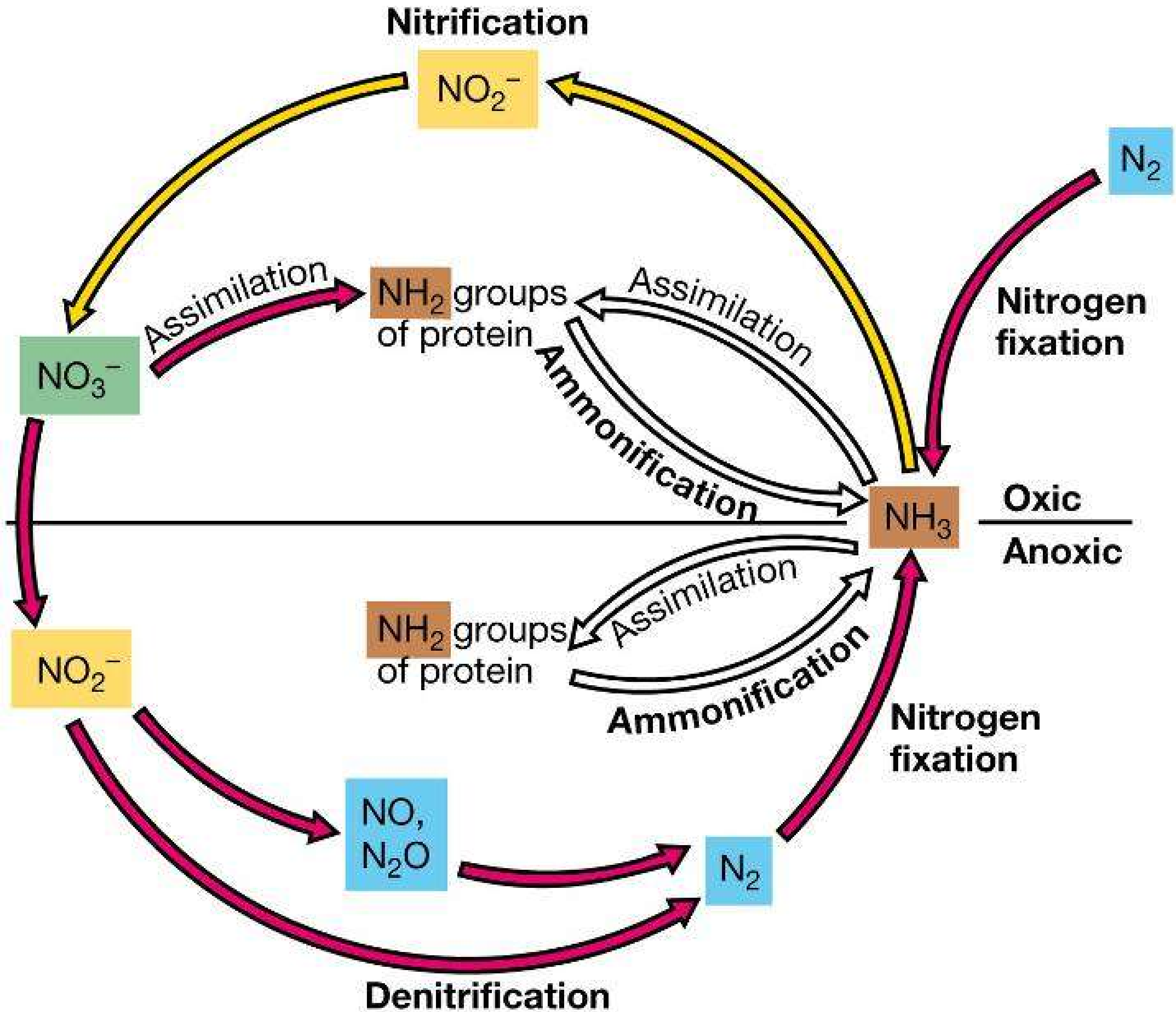

Redox cycle for nitrogen From Brock, Fig.19.29

Important nitrogen-containing microbial compounds for synthesis and degradation

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- **Purines, pyrimidines, nucleotides, RNA, DNA**
- **N-Acetylglucosamine, murein, chitin, glycoproteins**
- **Amino acids, peptides, proteins**
- **Ethanolamine, choline, phospolipids**
- **Cofactors, *e.g.* ATP, GTP, CoA, ThPP, NAD, FAD, heme & other tetrapyrroles, biotin, pantothenic acid, PLP, THF**
- **Urea, uric acid, carbamoylphosphate**
- **Secondary metabolites, antibiotics, chelators, toxins, etc**

Ammonia (NH_3) or Ammonium (NH_4^+):

the preferred nitrogen source for assimilation in microbes

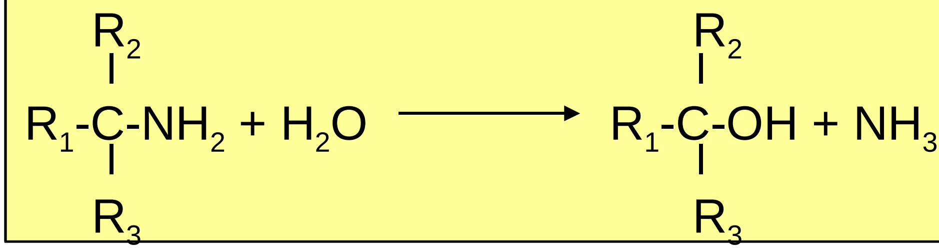
Three biological pathways for ammonia formation in nature

- **Degradation of organic compounds, primarily by deamination reactions**
- **Nitrate reduction, catalyzed by the assimilatory nitrate reductase**
- **Nitrogen fixation, catalyzed by nitrogenase**

Two biological pathways for ammonia removal in nature

- **Assimilation (=incorporation into carbon compounds)**
- **Nitrification (=ammonium oxidation)**

Release of ammonia from organic compounds by deamination



Assimilation of nitrate

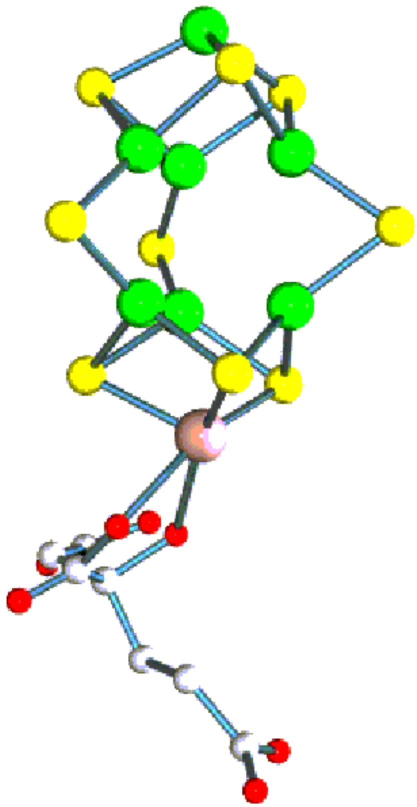
$$NO_3^- + 5\ NADH + H^+ \longrightarrow NH_4^+ + 5\ NAD^+ + 3\ H_2O$$

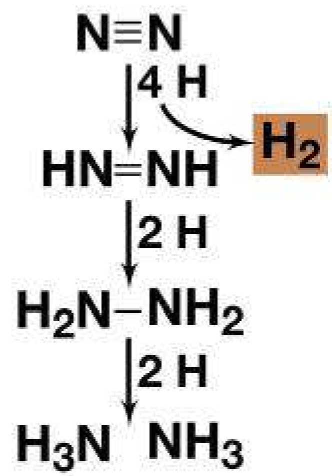
- Nitrate is used as a nitrogen source
- The catalyzing enzyme, nitrate reductase, is soluble (cytoplasmic)

