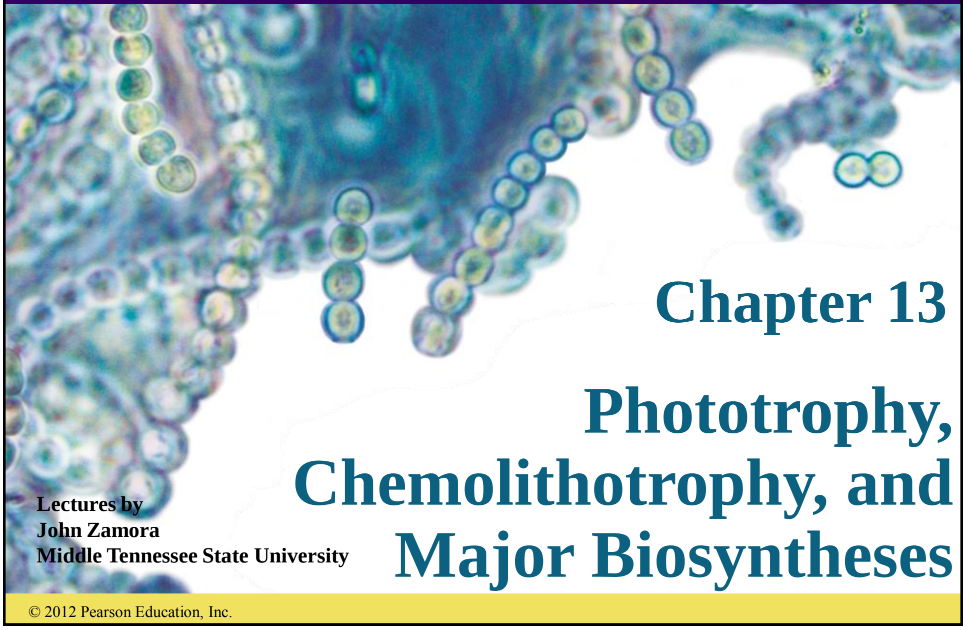
LECTURE PRESENTATIONS
For BROCK BIOLOGY OF MICROORGANISMS, THIRTEENTH EDITION
Michael T. Madigan, John M. Martinko, David A. Stahl, David P. Clark



Chapter 13

Phototrophy, Chemolithotrophy, and Major Biosyntheses

Lectures by
John Zamora
Middle Tennessee State University

Phototrophy, Chemolithotrophy and Major Biosyntheses

- This chapter focuses on metabolic diversity of:
  - *phototrophs*; use light as energy source,
  - *chemolithotrophs*; use inorganic compounds
- Two important biosyntheses are discussed:
  - *Autotrophy*, fixation of carbon dioxide (CO_2) into cell material, and
  - *Nitrogen fixation*, reduction of atmospheric nitrogen (N_2) to ammonia (NH_3) to supply the cell's nitrogen requirements.

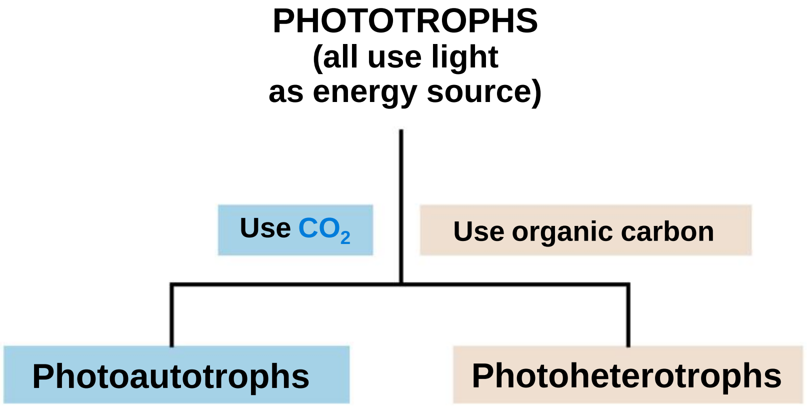
I. Phototrophy

- 13.1 Photosynthesis
- 13.2 Chlorophylls and Bacteriochlorophylls
- 13.3 Carotenoids and Phycobilins
- 13.4 Anoxygenic Photosynthesis
- 13.5 Oxygenic Photosynthesis

