

# Environmental Microbiology

Phototrophy, Chemolithotrophy, and Major  
Biosyntheses

prepared by Prof. Bulent Içgen

# Phototrophy, Chemolithotrophy and Major Biosyntheses

metabolic diversity of:

- **phototrophs**; use light as energy source,
- **chemolithotrophs**; use inorganic compounds
- Two important biosyntheses are discussed:
- **Autotrophy**, fixation of carbon dioxide ( $\text{CO}_2$ ) into cell material, and
- **Nitrogen fixation**, reduction of atmospheric nitrogen ( $\text{N}_2$ ) to ammonia ( $\text{NH}_3$ ) to supply the cell's nitrogen requirements.

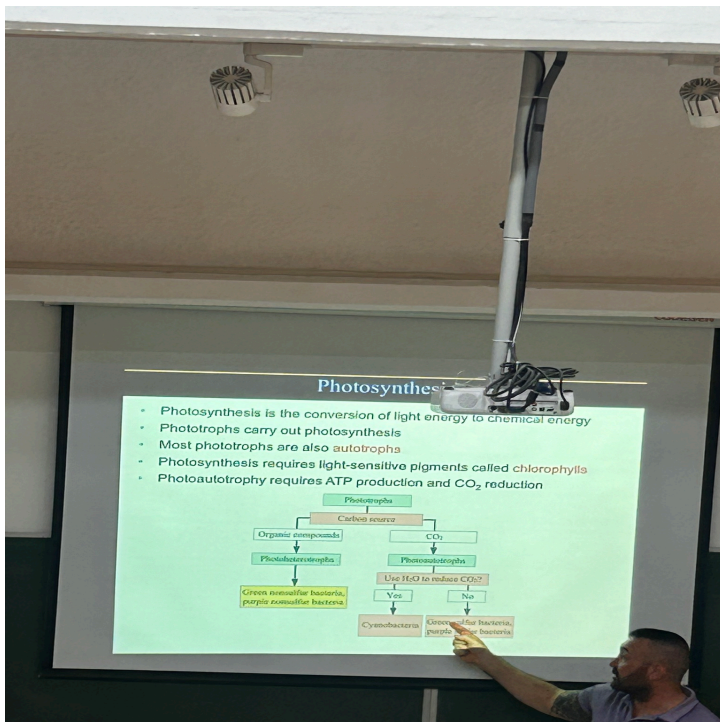
# Phototrophy

*They should have pigments to trap the light*

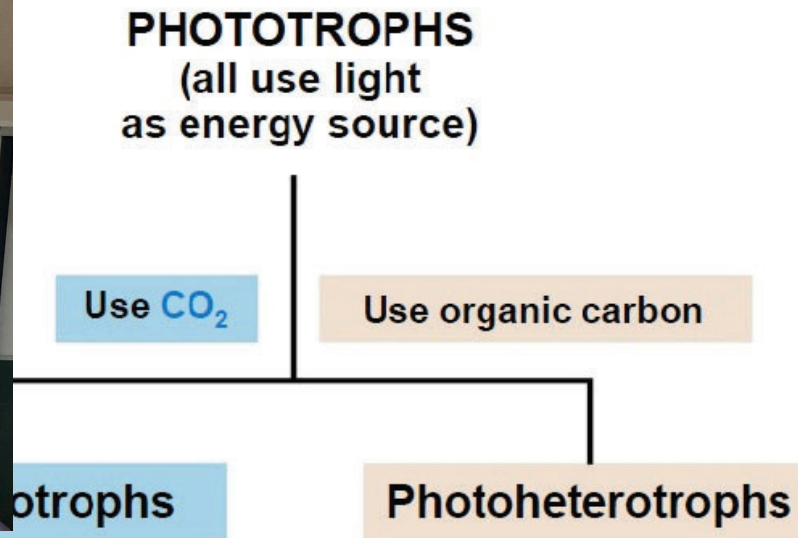
- Photosynthesis
- Chlorophylls and Bacteriochlorophylls *(main pigments)*  
*of green and purple bacteria*
- Carotenoids and Phycobilins *→ accessory pigments ⇒ to get more light.*
- Anoxygenic Photosynthesis
- Oxygenic Photosynthesis

# Photosynthesis

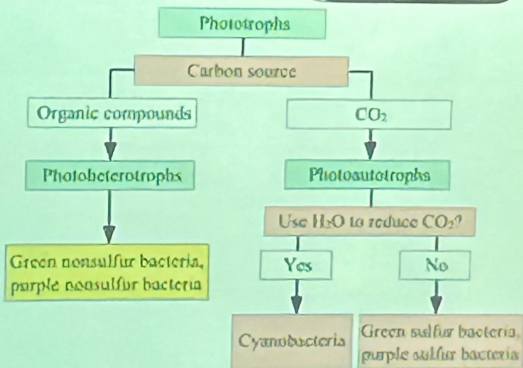
- Photosynthesis is the conversion of light energy to chemical energy
- Phototrophs carry out photosynthesis
- Most phototrophs are also **autotrophs**
- Photosynthesis requires light-sensitive pigments called **chlorophylls**
- Photoautotrophy requires ATP production and CO<sub>2</sub> reduction



of phototrophic organisms in terms of energy and carbon sources.



