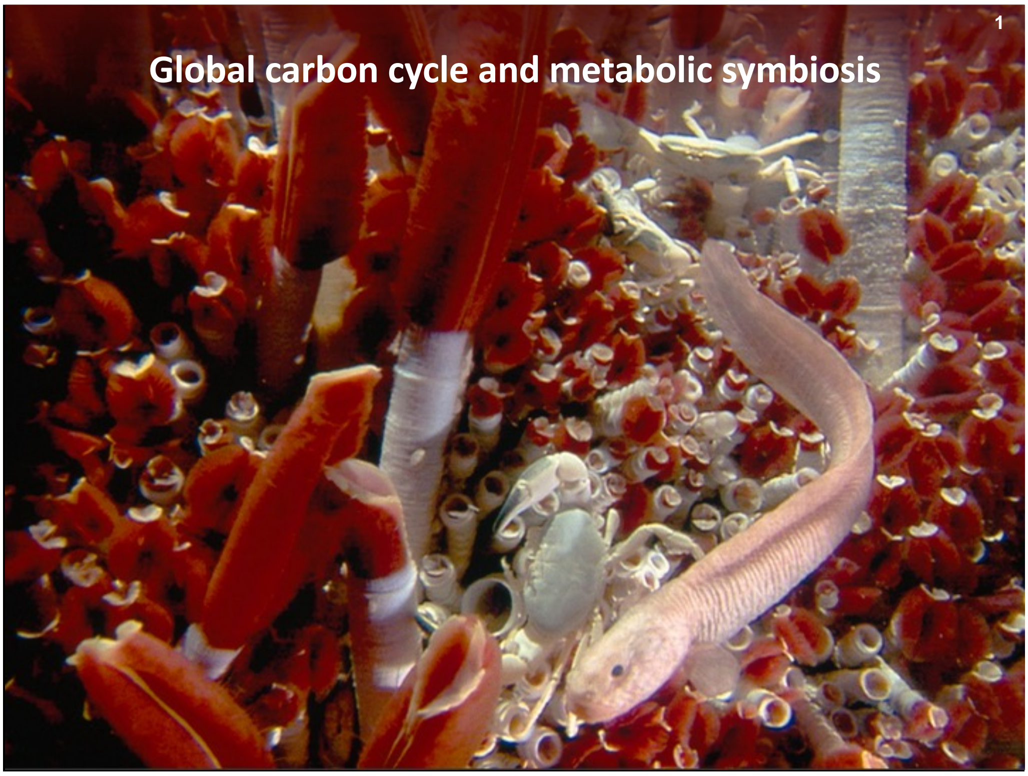


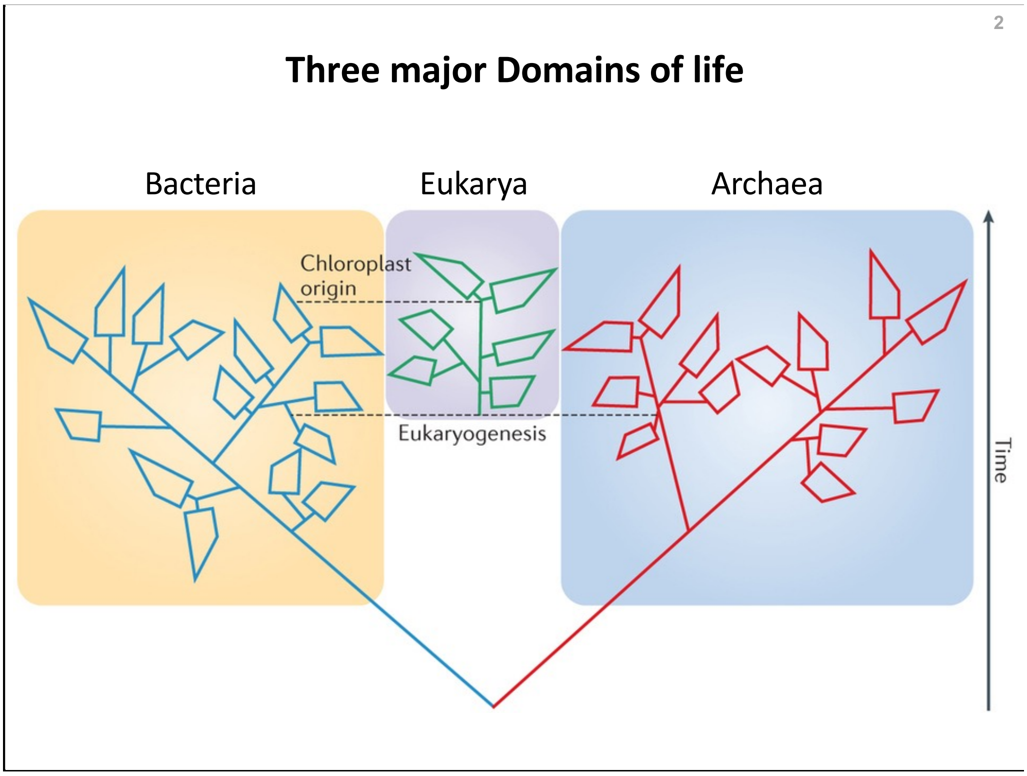
SOURCE: http://photography.nationalgeographic.com/photography/wallpaper/deep-sea-tubeworms-photography.html

SOURCE: http://environment.nationalgeographic.com/environment/habitats/deep-sea-vents/

SOURCE: http://news.nationalgeographic.com/news/2002/10/1028_021028_TVtubeworm.html

“Deep-sea hydrothermal vent giant tubeworms. With the help of high-intensity lighting, high-resolution cameras, and the deep-sea submarine *Alvin*, Kristof and his crew captured dramatic imagery in the dark, superheated depths of the Mid-Atlantic Ridge. At these nadirs, chemosynthesis - the thermal and chemical energy given off by deep-sea vents - gives life to creatures like the eelpout fish and tubeworms pictured here.”

Along the Galápagos Rift is a hotbed of volcanoes and hydrothermal vents that spew boiling soups of metals, salts, and poisonous gas. These seemingly inhospitable vents are home to some of the toughest, most exotic life on Earth, including clams and mussels as big as dinner plates and bright-white tubeworms two meters long. The giant tubeworms, discovered during a path-breaking exploration of the rift in **1977**, forced a rethinking of life on the ocean floor. The rift lies 500 kilometers north of the Galápagos Islands, and the seafloor extends 2,500 meters below the waves. "The rule of thumb was that only small organisms could occupy the deep sea," says Tim Shank, a biologist at the Woods Hole Oceanographic Institution in Massachusetts and co-director of a recent expedition to the Galápagos Rift organized to mark the 25th anniversary of the hydrothermal vent discovery. "We knew there were microbial communities that could survive without sunlight or oxygen, but tubeworms are massive life-forms," he added. The recent expedition focused primarily on tubeworms, which have developed unique adaptations to their environment. These worms live in pitch-black ocean depths in water laced with acid and toxic gas – harsh conditions that may resemble those in which life first evolved.



SOURCE: McInerney JO, O'Connell MJ, Pisani D (2014) The hybrid nature of the Eukaryota and a consilient view of life on Earth. *Nature Rev Microbiol* 12(6): 449-455 PMID: 24814066.

Figure 2. Schematic representation of the flow of genetic material from the two major prokaryotic groups into the base of the eukaryotes and the separate flow of genetic material from cyanobacteria into chloroplast-containing eukaryotes.

Symbiosis: different forms of intimacy

”Living together of two dissimilar organisms, usually in intimate association, and usually to the benefit of at least one of them."
- Heinrich Anton de Bary (1879)

Parasitism	the symbiont benefits the host is harmed
Commensalism	the symbiont benefits the host is not harmed
⇨ **Mutualism**	the symbiont benefits the host also benefits

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019.

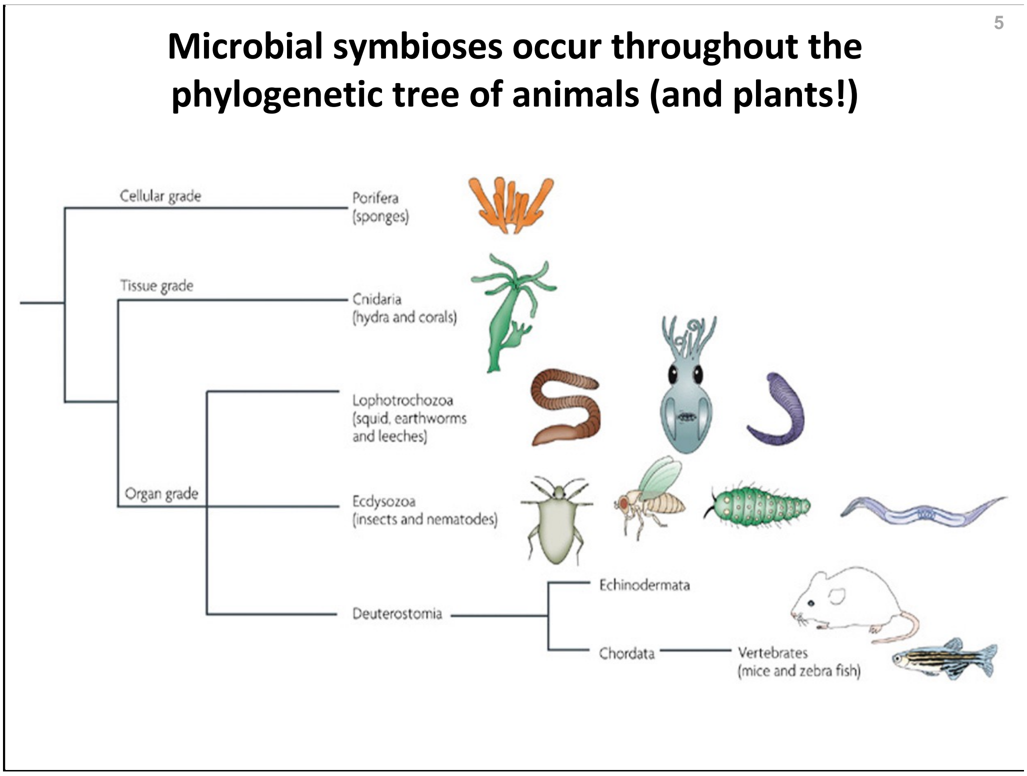
SOURCE: Relman DA (2008) 'Til death do us part': coming to terms with symbiotic relationships. *Nature Rev Microbiol* 6(10): 721-724 PMID: 19086265.

Symbiosis: different degrees of intimacy

Epibiotic (local or diffuse)	The symbiont is located on the host surface and does not penetrate into the tissue
Endobiotic (extracellular)	The symbiont is located inside the host tissue but is outside the host cell cytoplasm
Endobiotic (intracellular)	The symbiont is located inside the host tissue and is inside the host cell cytoplasm
Endobiotic (organellar)	The symbiont is fully integrated into host cells (e.g., mitochondria and chloroplasts)

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019.

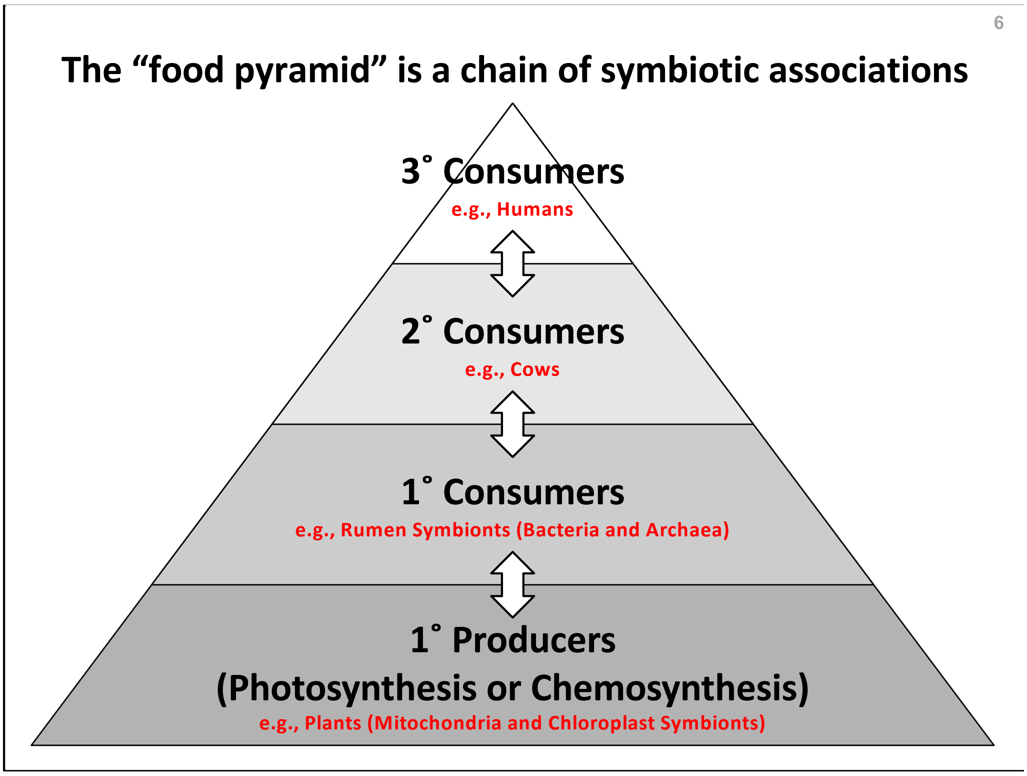
SOURCE: Relman DA (2008) 'Til death do us part': coming to terms with symbiotic relationships. *Nature Rev Microbiol* 6(10): 721-724 PMID: 19086265.