13.1 Photosynthesis

- *Photosynthesis* is the conversion of light energy to chemical energy
  - *Phototrophs* carry out photosynthesis (Figure 13.1)
  - Most phototrophs are also *autotrophs*
- Photosynthesis requires light-sensitive pigments called *chlorophylls*
- Photoautotrophy requires ATP production and CO_2 reduction

Figure 13.1 Classification of phototrophic organisms in terms of energy and carbon sources.



PHOTOTROPHS
(all use light
as energy source)

Use CO_2

Use organic carbon

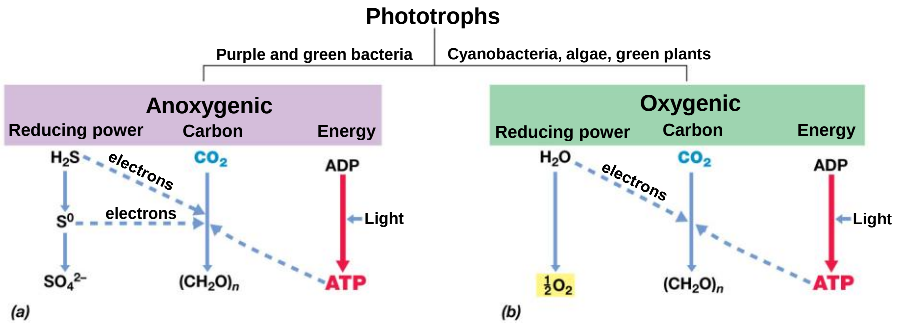
Photoautotrophs

Photoheterotrophs

13.1 Photosynthesis

- Photoautotrophy
  - – Oxidation of H_2O produces O_2 (oxygenic photosynthesis; Figure 13.2)
  - – Oxygen not produced (anoxygenic photosynsthesis; Figure 13.2)

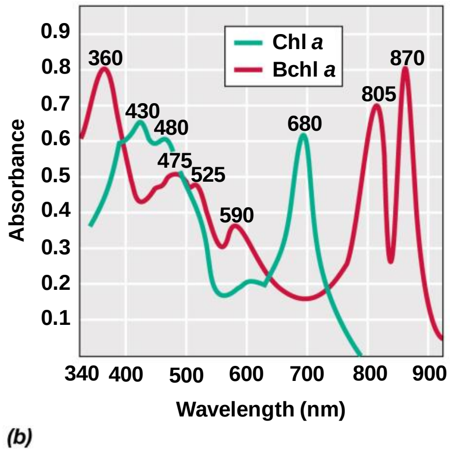
Figure 13.2 Patterns of photosynthesis.



13.2 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 (Figure 13.3b)

Figure 13.3b Spectra of chlorophyll a and bacteriochlorophyll a

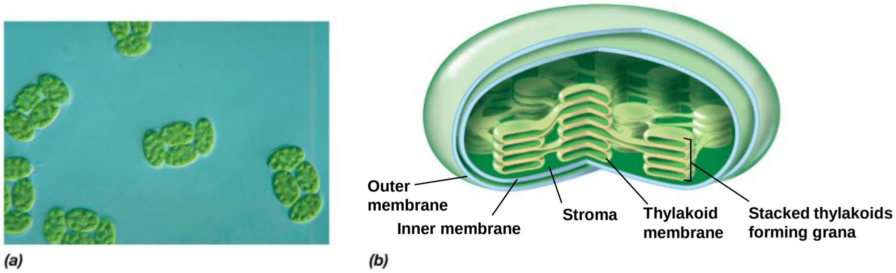


- Absorption spectrum (green curve) of cells of the green alga *Chlamydomonas*.
- The peaks at 680 and 430 nm are due to chlorophyll a, and the peak at 480 nm is due to carotenoids.
- Absorption spectrum (red curve) of cells of the phototrophic purple bacterium *Rhodopseudomonas palustris*.
- Peaks at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

