Environmental Microbiology Course 7

Nitrogen Cycle

Assoc. Prof. Dr. Emrah ŞefikAbamor

Nitrogen

- 80 % of atmosphere is N_2
- Living organisms can not directly incorporate N_2 into biological molecules
- Living organisms need nitrogen
  - Proteins
  - DNA
  - RNA
  - Other molecules

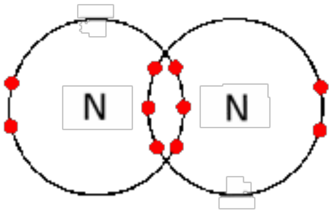
Components of the Nitrogen Cycle

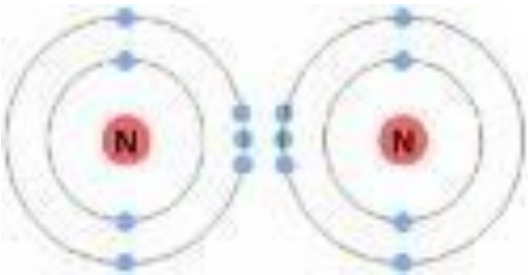
- N_2 (nitrogen gas)
  - In the atmosphere; <u>cannot</u> be used by plants or animals
- NH_3 (ammonia)
  - Found in soil; <u>cannot</u> be used by plants or animals
- NO_2 (nitrite)
  - Found in soil; <u>cannot</u> be used by plants or animals

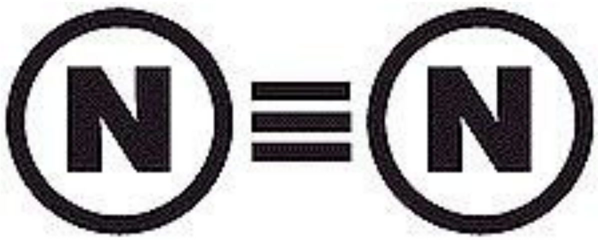
Components of the Nitrogen Cycle (Continued)

- NO_3^- (nitrate) & NH_4^+ (ammonium)
  - Only compounds of nitrogen that plants can use
- Various nitrogen compounds such as amino acids
  - Many different compounds that living things need for survival

Nitrogen's triple bond







- Although the majority of the air we breathe is N_2, most of the nitrogen in the atmosphere is **unavailable** for use by organisms.

- This is because the strong **triple bond** between the N atoms in N_2 molecules makes it relatively inert (like a noble gas).

NITROGEN CYCLE

- Is the cycling of nitrogen between living organisms and their environment.
- Is the process of converting nitrogen into compounds that can be used by plants and animals.
- Is a complex process with four important stages:
  - Nitrogen Fixation
  - Ammonification/Decay
  - Nitrification
  - Denitrification

Nitrogen cycle

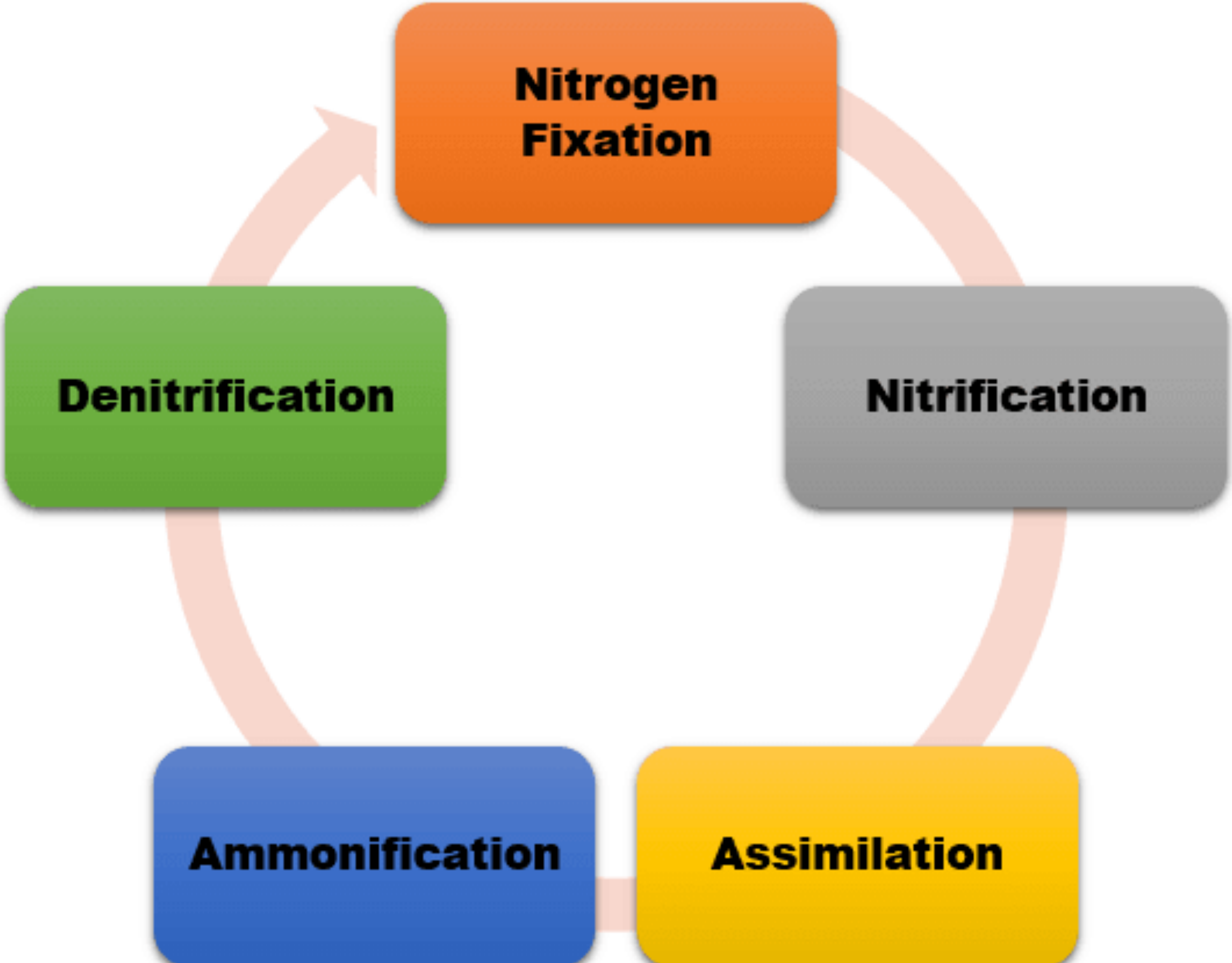
- Series of natural processes by which nitrogen passes through successive stations in air, soil, and organisms.

- The atmospheric nitrogen occur in an inert form (N2)/can't be used by most of the orangnims.

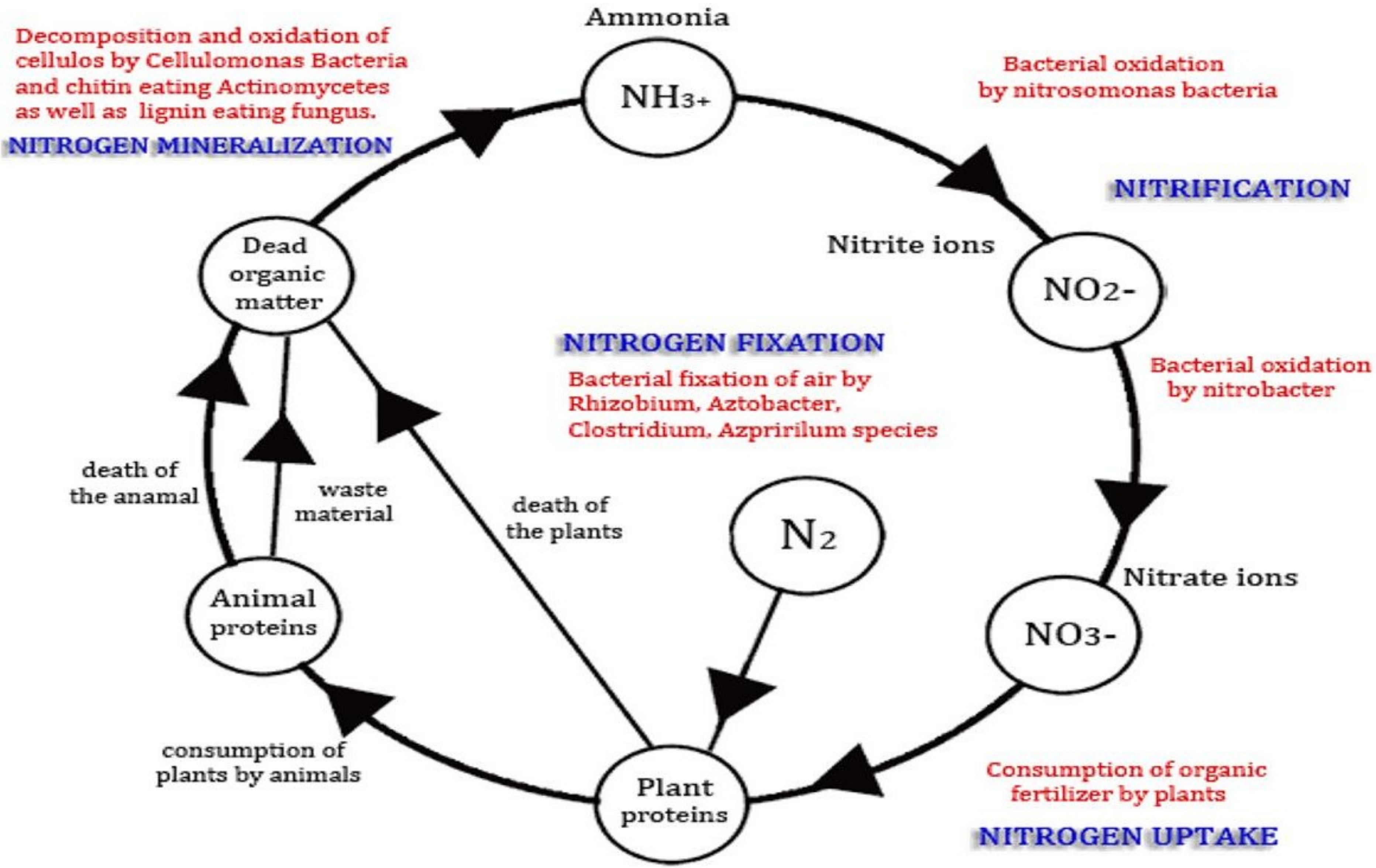
- Atmospheric nitrogen is fixed by nitrogen cycle.

Which principally invovles:-

- Nitrogen fixation.
- Nitrification.
- Assimilation.
- Ammonification.
- Dentrification.