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Ruby EG (2008) Symbiotic conversations are revealed under genetic interrogation. *Nature Rev Microbiol* 6(10): 752-762 PMID: 18794913.

Figure 1. Microbial symbioses occur throughout the phylogeny of animals. Experimentally accessible associations occur in all the main phylogenetic groups. These associations span the breadth of animal diversity, and are represented in cellular-grade, tissue-grade, and organ-grade levels of developmental and morphological complexity.



A **"food pyramid"** or **"energy pyramid"** is a graphical model of energy flow in a community. The different levels represent different groups of organisms that might compose a "generic" food chain. From the bottom-up, they are as follows:

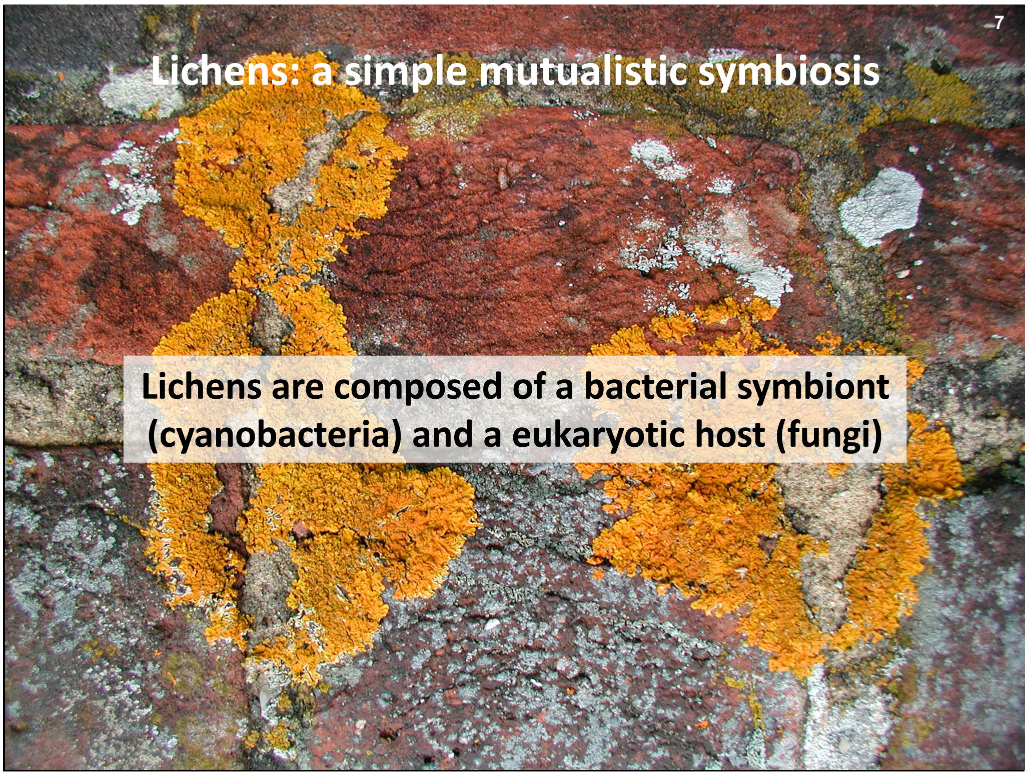
Producers — bring energy from nonliving sources (e.g., sunlight, chemicals) into the community

Primary consumers — eat the producers, which makes them herbivores in most communities

Secondary consumers — eat the primary consumers, which makes them carnivores

Tertiary consumers — eat the secondary consumers

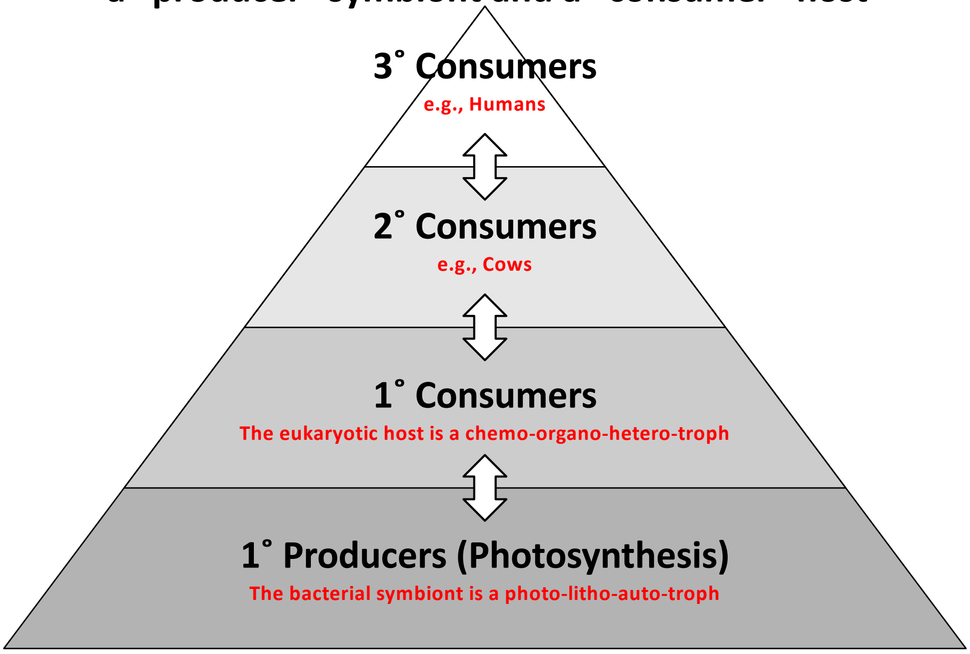
In some food chains, there is a fourth consumer level and even (rarely) a fifth. Typically, about 10% of the energy is transferred from one trophic level to the next, thus preventing a large number of consumer levels. Energy pyramids are necessarily upright in healthy ecosystems, that is, there must always be more energy available at a given level of the pyramid to support the energy and biomass requirement of the next level.



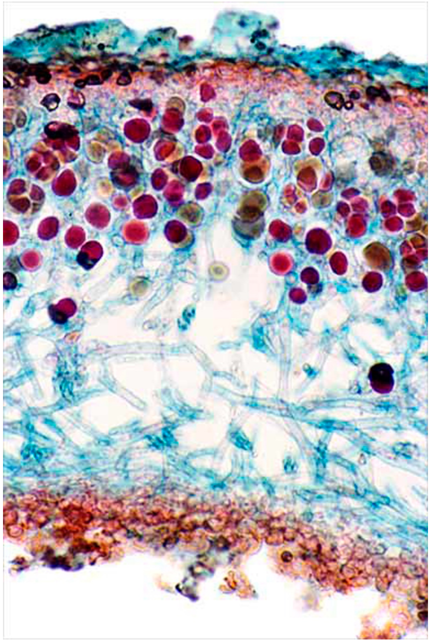
SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019.

IMAGE SOURCE: http://upload.wikimedia.org/wikipedia/commons/7/77/N2_Lichen.jpg

Lichens are a mutualistic symbiosis between a “producer” symbiont and a “consumer” host



Structure of a lichen (cross-section)



Outer cortex (fungus)
protects from intense sunlight and dessication

Phototrophic layer (cyanobacteria)
carbon and nitrogen fixation

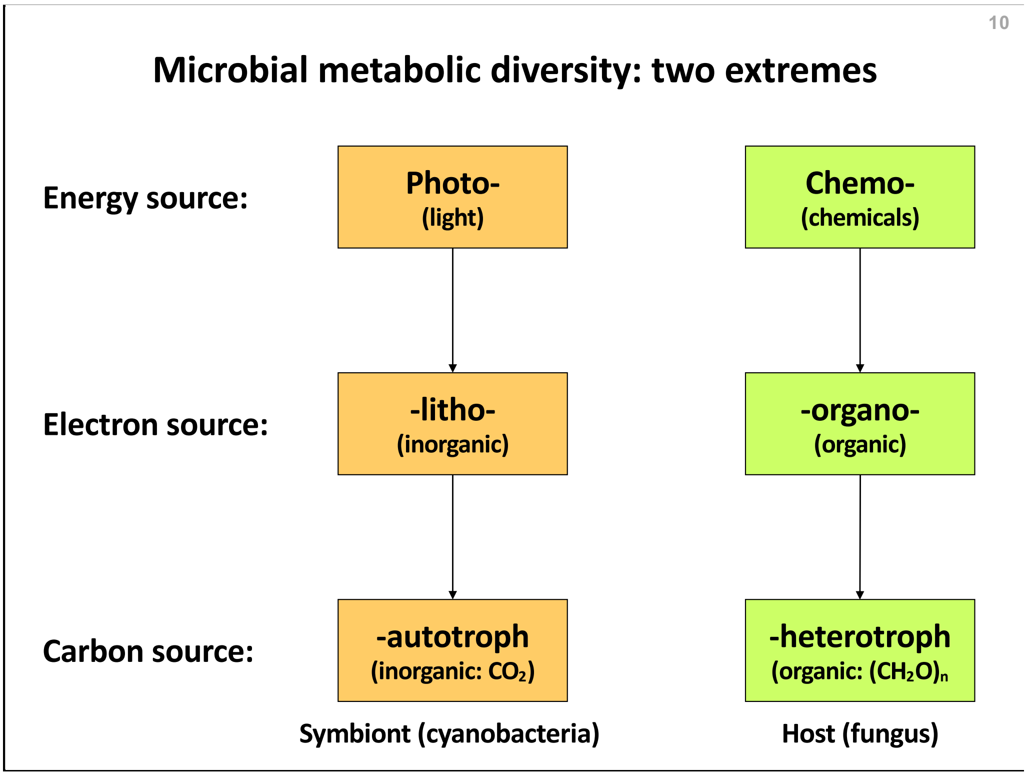
Medulla (fungus)
uptake and transfer of inorganic nutrients and water

Inner cortex (fungus)
attachment to substratum via 'rhizinae'

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 23: Microbial Symbioses with Microbes, Plants, and Animals (pp. 732-764), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: http://www.biology.ed.ac.uk/research/groups/jdeacon/FungalBiology/lichen.htm

Figure 2. When we look at a cross-section of one of the lichen lobes we see a clear zonation of the tissues. There is an **outer cortex** of tightly packed fungal cells, a **phototrophic layer** beneath this containing cyanobacteria or algae, then a more loosely arranged **medulla** with air spaces, an **inner cortex**, and structures termed **rhizinae**, which serve to attach the lichen to a rock surface. A similar arrangement of the tissues is found in most lichens and is functionally significant. The photosynthetic cells are protected from exposure to severe sunlight by the surface tissues, and the air spaces (resulting from the surrounding hydrophobic fungal hyphae) allow gaseous exchange.



SOURCE: Karl DM (2007) Microbial oceanography: paradigms, processes and promise. *Nature Rev Microbiol* 5(10): 759-769 PMID:17853905.

Energy Source. Phototrophs use light as the primary energy source. Chemotrophs use chemicals (inorganic, organic, or both) as the primary energy source.

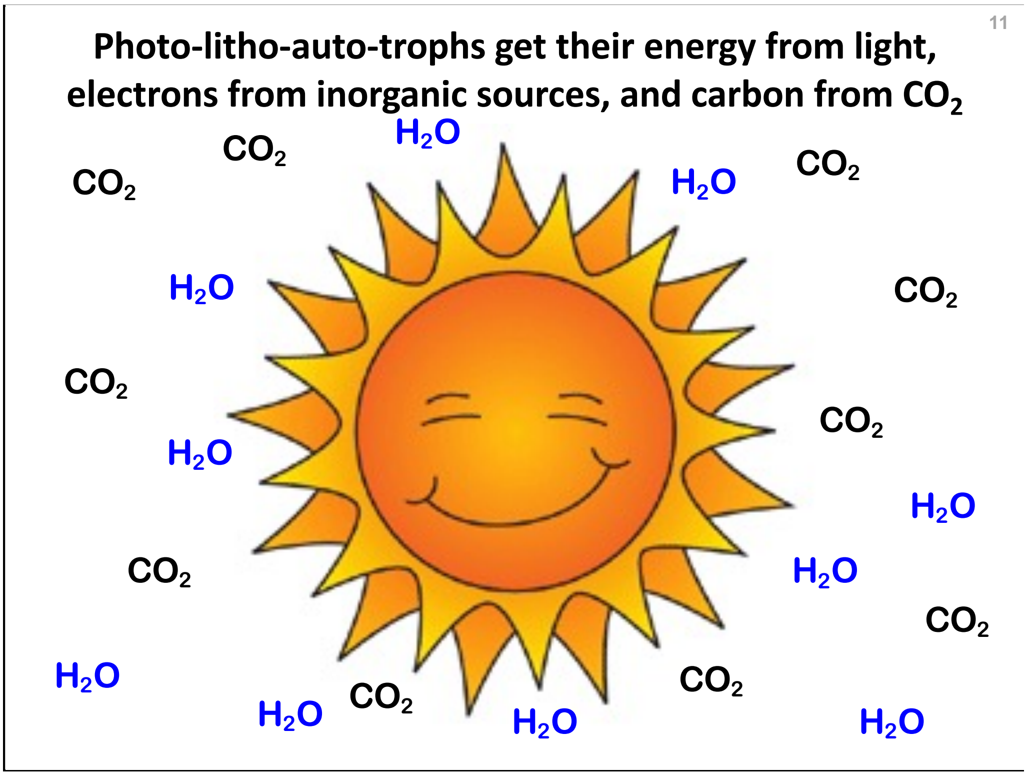
Electron Source. Lithotrophs use inorganic compounds as the electron source. Organotrophs use organic compounds as the electron source.

Carbon Source. Autotrophs use an inorganic compound (CO_2 or HCO_3^-) as the carbon source. Heterotrophs use organic compounds $(CH_2O)_n$ as the carbon source.