13.2 Chlorophylls and Bacteriochlorophylls

- Cyanobacteria produce chlorophyll *a* (Figure 13.4)
- Prochlorophytes (oxygenic phototrophs) produce chlorophyll *a* and *b*
- Anoxygenic phototrophs produce bacteriochlorophylls
- Chlorophyll pigments are located within special membranes
  - In eukaryotes, called *thylakoids* (Figure 13.5)
  - In prokaryotes, pigments are integrated into cytoplasmic membrane

Figure 13.5 The chloroplast



(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.

13.2 Chlorophylls and Bacteriochlorophylls

- *Reaction centers* participate directly in the conversion of light energy to ATP
- *Antenna pigments* funnel light energy to reaction centers
- *Chlorosomes* function as massive antenna complexes
  - Found in green sulfur bacteria and green nonsulfur bacteria

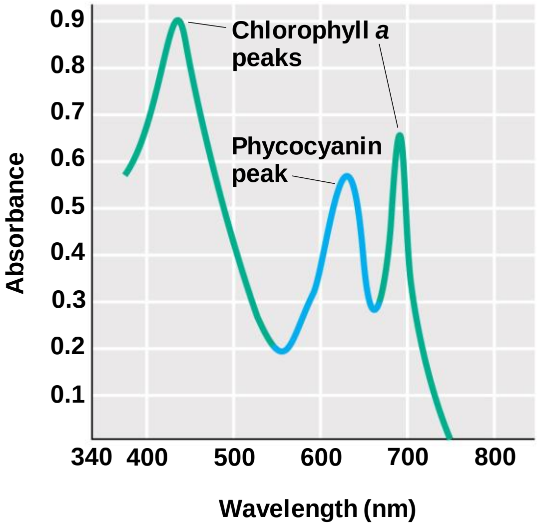
13.3 Carotenoids and Phycobilins

- 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

13.3 Carotenoids and Phycobilins

- *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 (Figure 13.11)

Figure 13.11 Absorption spectrum of a cyanobacterium that contains phycocyanin as an accessory pigment



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

13.4 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

13.4 Anoxygenic Photosynthesis

- Reducing power for CO_2 fixation comes from reductants (e^- donor) present in the environment (i.e., H_2S, Fe^{2+}, or NO_2^-)
  - Requires reverse electron transport for NADH production in purple phototrophs
  - Electrons are transported in the membrane through a series of proteins and cytochromes

---

13.4 Anoxygenic Photosynthesis

- For a purple bacterium (Figure 13.16) to grow autotrophically, the formation of ATP is not enough

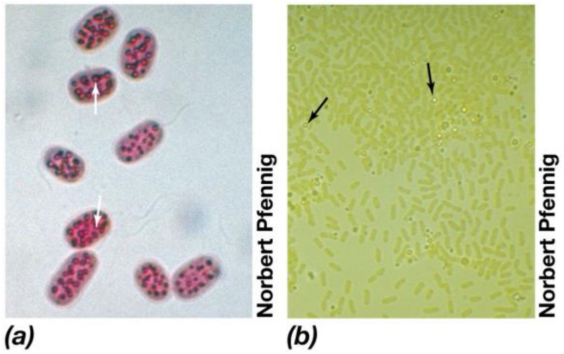


Figure 13.16 Phototrophic purple and green sulfur bacteria

(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.