Photosynthesis requires light-sensitive pigments called chlorophyll  
Photoautotrophy requires ATP production

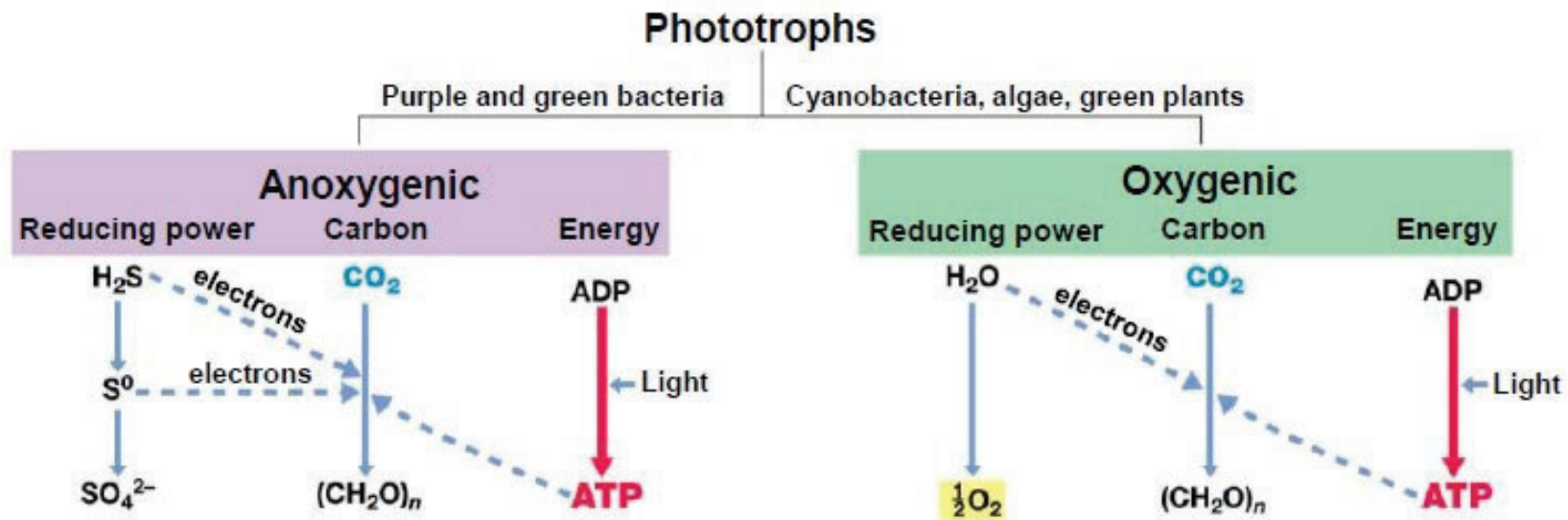


Plan  
Pigm

# Photosynthesis

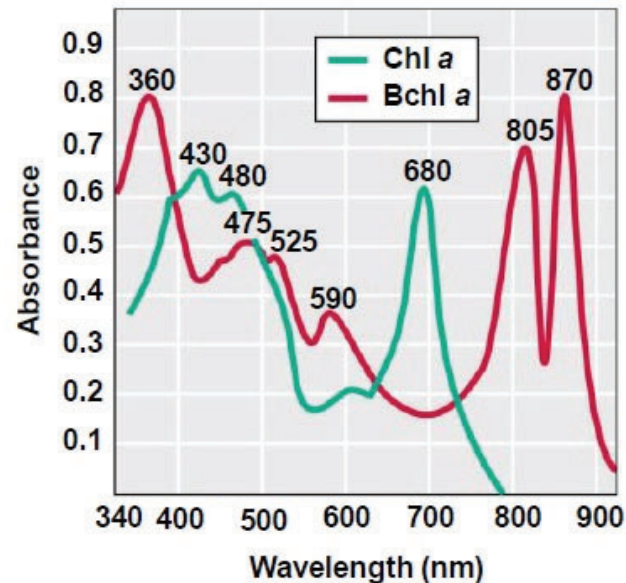
## Photoautotrophy

- Oxidation of  $\text{H}_2\text{O}$  produces  $\text{O}_2$  (**oxygenic photosynthesis**)
- Oxygen not produced (**anoxygenic photosynthesis**)



# Chlorophylls and Bacteriochlorophylls

- Organisms must produce some form of **chlorophyll** (or **bacteriochlorophyll**) to be photosynthetic
  - Chlorophyll is related to **porphyrins**
  - Number of different types of chlorophyll exist
- Different chlorophylls have different absorption spectra



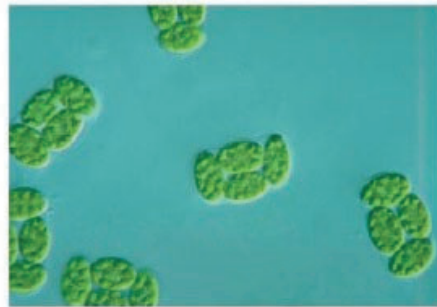
- Absorption spectrum (green curve) of cells of the green alga *Chlamydomonas*.
- The peaks at 680 and 430 nm are due to chlorophyll a, and the peak at 480 nm is due to carotenoids.
- Absorption spectrum (red curve) of cells of the phototrophic purple bacterium *Rhodospseudomonas palustris*.
- Peaks at 870, 805, 590, and 360 nm are due to bacteriochlorophyll a, and peaks at 525 and 475 nm are due to carotenoids.