Photolithoautotrophs (photo-litho-auto-trophs) represent one metabolic extreme. They use light as the energy source, inorganic compounds (e.g., H_2O or H_2S) as the electron source, and CO_2 (inorganic compound) as the carbon source.

Chemoorganoheterotrophs (chemo-organo-hetero-trophs) represent the other metabolic extreme. They use chemicals as the energy source, organic compounds (e.g., sugars) as the electron source, and $(CH_2O)_n$ (organic compounds) as the carbon source. Often, a single organic compound (e.g., glucose) serves as the source of both electrons and carbon.

Between these two extremes (photolithoautotrophy and chemoorganoheterotrophy) all combinations are possible, as indicated by the arrows. Take home lesson: microbial metabolism is highly modular and highly diverse!



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Photo-litho-auto-trophs use:

light as the energy source (photo-),

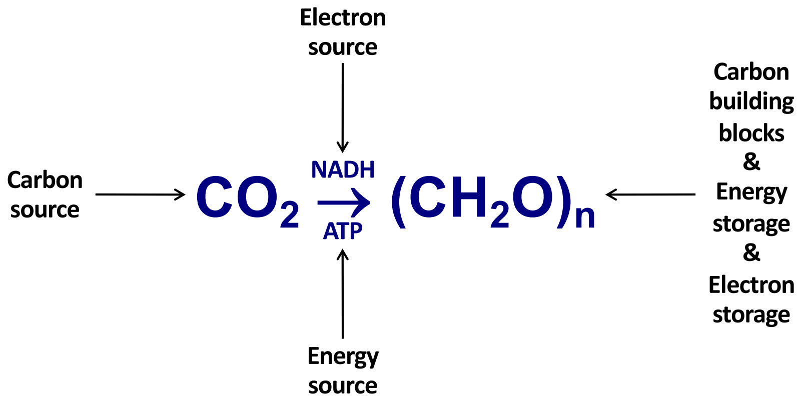
inorganic molecules (e.g., H_2O or H_2S) as the electron source (-litho-),

and inorganic carbon (CO_2) as the carbon source (-auto-)

to fulfill their nutritional needs (-troph).

12

Primary production: all life on Earth depends on this deceptively simple chemical transformation



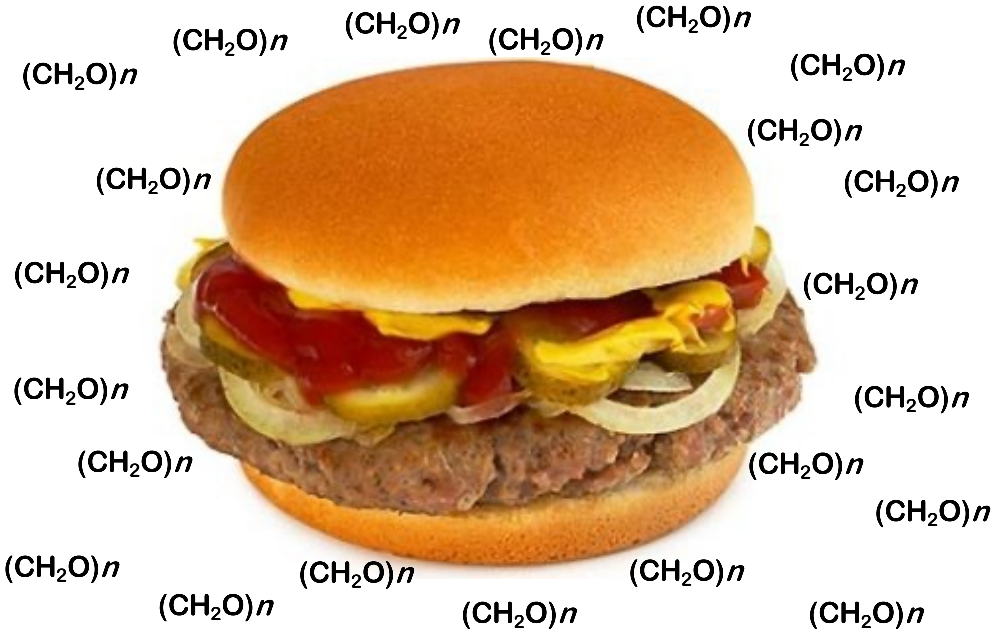
"The most important biological process on Earth is photo-synthesis, conversion of light energy to chemical energy"

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Energy Production and CO_2 Assimilation. Photoautotrophy requires two distinct sets of reactions: (1) **ATP production**, and (2) **CO_2 reduction** to cell material. For autotrophic growth, energy is supplied from ATP, and electrons for the reduction of CO_2 come from NADH (or NADPH). These are produced by the reduction of NAD^+ (or $NADP^+$) by electrons originating from various electron donors. Some phototrophic bacteria obtain reducing power from electron donors in their environment, such as reduced sulfur sources H_2S, S^0, $S_2O_3^{2-}$ or from H_2. By contrast, green plants, algae, and cyanobacteria use H_2O, an inherently poor electron donor, as the source of reducing power to convert $NAD(P)^+$ to NAD(P)H. The oxidation of H_2O produces molecular oxygen O_2 as a by-product. Because oxygen is produced, primary production in these organisms is called **oxygenic photosynthesis**. However, in many phototrophic bacteria electron donors other than water are oxidized and oxygen is not produced, and in these cases the process is called **anoxygenic photosynthesis**. The production of NAD(P)H from substances such as H_2S by anoxygenic phototrophs may or may not be directly driven by light, depending on the organism. By contrast, the oxidation of H_2O to O_2 by oxygenic phototrophs is always driven by light. Oxygenic phototrophs thus require light for *both* reducing power and energy conservation.

13

Chemo-organo-hetero-trophs get their energy, electrons, and carbon from organic compounds



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Chemo-organo-hetero-trophs use:

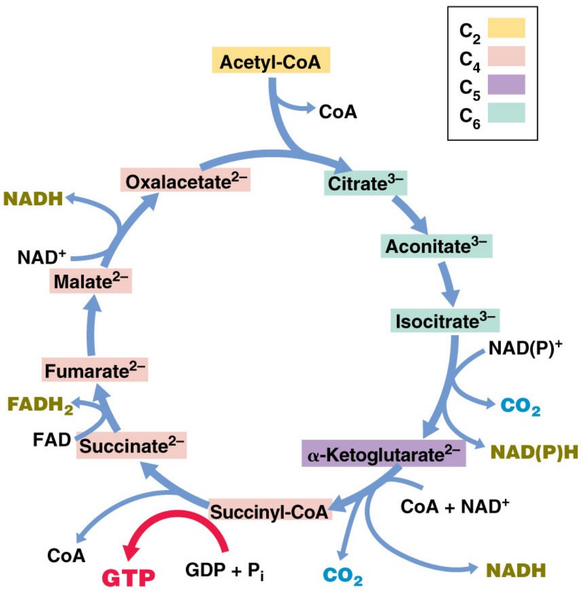
organic compounds $(CH_2O)n$ as the energy source (chemo-),

organic compounds $(CH_2O)n$ as the electron source (-organo-),

and organic compounds $(CH_2O)n$ as the carbon source (-hetero-)

to fulfill their nutritional needs (-troph).

Catabolism of organic substrates through the citric acid cycle generates high-energy electron donors



<u>Energy Yield:</u>

1 GTP → 1 ATP

3 NADH → 9 ATP

1 $FADH_2$ → 2 ATP

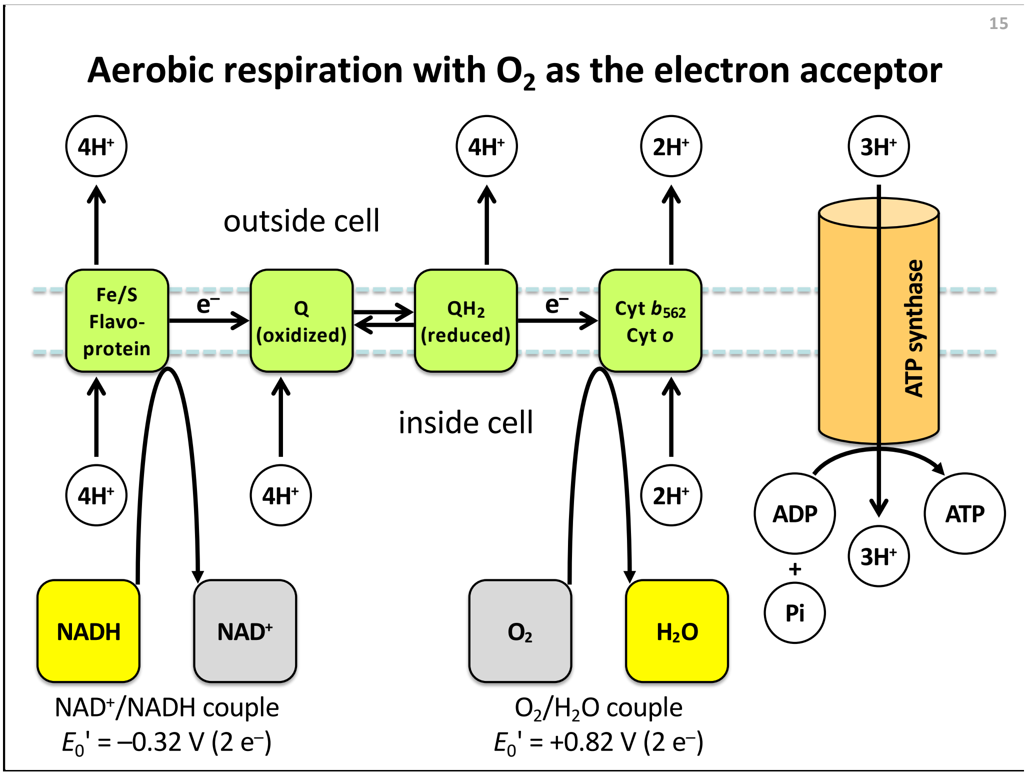
= 12 ATP for each Acetyl-CoA unit oxidized to 2 CO_2

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Figure 4.21. The citric acid cycle (also known as the TCA cycle or Krebs cycle) begins when the two-carbon compound acetyl-CoA condenses with the four-carbon compound oxaloacetate to form the six-carbon compound citrate. Through a series of oxidations and transformations, this six-carbon compound is ultimately converted back to the four-carbon compound oxaloacetate, which then begins another cycle with addition of the next molecule of acetyl-CoA. With each round of the citric acid cycle, two carbons enter as acetyl-coA (highly reduced form of carbon) and two carbon leave as CO_2 (highly oxidized form of carbon). In the process, the reducing potential stored in acetyl-CoA is transferred to the electron carriers NADH and $FADH_2$. NADH and $FADH_2$ serve as fuel for the respiratory electron transport chain to generate a proton motive force across the cytoplasmic membrane. This proton motive force can then be used by the membrane-embedded ATP synthase to generate ATP.

The NAD^+/NADH redox couple has a reduction potential of $E_0' = –0.32$ V (2 $e^–$). Oxidation of NADH generates 3 molecules of ATP per molecule of NADH oxidized.

The FAD/$FADH_2$ redox couple has a reduction potential of $E_0' = –0.22$ V (2 $e^–$). Oxidation of $FADH_2$ generates 2 molecules of ATP per molecule of NADH oxidized



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Figure 3.22. Aerobic respiration with oxygen as the terminal electron acceptor. Electron transport processes in the membrane of *Escherichia coli* when molecular oxygen (O_2) is used as the electron acceptor and NADH is the electron donor. $FADH_2$ can also serve as an electron donor to the electron transport chain at the level of the Q cycle (not shown). Transfer of electrons along the electron transport chain, from decreasingly electropositive donors to increasingly electronegative acceptors, generates a proton motive force across the cytoplasmic membrane. This proton motive force can then be used by the membrane-embedded ATP synthase to generate ATP.

The NAD^+/NADH redox couple has a reduction potential of $E_0' = –0.32$ V (2 e^-).

The FAD/$FADH_2$ redox couple has a reduction potential of $E_0' = –0.22$ V (2 e^-).

The O_2/H_2O redox couple has a reduction potential of $E_0' = +0.82$ V (2 e^-).

Abbreviations: Fp, flavoprotein; Fe/S, iron-sulfur cluster; Q, ubiquinone pool; QH_2 reduced ubiquinone; Cyt b_{556}, cytochrome b_{556}; Cyt *o*, cytochrome *o*.

Components of the electron transport chain are colored green. ATP synthase is colored orange. Reduced forms of electron donors/acceptors are colored red. Reduced forms of electron donors/acceptors are colored grey.