The nitrogen fixation reaction

Nitrogenase FeMo Cofactor

($MoFe_7S_9$ + Homocitrate)





$$N_2 + 10H^+ + 8e^- + 16ATP \rightarrow 2NH_4^+ + H_2 + 16ADP + 16P_i$$

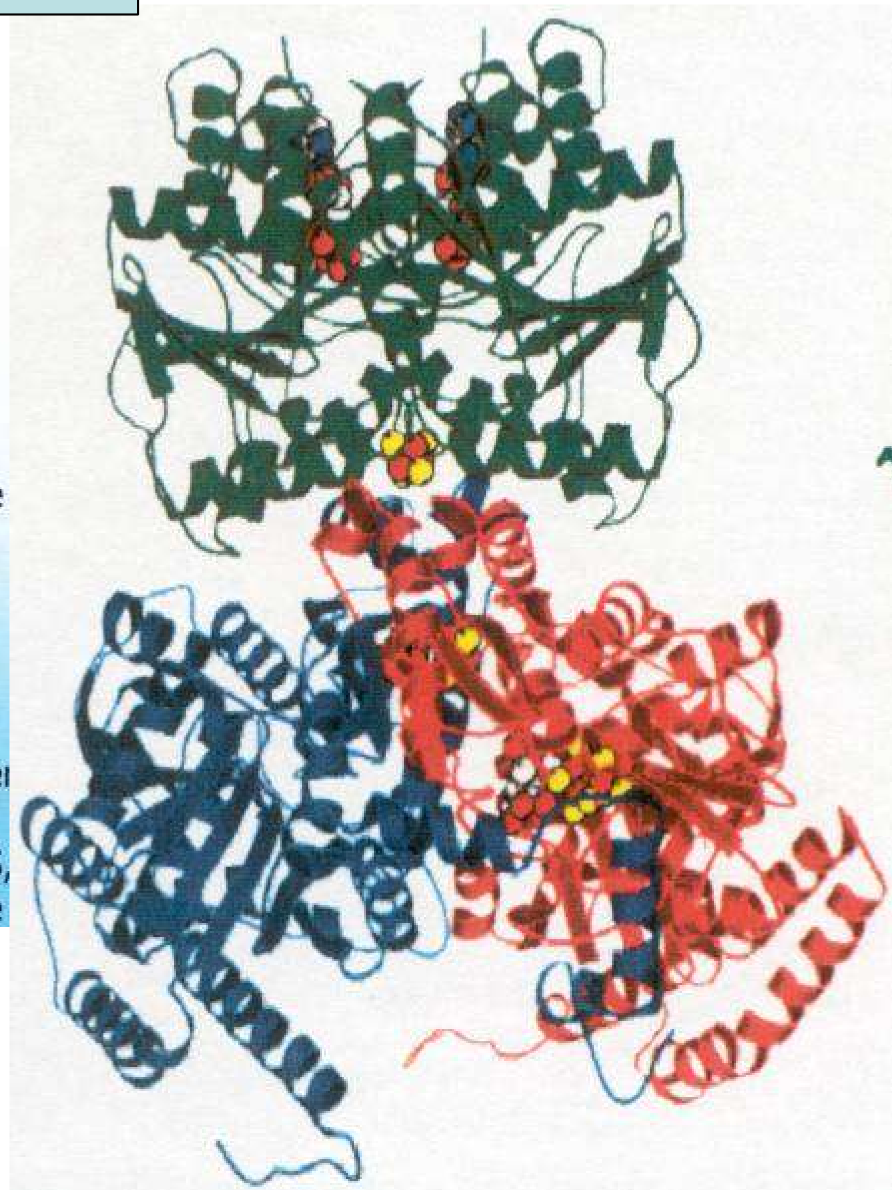
Nitrogenase complex

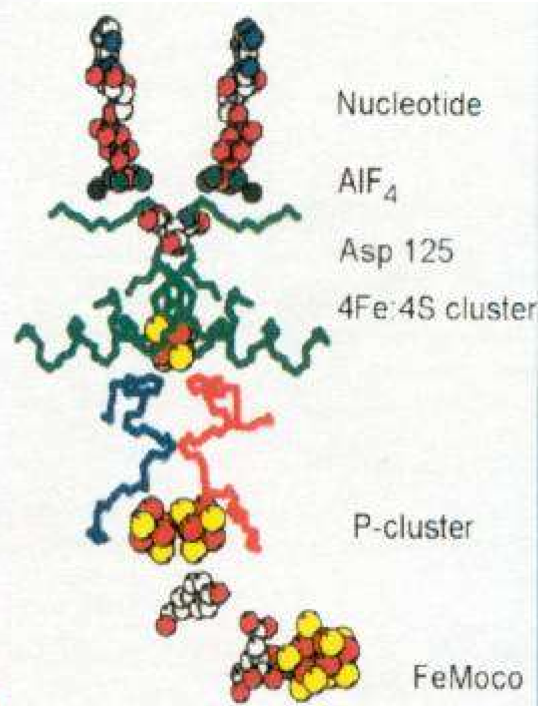
Fe-protein (NifH)

- α_2 homodimer
- ~60 kDa
- 4Fe:4S cluster per dimer
- MgATP/MgADP binding site

FeMo-protein (NifDK)

- $\alpha_2\beta_2$ double dimer
- ~240 kDa
- 8Fe:7S cluster per $\alpha\beta$ dimer ("P cluster")
- FeMo cofactor: 7Fe:1Mo:9S, homocitrate





A small selection of nitrogen-fixing microorganisms (Diazotrophs)

I. Free-living Bacteria and Archaea

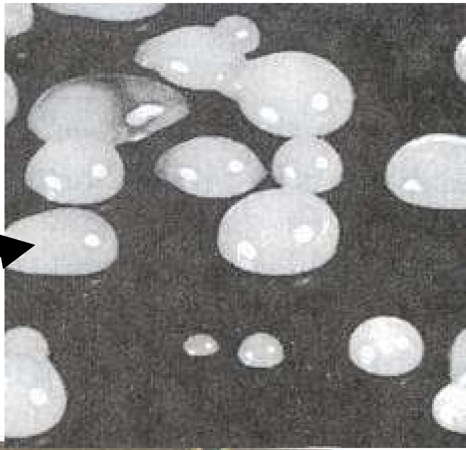
- **Free-living anaerobes**

Clostridium, Desulfovibrio, Methanosarcina, Methanococcus

Chromatium, Chlorobium, Rhodobacter, Rhodospirillum, Heliobacterium

- **Free-living facultative anaerobes**

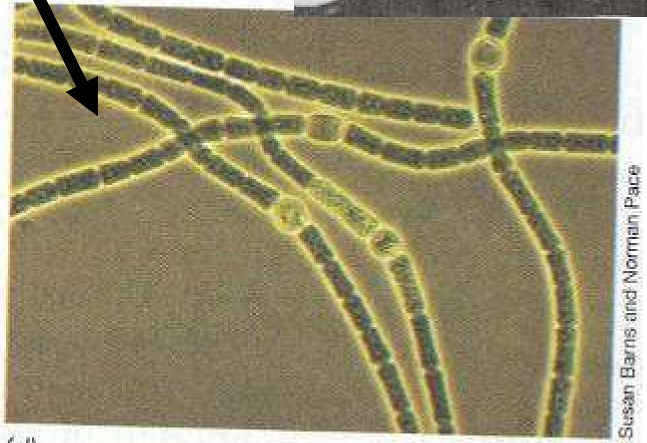
Klebsiella pneumoniae, Citrobacter freundii, Bacillus polymyxa



- **Free-living aerobes or microaerophiles**

Azotobacter spp., *Azospirillum, Acetobacter, Beijerinckia,*

Thiobacillus, some Cyanobacteria (e.g. *Anabaena variabilis*)



II. Symbiotic Bacteria

- **Legume symbionts**

Rhizobium, Bradyrhizobium, Azorhizobium

- **Non-legume symbionts**

Frankia spp.