# Chlorophylls and Bacteriochlorophylls

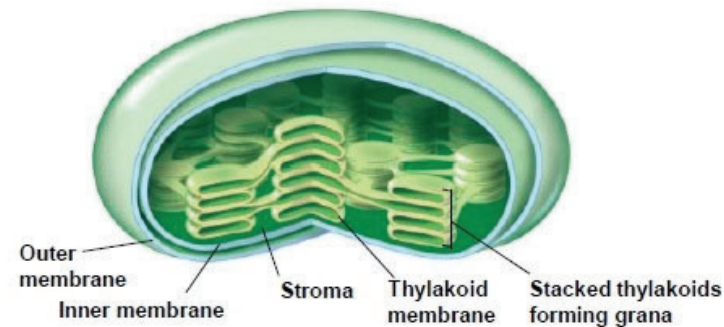
- Cyanobacteria produce chlorophyll a
- Prochlorophytes (oxygenic phototrophs) produce chlorophyll a and b
- Anoxygenic phototrophs produce **bacteriochlorophylls** (in inner membrane)
- Chlorophyll pigments are located within special membranes
  - In eukaryotes, called **thylakoids**
  - In prokaryotes, pigments are integrated into cytoplasmic membrane

Chloroplast → we have thylakoids

Green-purple bacteria  
bryce



(a)

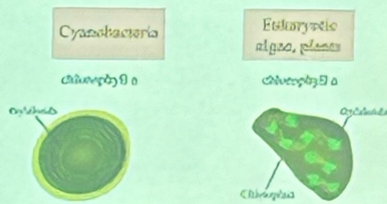


(b)

(a) Photomicrograph of cells of the green alga *Makinoella*. Each of the four cells in a cluster contains several chloroplasts. (b) Details of chloroplast structure, showing how the convolutions of the thylakoid membranes define an inner space called the stroma and form membrane stacks called grana.

## Chlorophylls and Bacteri

- Cyanobacteria produce chlorophyll a
- Prochlorophytes (oxygenic phototrophs) produce chlorophyll a and b
- Anoxygenic phototrophs produce bacteriochlorophylls
- Chlorophyll pigments are located within special membranes
  - In eukaryotes, called thylakoids
  - In prokaryotes, pigments are integrated into cytoplasmic membranes



ORGANISM TYPE	Prokaryote	Eukaryote
PHOTOSYNTHESIS TYPE	O <sub>2</sub> (Oxygenic)	O <sub>2</sub> (Oxygenic)
PHOTOSYNTHETIC MEMBRANE	Plasma Membrane	Thylakoids

# Carotenoids and Phycobilins

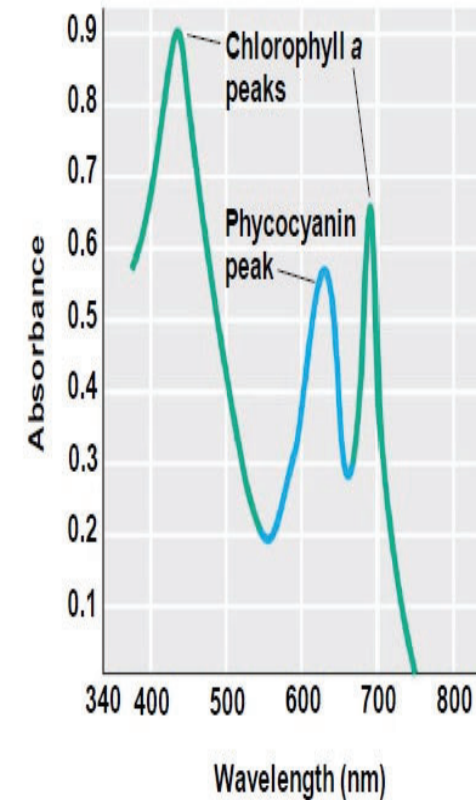
• Phototrophic organisms have **accessory pigments** in addition to chlorophyll, including carotenoids and phycobiliproteins

## Carotenoids

- Always found in phototrophic organisms
- Typically yellow, red, brown, or green
- Energy absorbed by carotenoids can be transferred to a reaction center
- Prevent photooxidative damage to cells

**Phycobiliproteins** are main antenna pigments of cyanobacteria and red algae

- Form into aggregates within the cell called phycobilisomes
- Allow cell to capture more light energy than chlorophyll alone



The presence of phycocyanin broadens the wavelengths of usable light energy (between 600 and 700 nm)

# Anoxygenic Photosynthesis

- Anoxygenic photosynthesis is found in four phyla of Bacteria
- Photosynthesis apparatus embedded in membranes
- Electron transport reactions occur in the reaction center of anoxygenic phototrophs

Reducing power for CO<sub>2</sub> fixation comes from reductants (e<sup>-</sup> donor) present in the environment (i.e., H<sub>2</sub>S, Fe<sup>2+</sup>, or NO<sub>2</sub><sup>-</sup>)

–Requires reverse electron transport for NADH production in purple phototrophs

–Electrons are transported in the membrane through a series of proteins and cytochromes

# Photosynthesis

## PURPLE BACTERIA

Purple Non-Sulfur Bacteria

Purple Sulfur Bacteria

CARBON SOURCE:

Organic compounds

CO<sub>2</sub>

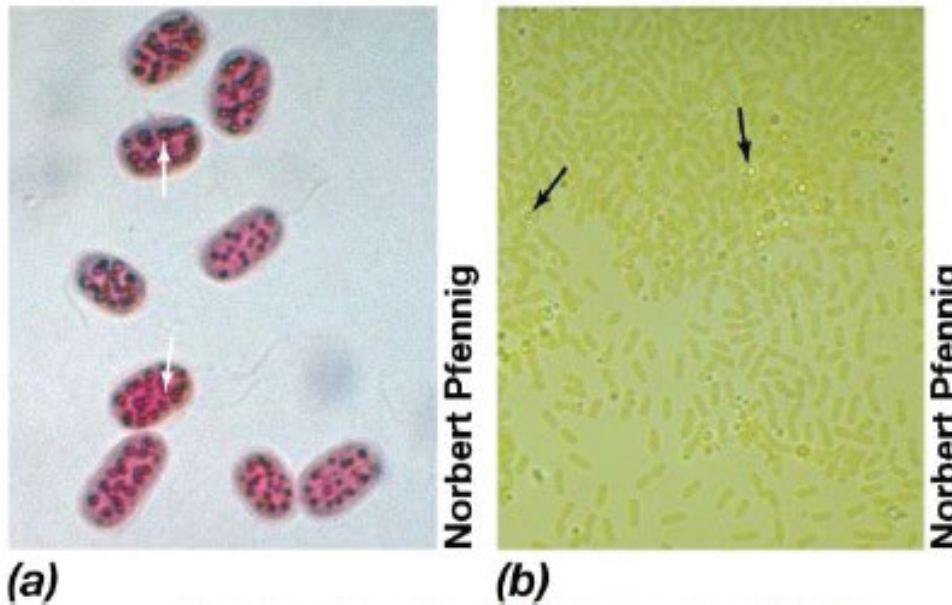
REDUCING POWER:

Organic compounds

H<sub>2</sub>S or S<sub>2</sub>O<sub>3</sub><sup>2-</sup> (thiosulfate)

# Anoxygenic Photosynthesis

For a purple bacterium to grow autotrophically, the formation of ATP is not enough



(a)

(b)

Phototrophic purple and green sulfur bacteria

(a) Purple bacterium. The sulfur granules are deposited inside the cell (arrows). (b) Green bacterium. The refractile bodies are sulfur granules deposited outside the cell (arrows). In both cases the sulfur granules arise from the oxidation of  $H_2S$  to obtain reducing power.

# Oxygenic Photosynthesis

- Oxygenic phototrophs use light to generate ATP and NADPH
- The two light reactions are called photosystem I and photosystem II
- ATP can also be produced by cyclic photophosphorylation

# Chemolithotrophy

- The Energetics of Chemolithotrophy
- Hydrogen Oxidation
- Oxidation of Reduced Sulfur Compounds
- Iron Oxidation
- Nitrification
- Anammox

# The Energetics of Chemolithotrophy

- Chemolithotrophs are organisms that obtain energy from the oxidation of inorganic compounds
- Although lacking photosynthetic pigments, they use CO<sub>2</sub> as their sole carbon source
- Most chemolithotrophic bacteria are also autotrophs.
- Mixotrophs are chemolithotrophs that require organic carbon as a carbon source

# The Energetics of Chemolithotrophy

Inorganic compound source = volcanic activity, agriculture and mining operations  
↓  
around volcanic area  
soil is very productive  
due to high inorganic  
compounds high concentration

- Many sources of reduced molecules exist in the environment
- Volcanic activity is a major source of reduced sulfur compounds, primarily  $\text{H}_2\text{S}$  and  $\text{S}^0$ .
- Agricultural and mining operations, burning of fossil fuels and industrial wastes add reduced nitrogen and iron compounds to the environment.
- Biological sources are production of  $\text{H}_2\text{S}$ ,  $\text{H}_2$ ,  $\text{NH}_3$
- The oxidation of different reduced compounds yields varying amounts of energy

# The Energetics of Chemolithotrophy

**Table 13.1** Energy yields from the oxidation of various inorganic electron donors<sup>a</sup>

Electron donor	Chemolithotrophic reaction	Group of chemolithotrophs	$E_0'$ of couple (V)	$\Delta G^{0'}$ (kJ/reaction)	Number of electrons/reaction
Phosphite <sup>b</sup>	$4 \text{HPO}_3^{2-} + \text{SO}_4^{2-} + \text{H}^+ \rightarrow 4 \text{HPO}_4^{2-} + \text{HS}^-$	Phosphite bacteria	-0.69	-91	2
Hydrogen <sup>b</sup>	$\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$	Hydrogen bacteria	-0.42	-237.2	2
Sulfide <sup>b</sup>	$\text{HS}^- + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{S}^0 + \text{H}_2\text{O}$	Sulfur bacteria	-0.27	-209.4	2
Sulfur <sup>b</sup>	$\text{S}^0 + 1\frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2 \text{H}^+$	Sulfur bacteria	-0.20	-587.1	6
Ammonium <sup>c</sup>	$\text{NH}_4^+ + 1\frac{1}{2} \text{O}_2 \rightarrow \text{NO}_2^- + 2 \text{H}^+ + \text{H}_2\text{O}$	Nitrifying bacteria	+0.34	-274.7	6
Nitrite <sup>b</sup>	$\text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^-$	Nitrifying bacteria	+0.43	-74.1	2
Ferrous iron <sup>b</sup>	$\text{Fe}^{2+} + \text{H}^+ + \frac{1}{4} \text{O}_2 \rightarrow \text{Fe}^{3+} + \frac{1}{2} \text{H}_2\text{O}$	Iron bacteria	+0.77	-32.9	1

<sup>a</sup>Data calculated from  $E_0'$  values in Appendix 1; values for  $\text{Fe}^{2+}$  are for pH 2, and others are for pH 7. At pH 7 the value for the  $\text{Fe}^{3+}/\text{Fe}^{2+}$  couple is about +0.2 V.

<sup>b</sup>Except for phosphite, all reactions are shown coupled to  $\text{O}_2$  as electron acceptor. The only known phosphite oxidizer couples to  $\text{SO}_4^{2-}$  as electron acceptor.  $\text{H}_2$  and most sulfur compounds can be oxidized anaerobically using one or more electron acceptors, and  $\text{Fe}^{2+}$  can be oxidized at neutral pH with  $\text{NO}_3^-$  as electron acceptor.

<sup>c</sup>Ammonium can also be oxidized with  $\text{NO}_2^-$  as electron acceptor (anammox, Section 13.11).