Nitrogen Fixation
Nitrification
Assimilation
Ammonification
Denitrification

NITROGEN CYCLE



Nitrogen fixation:-

- Deposits into soil & surface water ⟹ precipitation.
- Once settled; undergo changes

$$N_2 + 8\,H^+ + 8\,e^- \longrightarrow 2\,NH_3 + H_2$$

Conversion via Microorganisms:

- Bacteria in symbiotic relation with plants.
- Free aerobic bacteria/alga.
- Cyanobactetria

Nitrification

- The conversion of ammonia to nitrates is performed primarily by soil-living bacteria and other nitrifying bacteria.
- The primary stage of nitrification, the oxidation of ammonia (NH_3) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO_2^-).
- Other bacterial species, such as the *Nitrobacter*, are responsible for the oxidation of the nitrites into nitrates (NO_3^-).
- Nitrites need to be converted to nitrates because accumulated nitrites are toxic to plant life

Nitrification...

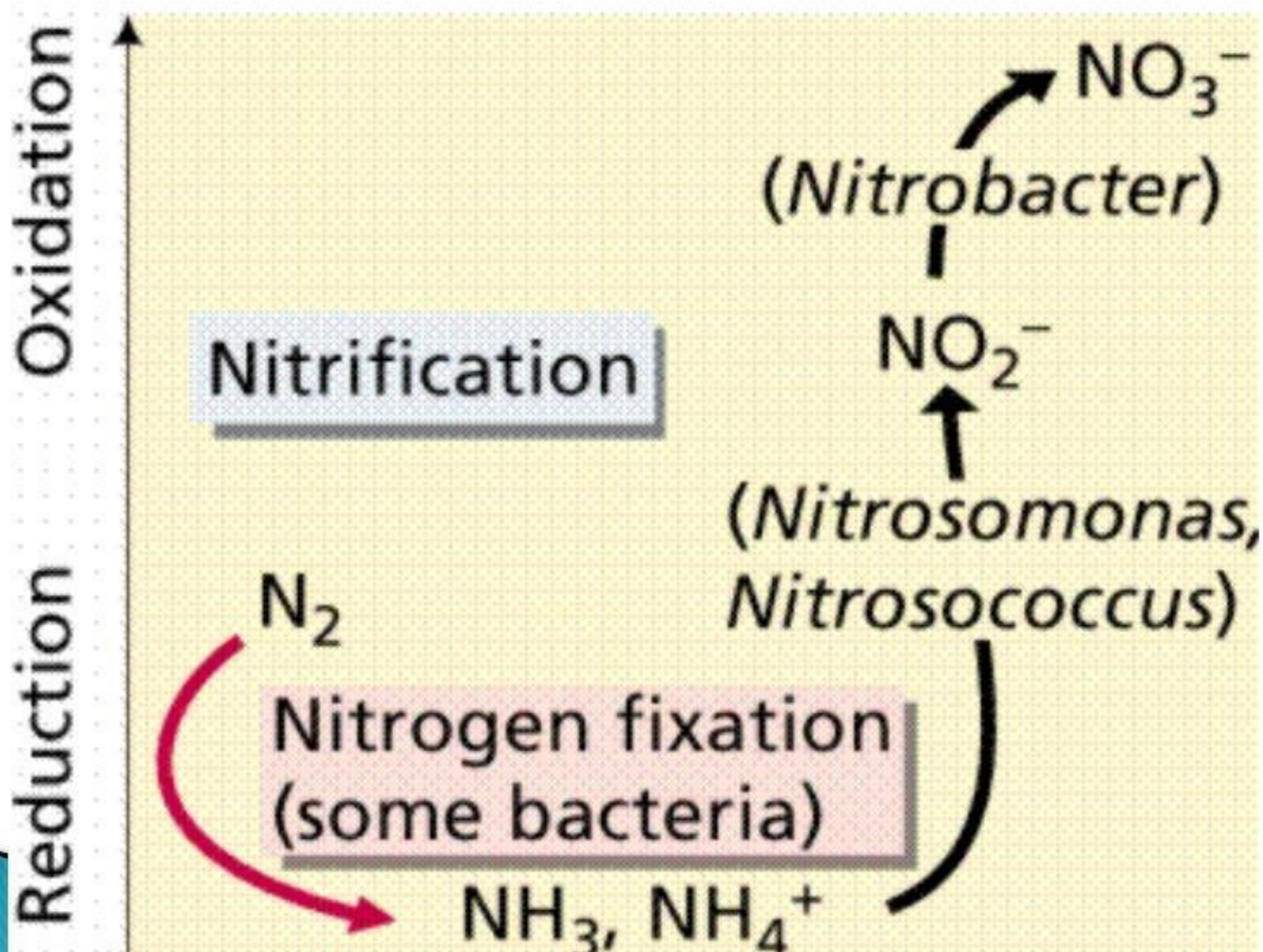
- This involves two oxidation processes
- The ammonia produced by ammonification is an energy rich substrate for *Nitrosomas* bacteria
- They oxidise it to nitrite:

$$NH_3 + 1^1/_2O_2 \rightarrow NO_2^- + H_2O$$

- This in turn provides a substrate for *Nitrobacter* bacteria oxidise the nitrite to nitrate:

$$NO_2^- + ^1/_2O_2 \rightarrow NO_3^-$$

Nitrification…



② Nitrification

- A relatively simple process, nitrification converts **ammonia** into **nitrate** – which is a plants preferred form of nitrogen.
- The process is carried out by **nitrifying bacteria** in two steps:

$NH_4^+ \rightarrow NO_2^-$

ammonia → nitrite ions

$NO_2^- \rightarrow NO_3^-$

nitrite ions → nitrate ions

The ammonia that starts the nitrification process comes from:

1. Nitrogen-fixing bacteria
2. Haber process
3. Animal waste
4. Decomposition of dead organisms

Industrial N-Fixation

- The Haber-Bosch Process

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad - 92kJ$$

- The Haber process uses an iron catalyst
- High temperatures (500°C)
- High pressures (250 atmospheres)
- The energy require comes from burning fossil fuels (coal, gas or oil)
- Hydrogen is produced from natural gas (methane) or other hydrocarbon

❸ Assimilation

- Plants require nitrogen in the form of dissolved nitrates NO_3^-.
- Once the processes of **nitrogen-fixation** and **nitrification** have been carried out, plants are able to absorb NO_3^- by active transport.

Absorbed nitrates are used to synthesise **amino acids** and **nucleic acids**.

The uptake and incorporation of nitrogen into plant tissues is called **assimilation**.

This nitrogen is now available to the rest of the food chain through feeding.

Assimilation:-

- Process by which plants/animals take up nitrates and ammonia.
- Plants take up NO_3^- and NH_4^+ through their roots and integrate them into various plant proteins /nucleic acids.
- Animals take up this nitrogen by consuming plant tissues.

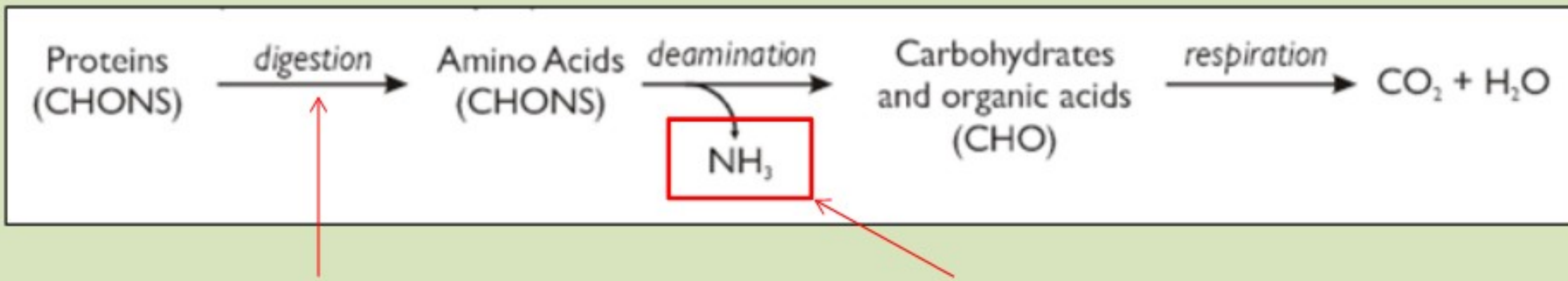
Ammonification

- When living organisms excrete waste or die their nitrogen is returned to the soil in the form of ammonium compounds by saprobiotic microorganisms (bacteria and fungi).
- Microbial saprophytes break down proteins in detritus to form ammonium ions (ammonification or deamination).
- This is where nitrogen returns to the non-living component of the ecosystem.

④ Ammonification

- When organisms die (and produce waste), **microbial saprophytes** release nitrogen locked in this material back to the environment.

- More specifically, the proteins in **detritus** is broken down in two stages:



Step 1:
The saprophytes release enzymes which digest proteins into amino acids.

Step 2:
The amino group is removed from the amino acids, which then dissolves in soil to form NH_4^+ ions.

Denitrification

- Denitrification is the reduction of nitrates back into the largely inert nitrogen gas (N_2), completing the nitrogen cycle.
- This process is performed by bacterial species such as *Pseudomonas* and *Clostridium*.
- They live deep in soil and in aquatic sediments where conditions are anaerobic. These anaerobic bacteria can also live in aerobic conditions

Denitrification:-

- ‘Reduction of NO3+ ⟶ N2 (anaerobic bacteria)’
- Pseudomonas/Clostridium (anaerobic conditions)