Ecologic and agronomic importance of symbiotic nitrogen fixation

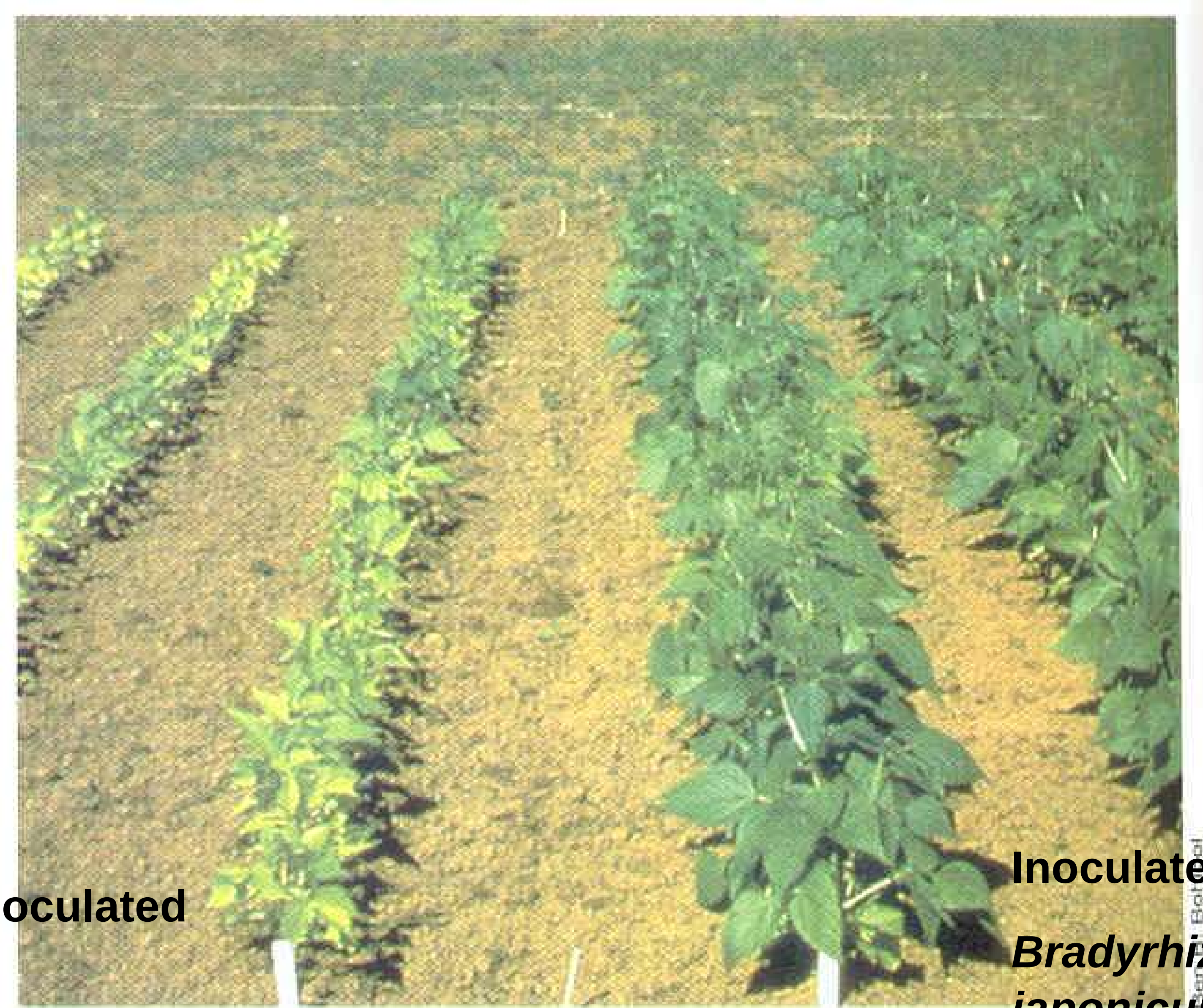
Table 1: N input of diazotrophs in agricultural soil (kg/ha yr)

Azotobacter vinelandii	
(free-living diazotroph)	**1**
Rhizobium leguminosarum **bv.** ***trifolii***	
(symbiotic diazotroph)	**300**

Table 2: Worldwide production of important crop plants

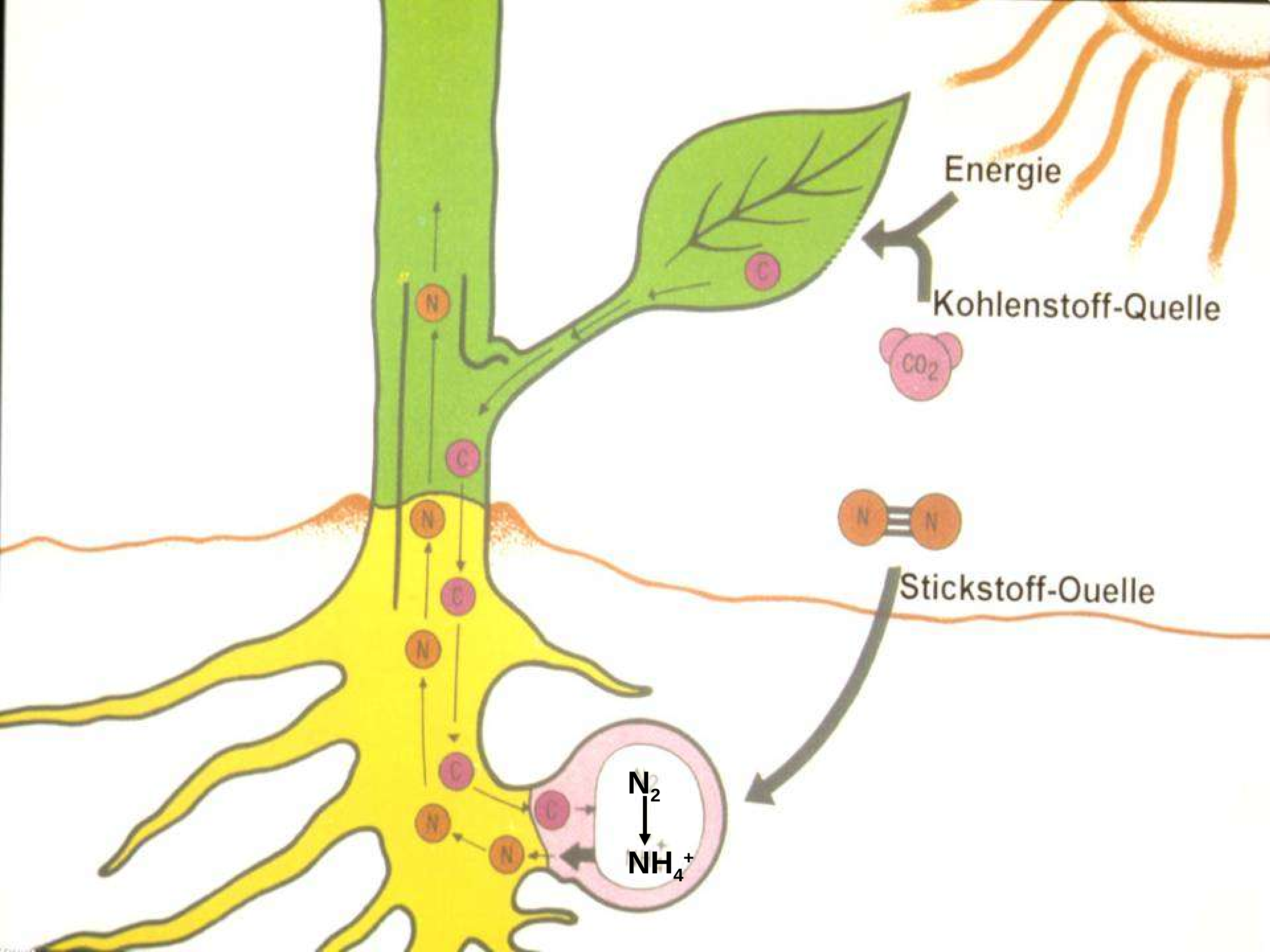
	Production (Mio. t)	Acreage (Mio. ha)
Wheat	**445**	**237**
Rice	**400**	**145**
Maize	**292**	**131**
Soybean	**83**	**53**