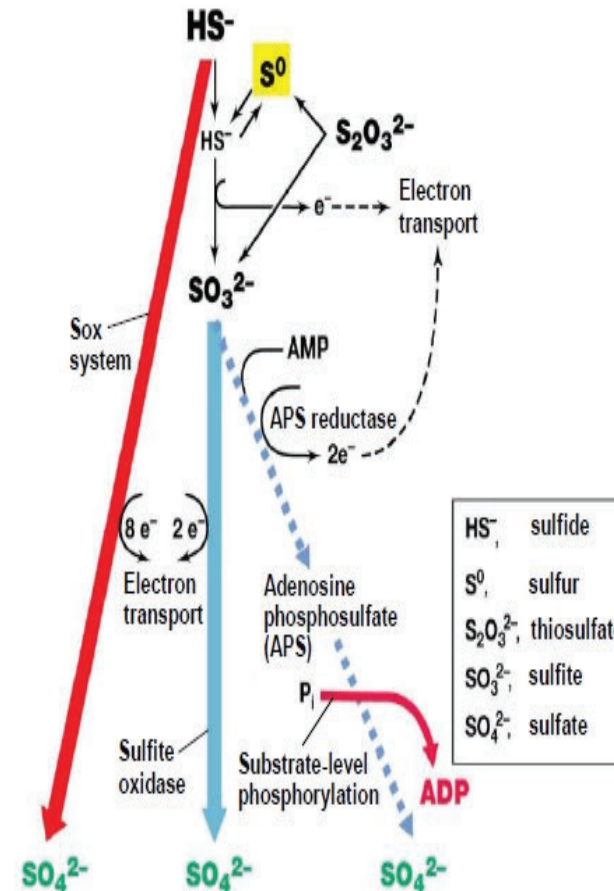
# Hydrogen Oxidation

- Anaerobic H<sub>2</sub>-oxidizing Bacteria and Archaea are known
- Catalyzed by hydrogenase
- Calvin cycle and hydrogenase enzymes allow chemolithotrophic growth

# Oxidation of Reduced Sulfur Compounds

- Many reduced sulfur compounds are used as electron donors
- Discovered by Sergei Winogradsky
- $\text{H}_2\text{S}$ ,  $\text{S}^0$ ,  $\text{S}_2\text{O}_3^{2-}$  are commonly used
- One product of sulfur oxidation is  $\text{H}^+$ , which lowers the pH of its surroundings
- Sox system oxidizes reduced sulfur compounds directly to sulfate
- Usually aerobic, but some organisms can use nitrate as an electron acceptor

Oxidation of reduced sulfur compounds by sulfur chemolithotrophs



# Iron Oxidation

- Ferrous iron ( $\text{Fe}^{2+}$ ) oxidized to ferric iron ( $\text{Fe}^{3+}$ )
- Ferric hydroxide precipitates in water
- Many Fe oxidizers can grow at  $\text{pH} < 1$

Often associated with acidic pollution from coal mining activities

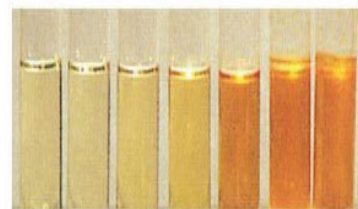
- Some anoxygenic phototrophs can oxidize  $\text{Fe}^{2+}$  anaerobically using  $\text{Fe}^{2+}$  as an electron donor for  $\text{CO}_2$  reduction

Iron-oxidizing bacteria



Bill Strode

(a)



T. D. Brock

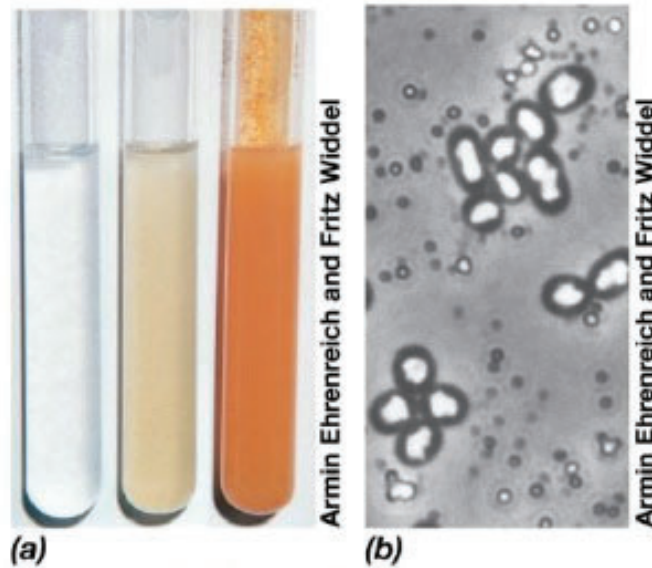
(b)

(a) Acid mine drainage showing the confluence of a normal river and a creek draining a coal-mining area. The acidic creek is very high in  $\text{Fe}^{2+}$ . At low pH values,  $\text{Fe}^{2+}$  does not oxidize spontaneously in air, but *Acidithiobacillus ferrooxidans* carries out the oxidation; insoluble  $\text{Fe}(\text{OH})_3$  and complex ferric salts precipitate.

(b) Cultures of *A. ferrooxidans*. Shown is a dilution series, with no growth in the tube on the left and increasing amounts of growth from left to right. Growth is evident from the production of  $\text{Fe}^{3+}$ , which readily complexes to form  $\text{Fe}(\text{OH})_3$  and protons.

# Iron Oxidation

Ferrous iron can be oxidized under anoxic conditions by certain anoxygenic phototrophic bacteria



(a) Oxidation in anoxic tube cultures. Brown-red color is due to  $Fe(OH)_3$

(b) Phase-contrast micrograph of an  $Fe^{2+}$  oxidizing purple bacterium. The bright refractile areas within cells are gas vesicles. The granules outside the cells are iron precipitates.