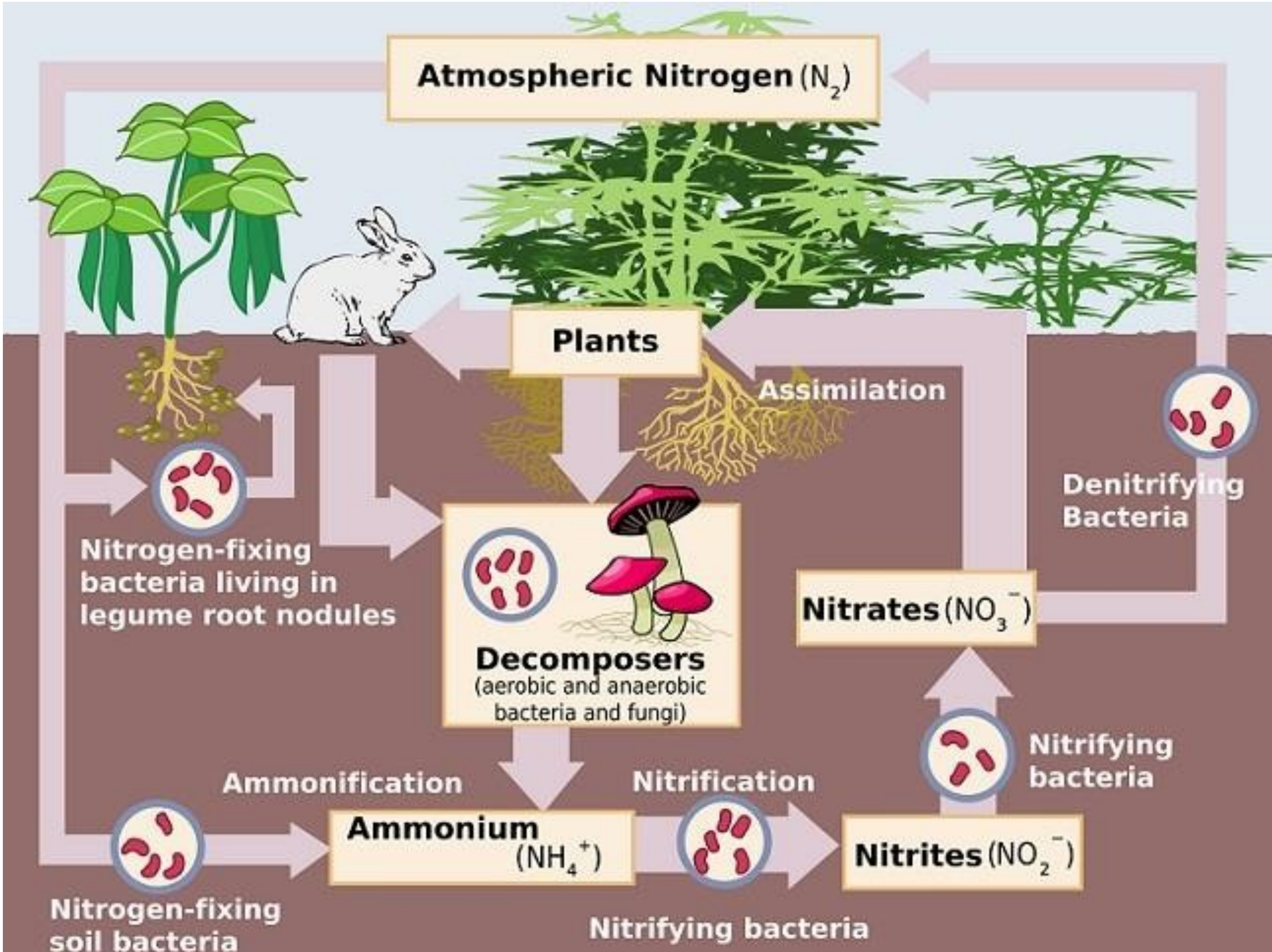
↓

1) $NO_3^- \longrightarrow NO_2^- \longrightarrow NO + N_2O \longrightarrow N_2$

2) $2\ NO_3^- + 10\ e^- + 12\ H^+ \longrightarrow N_2 + 6\ H_2O$

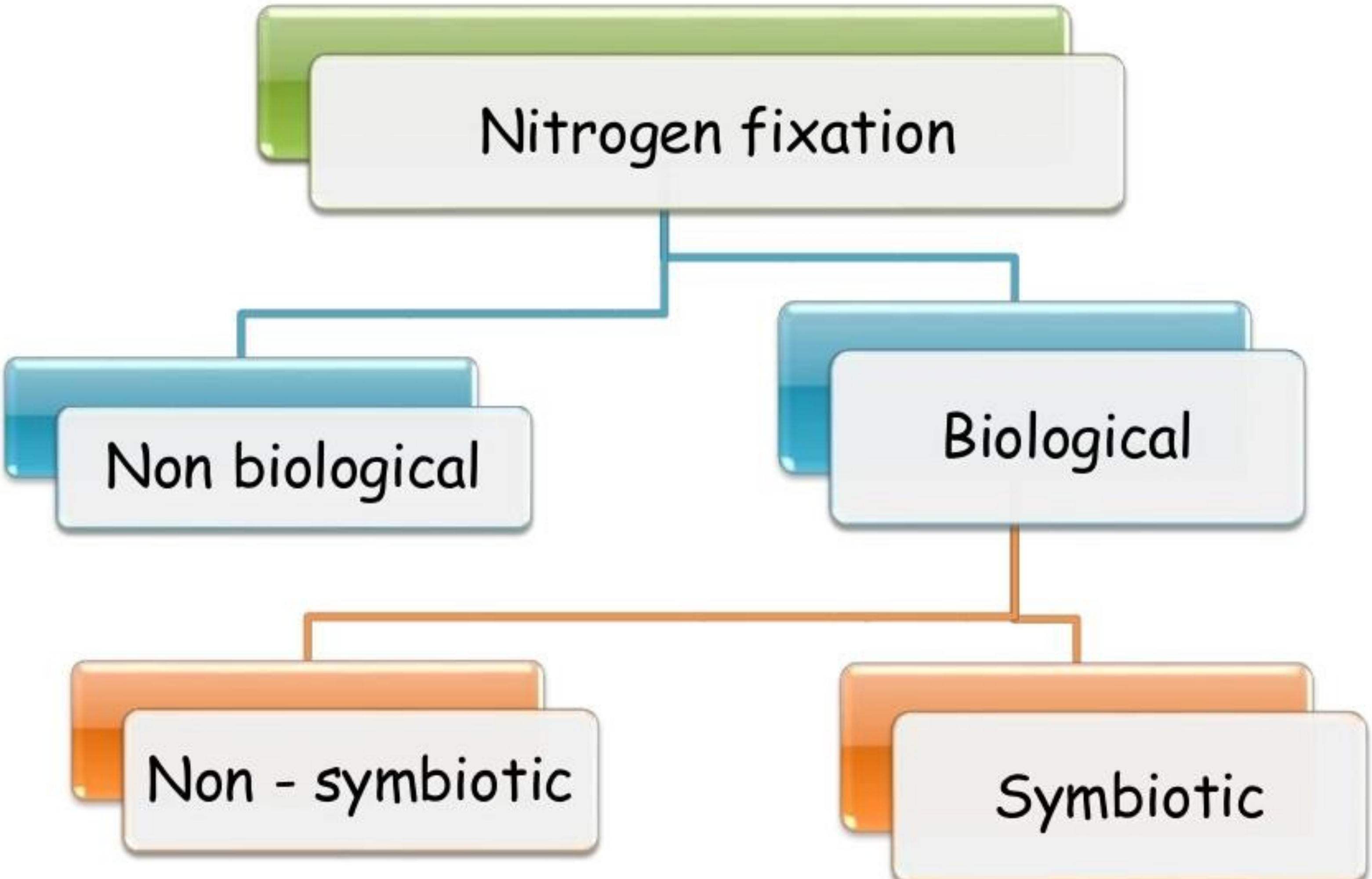
ANAMMOX

- In this biological process, nitrite and ammonium are converted directly into dinitrogen gas. This process contributes up to 50% of the dinitrogen gas produced in the oceans. It is thus a major sink for fixed nitrogen and so limits oceanic primary productivity. The overall catabolic reaction is:
- $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$. (*Brocadia*)
- The bacteria that perform the anammox process belong to the bacterial phylum planctomycetes of which *Planctomyces* and *Pirellula* are the best known genera.



Atmospheric Nitrogen (N_2)
Plants
Assimilation
Denitrifying Bacteria
Nitrogen-fixing bacteria living in legume root nodules
Decomposers
(aerobic and anaerobic bacteria and fungi)
Nitrates (NO_3^-)
Nitrifying bacteria
Ammonification
Nitrification
Ammonium (NH_4^+)
Nitrites (NO_2^-)
Nitrogen-fixing soil bacteria
Nitrifying bacteria

TYPES OF NITROGEN FIXATION



NON BIOLOGICAL FIXATION

- The micro-organisms do not take place
- Found in rainy season during lightning

1. $N_2 + O_2 \xrightarrow{\text{lightning}} 2\,NO$ (Nitric oxide)
2. $2NO + O_2 \xrightarrow{\text{oxidation}} 2NO_2$ (Nitrogen per oxide)
3. $2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$
4. $4NO_2 + 2H_2O + O_2 \longrightarrow 4HNO_3$ (Nitric acid)
5. $CaO + 2HNO_3 \longrightarrow Ca\,(NO_3)_2 + H_2O$ (Calcium nitrate)
6. $HNO_3 + NH_3 \longrightarrow NH_4NO_3$ (Ammonium nitrate)
7. $HNO_2 + NH_3 \longrightarrow NH_4NO_2$ (Ammonium nitrite)

BIOLOGICAL FIXATION

- Fixation of atmospheric Nitrogen into nitrogenous salts with the help of micro-organisms
- Two types
  - Symbiotic
  - Non-symbiotic

NON-SYMBIOTIC

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Bacteria: special type (nitrogen fixing bacteria) types -
  - Free living aerobic : *Azotobacter, Beijerenckia*
  - Free living anaerobic : *Clostridium*
  - Free living photosynthetic : *Chlorobium, Rhodopseudomonas*
  - Free living chemosynthetic :*Desulfovibro,Thiobacillus*

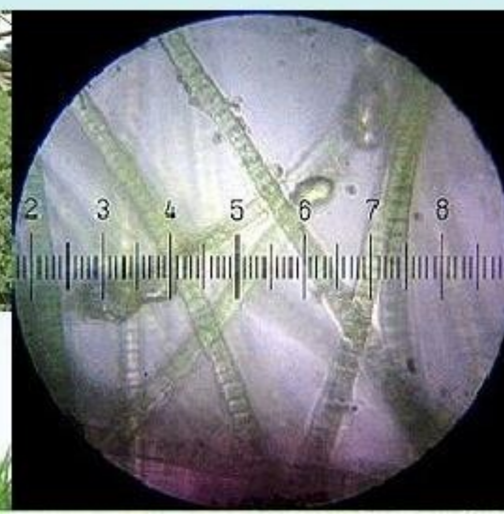
CONTD..