Gibbs free energy change (ΔG^{o}') for redox reactions

Nernst equation: $\Delta G^{o\prime} = -n * F * \Delta E_0'$

where:

n = number of electrons transferred

F = Faraday's constant ≈ 100 kJ * V^{-1} * $mole^{-1}$

$\Delta E_0' = (E_0'_{acceptor}) - (E_0'_{donor})$

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

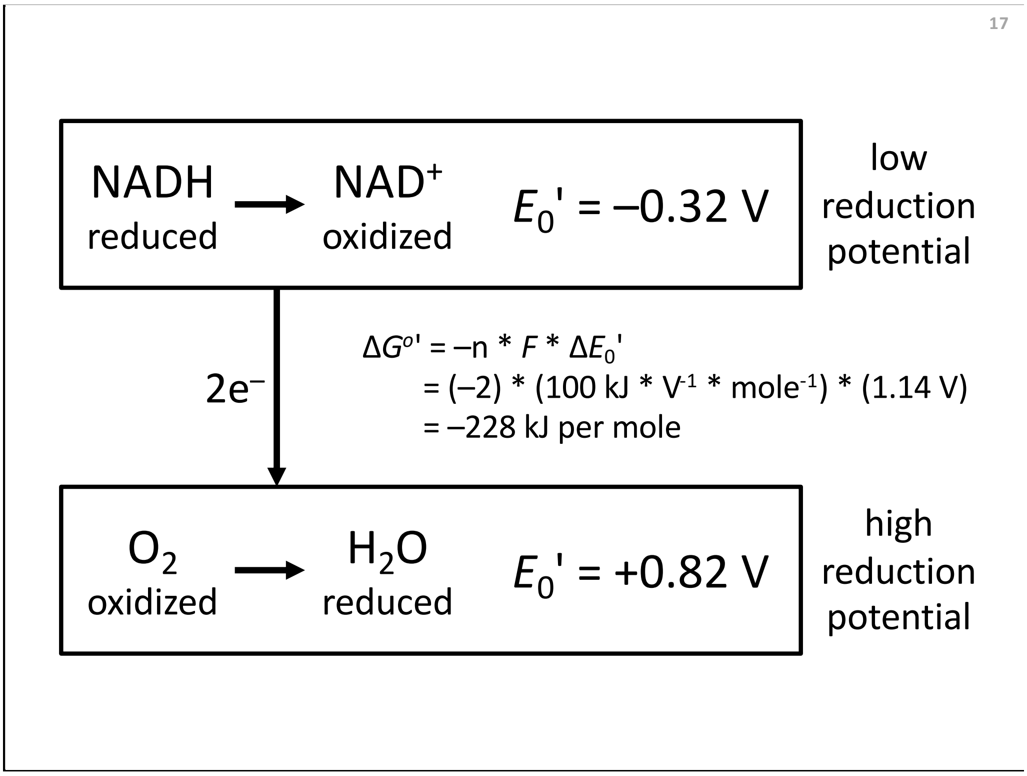
The Nernst equation, which expresses the Gibbs free energy change for reduction-oxidation (redox) reactions, is a "primary concept". You should memorize the Nernst equation and feel comfortable manipulating it.

This includes memorizing Faraday's constant: 96.5 kJ * V^{-1} * $mole^{-1}$ (although 100 kJ * V^{-1} * $mole^{-1}$ is precise enough for our purposes in this course). You should also remember that 1 joule = 1 kg * m^2 * s^{-2} in case you need to use these SI "basic" units in your calculations.

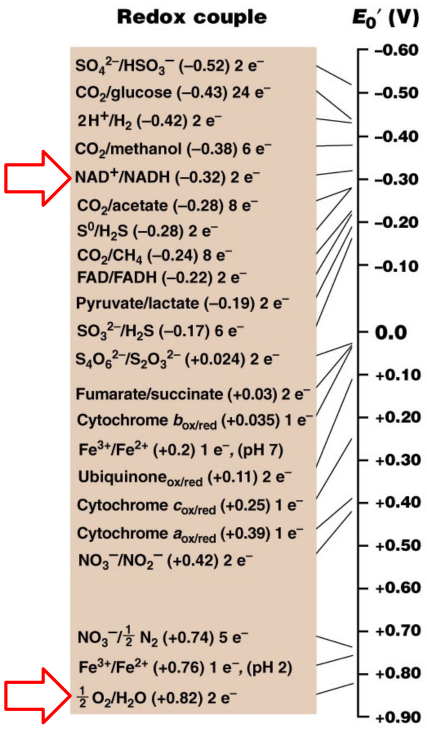
The change in free energy during a reaction is expressed as ΔG° ', the free energy change under standard conditions: pH 7.0 (approximate cytoplasmic pH), 25° C, 1 atmosphere of pressure, and all reactants and products at 1 M concentration.

A single **volt** is defined as the difference in electrical potential across a wire when an electric current of one ampere dissipates one watt (joules per second = N * m * s^{-1} = kg * m^2 * s^{-3}) of power. It is also equal to the potential difference between two points 1 meter apart in an electric field of 1 newton per coulomb. Additionally, it is the potential difference between two points that will impart one joule of energy per coulomb of charge that passes through it. It can be expressed in terms of the SI base units m, kg, s, and A as: kg * m^2 * A^{-1} * s^{-3}.

The **ampere** (symbol: A) is the SI unit of electric current (symbol: I) and is one of the seven SI base units. The ampere is a measure of the amount of electric charge passing a point in an electric circuit per unit time with 6.241×10^{18} electrons, or one **coulomb** per second constituting one ampere. The practical definition may lead to confusion with the definition of a coulomb (i.e., 1 ampere-second), but in practical terms this means that measures of a constant current (e.g., the nominal flow of charge per second through a simple circuit) will be defined in amperes (e.g., "a 20 mA circuit") and the flow of charge through a circuit over a period of time will be defined in coulombs (e.g., "a variable-current circuit that flows a total of 10 coulombs over 5 seconds"). In this way, amperes can be viewed as a "rate of flow" and coulombs viewed as an "amount of flow."



The NAD^+/NADH redox couple has a reduction potential of E_0' = –0.32 V (2 electrons get transferred).
The O_2/H_2O redox couple has a reduction potential of E_0' = +0.82 V (2 electrons get transferred).
Therefore, electrons naturally "transfer" from NADH as the electron donor (which gets oxidized to NAD^+) to O_2 as the electron acceptor (which gets reduced to H_2O).



$\Delta G^{o\prime} = -n * F * \Delta E_0'$

where:

n = number of e⁻ transferred

$F \approx 100$ kJ * V^{-1} * mole^{-1}

$\Delta E_0' = (E_0'_{\text{acceptor}}) - (E_0'_{\text{donor}})$

Example:

E_0' for e⁻ donor half-reaction:
E_0' for NAD^+/NADH = –0.32 V (2 e⁻)

E_0' for e⁻ acceptor half-reaction:
E_0' for O_2/H_2O E_0' = +0.82 V (2 e⁻)

$NADH + H^+ + \frac{1}{2}O_2 \rightarrow NAD^+ + H_2O$

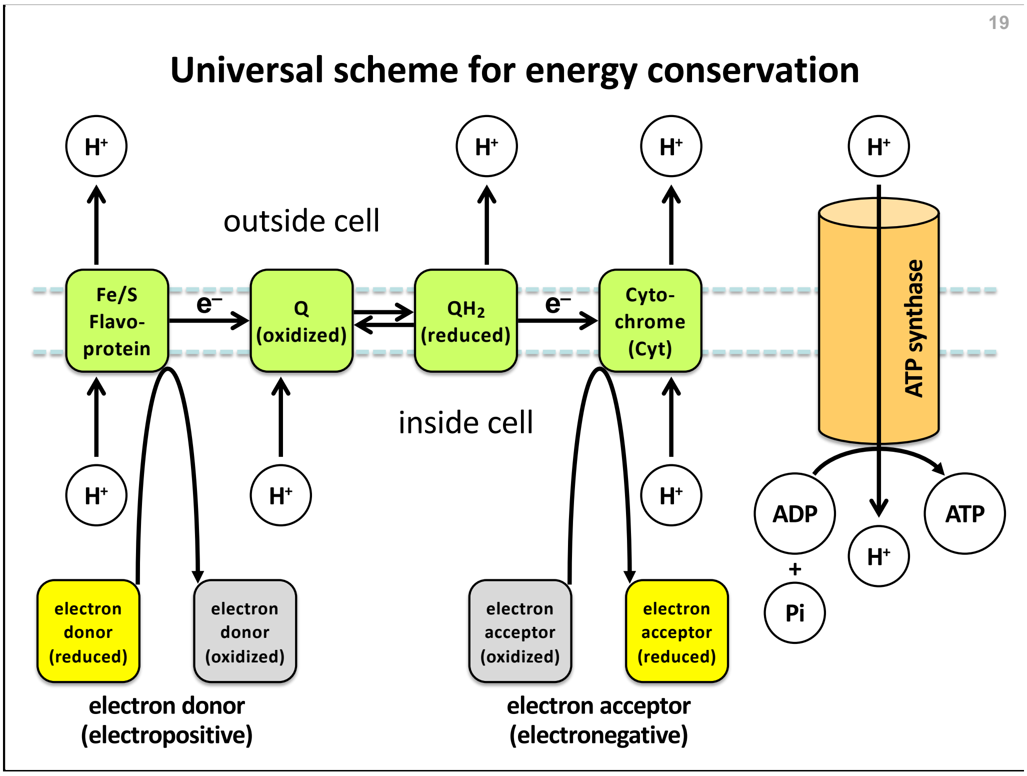
$\Delta G^{o\prime} = -228$ kJ per mole

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Figure 3.10. The redox tower. Redox couples are arranged from the strongest **electron donors** (most **electropositive**) at the top to the strongest **electron acceptors** (most **electronegative**) at the bottom. Electrons can be “caught” by acceptors at any intermediate level as long as the donor couple has a lower **reduction potential (*E_0'*)** than the acceptor couple. The greater the difference in reduction potential between electron donor and electron acceptor, the more free energy is released when they react.

Substances differ in their tendency to be electron donors or electron acceptors. This tendency is expressed in terms of their reduction potential (E_0', standard conditions), which is measured in volts (V) in reference to a standard redox couple: $2H^+/H_2$ (E_0' = –0.42 V for a 2-electron transfer). By convention, reduction potentials are given for half reactions written as reductions, with reactions at pH 7 because the cytoplasm of most cells is neutral or nearly neutral.

Voltage, also called electromotive force, is a quantitative expression of the potential difference in charge between two points in an electrical field. The greater the voltage, the greater the flow of electrical current (that is, the quantity of charge carriers that pass a fixed point per unit of time) through a conducting or semiconducting medium for a given resistance to the flow. Voltage is symbolized by an uppercase italic letter *V* or *E*. The standard unit is the volt, symbolized by a non-italic uppercase letter V. One volt will drive one coulomb (6.24×10^{18}) of charge carriers, such as electrons, through a resistance of one ohm in one second.



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 3: Microbial Metabolism (pp. 109-137), published by Pearson Education Inc., San Francisco © 2019.

Components of the electron transport chain (flavoprotein Fe/S; oxidized quinone (Q); reduced quinone (QH_2); cytochrome) are colored green. ATP synthase is colored orange. Reduced forms of electron donors/acceptors are colored red. Oxidized forms of electron donors/acceptors are colored grey.

The "universal scheme" for energy conservation in bacteria, as depicted on this slide, is a "primary concept". You should memorize this general scheme and understand the general principals underlying it. You do not need to memorize the specific components (or their names) of the electron transport chain, which vary from organism to organism. But you should understand that electrons pass from a strong electron donor (more electropositive) to a strong electron acceptor (more electronegative), and some of the energy released in this process is used to move protons across the membrane (from inside to outside). This generates a "proton motive force" that can be used by ATP synthase to make ATP.

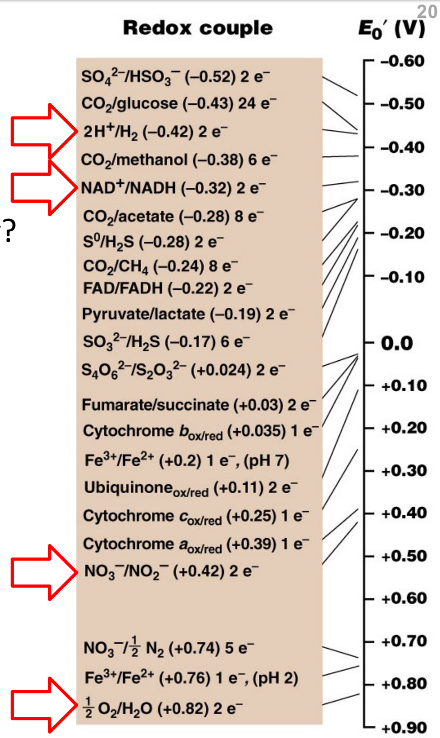
Which reaction yields the most energy?

A. H_2 reacting with NO_3^-

B. NADH reacting with NO_3^-

C. H_2 reacting with O_2

D. NADH reacting with O_2



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Answer: (C)

You have discovered an organism that can perform a two-electron-transfer reaction between the redox couples $^{1}/_{2}O_2/H_2O$ (E_0' = +0.82 V) and $2H^+/H_2$ (E_0' = –0.42 V). Which of the following statements is true (choose two):

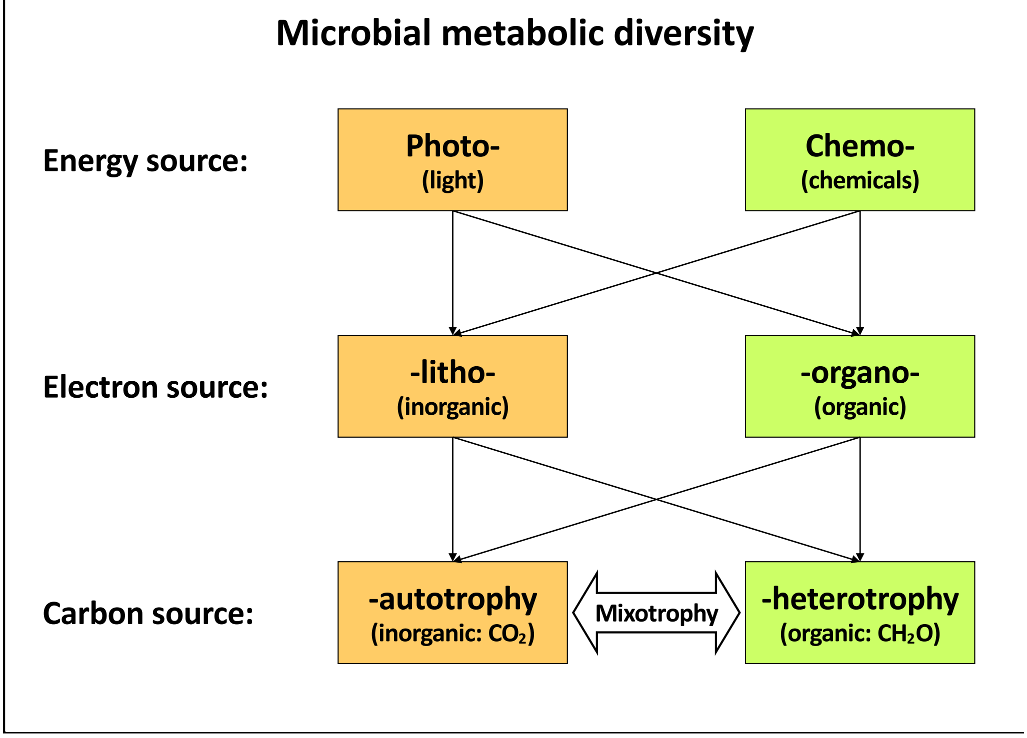
A. H_2O is the electron donor and H^+ is the electron acceptor.