13.5 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*

II. Chemolithotrophy

- 13.6 The Energetics of Chemolithotrophy
- 13.7 Hydrogen Oxidation
- 13.8 Oxidation of Reduced Sulfur Compounds
- 13.9 Iron Oxidation
- 13.10 Nitrification
- 13.11 Anammox

13.6 The Energetics of Chemolithotrophy

- *Chemolithotrophs* are organisms that obtain energy from the oxidation of inorganic compounds
- Although lacking photosynthetic pigments, they use CO_2 as their sole carbon source
- Most chemolithotrophic bacteria are also autotrophs.
- *Mixotrophs* are chemolithotrophs that require organic carbon as a carbon source

13.6 The Energetics of Chemolithotrophy

- Many sources of reduced molecules exist in the environment
- Volcanic activity is a major source of reduced sulfur compounds, primarily H_2S and 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 H_2S, H_2, NH_3
- The oxidation of different reduced compounds yields varying amounts of energy

13.6 The Energetics of Chemolithotrophy

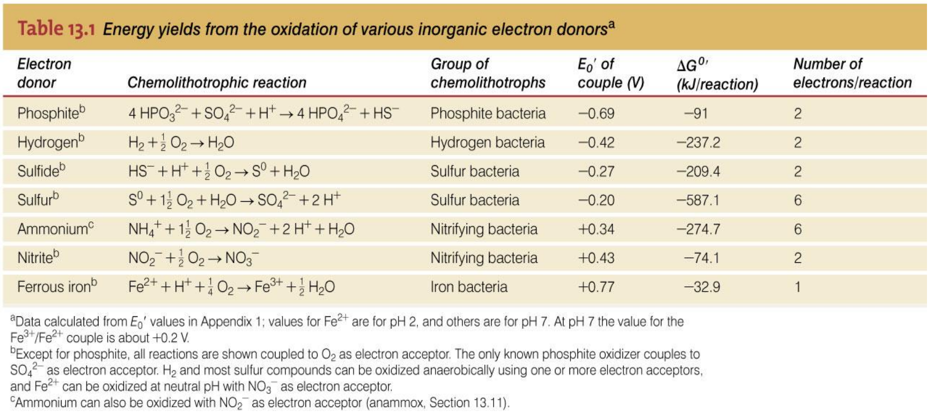


Table 13.1 *Energy yields from the oxidation of various inorganic electron donors*[a]

Electron donor	*Chemolithotrophic reaction*	*Group of chemolithotrophs*	*E_0' of couple (V)*	*$\Delta G^{0\prime}$ (kJ/reaction)*	*Number of electrons/reaction*
Phosphite[b]	$4\ HPO_3^{2-} + SO_4^{2-} + H^+ \rightarrow 4\ HPO_4^{2-} + HS^-$	Phosphite bacteria	−0.69	−91	2
Hydrogen[b]	$H_2 + \frac{1}{2}\ O_2 \rightarrow H_2O$	Hydrogen bacteria	−0.42	−237.2	2
Sulfide[b]	$HS^- + H^+ + \frac{1}{2}\ O_2 \rightarrow S^0 + H_2O$	Sulfur bacteria	−0.27	−209.4	2
Sulfur[b]	$S^0 + 1\frac{1}{2}\ O_2 + H_2O \rightarrow SO_4^{2-} + 2\ H^+$	Sulfur bacteria	−0.20	−587.1	6
Ammonium[c]	$NH_4^+ + 1\frac{1}{2}\ O_2 \rightarrow NO_2^- + 2\ H^+ + H_2O$	Nitrifying bacteria	+0.34	−274.7	6
Nitrite[b]	$NO_2^- + \frac{1}{2}\ O_2 \rightarrow NO_3^-$	Nitrifying bacteria	+0.43	−74.1	2
Ferrous iron[b]	$Fe^{2+} + H^+ + \frac{1}{4}\ O_2 \rightarrow Fe^{3+} + \frac{1}{2}\ H_2O$	Iron bacteria	+0.77	−32.9	1

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

[b] Except for phosphite, all reactions are shown coupled to O_2 as electron acceptor. The only known phosphite oxidizer couples to SO_4^{2-} as electron acceptor. H_2 and most sulfur compounds can be oxidized anaerobically using one or more electron acceptors, and Fe^{2+} can be oxidized at neutral pH with NO_3^- as electron acceptor.