- Free living fungi: yeasts and *Pillularia*
- Blue green algae:
  - unicellular – Gloeothece, Synechococcus
  - Filamentous (non heterocystous) -Oscillatoria
  - Filamentous (heterocystous) – Tolypothrix, Nostoc, Anabaena

Nitrogen-Fixing Bacteria

- symbiotic bacteria living on the roots of plants (mostly legumes and alders)
- cyanobacteria (formerly known as blue-green algae)
- archaebacteria (also known as archaea) in deep-sea hydrothermal vents

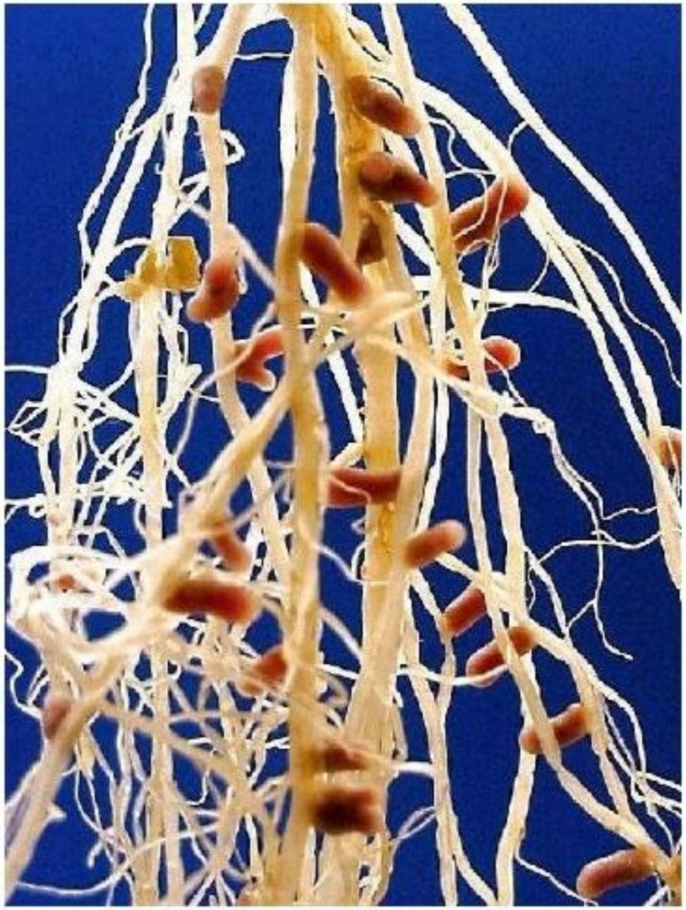




Symbiotic Nitrogen Fixing Organisms

- *Rhizobium* species - infect roots of legumes (Pea Family of Plants)
  - Alfalfa, peas, beans, clover, soybeans, & peanuts
  - Attach to root hair, "infection thread" forms
  - Bacteria enter through thread and penetrate root cells
  - Bacteria differentiate into BACTEROIDS
    - Thicker cell walls
      - Combination of plant & bacterial cell wall
    - Dense cytoplasm
    - Do not divide

Rhizobium



- These bacteria can infect the roots of leguminous plants, leading to the formation of lumps or nodules where the nitrogen fixation takes place.
- About 90% of legume species can become nodulated.

Rhizobia in Root Nodules

In root nodules the nitrogen-fixing form exists as irregular cells called bacteroids which are often club and Y-shaped.

Free Living Rhizobium

- In the soil the bacteria are free living and motile, feeding on the remains of dead organisms.
  - Free living *Rhizobium* cannot (or rarely) fix nitrogen

Origin of *Rhizobium*/Legume Mutualism

- Don't really know
  - An ancestral bacteria might have infected plant roots
    - Because the plant benefited from the fixed nitrogen, rather than excluding the bacteria the plant evolved to facilitate the relationship
    - Because the bacteria benefited from the sugar supplied by the plant they might have evolved to facilitate the relationship

Nodule Formation

- Root cells release chemicals into the soil.
  - encourage the growth of the bacterial population in the area around the roots (the rhizosphere).
- Bacterial cell wall and the root surface interactions are responsible for the rhizobia recognizing their correct host plant and attaching to the root hairs.
- Flavonoids secreted by the root cells activate the nod genes in the bacteria which then induce nodule formation.

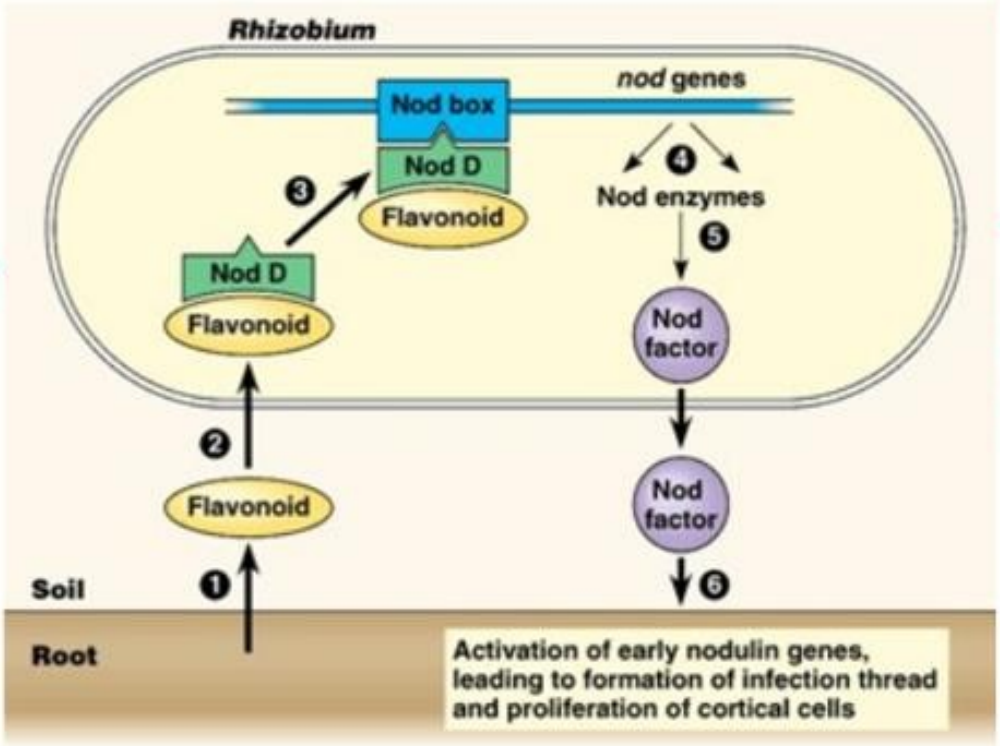
Nodule Formation

- Once bound to the root hair, the bacteria excrete nod factors. These stimulate the hair to curl.
- Rhizobia then invade the root through the hair tip where they induce the formation of an infection thread. This thread is constructed by the root cells and not the bacteria and is formed only in response to infection.

3. Flavanoid binds transcription factor (Nod D), complex bids DNA,

2. Bacterial receptor recognizes signal, and transports it across cell membrane,

1. Plant secretes a specific elicitor (flavonoid).

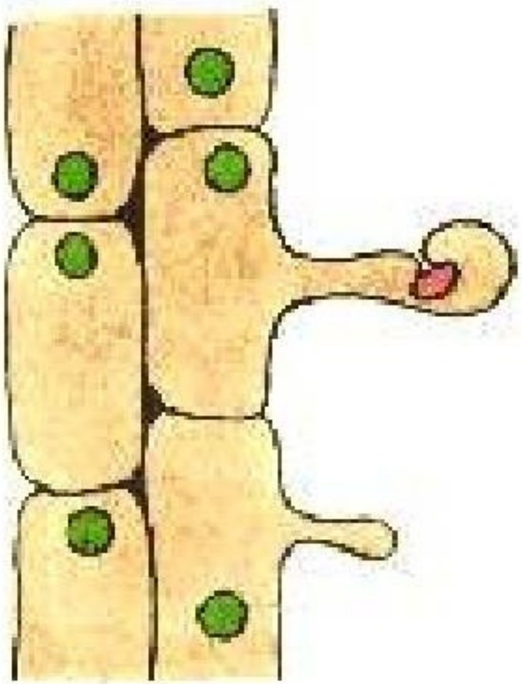


4. *nod* genes are expressed,

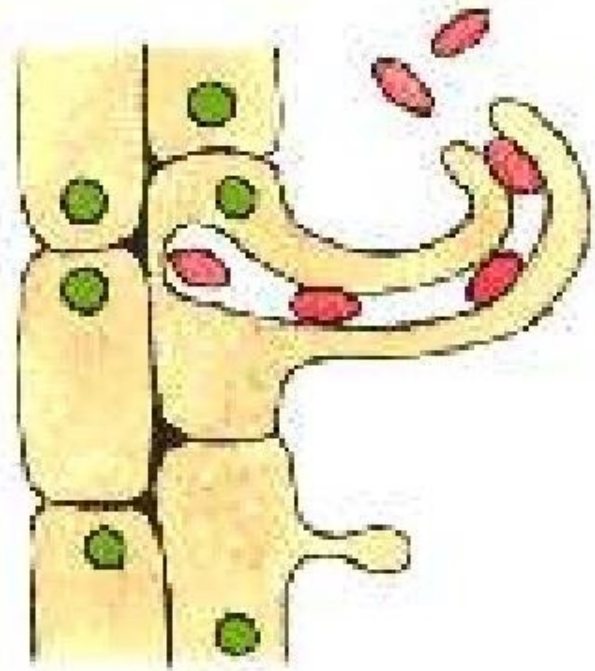
5. Nod factor is produced (a molecule with host specificity),

6. Nod factor is recognized by the host, in turn activating host genes for proper response.

Formation of Infection Thread



Root hair starts to curl

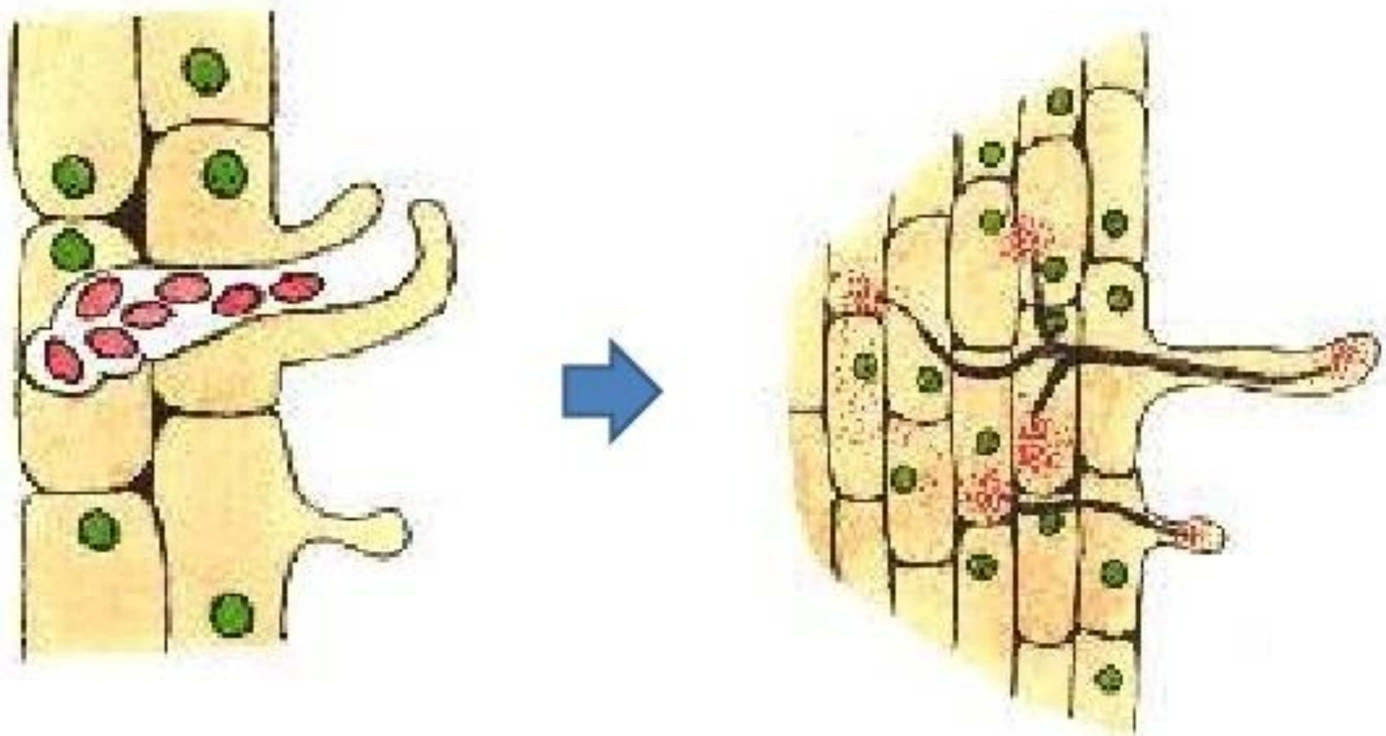


formation of an infection thread through which rhizobia enter root cells

Nodule Formation

- The infection thread grows through the root hair cells and penetrates other root cells nearby often with branching of the thread.
- The bacteria multiply within the expanding network of tubes, continuing to produce nod factors which stimulate the root cells to proliferate, eventually forming a root nodule.