$Fe^{2+}$  oxidation by anoxygenic phototrophic bacteria

# Nitrification

- $\text{NH}_3$  and  $\text{NO}_2^-$  are oxidized by nitrifying bacteria during the process of nitrification
- Two groups of bacteria work in concert to fully oxidize ammonia to nitrate
- One group (e.g. *Nitrosomonas*) oxidizes  $\text{NH}_3$  to nitrite ( $\text{NO}_2^-$ ), and another group (e.g. *Nitrobacter* and *Nitrospira*) oxidizes  $\text{NO}_2^-$  to  $\text{NO}_3^-$ .
- Key enzymes are ammonia monooxygenase, hydroxylamine oxidoreductase, and nitrite oxidoreductase

Discovered by the Russian microbiologist Winogradsky at the end of the 19th century.

- Winogradsky showed that the nitrifying bacteria were autotrophs obtaining all of their carbon from  $\text{CO}_2$ .
- Only small energy yields from this reaction
  - Growth of nitrifying bacteria is very slow

# Nitrification

## Ammonia-oxidizing bacteria

- $\text{NH}_3$  is oxidized by ammonia monooxygenase producing  $\text{NH}_2\text{OH}$  and  $\text{H}_2\text{O}$
- Hydroxylamine oxidoreductase then oxidizes  $\text{NH}_2\text{OH}$  to  $\text{NO}_2^-$
- Electrons and protons used to generate ATP

## Nitrite-oxidizing bacteria

- Nitrite ( $\text{NO}_2^-$ ) is oxidized by enzyme nitrite oxidoreductase to nitrate ( $\text{NO}_3^-$ )
- Electrons and protons used to generate ATP

# Nitrification

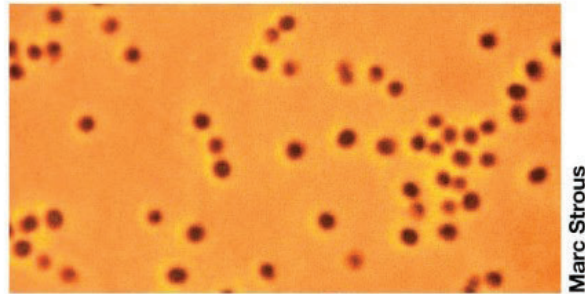
- Nitrifying bacteria play key ecological roles in the nitrogen cycle, converting  $\text{NH}_3$  into  $\text{NO}_3^-$ , a key plant nutrient.
- Also important in sewage and wastewater treatment, removing toxic amines and  $\text{NH}_3$  and releasing less toxic nitrogen compounds.
- $\text{NH}_3$  produced in the sediments from the decomposition of organic nitrogenous compounds is oxidized to  $\text{NO}_3^-$ , a more favorable N-source for algae and cyanobacteria.

# Anammox

- Anammox: <sup>anaerobic</sup> anoxic ammonia oxidation
- Performed by unusual group of obligate anaerobes
- Anammoxosome is compartment where anammox reactions occur
- Protects cell from reactions occurring during anammox
- Hydrazine is an intermediate of anammox
- Anammox is very beneficial in the treatment of sewage and wastewater
- Ammonia is oxidized with  $\text{NO}_2^-$  as the electron acceptor to yield  $\text{N}_2$  as follows:
  - $\text{NH}_4^+ + \text{NO}_2^- \longrightarrow \text{N}_2 + 2\text{H}_2\text{O} \quad \Delta G^{0'} = -357 \text{ kJ}$
  - The first anammox organism discovered, *Brocadia anammoxidans*, is a member of the *Planctomycetes* phylum of Bacteria.
  - Several other genera of anammox bacteria are known, including *Kuenenia*, *Anammoxoglobus*, *Jettenia*, and *Scalindua*

# Anammox

- Like classical nitrifying bacteria, anammox bacteria are also autotrophs.
- Grow with  $\text{CO}_2$  as their sole carbon source and use  $\text{NO}_2^-$  as an electron donor to produce cell material:
- $\text{CO}_2 + 2 \text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + 2 \text{NO}_3^-$



Marc Strous

Phase-contrast photomicrograph of cells of *Brocadia anammoxidans*. A single cell is about 1  $\mu\text{m}$  in diameter

In nature, the source of  $\text{NO}_2^-$  in the anammox reaction is presumably the aerobic ammonia-oxidizing bacteria.

- Two groups of ammonia oxidizers, nitrifiers (aerobic) and Anammox bacteria (anaerobic), live together in ammonia-rich habitats.
- The suspended particles in these habitats contain both oxic and anoxic zones in which ammonia oxidizers of different physiologies can coexist in close association.