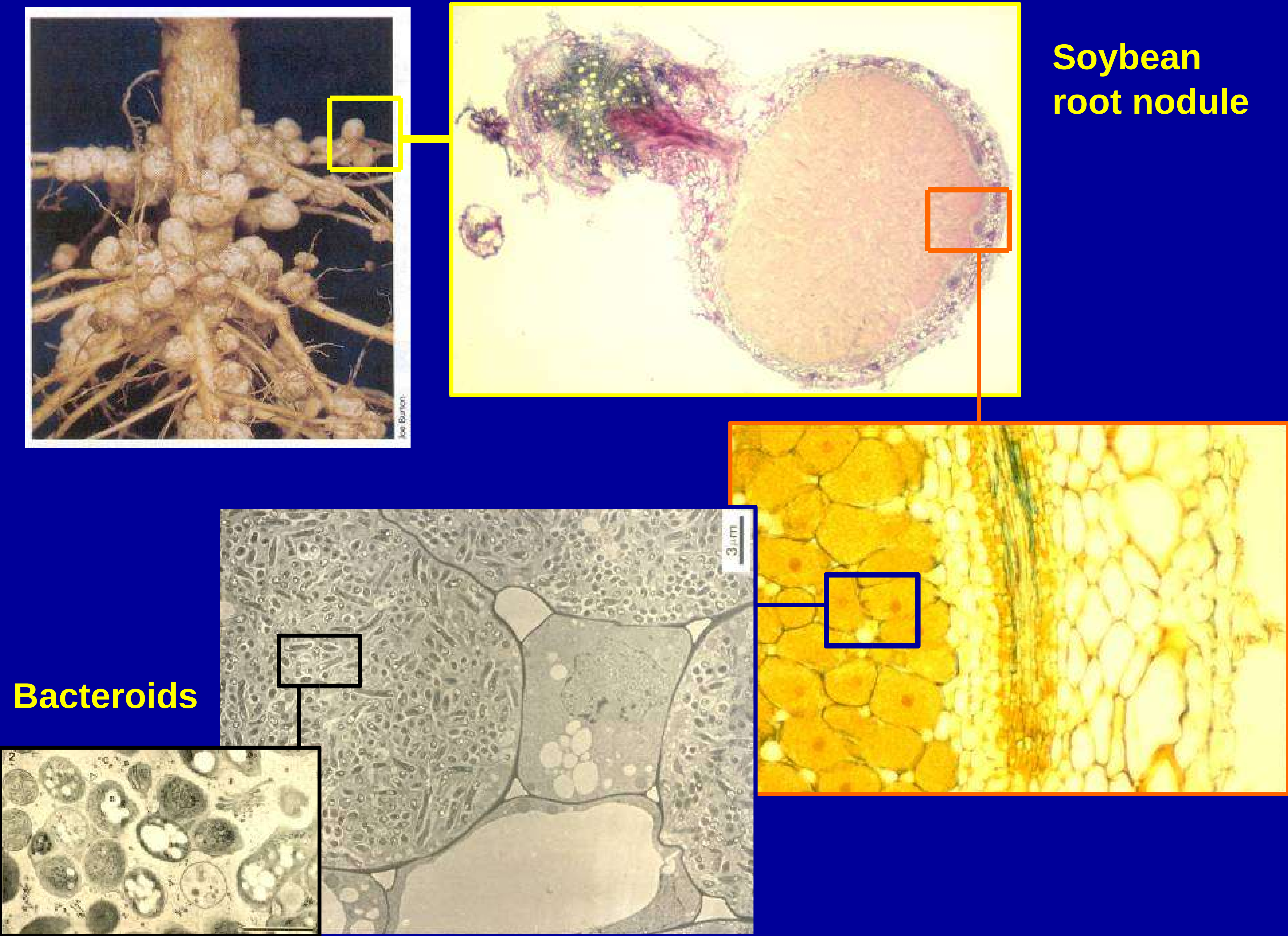
A field of soybean plants growing in nitrogen-poor soil



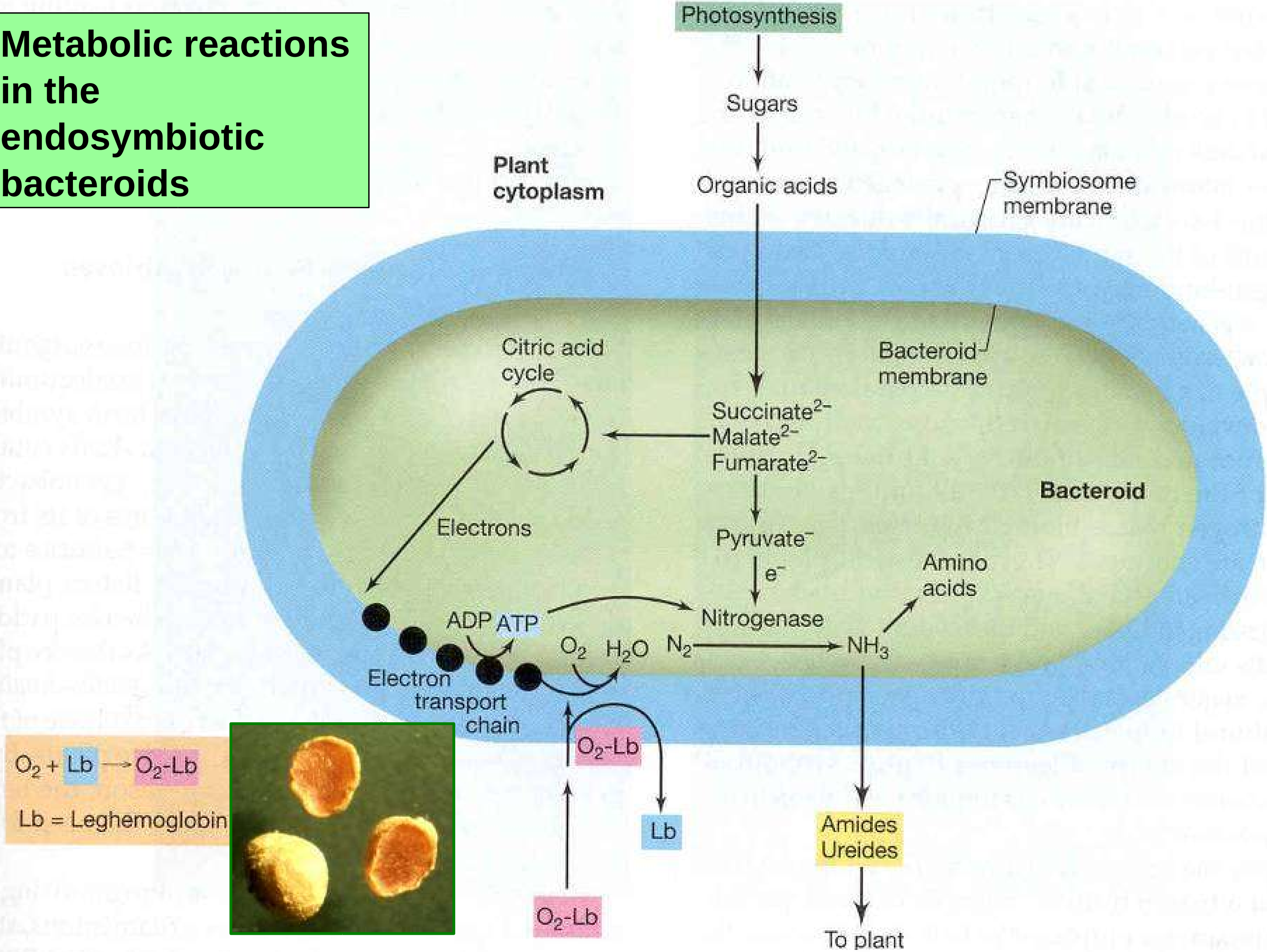
Not inoculated

Inoculated with *Bradyrhizobium japonicum*


Energie
Kohlenstoff-Quelle
CO_2
Stickstoff-Ouelle
N_2
NH_4^+


Soybean
root nodule
Bacteroids
3 µm

Metabolic reactions in the endosymbiotic bacteroids



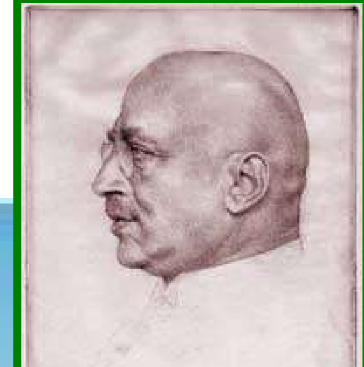
Technische Ammoniaksynthese (HABER-BOSCH)