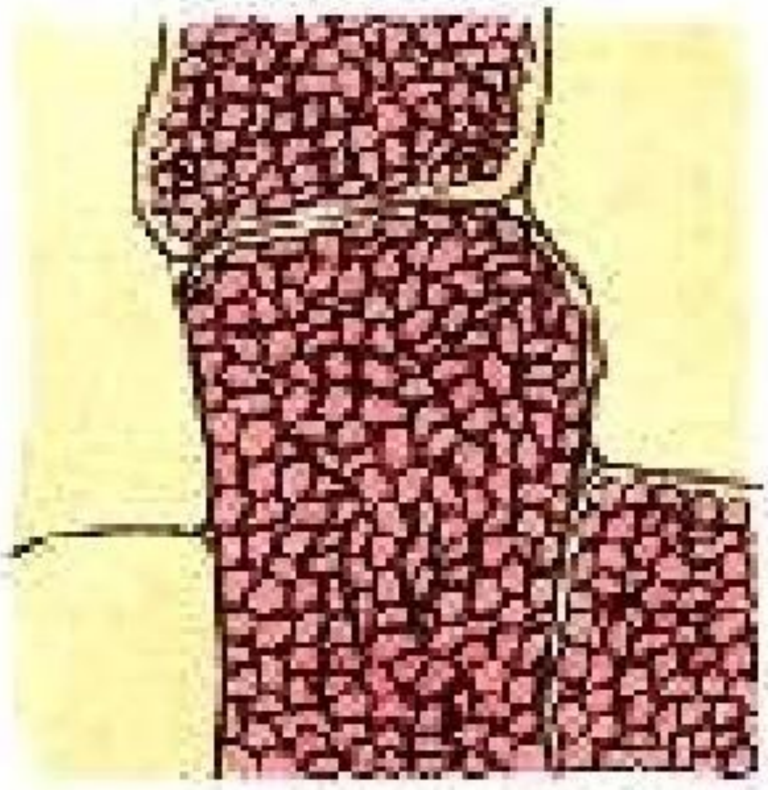
Formation of Bacteroids



infection thread spreads
into adjacent cells

bacteroids are released from
the infection thread

Bacteroids

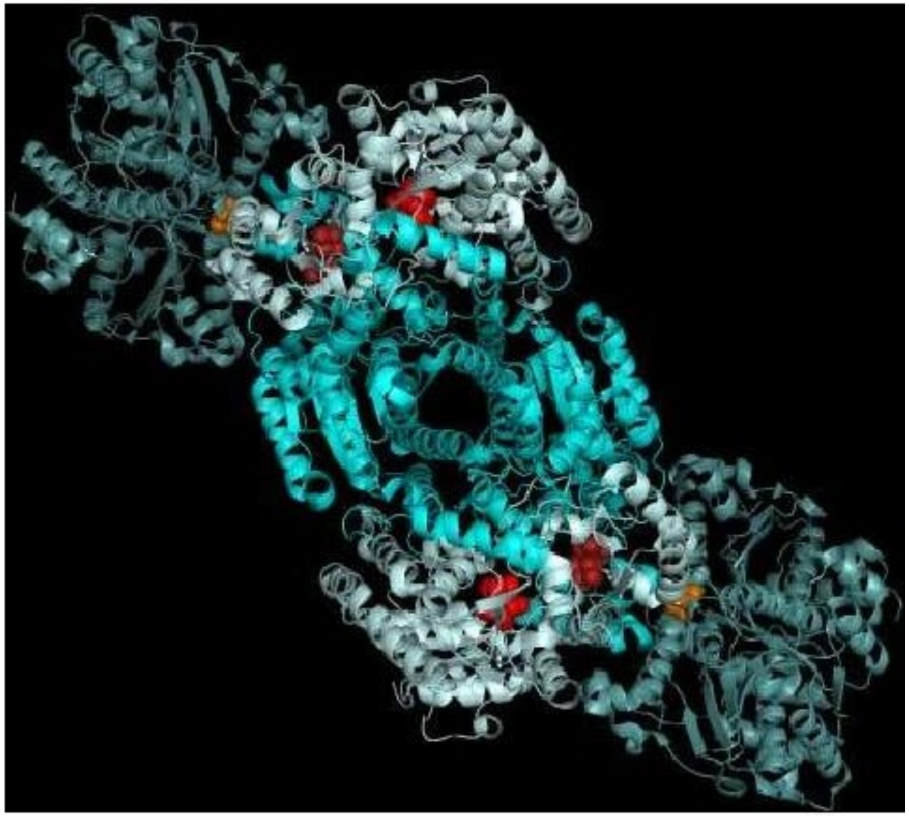


BIOCHEMISTRY OF NITROGEN FIXATION

- Basic requirements for Nitrogen fixation
  - Nitrogenase and hydrogenase enzyme
  - Protective mechanism against Oxygen
  - Ferrodoxin
  - Hydrogen releasing system or electron donor (Pyruvic acid or glucose/sucrose)
  - Constant supply of ATP
  - Coenzymes and cofactors TPP, CoA, inorganic phosphate and Mg^{+2}
  - Cobalt and Molybdenum
  - A carbon compound

Nitrogenase

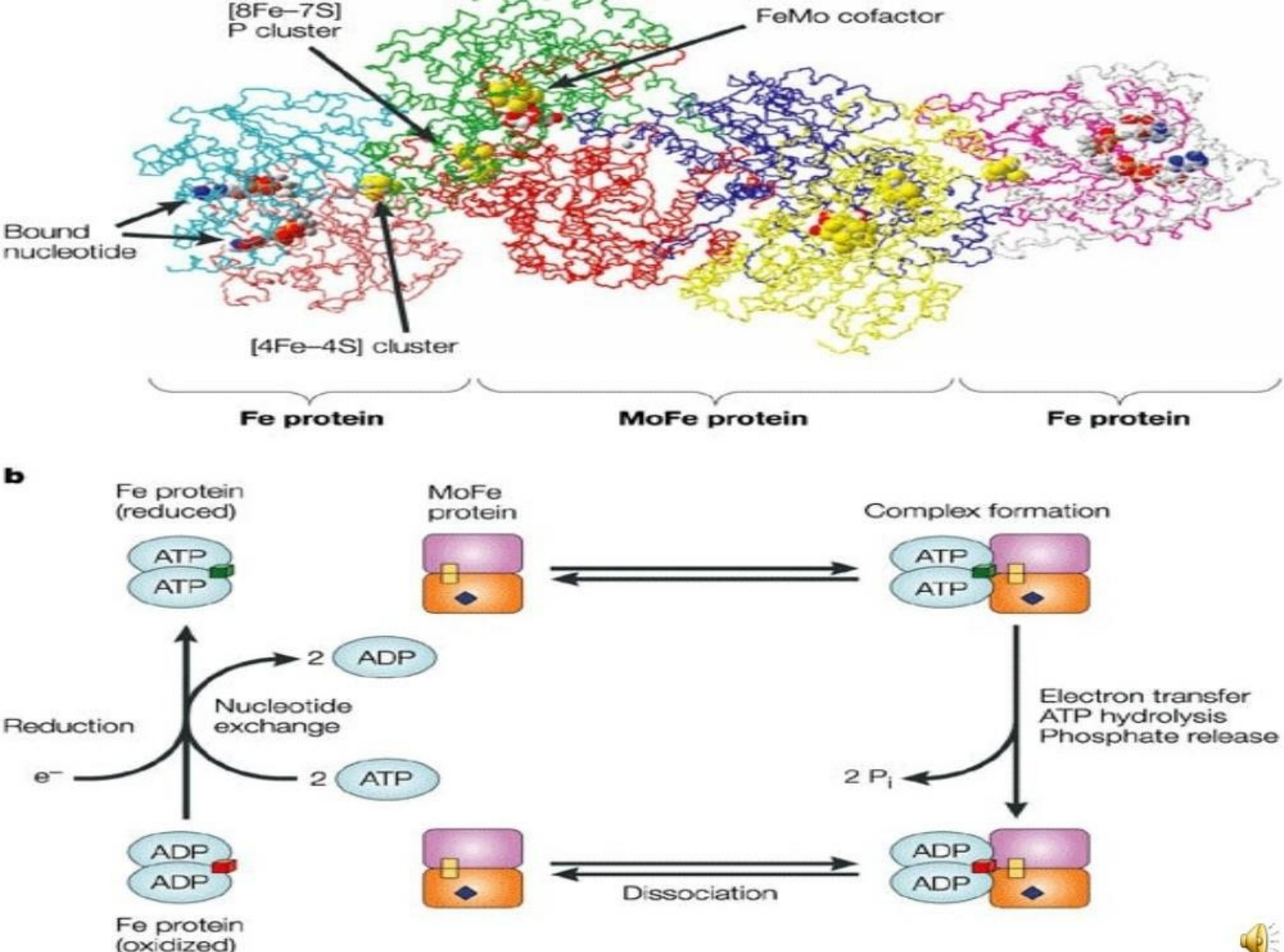
- Nitrogenase is the enzyme that catalyses the conversion of nitrogen gas to ammonia in nitrogen-fixing organisms.
- In legumes it only occurs within the bacteroids.
- The reaction requires hydrogen as well as energy from ATP.