[c] Ammonium can also be oxidized with NO_2^- as electron acceptor (anammox, Section 13.11).

13.7 Hydrogen Oxidation

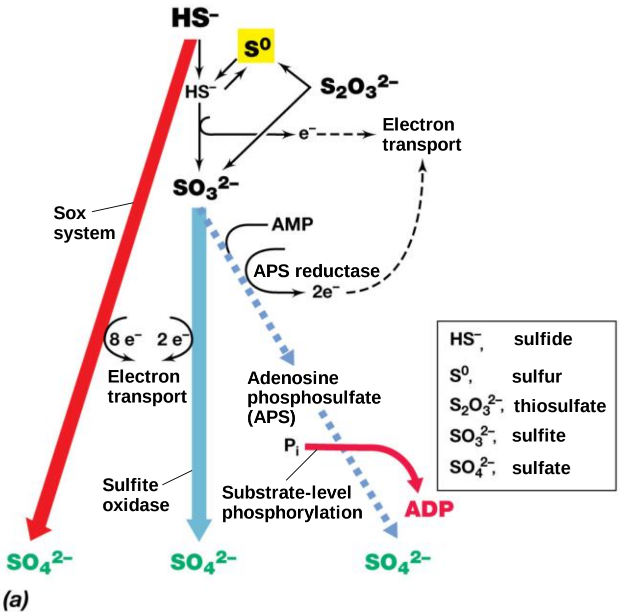
- Anaerobic H_2-oxidizing *Bacteria* and *Archaea* are known
- Catalyzed by *hydrogenase*
- Calvin cycle and hydrogenase enzymes allow chemolithotrophic growth

13.8 Oxidation of Reduced Sulfur Compounds

- Many reduced sulfur compounds are used as electron donors (Figure 13.22a)
- Discovered by *Sergei Winogradsky*
- H_2S, S^0, $S_2O_3^-$ are commonly used
- One product of sulfur oxidation is H^+, which lowers of 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

---

Figure 13.22a Oxidation of reduced sulfur compounds by sulfur chemolithotrophs

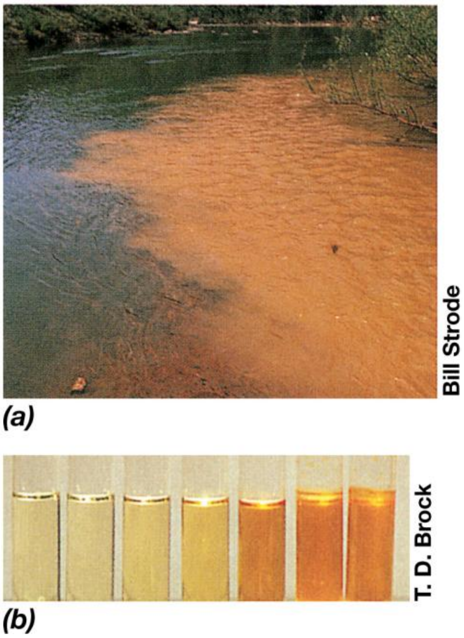


13.9 Iron Oxidation

- Ferrous iron (Fe^{2+}) oxidized to ferric iron (Fe^{3+})
- Ferric hydroxide precipitates in water
- Many Fe oxidizers can grow at $pH < 1$
  - Often associated with acidic pollution from coal mining activities (Figure 13.23)
- Some anoxygenic phototrophs can oxidize Fe^{2+} anaerobically using Fe^{2+} as an electron donor for CO_2 reduction