$$N_2 + 3\,H_2 \xrightarrow[500^{\circ}C,\ 200\ Atm]{[Fe/Al_2O_3]} 2\,NH_3$$

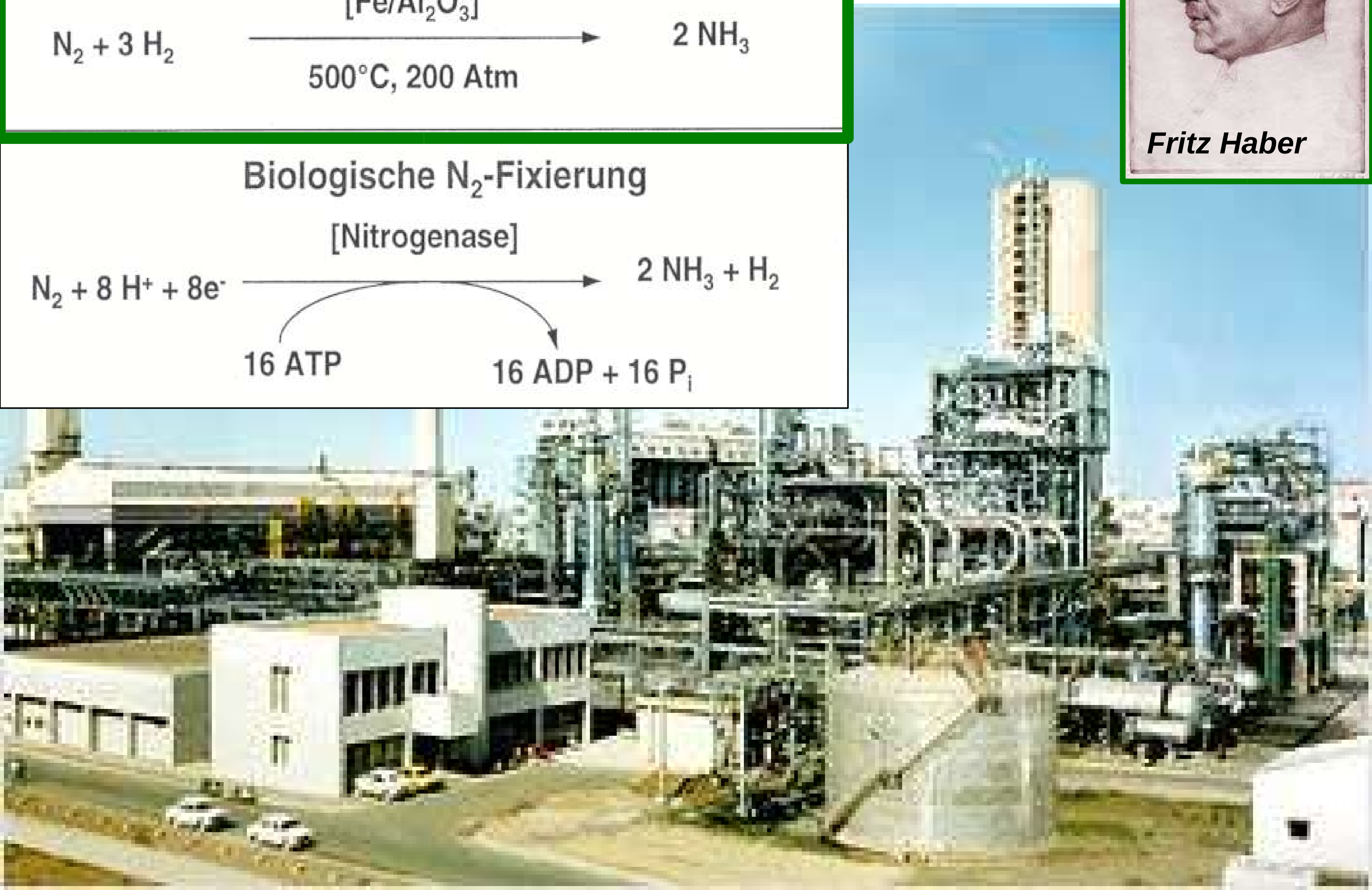
Biologische N_2-Fixierung

$$N_2 + 8\,H^+ + 8e^- \xrightarrow{[Nitrogenase]} 2\,NH_3 + H_2$$

$$16\ ATP \rightarrow 16\ ADP + 16\ P_i$$



Fritz Haber



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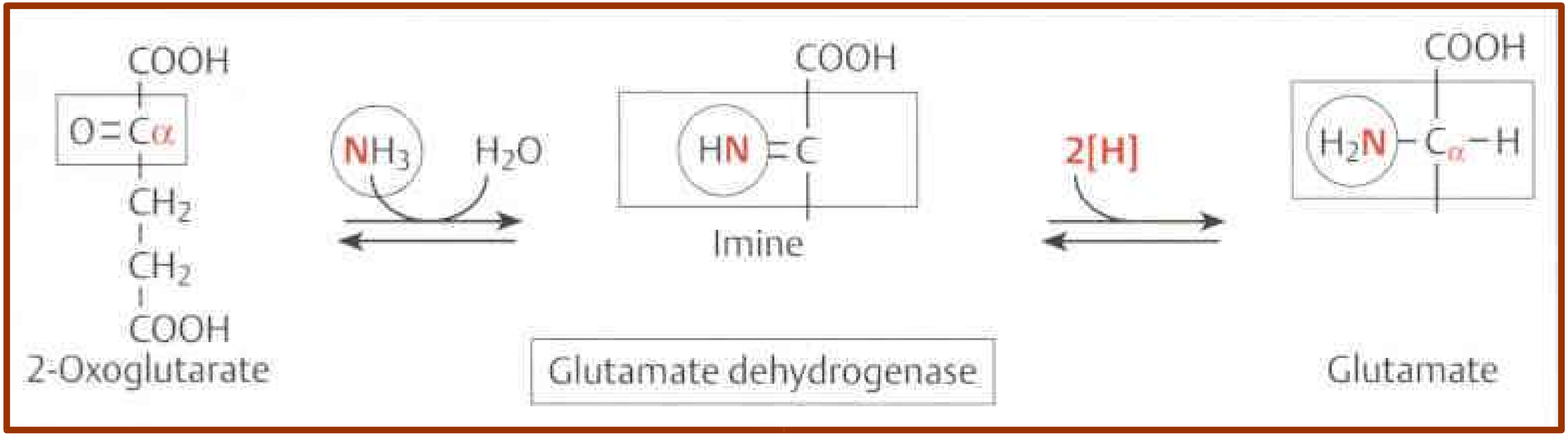
Bacterial ammonia assimilation pathway that operates at high NH_3 concentrations