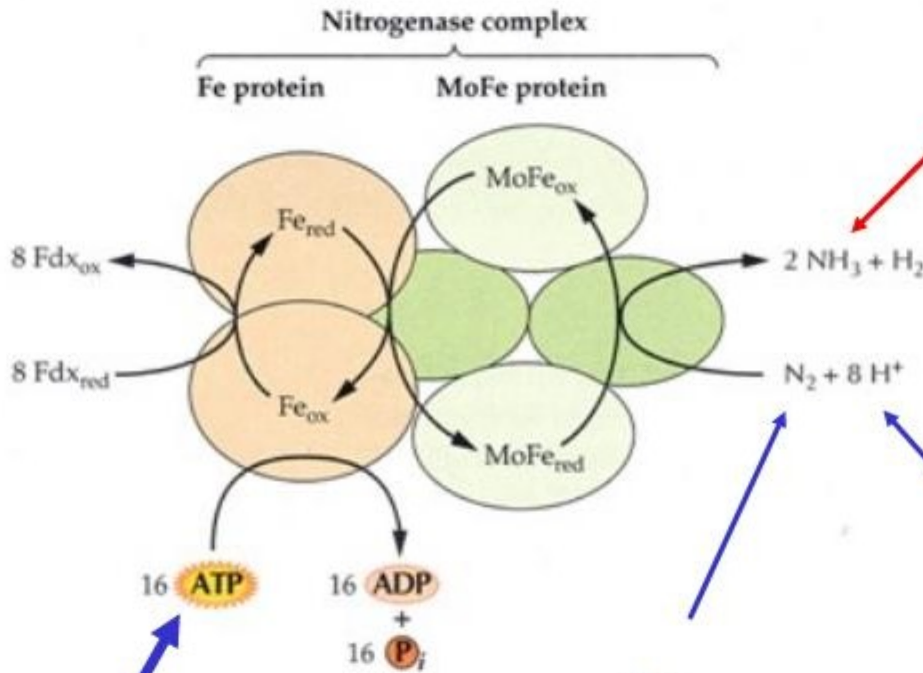
NITROGENASE ENZYME

- Plays key role
- Active in anaerobic condition
- Made up of two protein subunits
  - Non heme iron protein (Fe-protein or dinitrogen reductase)
  - Iron molybdenum protein (Mo Fe-protein or dinitrogenase)
- Fe protein reacts with ATP and reduces second subunit which ultimately reduces N_2 into ammonia

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$


[8Fe–7S]
P cluster
FeMo cofactor
Bound
nucleotide
[4Fe–4S] cluster
Fe protein
MoFe protein
Fe protein
b
Fe protein
(reduced)
ATP
ATP
MoFe
protein
Complex formation
ATP
ATP
2 ADP
Nucleotide
exchange
Reduction
e⁻
2 ATP
Electron transfer
ATP hydrolysis
Phosphate release
2 Pi
ADP
ADP
Fe protein
(oxidized)
Dissociation
ADP
ADP

Nitrogen Fixation Requires Energy



Ammonia and H_2 Output.

8 reduced ferredoxins

N_2

8 protons

16 ATPs for hydrolysis

CONTD..

- The reduction of N_2 into NH_3 requires 6 protons and 6 electrons
- 12 mols of ATP required
- One pair of electron requires 4 ATP
- The modified equation

$$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2$$

- Hydrogen produced is catalyzed into protons and electrons by hydrogenase

$$H_2 \xrightleftharpoons{\text{hydrogenase}} 2H^+ + 2e^-$$

Nitrogenase Enzyme Reactions

- Two molecules of ammonia are produced from one molecule of nitrogen gas.
- The reaction requires 16 molecules of ATP and a supply of electrons and protons (H ions) plus the enzyme nitrogenase.
- Nitrogenase consists of two proteins, an iron protein and a molybdenum-iron protein. The reaction occurs while N2 is bound to the nitogenase enzyme complex.

- The Fe protein is first reduced by electrons donated by ferredoxin.
- Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH.
- In two further cycles of this process (each requiring electrons donated by ferredoxin) HN=NH is reduced to H_2N-NH_2, and this in turn is reduced to 2 NH_3.
- Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process in generated by photosynthesis, respiration or fermentation.

PATHWAY OF NITROGEN FIXATION IN ROOT NODULES

- Glucose-6-phosphate acts as a electron donor

Sucrose (synthesized in leaves) → Sucrose (in roots) → Glucose and fructose → Glucose-6-phosphate

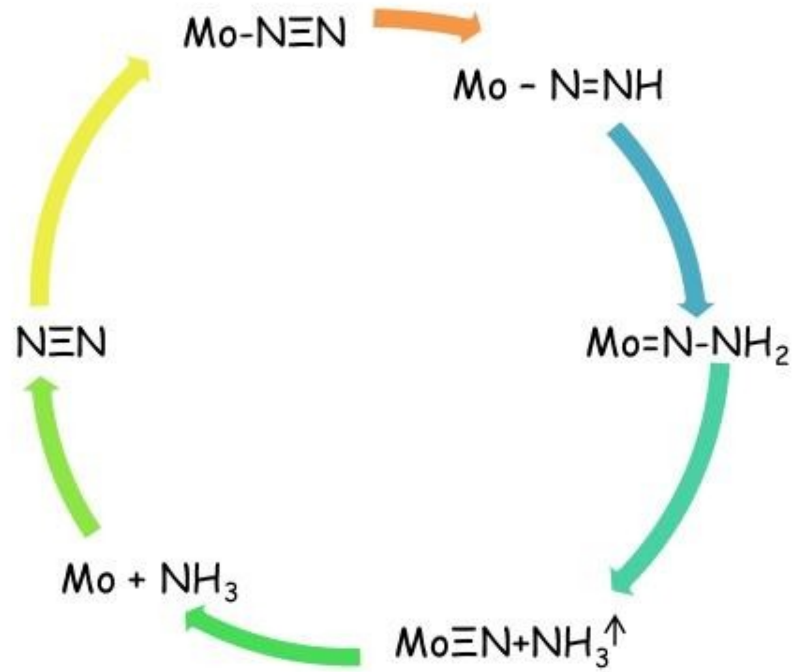
- Glucose-6-phosphate is converted to phosphogluconic acid

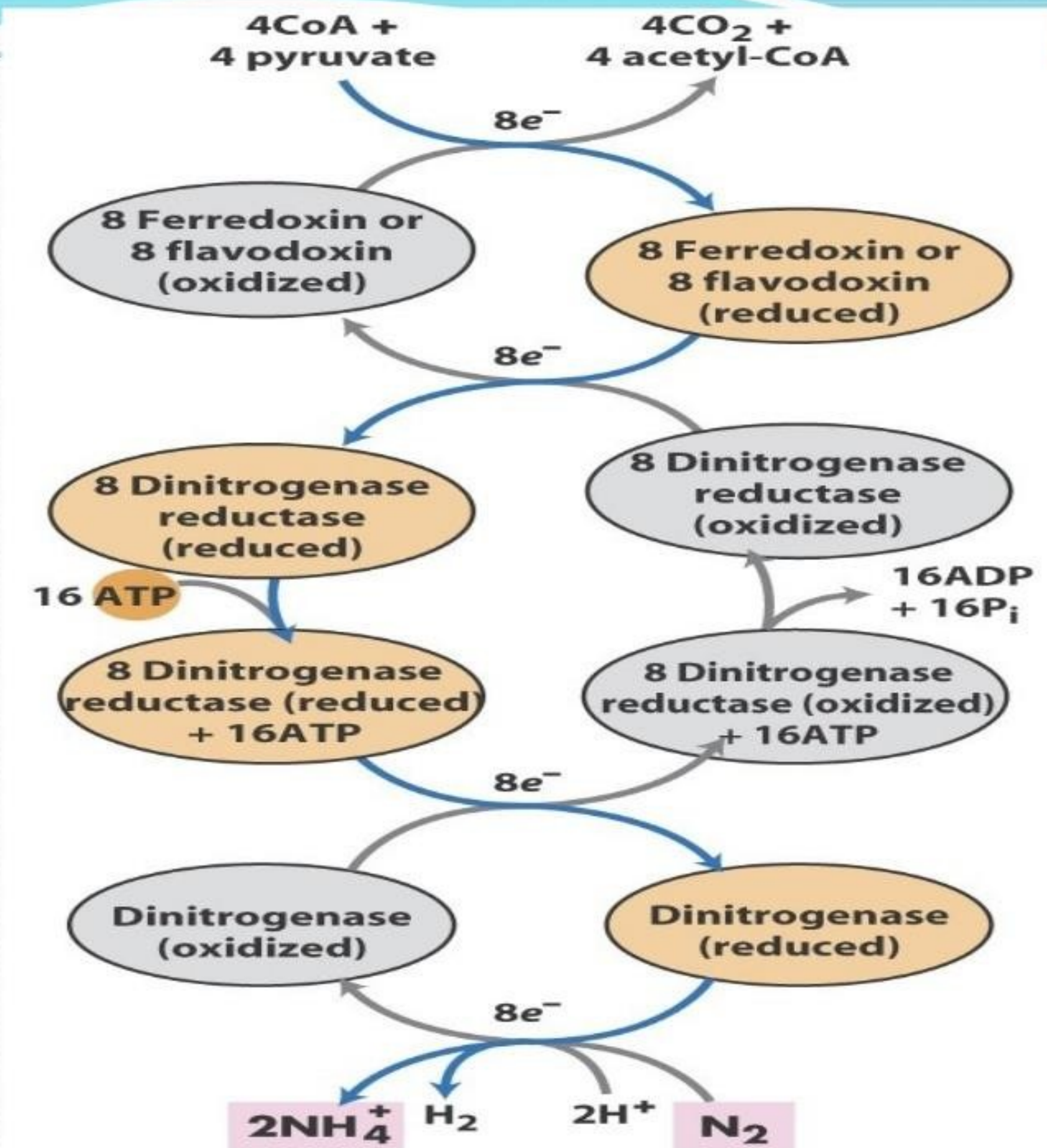
Glucose-6-phosphate + NADP+ + H_2O → 6-phosphogluconic acid + NADPH + H^+

- NADPH donates electrons to ferrodoxin. Protons released and ferrodoxin is reduced
- Reduced ferrodoxin acts as electron carrier. Donate electron to Fe-protein to reduce it. Electrons released from ferrodoxin thus oxidized

CONTD..