# Autotrophy and Nitrogen Fixation

## Major Biosyntheses: Autotrophy and Nitrogen Fixation

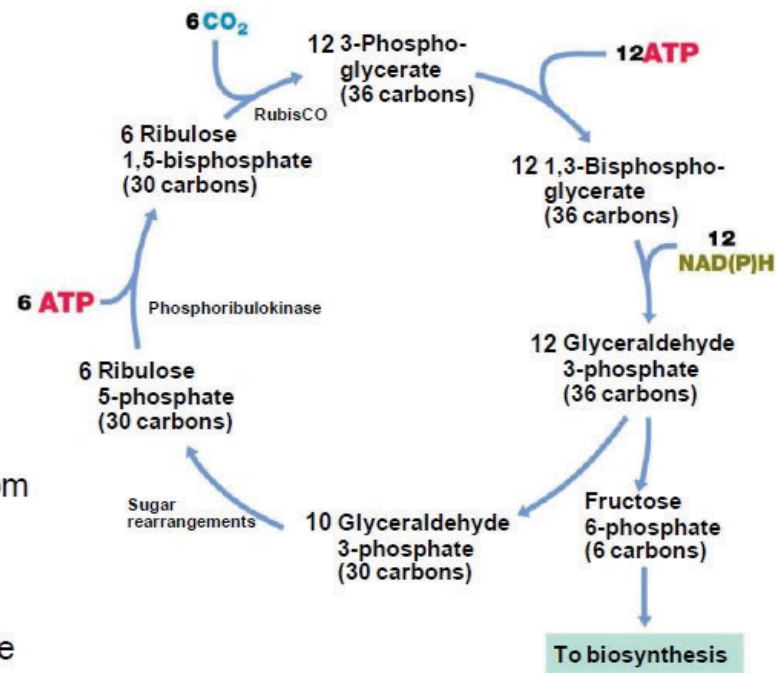
- The Calvin Cycle
- Other Autotrophic Pathways in Phototrophs
- Nitrogen Fixation and Nitrogenase
- Genetics and Regulation of Nitrogen Fixation

# The Calvin Cycle

## The Calvin cycle

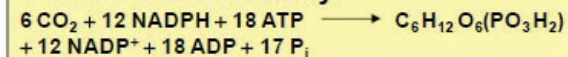
- Named for its discoverer Melvin Calvin
- Fixes CO<sub>2</sub> into cellular material for autotrophic growth
- Requires NADPH, ATP, ribulose biphosphate carboxylase (RubisCO), and phosphoribulokinase
- 6 molecules of CO<sub>2</sub> are required to make 1 molecule of glucose

The Calvin cycle



Shown is the production of one hexose molecule from CO<sub>2</sub>. For each six molecules of CO<sub>2</sub> incorporated, one fructose 6-phosphate is produced

### Overall stoichiometry:



# Other Autotrophic Pathways in Phototrophs

- Green sulfur bacteria use the reverse citric acid cycle to fix CO<sub>2</sub>
- Green nonsulfur bacteria use the hydroxypropionate pathway to fix CO<sub>2</sub>

# Nitrogenase and Nitrogen Fixation

- Utilization of  $N_2$  in cell synthesis is called **nitrogen fixation**.
- No eukaryotic organisms are known that fix  $N_2$
- Only certain prokaryotes can fix  $N_2$
- Some nitrogen fixers are free-living and others are symbiotic
- Reaction is catalyzed by **nitrogenase**
- Sensitive to the presence of oxygen
- A wide variety of nitrogenases use different metal cofactors
- In nitrogen fixation,  $N_2$  is first reduced to  $NH_3$  and then assimilated into organic forms, such as amino acids and nucleotides.
- It is of enormous agricultural importance, supporting the nitrogen needs of key crops, such as soybeans.

# Nitrogenase and Nitrogen Fixation

- Different physiological types of prokaryotes can fix nitrogen, including several that live in extreme environments.
- Below 0 °C and as high as 92 °C
- At pH 2 and pH 10
- Few microbial environments would be off limits to nitrogen-fixing bacteria.

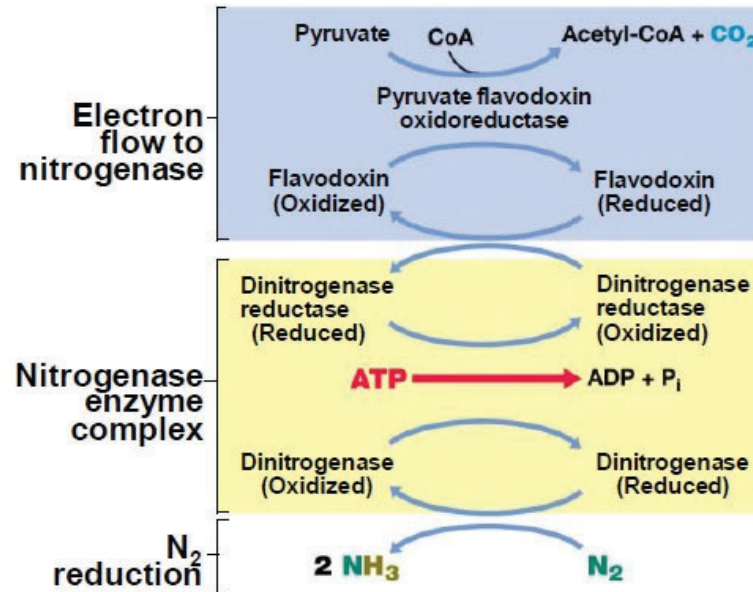
# Nitrogenase and Nitrogen Fixation

➤ Electron Flow in Nitrogen Fixation

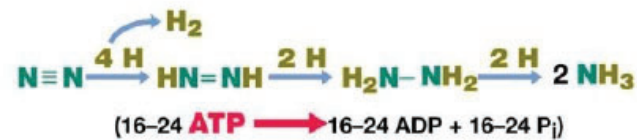
Electron donor  $\longrightarrow$  dinitrogenase reductase  $\longrightarrow$  dinitrogenase  $\longrightarrow$   $N_2$

➤ Ammonia is the final product

Nitrogenase function



(a)



(b)