B. H_2 is the electron donor and O_2 is the electron acceptor.

C. The reaction produces H_2.

D. The reaction produces H_2O.

Answer: (B) and (D)



SOURCE: Karl DM (2007) Microbial oceanography: paradigms, processes and promise. *Nature Rev Microbiol* 5(10): 759-769 PMID:17853905.

Energy Source. Phototrophs use light as the primary energy source. Chemotrophs use chemicals (inorganic, organic, or both) as the primary energy source.

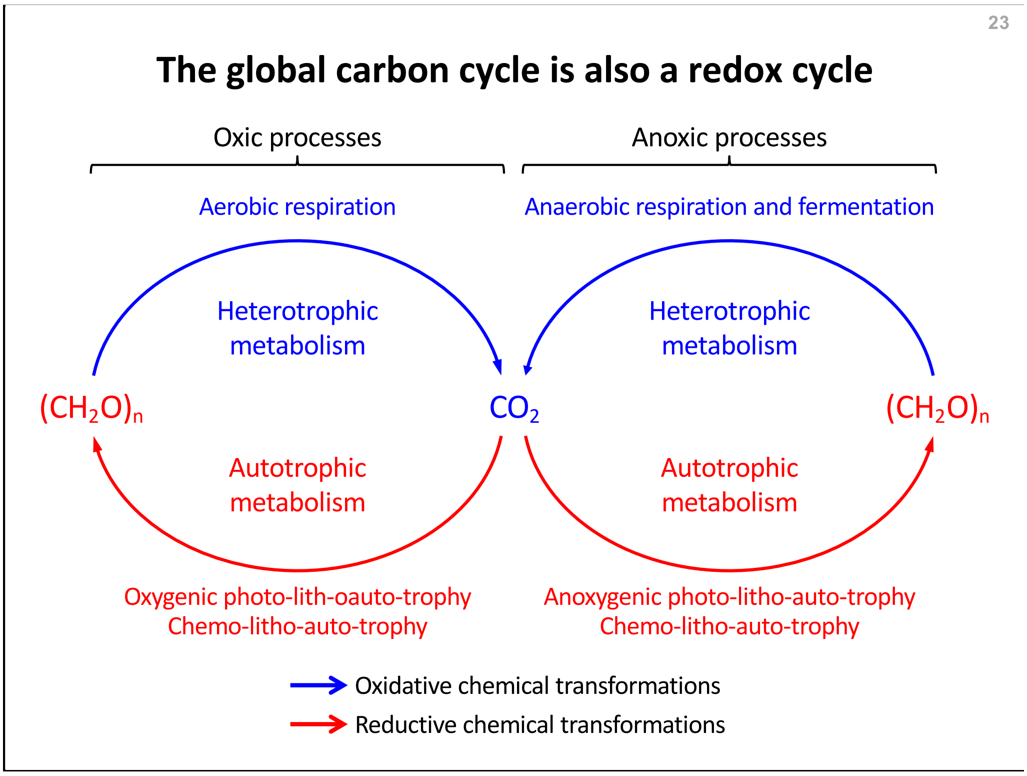
Electron Source. Lithotrophs use inorganic compounds as the electron source. Organotrophs use organic compounds as the electron source.

Carbon Source. Autotrophs use an inorganic compound (CO_2 or HCO_3^-) as the carbon source. Heterotrophs use organic compounds $(CH_2O)_n$ as the carbon source.

Photolithoautotrophs (photo-litho-auto-trophs) represent one metabolic extreme. They use light as the energy source, inorganic compounds (e.g., H_2O or H_2S) as the electron source, and CO_2 (inorganic compound) as the carbon source.

Chemoorganoheterotrophs (chemo-organo-hetero-trophs) represent the other metabolic extreme. They use chemicals as the energy source, organic compounds $(CH_2O)_n$ as the electron source, and organic compounds $(CH_2O)_n$ as the carbon source. Often, a single organic compound (e.g., glucose) serves as the source of both electrons and carbon.

Between these two extremes (photolithoautotrophy and chemoorganoheterotrophy) all combinations are possible, as indicated by the arrows. Take home lesson: microbial metabolism is highly modular and highly diverse!



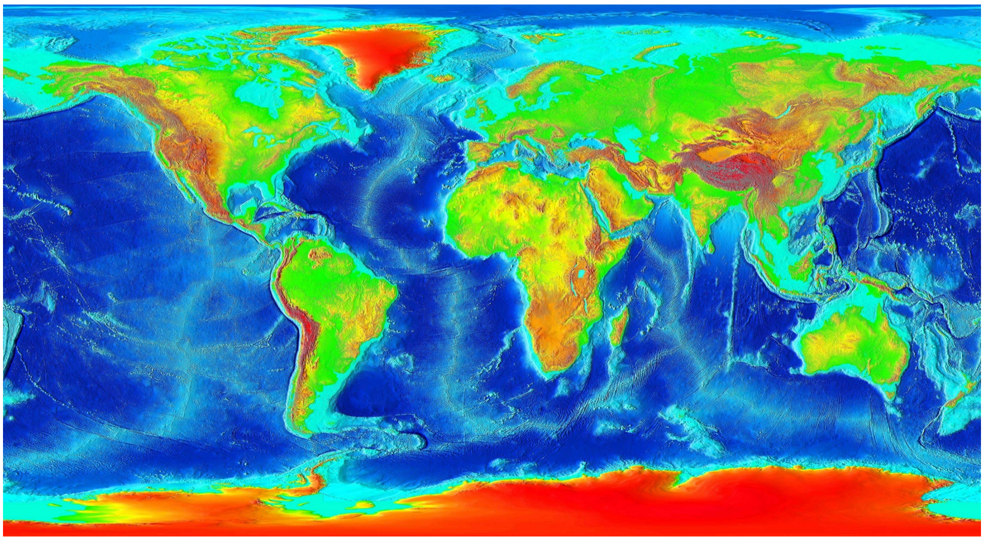
SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 21: Nutrient Cycles (pp. 687-707), published by Pearson Education Inc., San Francisco © 2019.

Figure 21.2. Reduction-oxidation (redox) cycle for carbon. The diagram contrasts **autotrophic** processes ($CO_2 \rightarrow$ organic compounds) and **heterotrophic** processes (organic compounds $\rightarrow CO_2$). Blue arrows indicate **oxidations**. Red arrows indicate **reductions**.

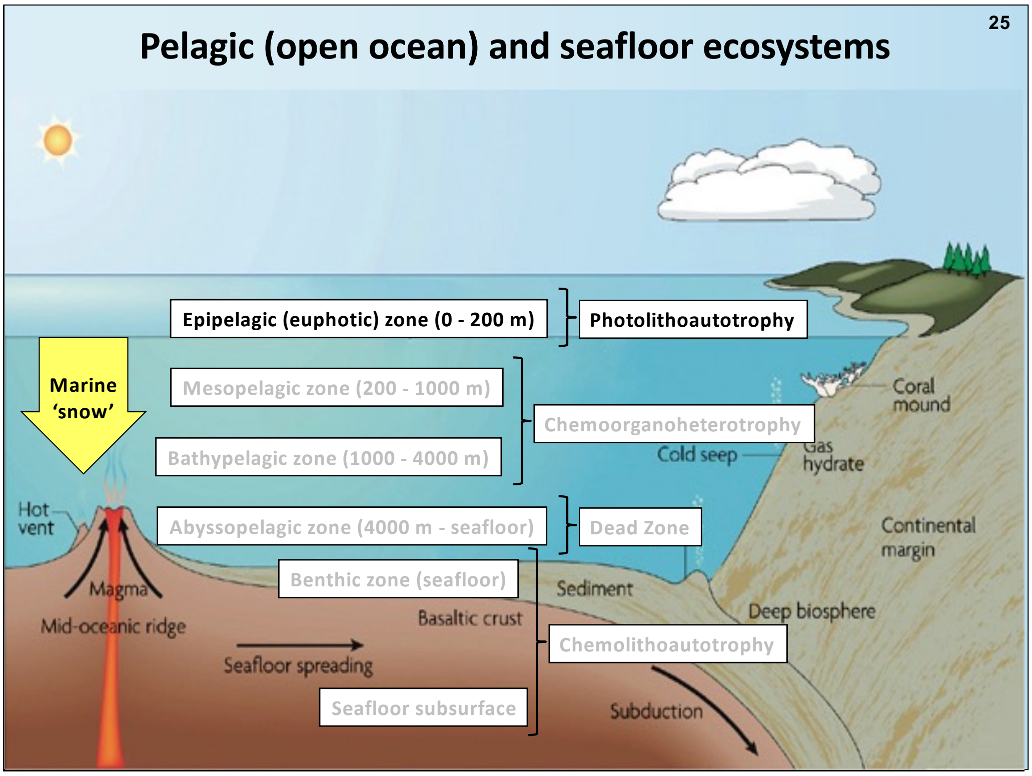
Photosynthesis. The redox cycle for carbon begins with photosynthesis: $CO_2 + H_2O \rightarrow (CH_2O)_n + O_2$. Here $(CH_2O)_n$ represents organic matter at the oxidation state of cell material, such as polysaccharides (the main form in which photosynthesized organic matter is stored in the cell). Phototrophic organisms also carry out respiration, both in the light and the dark. The overall equation for respiration is the reverse of oxygenic photosynthesis: $(CH_2O)_n + O_2 \rightarrow CO_2 + H_2O$ where $(CH_2O)_n$ again represents storage polysaccharides. If a phototrophic organism is to increase in cell number or mass, then the rate of photosynthesis must exceed the rate of respiration. If the organism grows, then some of the carbon fixed from CO_2 into polysaccharide has become the starting material for biosynthesis. In this way, autotrophic organisms build biomass from CO_2, and this biomass eventually supports the organic carbon needs of all heterotrophic organisms (including us humans!).

Decomposition. Photosynthetically fixed carbon is eventually degraded by microorganisms, and two major forms of carbon remain: methane (CH_4) and carbon dioxide (CO_2). These two gases are formed from the activities of methanogens and chemoorganotrophs, respectively. In anoxic habitats CH_4 is produced from the reduction of CO_2 with H_2 and from certain organic compounds such as acetate. However, virtually any organic compound can eventually be converted to CH_4 from the combined activities of methanogens and other bacteria (syntrophs); H_2 generated from the fermentative degradation of organic compounds by syntrophs gets consumed by methanogens and converted to CH_4. Methane produced in anoxic habitats is insoluble and flows to oxic environments where it is oxidized to CO_2 by methanotrophs. Hence, all organic carbon eventually returns to CO_2, and the carbon cycle is complete. The balance between the oxidative and reductive portions of the carbon cycle is critical: the metabolic products of some organisms are the substrates for others. Thus, the cycle needs to keep in balance if it is to continue as it has for billions of years. Any significant changes in levels of gaseous forms of carbon may have serious global consequences, as we are already experiencing in the form of global warming from the increasing CO_2 levels in the atmosphere from deforestation and the burning of fossil fuels. Total CO_2 released by microbial activities far exceeds that produced from decomposition by multicellular eukaryotes, and this is especially true of anoxic environments.

Oceans account for more than 70% of Earth's surface and 50% of Earth's primary production



Eukaryotes dominate primary production on land
Prokaryotes dominate primary production in oceans

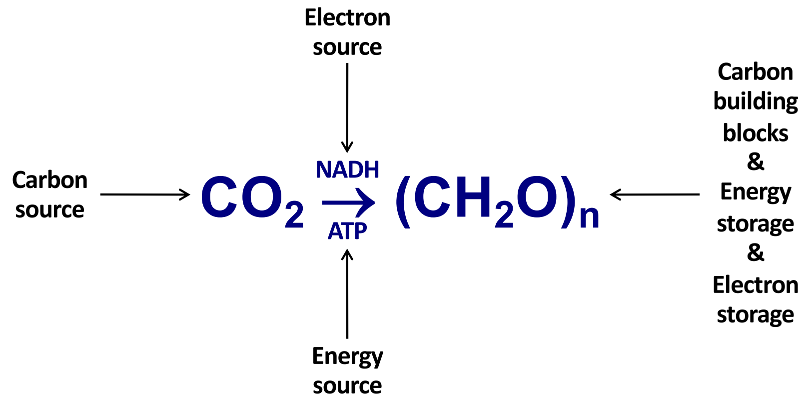


SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 20: Microbial Ecosystems (pp. 651-686), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Jorgensen B, Boetius A (2007) Feast and famine - microbial life in the deep sea bed. *Nature Rev Microbiol* 5(10): 770-781 PMID: 17828281.