---

Figure 13.23 Iron-oxidizing bacteria



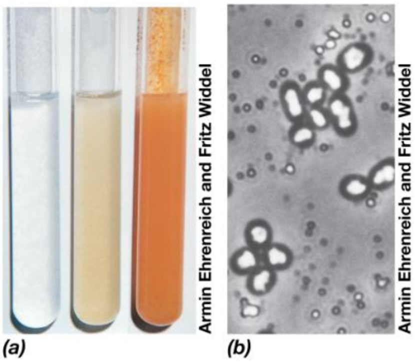
Bill Strode

T. D. Brock

(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 Fe^{2+}. At low pH values, Fe^{2+} does not oxidize spontaneously in air, but *Acidithiobacillus ferrooxidans carries out the* oxidation; insoluble $Fe(OH)_3$ 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 Fe^{3+}, which readily complexes to form $Fe(OH)_3$ and protons.

13.9 Iron Oxidation

- Ferrous iron can be oxidized under anoxic conditions by certain anoxygenic phototrophic bacteria (Figure 13.25)



(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.

Figure 13.25 Fe2+ oxidation by anoxygenic phototrophic bacteria

13.10 Nitrification

- NH_3 and 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 NH_3 to nitrite (NO_2^-), and another group (e.g. *Nitrobacter* and *Nitrospira*) oxidizes NO_2^- to NO_3^-.
- Key enzymes are *ammonia monooxygenase*, *hydroxylamine oxidoreductase,* and *nitrite oxidoreductase*

13.10 Nitrification

- 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 CO_2.
- Only small energy yields from this reaction
  - Growth of nitrifying bacteria is very slow

13.10 Nitrification

- Ammonia-oxidizing bacteria
  - NH_3 is oxidized by *ammonia monooxygenase* producing NH_2OH and H_2O
  - *Hydroxylamine oxidoreductase* then oxidizes NH_2OH to NO_2^-
  - Electrons and protons used to generate ATP

13.10 Nitrification

- Nitrite-oxidizing bacteria
  - Nitrite (NO_2^-) is oxidized by enzyme *nitrite oxidoreductase* to nitrate (NO_3^-)
  - Electrons and protons used to generate ATP

13.10 Nitrification

- Nitrifying bacteria play key ecological roles in the nitrogen cycle, converting NH_3 into NO_3^-, a key plant nutrient.
- Also important in sewage and wastewater treatment, removing toxic amines and NH_3 and releasing less toxic nitrogen compounds.
- NH_3 produced in the sediments from the decomposition of organic nitrogenous compounds is oxidized to NO_3^-, a more favorable N-source for algae and cyanobacteria.

13.11 Anammox

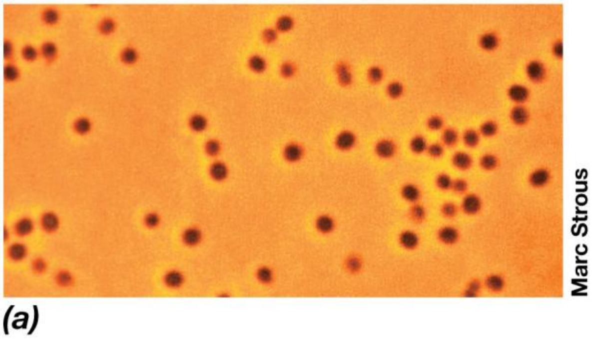
- *Anammox*: anoxic ammonia oxidation
  - Performed by unusual group of obligate aerobes (Figure 13.28a)
  - *Anammoxosome* is compartment where anammox reactions occur
    - Protects cell from reactions occurring during anammox
    - *Hydrazine* is an intermediate of anammox
- Anammox is very beneficial in the treatment of sewage and wastewater

13.11 Anammox

- Ammonia is oxidized with NO_2^- as the electron acceptor to yield N_2 as follows:

  $NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$ $\Delta G^{0\prime} = -357$ 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*.