# Free and Symbiotic Nitrogen Fixers

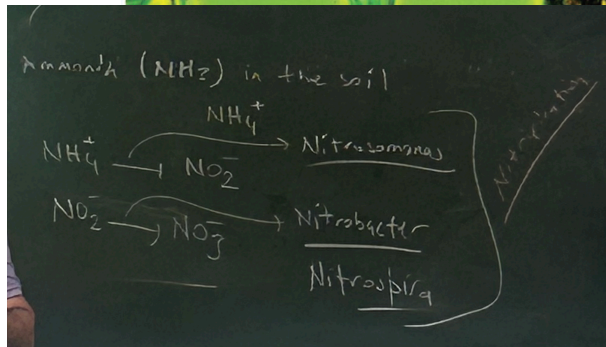
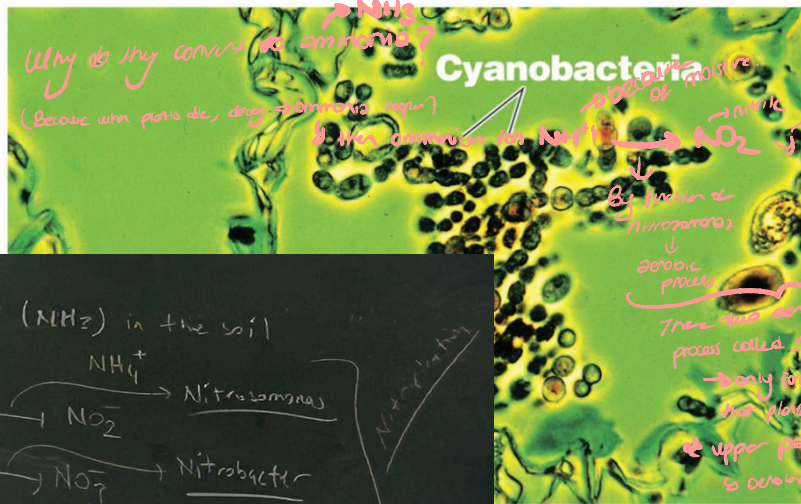
79% of the nitrogen in the air → not available for eukaryotic cells, so eukaryotic cells can not get nitrogen directly only way → prokaryotic cells

## Free Nitrogen Fixers

Free N<sub>2</sub> fixer (non-symbiotic) → they fix atmospheric N<sub>2</sub>.

- In rhizosphere
  - *Azotobacter*
  - *Beijerinckia*
  - *Clostridium pasteurianum*
  - Cyanobacteria: **heterocysts**

forms some cells more thick cell wall. → change their structure. have very thick walls → they protect their nitrogen.

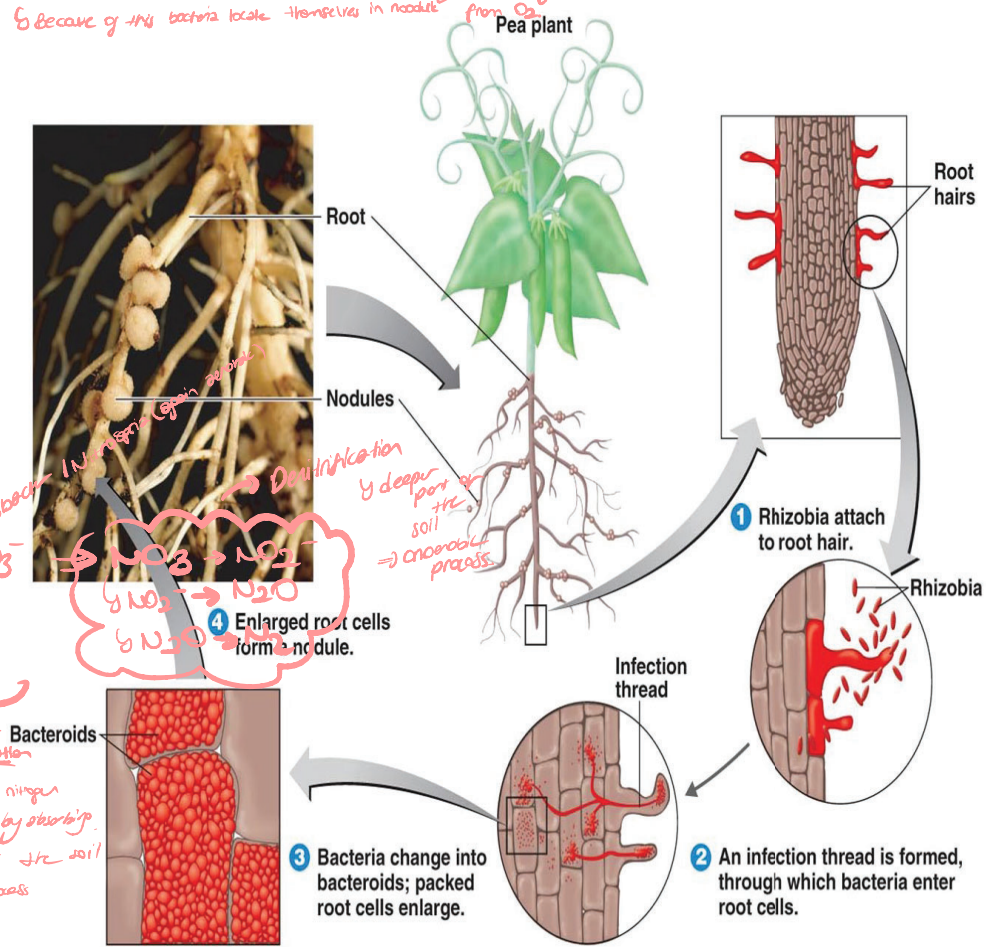


## Symbiotic Nitrogen Fixers

on the root of the legume (bacteria) (beans, peas, vicia)

structure of nodule → bacteria accumulate in which? → *Rhizobium* (leafy group) → *Bradyrhizobium* (slim group)

Nitrogen fixation (Enzyme: Nitrogenase) → to protect oxygen from pea plant



LM 10 μm

