen in the soil and root nodules of legume crops and make it available to the plant.
- They solubilise the insoluble form of phosphate like tricalcium, iron and aluminium phosphate into available forms.
- They scavenge phosphate from soil layers.
- They produce hormones and antimetabolites which promote root growth.
- They decompose organic matter and help in mineralization in soil.
- When applied to seed or soil, biofertilizers increase the availability of nutrients and improve the yield by 10 to 25% without adversely affecting the soil and environment

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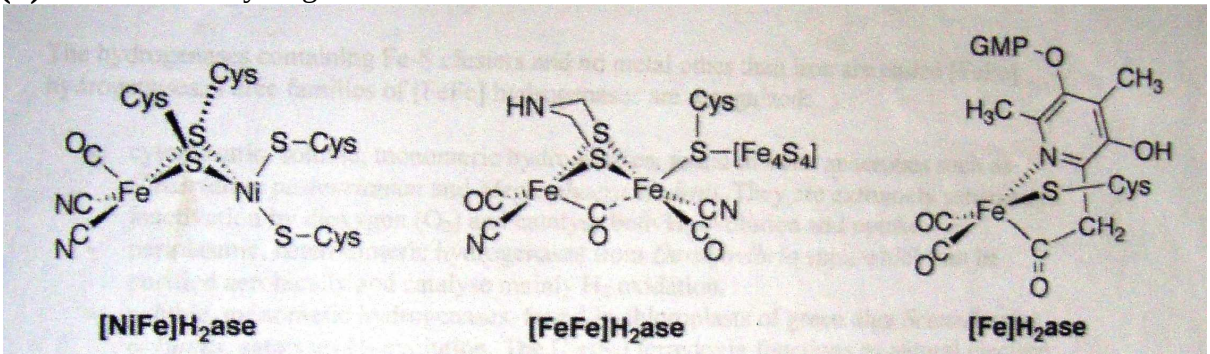
(v). Structure of Hydrogenase



The active site structures of the three types of hydrogenase enzymes.

Application of hydrogenase

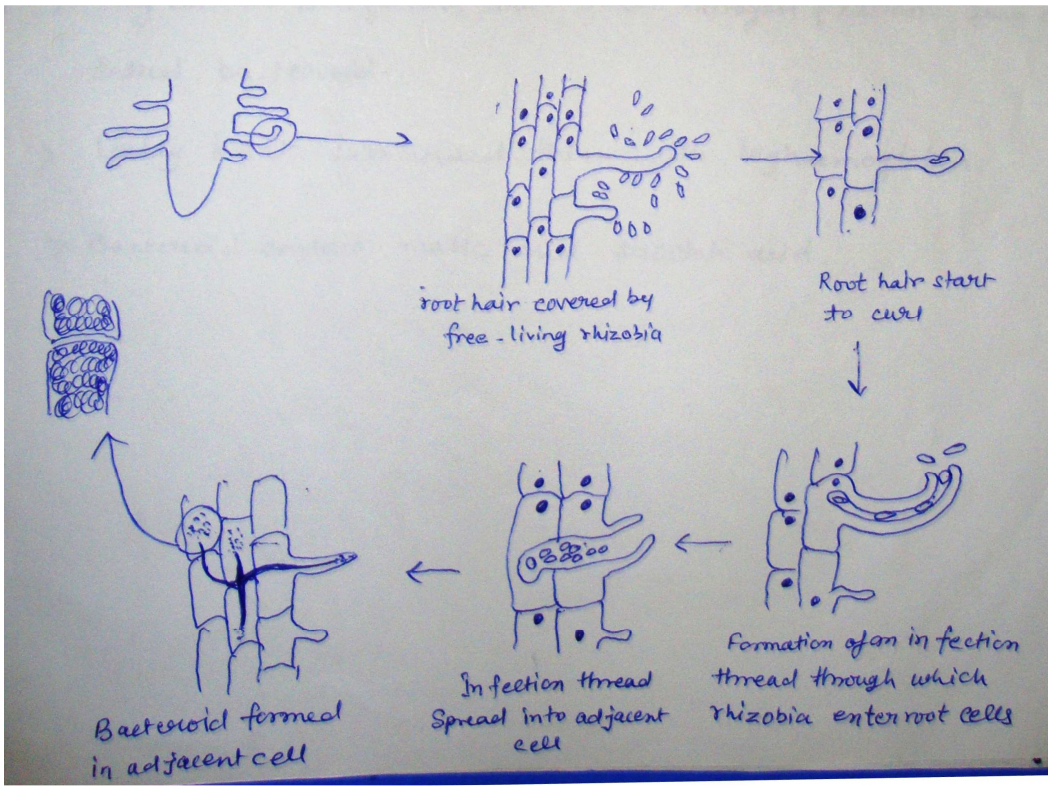
Hydrogenase were first discovered in the 1930s, and they have since attracted interest from many researchers including inorganic chemists who have synthesized a variety of hydrogenase mimics. Understanding the catalytic mechanism of hydrogenase might help scientists design clean biological energy sources, such as algae, that produce hydrogen.
The enzyme catalyses the reversible oxidation of molecular hydrogen (H_2) as:-

$H_2 + A_{ox} = 2H^+ + A_{red}$

$2H^+ + D_{red} = H_2 + D_{ox}$

Hydrogen uptake is coupled to the reduction of electron acceptors such as oxygen, nitrate, sulphate, carbon dioxide and fumarate. On the other hand proton reduction is coupled to the oxidation of electron donors such as ferredoxin (FNR), and serve to dispose exess electron in cells (essential in pyruvate fermentation). Both low-molecular weight compounds and protein such as FNRs, cytochorme c_3 and cytochrome c_6 can act as physiological electron donors or acceptors for hydrogenases.

(vi). Nodule formation



Leguminated Plant used in agriculture

It has been the practice from the time immemorial that legumes are grown in different cropping systems associated with rice. Promising cropping system includes the raising of pre-rice legumes and post –rice legumes. Besides the above two systems, legumes are grown along with rice as bund, border and instiu crops. Several multipurpose cover legumes as short duration crops are grown after the harvest of the second rice crop of the first year and before the planting of the first crop of the subsequent year. Grain legumes admirably fit into the system in which several pulses such as green gram, black gram, soybean etc. Which not only yield forage or green manure, but also add nitrogen to the soil, are included. The legumes associated with rice cropping systems, by their ability to fix atmospheric nitrogen in the root nodules have the potential to accumulate nitrogen varying from 10 to 200 Kg N/ha and even upto 450 Kg N/ha.

Various legumes associated with crop bring about the following benefits.

(a) Soil fertility enhancement
(b) Nitrogen addition
(c) Increased N uptake in field
(d) Suppression of insect pests, disease and weeds

(vii). Molecular basis of *Rhizobia*-Host relation

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Nodule formation : Nod gene, Not protein and Nod factors

- 10 nod genes were identified in *Rhizobium leguminosarum*.
- The nod ABC genes encode protein that produce oligosaccharides called nod factors; these induce root hair curling and trigger cell division in pea plant eventually leading to the formation of nodule.
- Nod gene triggered by flavonoids
- Nod gene encode protein Nod protein which control other nod gene.