Figure 13.28a Anammox



Phase-contrast photomicrograph of cells of *Brocadia anammoxidans.* A single cell is about 1 m in diameter

13.11 Anammox

- Like classical nitrifying bacteria, anammox bacteria are also autotrophs.
- Grow with CO_2 as their sole carbon source and use NO_2^- as an electron donor to produce cell material:

$$CO_2 + 2\ NO_2^- + H_2O \rightarrow CH_2O + 2\ NO_3^-$$

13.11 Anammox

- In nature, the source of NO_2^- in the anammox reaction is presumably the aerobic ammonia-oxidizing bacteria.
- Two groups of ammonia oxidizers, nitrifiers (aerobic) and Anammox bacteria (anerobic), 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.

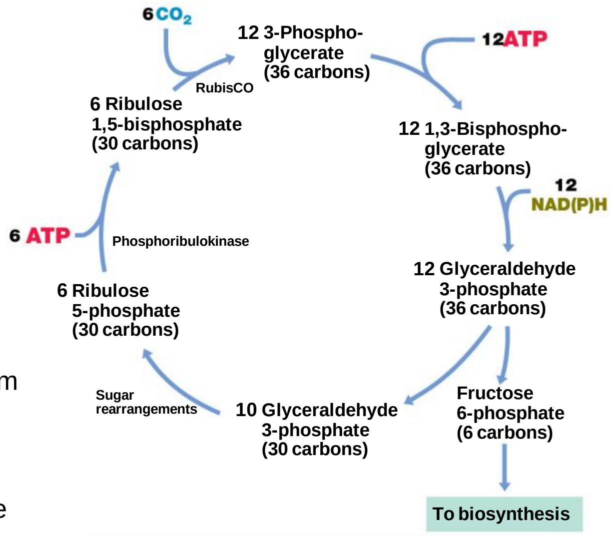
III. Major Biosyntheses: Autotrophy and Nitrogen Fixation

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

13.12 The Calvin Cycle

- *The Calvin cycle*
  - Named for its discoverer Melvin Calvin
  - Fixes CO_2 into cellular material for autotrophic growth
  - Requires *NADPH, ATP, ribulose bisphophate carboxylase (RubisCO),* and *phosphoribulokinase*
  - 6 molecules of CO_2 are required to make 1 molecule of glucose (Figure 13.30)

Figure 13.30 The Calvin cycle



6 CO_2

12 3-Phospho-glycerate (36 carbons)

12 ATP

RubisCO

6 Ribulose 1,5-bisphosphate (30 carbons)

12 1,3-Bisphospho-glycerate (36 carbons)

12 NAD(P)H

6 ATP

Phosphoribulokinase

12 Glyceraldehyde 3-phosphate (36 carbons)

6 Ribulose 5-phosphate (30 carbons)

Sugar rearrangements

10 Glyceraldehyde 3-phosphate (30 carbons)

Fructose 6-phosphate (6 carbons)

To biosynthesis

Shown is the production of one hexose molecule from CO_2. For each six molecules of CO_2 incorporated, one fructose 6-phosphate is produced

Overall stoichiometry:

$$6\,CO_2 + 12\,NADPH + 18\,ATP \longrightarrow C_6H_{12}O_6(PO_3H_2) + 12\,NADP^+ + 18\,ADP + 17\,P_i$$

13.13 Other Autotrophic Pathways in Phototrophs

- Green sulfur bacteria use the *reverse citric acid cycle* to fix CO_2
- Green nonsulfur bacteria use the *hydroxypropionate pathway* to fix CO_2

13.14 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

13.14 Nitrogenase and Nitrogen Fixation

- 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.

13.14 Nitrogenase and Nitrogen Fixation

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

13.14 Nitrogenase and Nitrogen Fixation

- Electron Flow in Nitrogen Fixation (Figure 13.34)
  - Electron donor → dinitrogenase reductase → dinitrogenase → N_2
  - Ammonia is the final product

Figure 13.34 Nitrogenase function