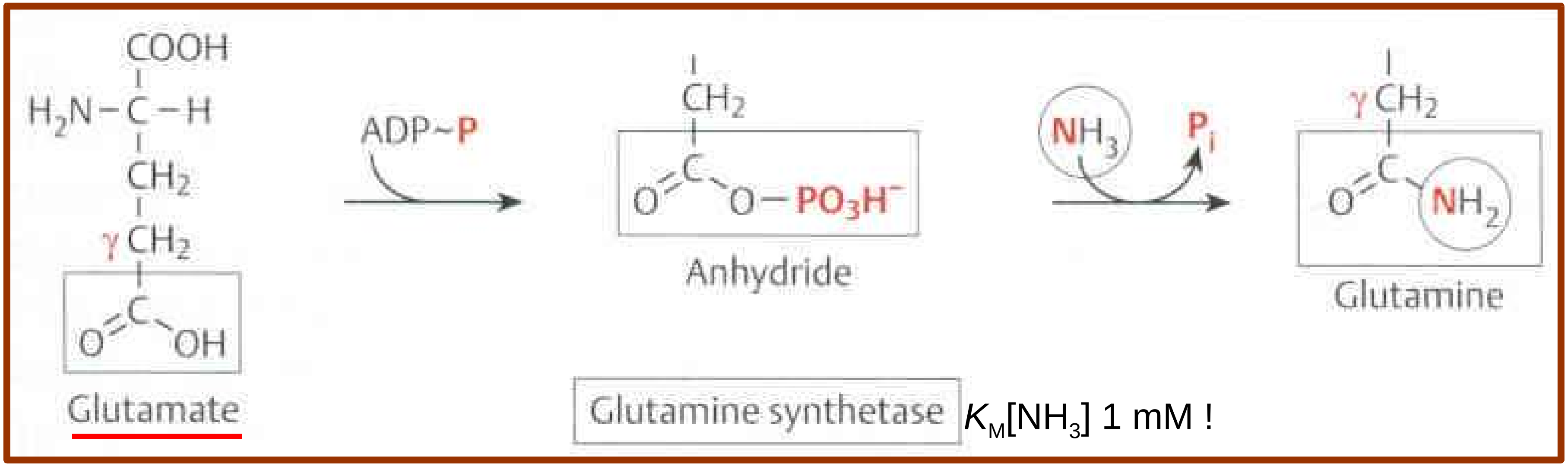


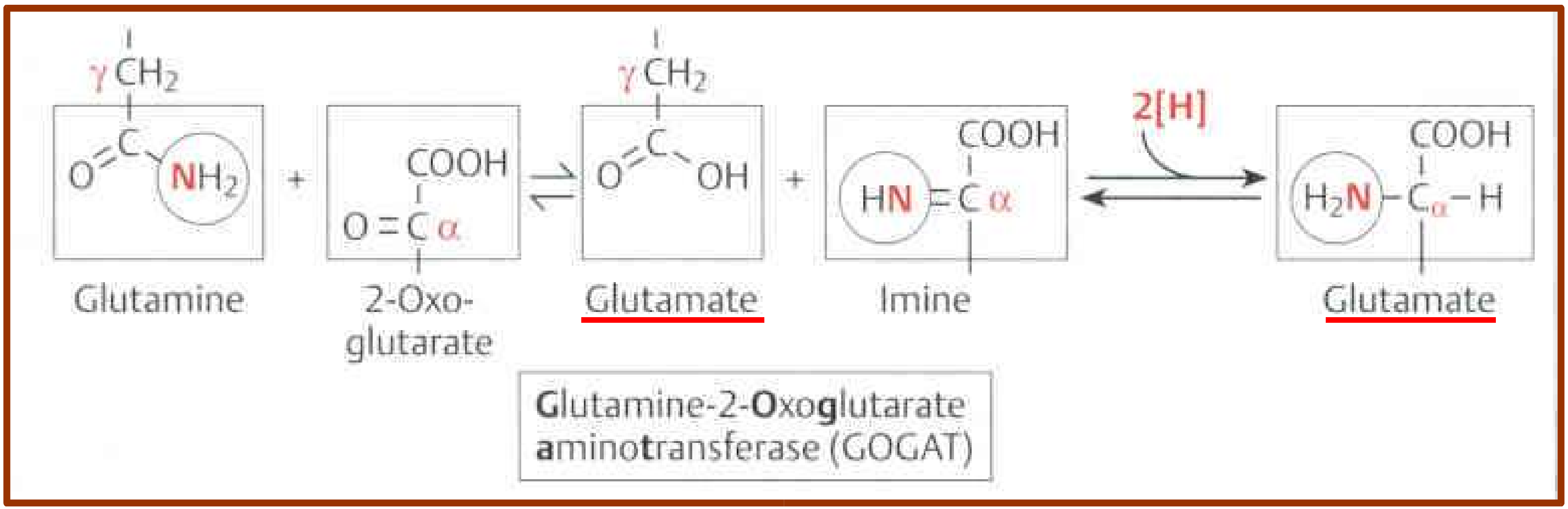
$K_M[NH_3]$ 50 mM !

Few bacteria use analogous reactions, catalyzed by alanine dehydrogenase or aspartate dehydrogenase, i.e., these enzymes use pyruvate or oxaloacetate as substrates, leading to the formation of alanine or aspartate

Bacterial ammonia assimilation pathway that operates at low NH_3 concentrations (<1 mM)

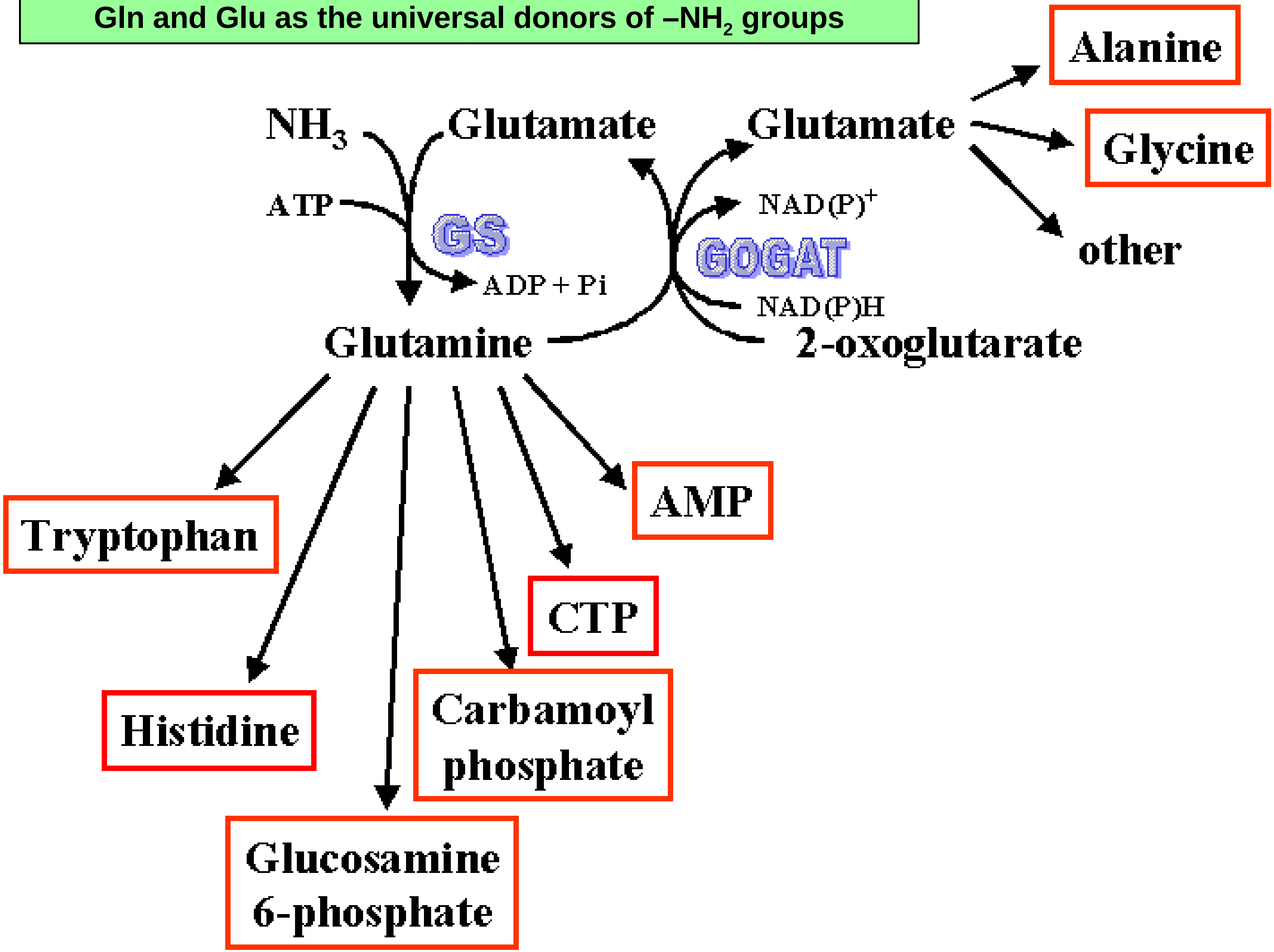
(two sequential enzymatic reactions)





NOTE: 2 Glutamates are formed in the 2nd reaction, of which one is recycled in the 1st reaction!

Gln and Glu as the universal donors of $-NH_2$ groups



Nitrification reactions

Nitrosifying bacteria

(*Nitrosomonas*, *Nitrosococcus*)

1. $NH_3 + O_2 + 2\,e^- + 2\,H^+ \rightarrow NH_2OH + H_2O$
2. $NH_2OH + H_2O + \frac{1}{2}\,O_2 \rightarrow NO_2^- + 2\,H_2O + H^+$