Figure 1. Vertical section of the seabed and seafloor structures. This figure shows the plate-tectonic conveyer belt. At the mid-oceanic ridge new basaltic crust is formed continuously. The ocean plates move towards the continents where they are subducted again. Sinking particles form thick sediment piles on the ageing crust and on the continental margins. Plate tectonics and gravitational and hydrological forces cause a range of structures to form on the seabed, including hydrothermal vents at mid-oceanic ridges and subduction zones, gas-seeping mud volcanoes on continental margins, and carbonate mounds inhabited by cold-water corals.

Primary production: all life on Earth depends on this deceptively simple chemical transformation

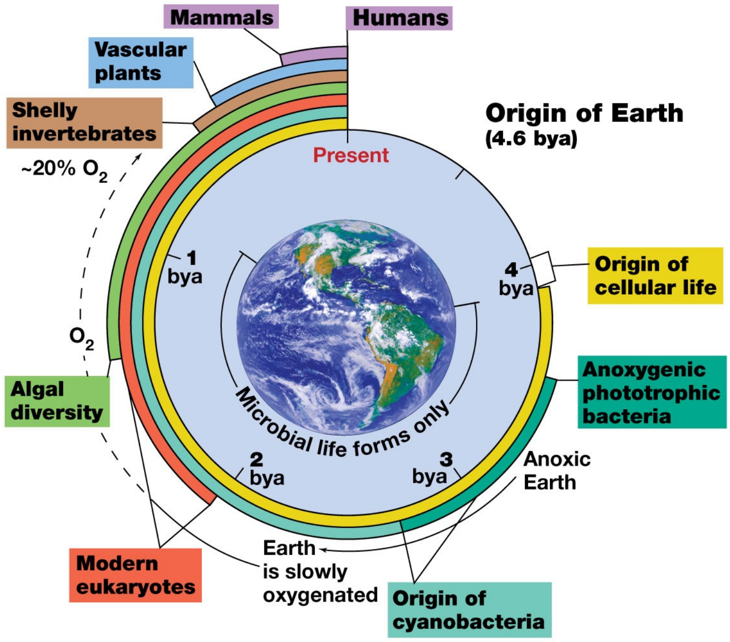


CO2 fixation requires an electron source to make NADH and an energy source to make ATP

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

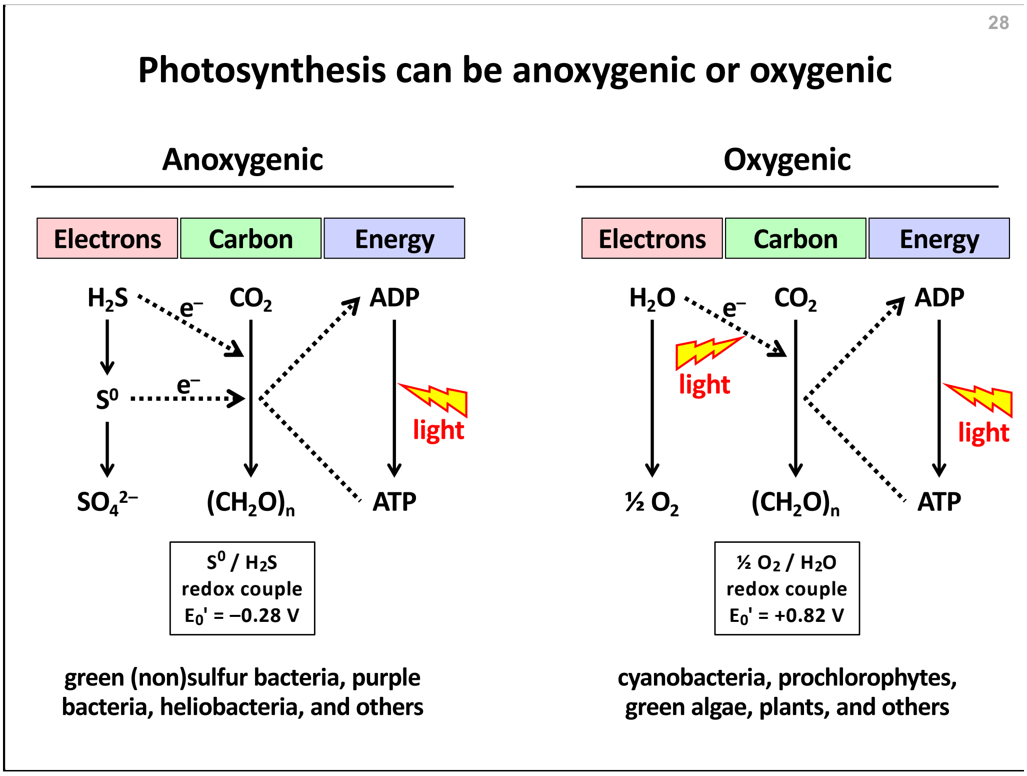
Energy Production and CO_2 Assimilation. Photoautotrophy requires two distinct sets of reactions: (1) **ATP production**, and (2) **CO_2 reduction** to cell material. For autotrophic growth, energy is supplied from ATP, and electrons for the reduction of CO_2 come from NADH (or NADPH). These are produced by the reduction of NAD^+ (or $NADP^+$) by electrons originating from various electron donors. Some phototrophic bacteria obtain reducing power from electron donors in their environment, such as reduced sulfur sources H_2S, S^0, $S_2O_3^{2-}$ or from H_2. By contrast, green plants, algae, and cyanobacteria use H_2O, an inherently poor electron donor, as the source of reducing power to convert $NAD(P)^+$ to NAD(P)H. The oxidation of H_2O produces molecular oxygen O_2 as a by-product. Because oxygen is produced, primary production in these organisms is called **oxygenic photosynthesis**. However, in many phototrophic bacteria electron donors other than water are oxidized and oxygen is not produced, and in these cases the process is called **anoxygenic photosynthesis**. The production of NAD(P)H from substances such as H_2S by anoxygenic phototrophs may or may not be directly driven by light, depending on the organism. By contrast, the oxidation of H_2O to O_2 by oxygenic phototrophs is always driven by light. Oxygenic phototrophs thus require light for *both* reducing power and energy conservation.

A very brief history of life on Earth...



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 1: The Microbial World (pp. 37-69), published by Pearson Education Inc., San Francisco © 2019.

Figure 1.5. A summary of life on Earth through time and origin of the cellular domains. At the time of its origin, Earth was sterile. Cellular life was present on Earth by about 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 billion years ago, but current levels of oxygen in the atmosphere were not achieved until about 500-800 million years ago. Eukaryotes arose about 2 billion years ago. Eukaryotes are nucleated cells (by definition) and include both microbial organisms (the overwhelming majority) and multicellular organisms (a tiny minority).

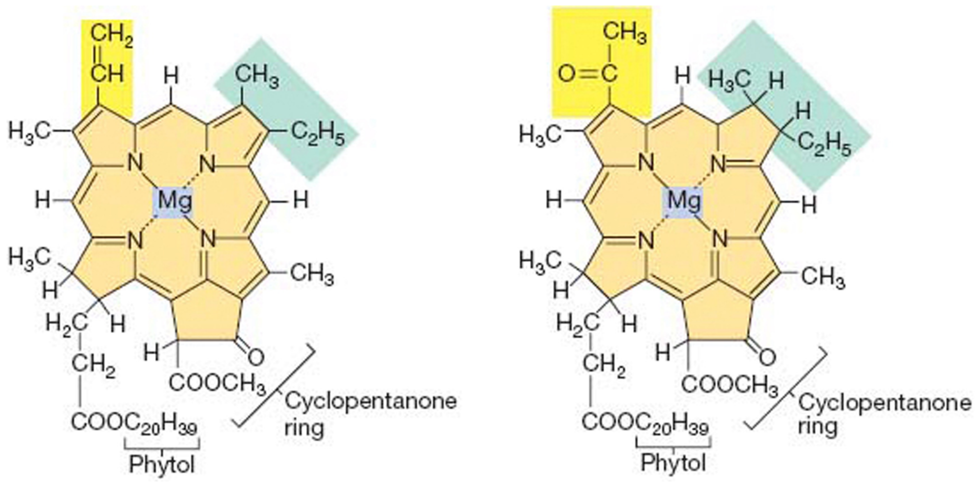


SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.1. Patterns of photosynthesis. Energy and reducing power synthesis in anoxygenic phototrophs (left) and oxygenic phototrophs (right). Both types of phototrophs obtain their energy from light (hv). Oxygenic phototrophs produce molecular oxygen (O_2), while anoxygenic phototrophs do not.

(Bacterio)chlorophyll harvests light energy

The polyene network absorbs visible light with ε ≥ 10^5 cm^{-1} M^{-1} !



Chlorophyll a (oxygenic photosynthesis)

Bacteriochlorophyll a (anoxygenic photosynthesis)

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.2. Structures and spectra of chlorophyll *a* (left) and bacteriochlorophyll *a* (right). The two molecules are identical except for those portions contrasted in yellow and green. The only organisms that can perform photosynthesis are ones that produce some form of chlorophyll (oxygenic phototrophs) or bacteriochlorophyll (anoxygenic phototrophs). Chlorophyll is a porphyrin, as are the cytochromes. But unlike the cytochromes, chlorophyll contains magnesium instead of iron at the center of the porphyrin ring. Chlorophyll also contains specific side chains on the porphyrin ring, as well as a hydrophobic alcohol side chain that helps to anchor the chlorophyll into the photosynthetic membranes. Chlorophyll *a* is the principal chlorophyll of higher plants, most algae, and the cyanobacteria. Chlorophyll *a* is green because it *absorbs* red and blue light preferentially and *transmits* green light. The spectral properties of any pigment can best be expressed by its *absorption spectrum,* a measure of the absorbance of the pigment at different wavelengths. The absorption spectrum of cells containing chlorophyll *a* shows strong absorption of red light (maximum absorption at a wavelength of 680 nm) and blue light (maximum absorption at 430 nm). Light causes an electron from an occupied orbital to be promoted to a higher energy empty orbital. The excited state chlorophyll molecule has extra energy that it can transfer to other molecules and provide the energy necessary for an endothermic reaction. The high energy electron makes excited chlorophyll a reducing agent.

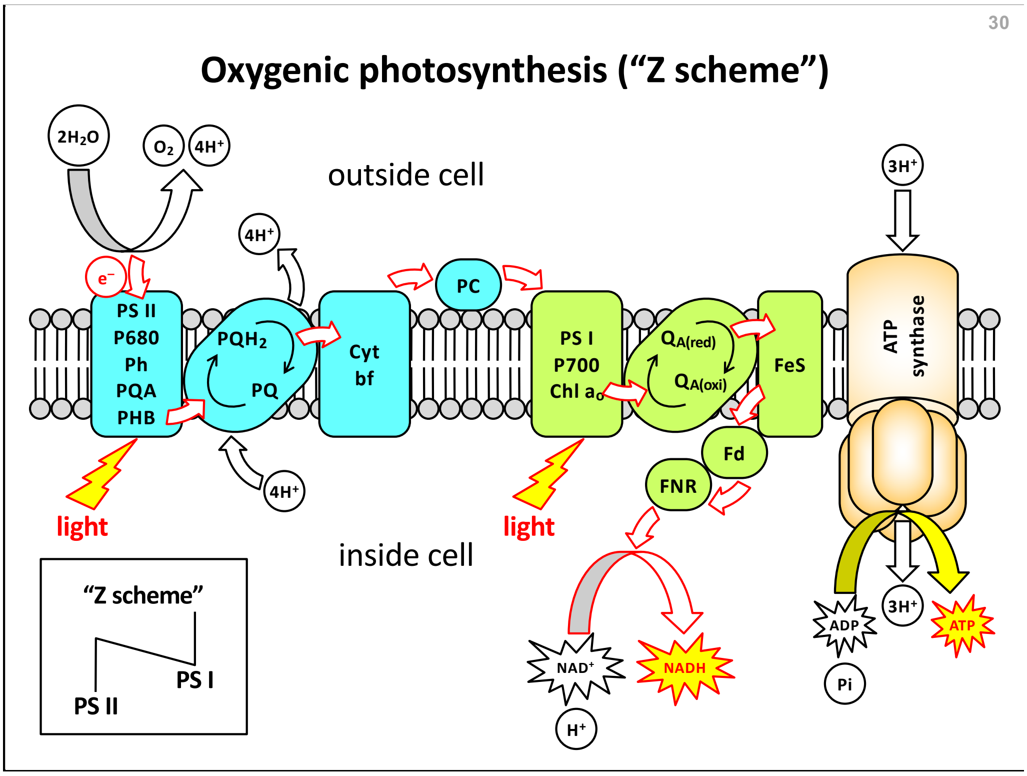


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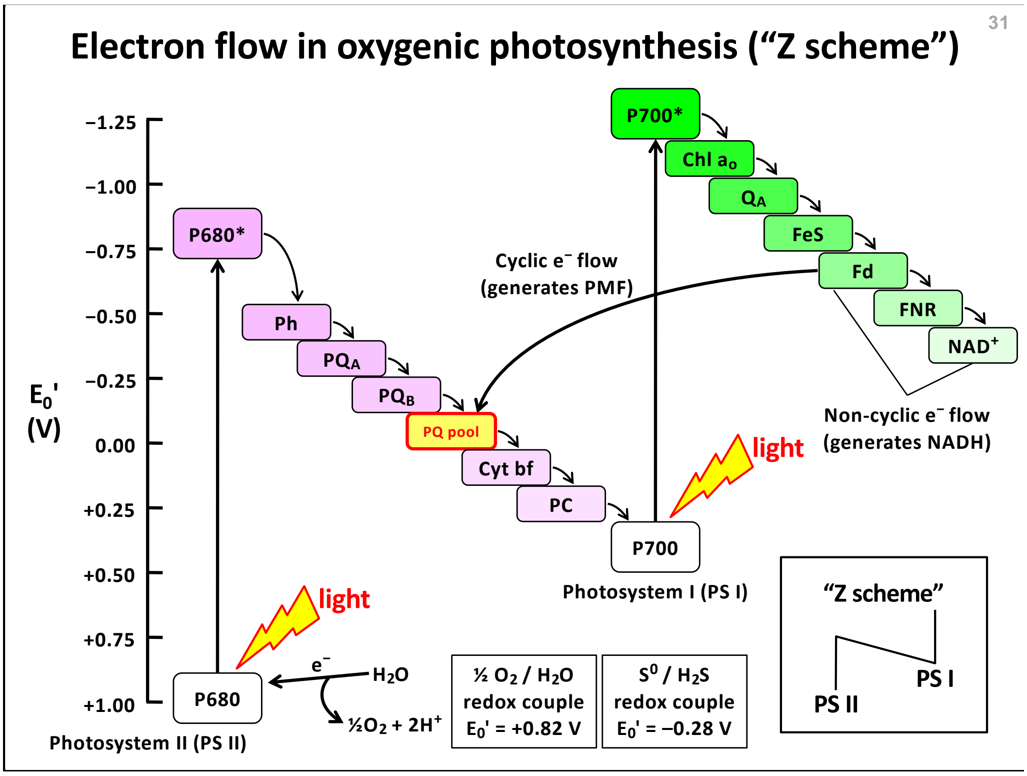
Figure 14.15. Electron flow in oxygenic photosynthesis: the 'Z scheme'. Electrons flow through two photosystems, PS I and PS II. Compare with anoxygenic photosynthesis (Figure 13.14). Red arrows indicate electron transfers.