- Reduced Fe-protein combines with ATP in the presence of Mg^{+2}
- Second sub unit is activated and reduced
- It donates electrons to N_2 to NH_3
- Enzyme set free after complete reduction of N_2 to NH_3





4CoA +
4 pyruvate
4CO2 +
4 acetyl-CoA
8e⁻
8 Ferredoxin or
8 flavodoxin
(oxidized)
8 Ferredoxin or
8 flavodoxin
(reduced)
8e⁻
8 Dinitrogenase
reductase
(reduced)
8 Dinitrogenase
reductase
(oxidized)
16 ATP
16ADP
+ 16Pi
8 Dinitrogenase
reductase (reduced)
+ 16ATP
8 Dinitrogenase
reductase (oxidized)
+ 16ATP
8e⁻
Dinitrogenase
(oxidized)
Dinitrogenase
(reduced)
8e⁻
2NH4+
H2
2H+
N2

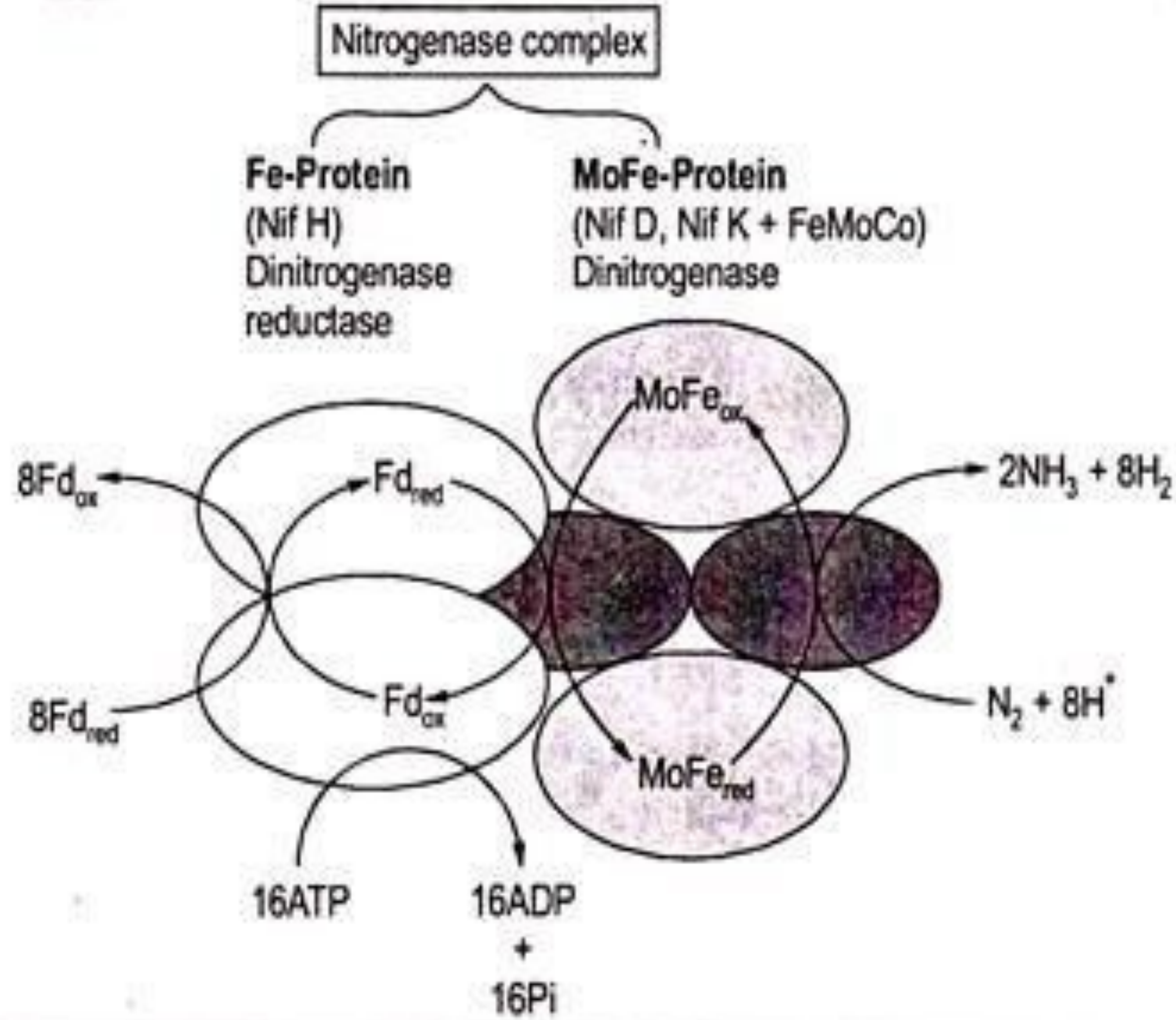


Fig. 10.4 : Diagrammatic structure of the nitrogenase enzyme complex, showing the flow of reducing power to the substrate in biological nitrogen fixation. The Fe-protein encoded by *nifH*, accepts electrons from ferredoxin and transfers it singly at very low potential to the MoFe-protein, accompanied by net hydrolysis of ATP. The MoFe-protein, an $\alpha_2 \beta_2$ heterotetramer encoded by *nifD* and *nifK*, reduces H^+ and N_2 gas in a stepwise manner leading to the formation of H_2 and ammonia

Nitrogenase

- An enzyme present in *Rhizobium* bacteria that live in root nodules of leguminous plants
- Some free-living soil and aquatic bacteria also possess nitrogenase
- Nitrogenase reaction:

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \longrightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

Nitrogenase is Oxygen Sensitive

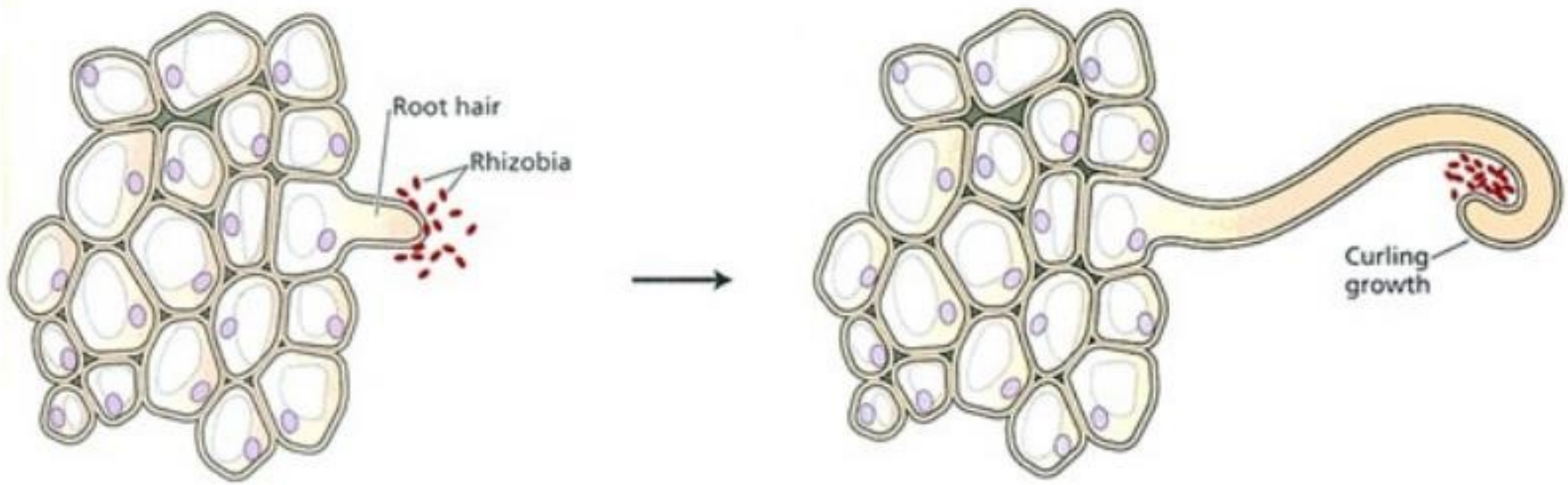
- The nitrogenase complex is sensitive to oxygen, becoming inactivated when exposed to it.
- Free Living Bacteria
  - have a variety of different mechanisms for protecting the nitrogenase complex, including high rates of metabolism and physical barriers.
  - E.g., *Azotobacter* overcomes this problem by having the highest rate of respiration of any organism, thus maintaining a low level of oxygen in its cells.

Rhizobia in Nodules

- Rhizobia controls oxygen levels in the nodule with leghaemoglobin.
  - This red, iron-containing protein has a similar function to that of haemoglobin; binding to oxygen.
  - provides sufficient oxygen for the bacteroids but prevents the accumulation of free oxygen that would destroy the activity of nitrogenase.
- Leghaemoglobin is formed through the interaction of the plant and the rhizobia
  - neither can produce it alone.

Insides of nodules are red because of leghaemoglobin

Rhizobium Infection I



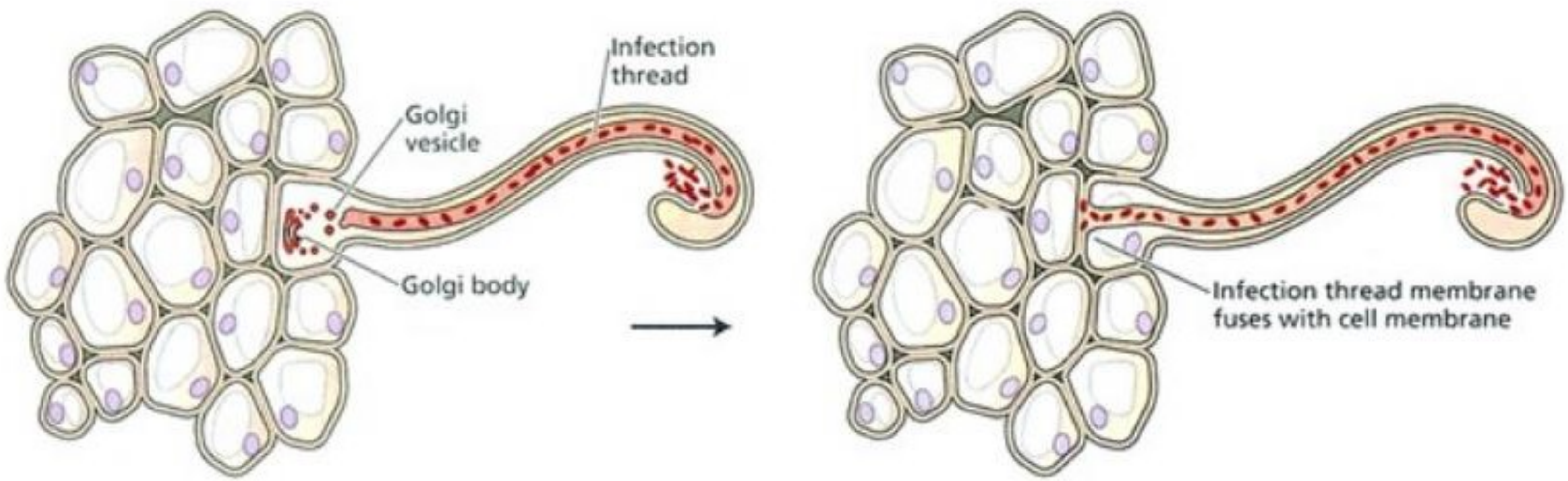
1. Emerging root hair sends chemical attractants (elicitors),