Sum: $NH_3 + 1\frac{1}{2}\,O_2 \rightarrow NO_2^- + H_2O$

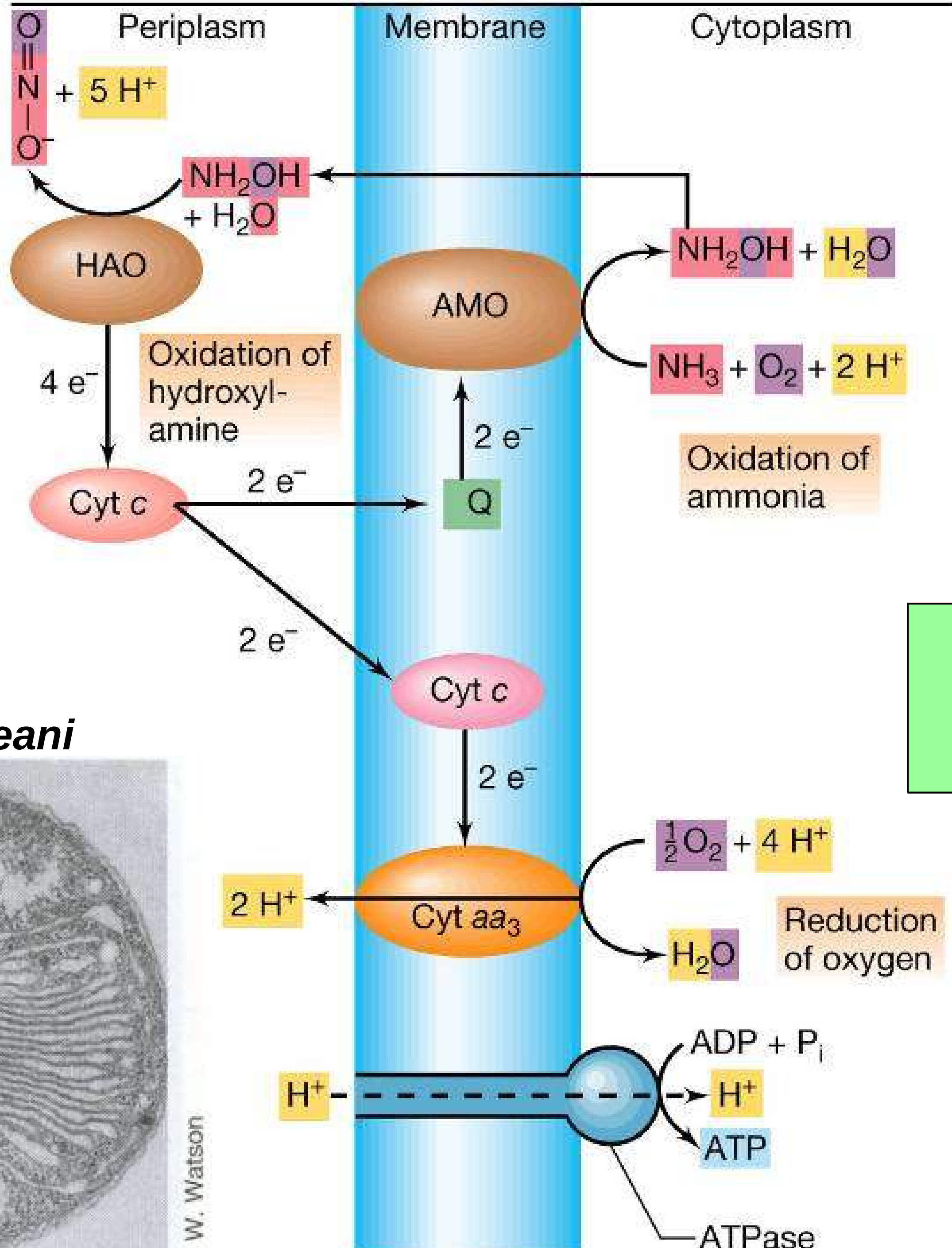
$\Delta G^{0\prime} = -275$ kJ/reaction

Nitrifying bacteria

(*Nitrobacter*, *Nitrospira*)

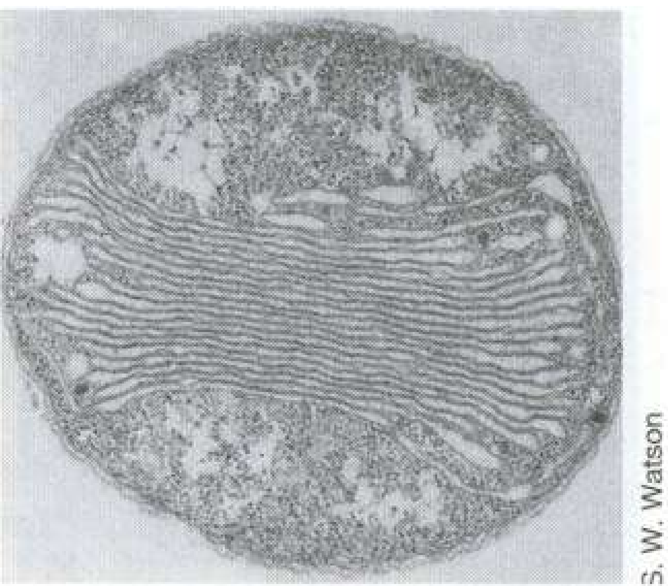
$NO_2^- + \frac{1}{2}\,O_2 \rightarrow NO_3^-$