Abbreviations:

PSII, photosystem II; **P680**, reaction center chlorophyll of PSII; **Ph**, pheophytin; **PQ**, plastoquinones (A and B); **Cyt *bf***, cytochrome *bf*; **PC**, plastocyanin; **PSI**, photosystem I; **P700**, reaction center chlorophyll of PSI; **Chl a$_o$**, chlorophyll a$_o$, **Q_{Aoxi}**, quinone A oxidized form; **Q_{Ared}**, quinone A reduced form; **FeS**, non-heme iron-sulfur protein; **Fd**, ferredoxin; **FNR**, ferredoxin NADP reductase; **Pi**, inorganic phosphate; **e⁻**, electron



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.14. Electron flow in oxygenic photosynthesis: the 'Z scheme'. Electrons flow through two photosystems, PS I and PS II. Compare with anoxygenic photosynthesis (Figure 14.12).

Abbreviations: **PSII**, photosystem II; **P680**, reaction center chlorophyll of PSII; **Ph**, pheophytin; **PQ**, plastoquinones (A and B); **Cyt *bf***, cytochrome *bf*; **PC**, plastocyanin; **PSI**, photosystem I; **P700**, reaction center chlorophyll of PSI; **Chl a_o**, chlorophyll a_o, **Q_{Aoxi}**, quinone A oxidized form; **Q_{Ared}**, quinone A reduced form; **FeS**, non-heme iron-sulfur protein; **Fd**, ferredoxin; **FNR**, ferredoxin NADP reductase.

Electron donor: H_2O, water.

Remember: $\Delta G_0' = -n * F * \Delta E_0'$ where n = the number of electrons transferred and F (Faraday's Constant) = 96.5 kJ * V^{-1} * $mole^{-1}$ (although 100 kJ * V^{-1} * $mole^{-1}$ is precise enough for our purposes in this course.)

Which of the following statements is true:

A. Oxygenic photosynthesis requires two inputs of light energy.

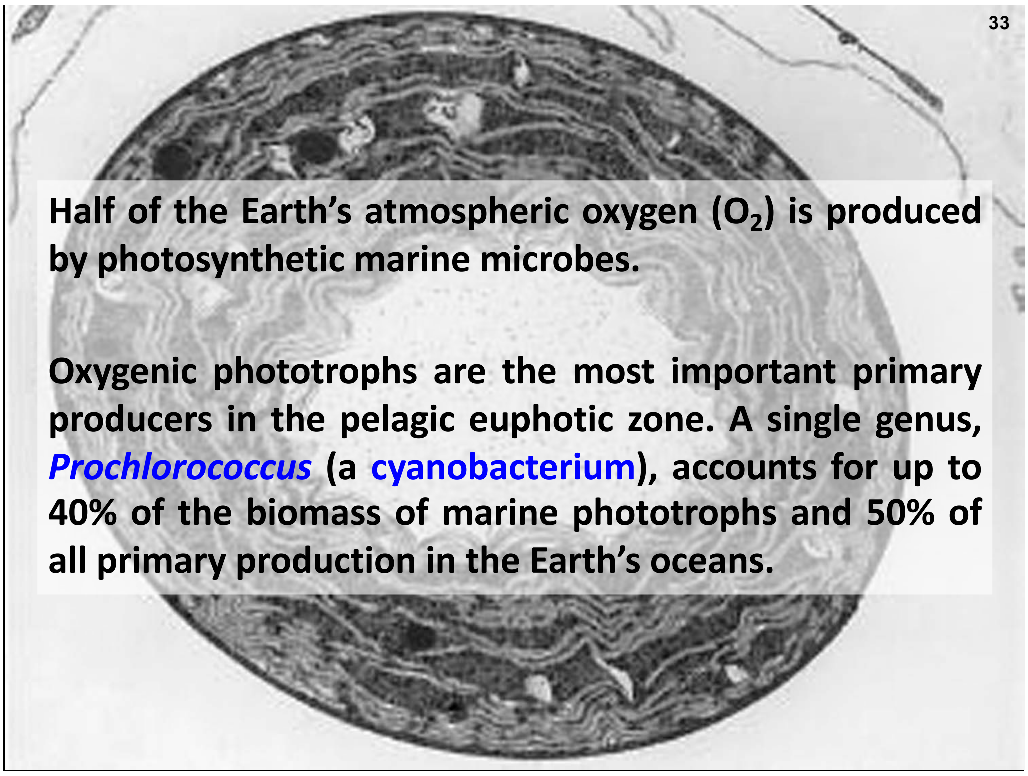
B. Oxygenic photosynthesis uses H_2O as the electron donor.

C. Oxygenic photosynthesis generates O_2.

D. Oxygenic photosynthesis evolved more recently than anoxygenic photosynthesis.

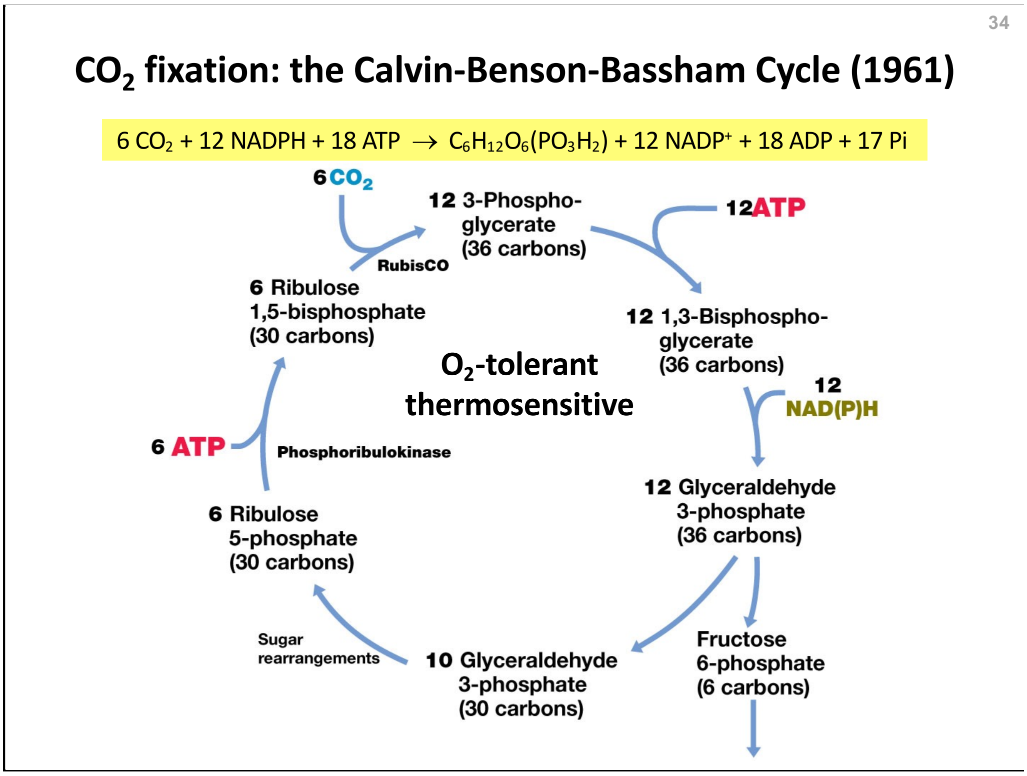
E. All of the above.

Answer: (E)



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 15: Functional Diversity of Microorganisms (pp. 487-529), published by Pearson Education Inc., San Francisco © 2019.

Figure 18.28. Electron micrograph of the prochlorophyte *Prochloron*. Note the extensive internal thylakoid membranes where photosynthesis takes place. Cells are about 10 µm in diameter. *Prochloron* is a much larger relative of *Prochlorococcus* (0.5-0.8 µm diameter).

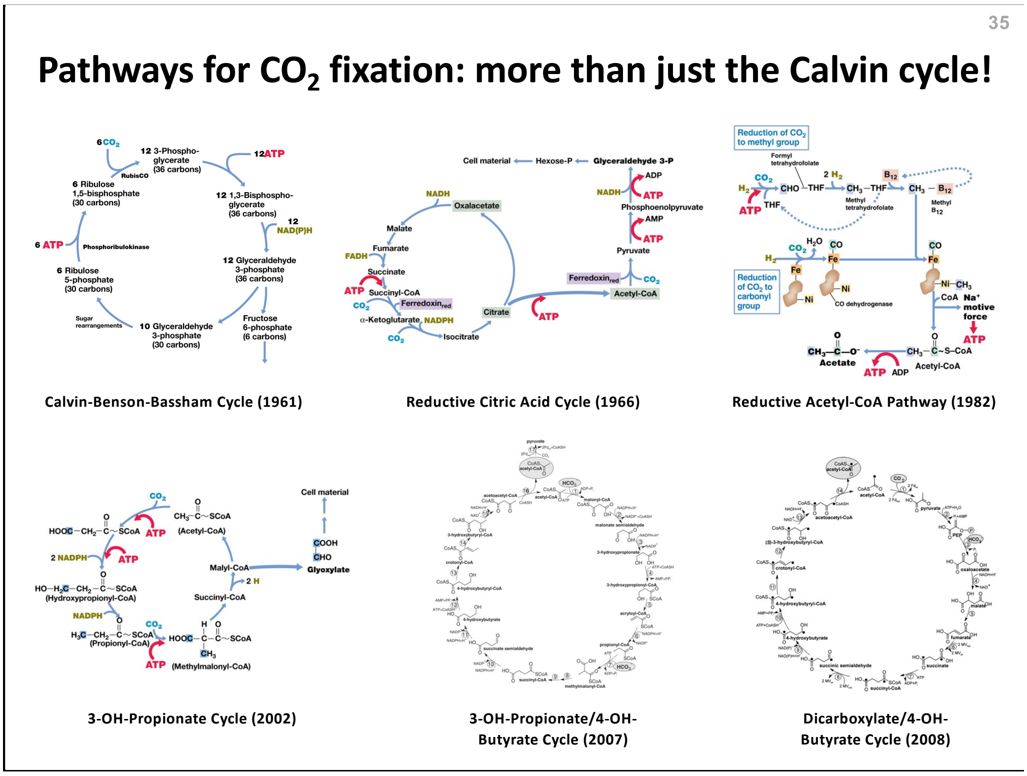


SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Berg IA (2011) Ecological aspects of the distribution of different autotrophic CO_2 fixation pathways. *Appl Environ Microbiol* 77(6): 1925-1936 PMID: 21216907.

Figure 14.18. The Calvin-Benson-Bassham cycle (a.k.a. "Calvin cycle"). Shown is the production of one hexose molecule (fructose 6-phosphate) from six CO_2 molecules. In phototrophs, ATP comes from photo-phosphorylation and NAD(P)H comes from light energy or reverse electron flow. The Calvin cycle operates in some thermophiles but never in hyperthermophiles; its upper temperature limit appears to be about 70˚C. This may be explained by the heat instability of some intermediates of the cycle, mainly of glyceraldehyde-3-phosphate, producing toxic methylglyoxal at high temperatures. Energetically, it is an expensive way to fix carbon, but this is presumably not a problem for the phototrophic microbes that use this cycle because they have access to an abundant energy source (light). The Calvin cycle is the quantitatively most important mechanism of autotrophic CO_2 fixation in nature. The success of the Calvin cycle was probably due to the complete robustness of its enzymes to molecular oxygen, which is generated when water (the electron source for oxidative photosynthesis) is oxidized during photosynthesis.

In aerobic respiration, one molecule of glucose yields 38 ATP molecules: 8 produced during glycolysis, 6 from the link reaction, and 24 from the citric acid cycle (a.k.a. TCA cycle or Krebs cycle). The net gain is 36 ATP, as 2 of the ATP molecules produced from glycolysis are used up in the re-oxidation of the hydrogen carrier molecule NAD^+. Oxidation of 1 NAD(P)H molecule gives rise to 3 ATP, so the energetic cost of producing one molecule of fructose-6-phosphate is: 26 ATP (from 12 NADPH) + 18 ATP = 54 ATP.