2. Bacteria respond with a recognition signal,

3. Root hair grows and curls around the bacterial colony,

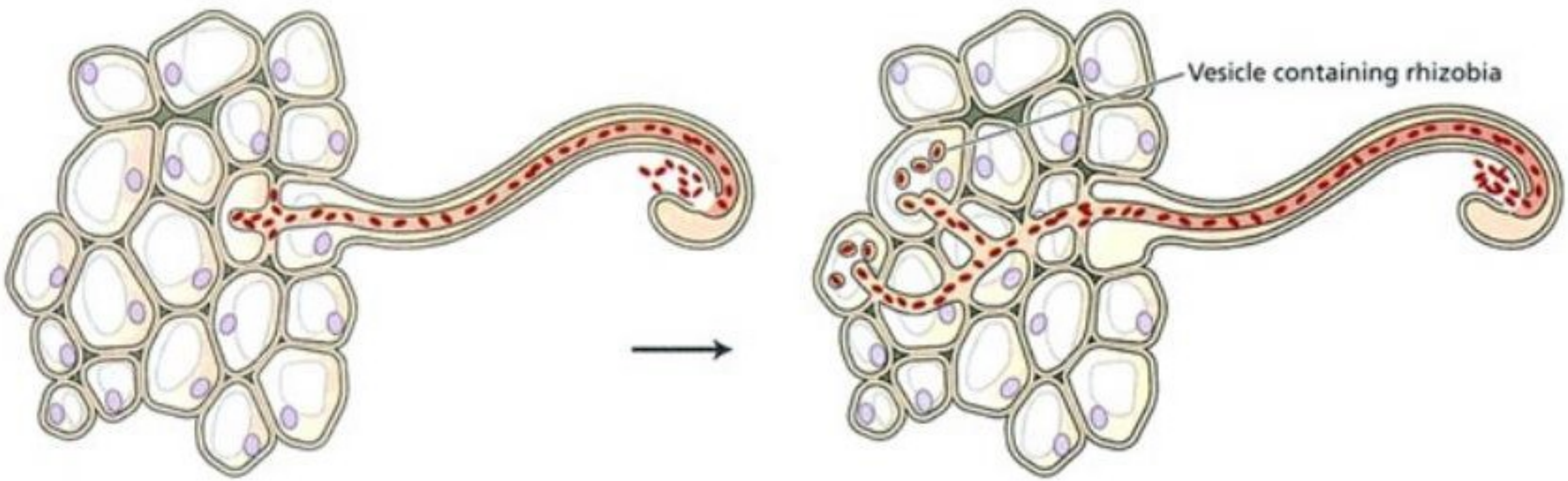
4. Bacteria proliferate within the curl.

Rhizobium Infection II



5. Plant cell wall is degraded,
6. Plant plasma membrane invaginates root hair cell,
7. Infection thread reaches root hair plasma membrane, fuses,
8. Bacteria enter the apoplast.

Rhizobium Infection III



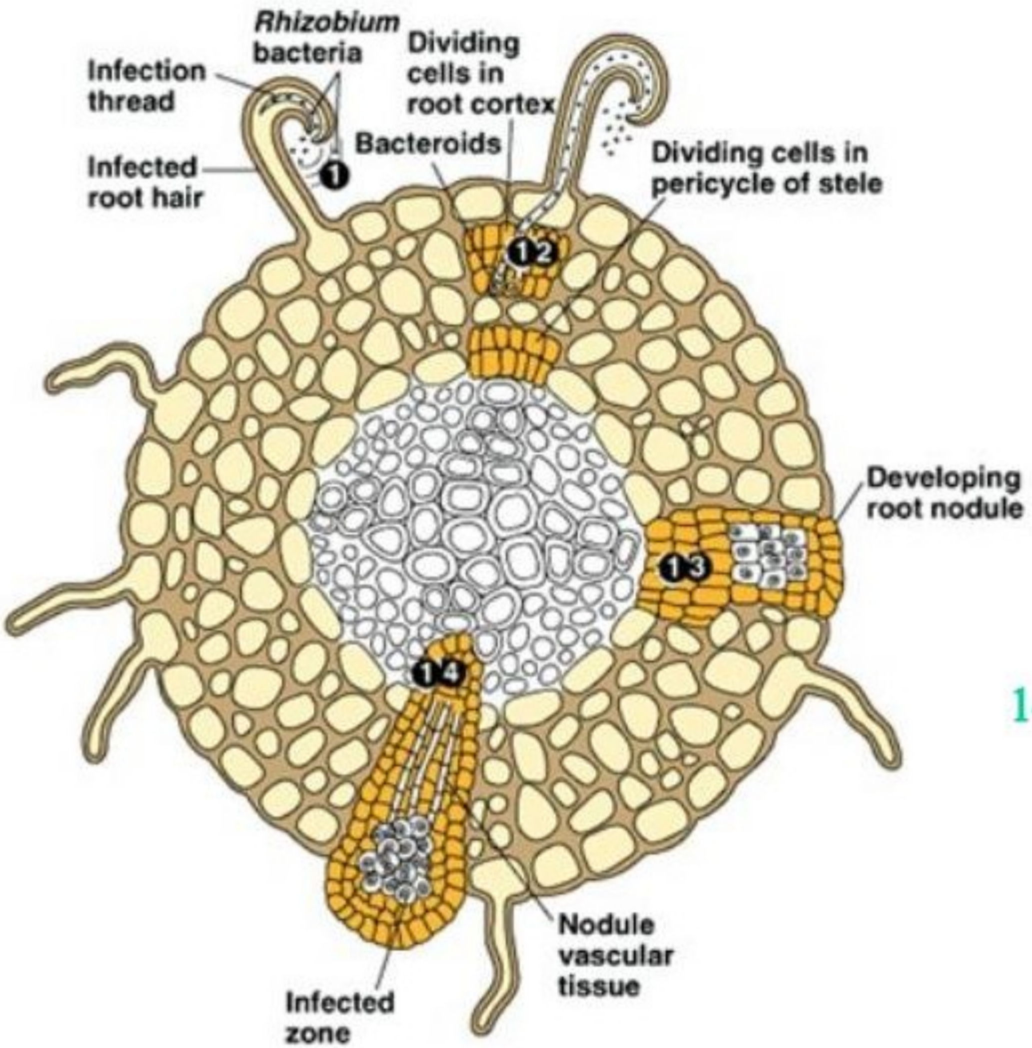
9. New infection threads form,

10. Threads form toward "target cells",

11. Bacteria "bleb" off of the infection thread, into the cytosol,

12. Bacteria are surrounded by a plant membrane.

Nodule Formation



12. Bacteria are surrounded by a plant membrane.

- bacterial induce plant cell division in infected and surrounding cells,
- cells in the pericycle begin dividing, (similar to lateral root formation).

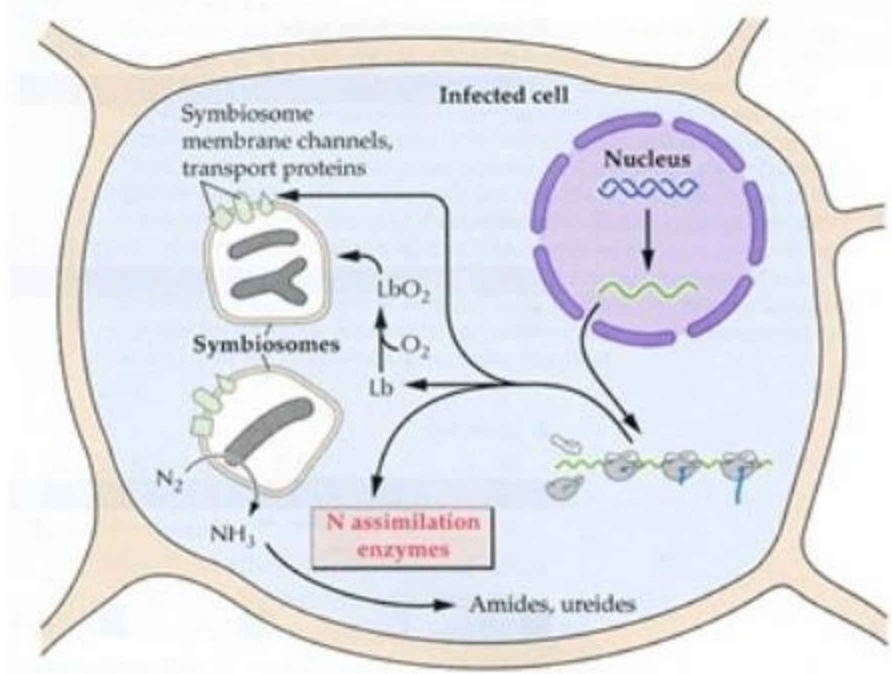
13. Affected pericycle and cortical derived cells continue to divide until the regions fuse.

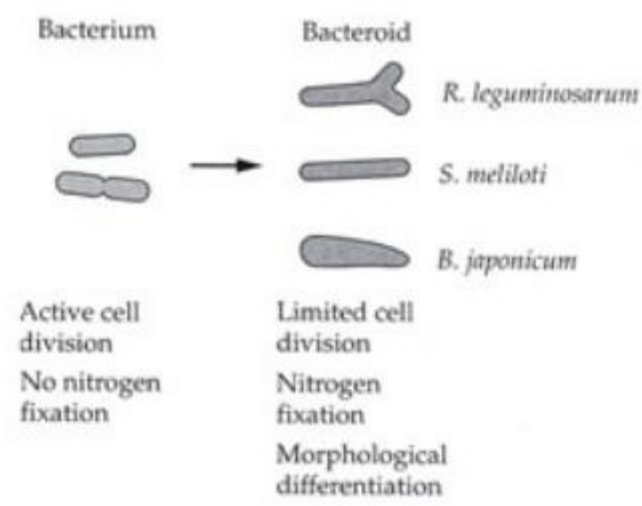
14. Vasculature forms between the nodule and the plant stele,

- nitrogenous compounds are carried to the plant,
- nutrients to the bacteria.

Nitrogen Assimilation II

- Symbiosome,
  - plant membrane, surrounding…
  - one or more bacteria,
    - once inside a symbiosome, bacteria differentiate into bacteroids,
    - bacteroids may differentiate,
- Host Cell Synthesizes,
  - transport proteins for the symbiosome membrane,
  - leghemoglobin, an oxygen binding molecule,
  - N assimilation enzymes.





ii. Symbiotic: symbiotic relationship between bacteria (or actinomycete) and plant root that takes place in the plant nodules on the roots. Nitrogen fixation rates can range from 40 - 300 kg N/ha/yr. Classic examples are

Alfalfa & Rhizobium (bacteria)

Alnus (red alder) & Frankia (actinomycete)

Robinia (black locust) & Rhizobium (bacteria)