$\Delta G^{0\prime} = -74.1$ kJ/reaction

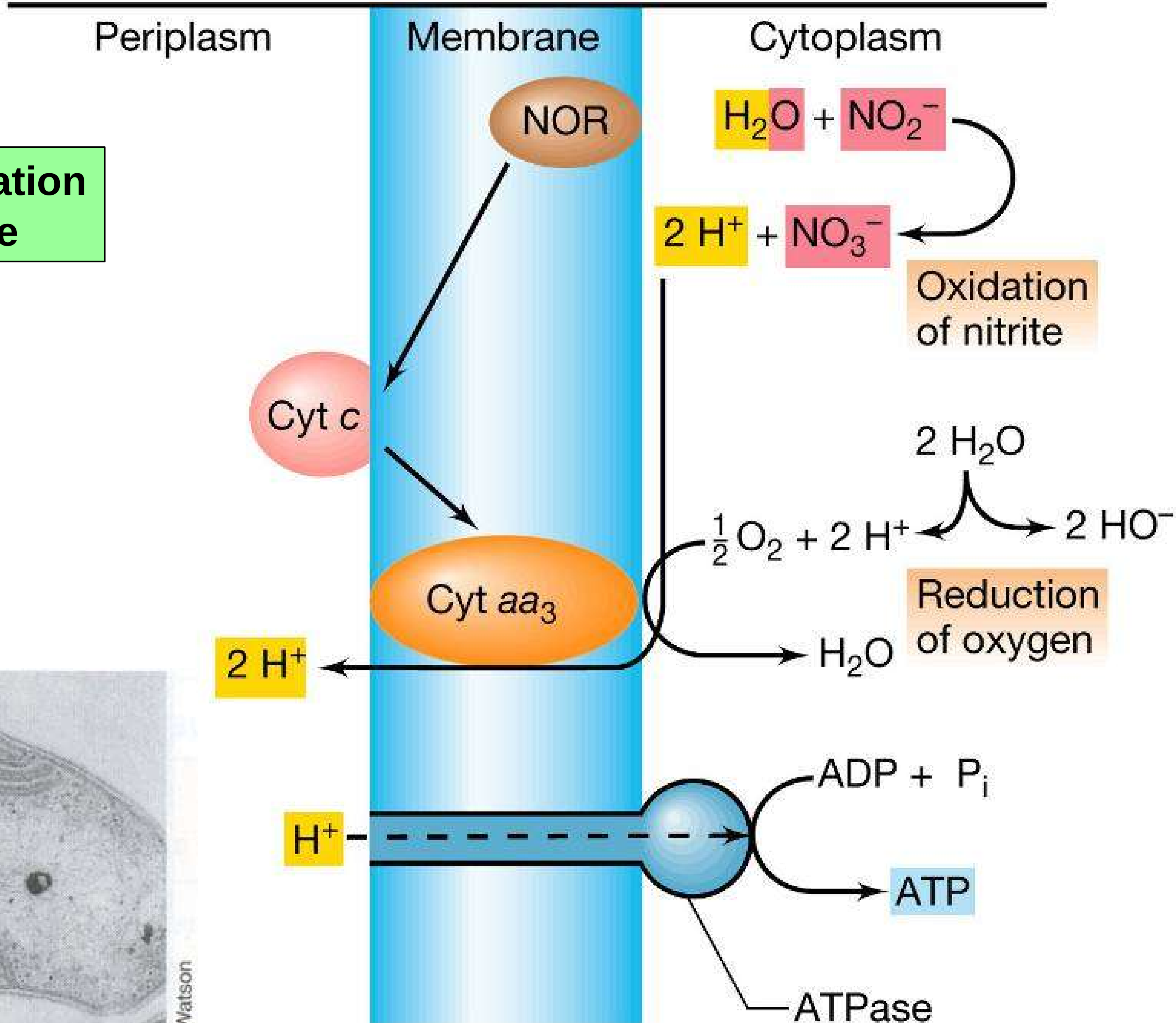


Ammonia oxidation to nitrite

Nitrosococcus oceani



Nitrite oxidation to nitrate



Nitrobacter winogradskyi

Globale Stickstoffbilanz

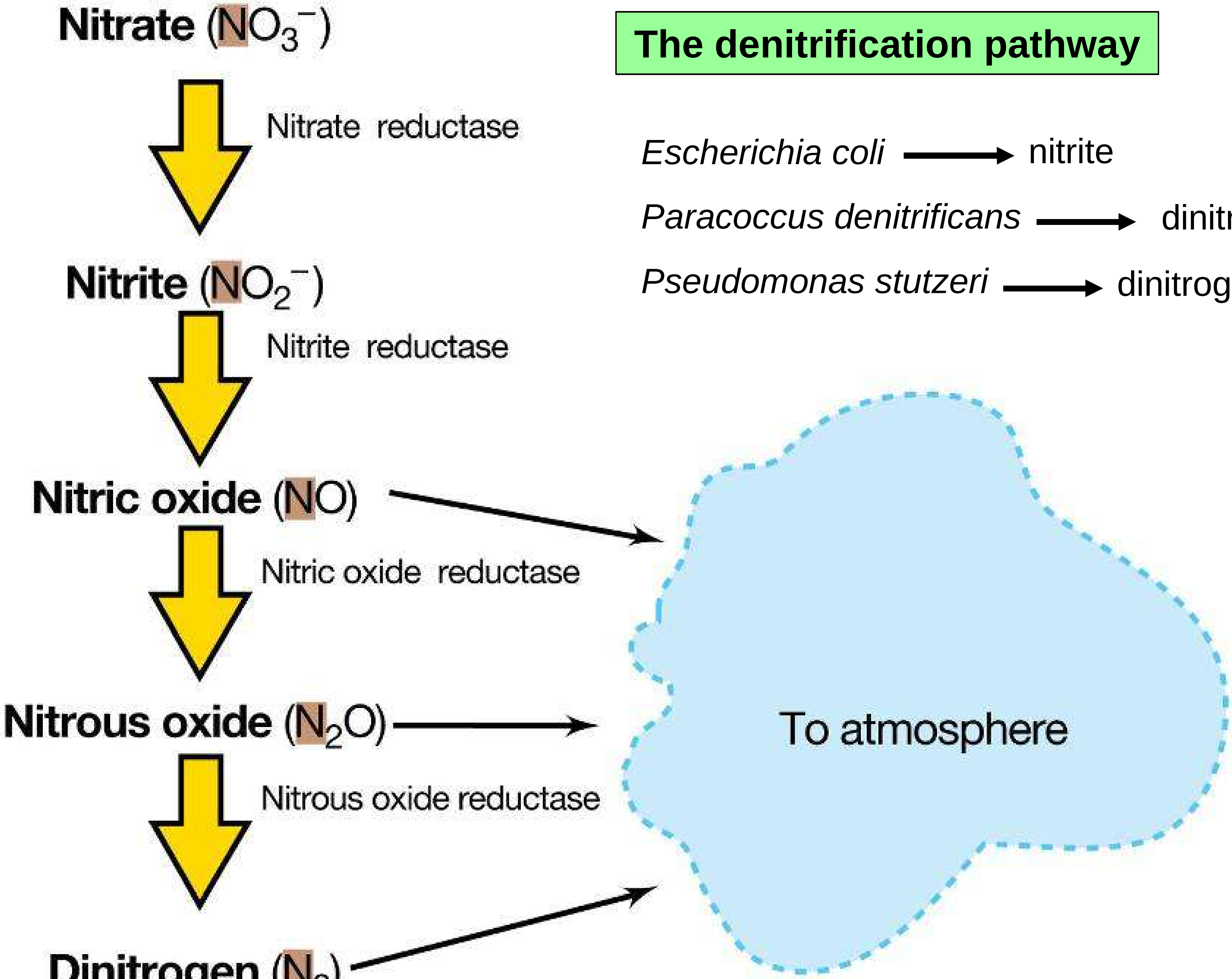
Prozess	Soll	Haben
	$\times 10^6$ t N/Jahr	
NH_3-Niederschlag		+ 140
NO_3^-/NO_2^--Niederschlag		+ 60
Biolog. N_2-Fixierung		+ 180
Industr. N-Düngerproduktion		+ 70
Denitrifikation	- 250	
NH_3-Diffusion	- 150	
Nutzpflanzenproduktion	- 55	
	- 455	+ 450

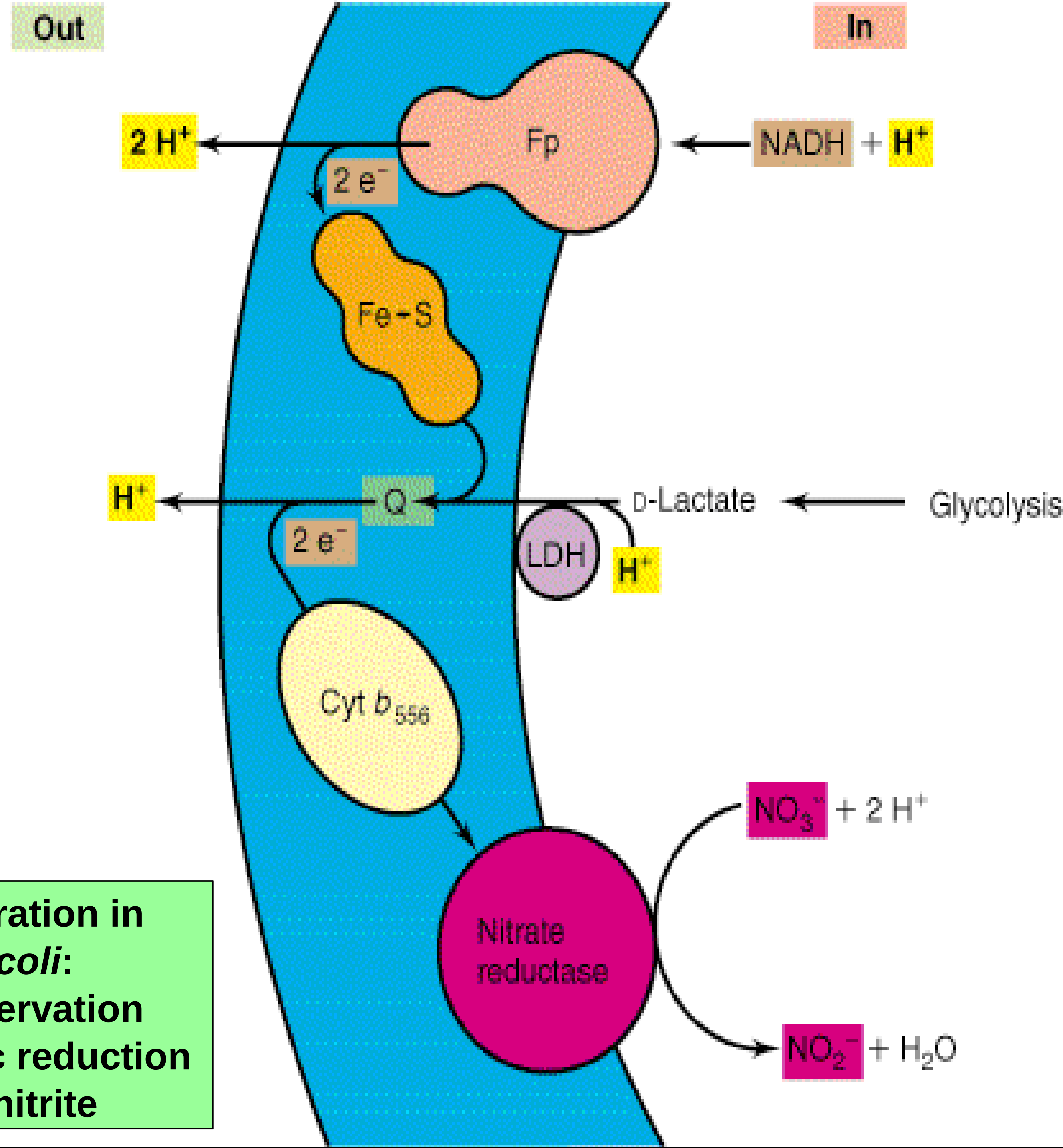
The denitrification pathway

Escherichia coli → nitrite

Paracoccus denitrificans → dinitrogen

Pseudomonas stutzeri → dinitrogen





Nitrate respiration in *Escherichia coli*: energy conservation by anaerobic reduction of nitrate to nitrite