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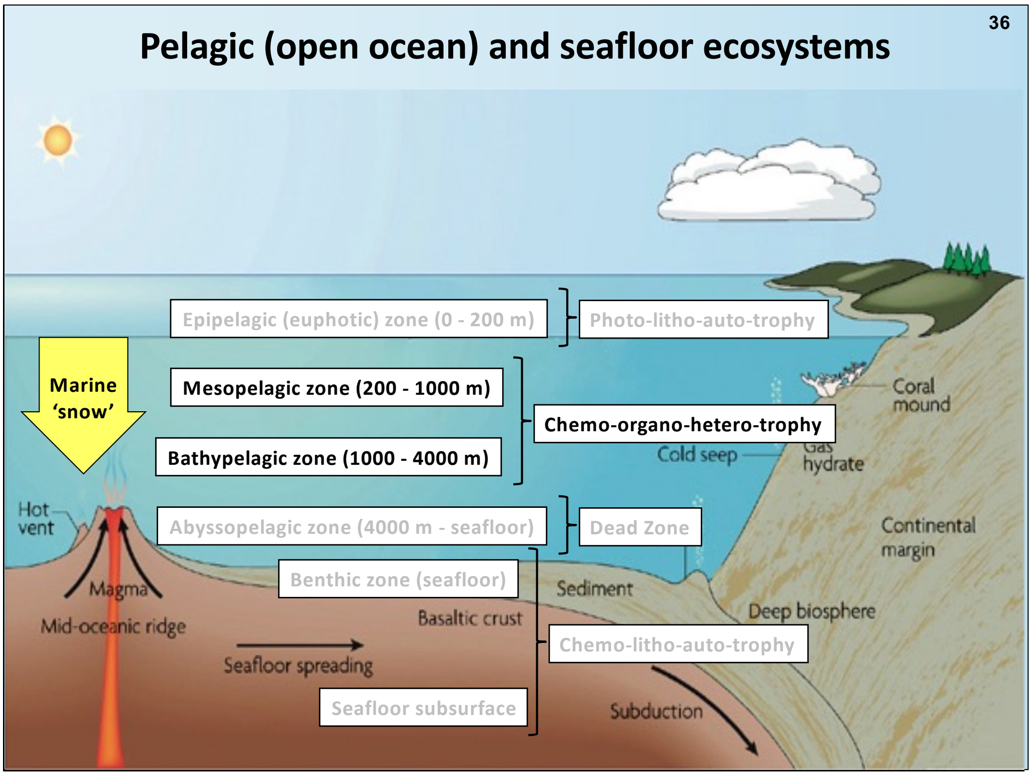
Correa SS, Schultz J, Lauersen KJ, Rosado AS (2022) Natural carbon fixation and advances in synthetic engineering for redesigning and creating new fixation pathways. *J Adv Res* S2090-1232(22)00165-5 PMID: 35918056.

This slide illustrates the six currently known pathways for fixation of carbon from carbon dioxide (CO_2) or bicarbonate (HCO_3^-). You do not need to memorize these pathways! The purpose of this slide is to make you aware of the existence of these pathways and their diversity.

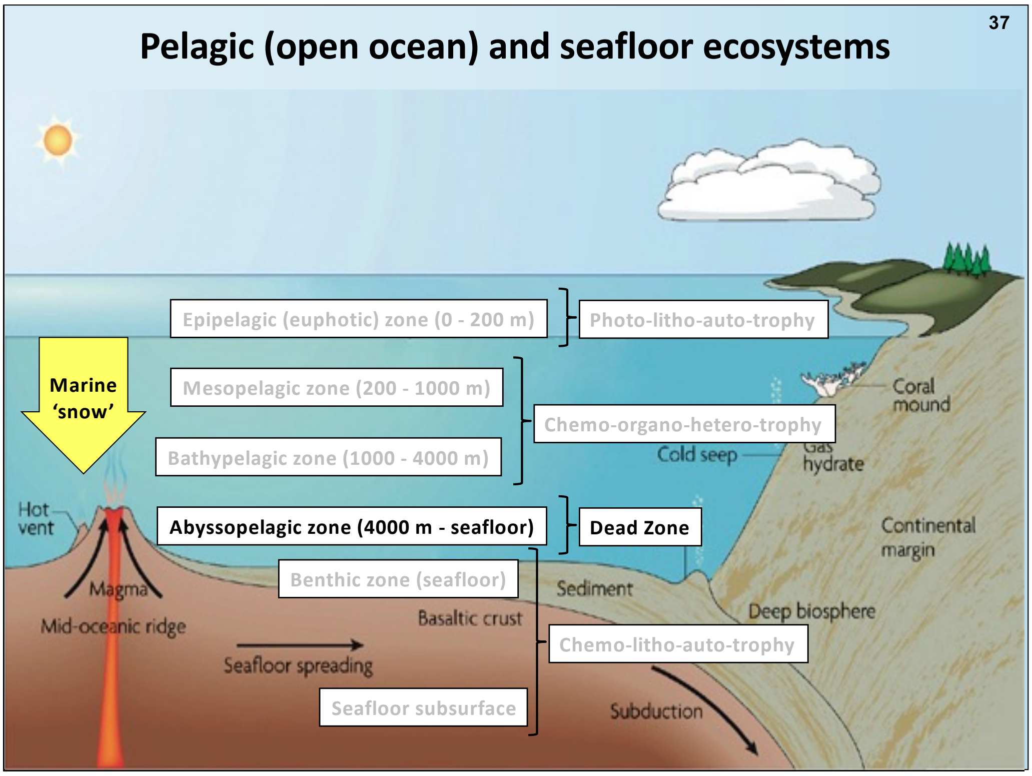
However, you should know the general biological functions of carbon fixation, as well as the biochemical requirements for carbon fixation: a source of electrons (usually in the form of NADH or NADPH) for reduction of CO_2 or HCO_3^-; a source of energy (in the form of ATP) to drive these energetically unfavorable reductive reactions; and a source of highly oxidized carbon (CO_2 or HCO_3^-) to convert to more highly reduced carbon compounds (like sugars). Why so many pathways? Because the different pathways are found in microorganism that are adapted to living in different ecological niches with different chemical and physical properties, such as oxygen, temperature, and acidity. Some of the pathways are sensitive to oxygen so they are found only in microbes that live in anaerobic environments (e.g., the Reductive Acetyl-CoA Pathway), while other pathways are insensitive to oxygen (e.g., the Calvin-Benson-Bassham Cycle). Some of the pathways are sensitive to high temperatures so they are found only in microbes that live in relatively cool environments, while other pathways function optimally at high temperatures so they are found in organisms that are adapted to living in hot environments, such as deep-sea hydrothermal vents (which we will discuss later in this lecture). Some of the pathways are sensitive to acidic pH so they are found only in microbes that live in relatively pH-neutral environments, while other pathways function optimally at acidic pH. And so on…



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 20: Microbial Ecosystems (pp. 651-686), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Jorgensen B, Boetius A (2007) Feast and famine - microbial life in the deep sea bed. *Nature Rev Microbiol* 5(10): 770-781 PMID: 17828281.

Figure 1. Vertical section of the seabed and seafloor structures. This figure shows the plate-tectonic conveyer belt. At the mid-oceanic ridge new basaltic crust is formed continuously. The ocean plates move towards the continents where they are subducted again. Sinking particles form thick sediment piles on the ageing crust and on the continental margins. Plate tectonics and gravitational and hydrological forces cause a range of structures to form on the seabed, including hydrothermal vents at mid-oceanic ridges and subduction zones, gas-seeping mud volcanoes on continental margins and carbonate mounds inhabited by cold-water corals.



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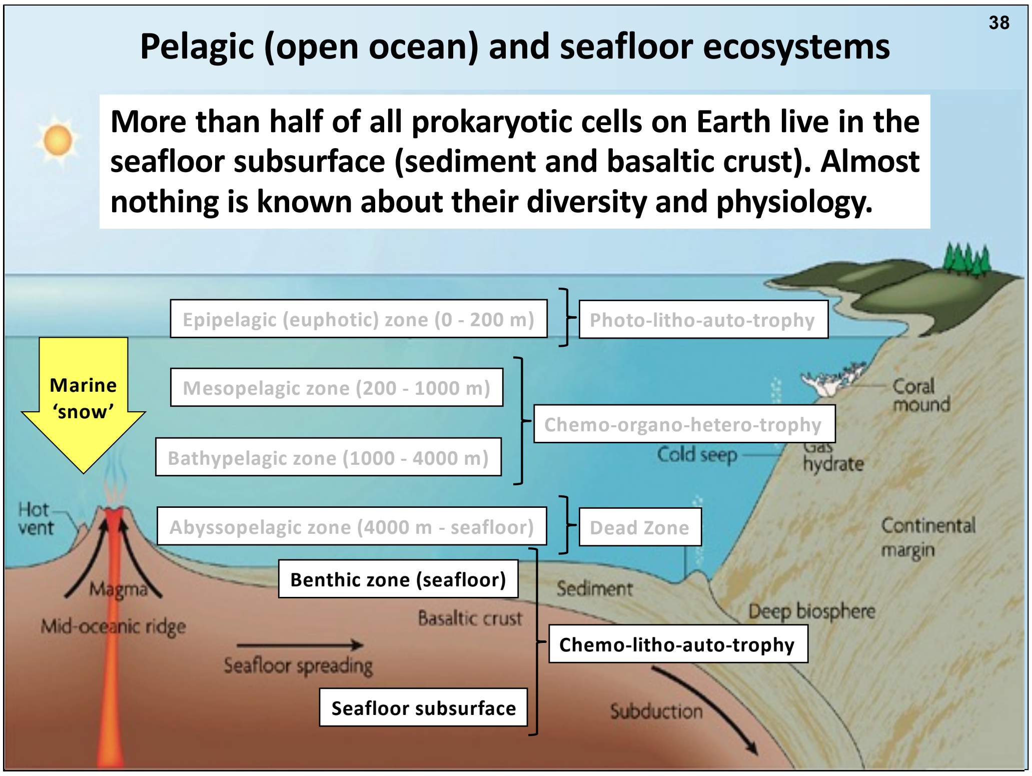

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Deep-sea microbes are always hungry. **Deep-sea prokaryotes grow extremely slowly due to severe nutrient limitation. The doubling time of seafloor subsurface prokaryotes is estimated to be 100's or even 1000's of years.**

Deep-sea microbes like cold. **They are psychrophilic. The temperature of seawater is 2-3˚C from 100 m down to the bottom. Exception: hydrothermal vent microbes are thermophilic.**

Deep-sea microbes like pressure. **They are barophilic. The pressure exerted by a water column increases by about 1 atm for every 10 m. Microbes on the seafloor (2,500-5,000 m) must withstand pressures of 250-500 atmospheres (atm)!**

What a life, huh!?

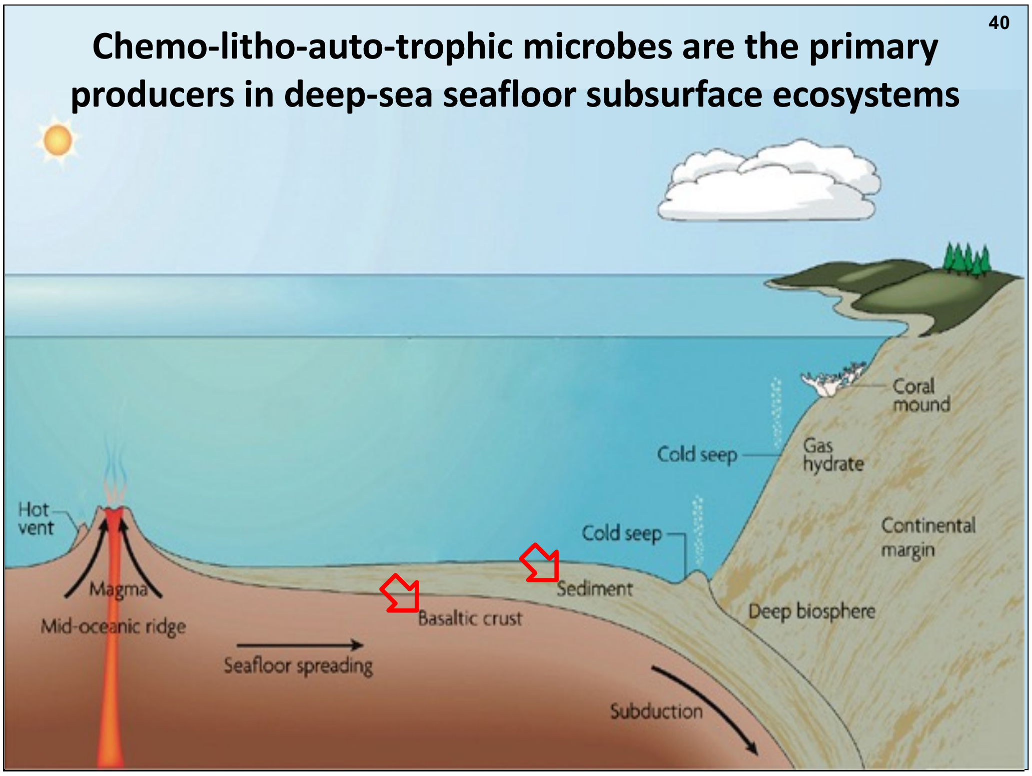
Pressure: **~ 30 psi**
~ 2 atm
~ 200 kPa

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 20: Microbial Ecosystems (pp. 651-686), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Schippers A, Neretin LN, Kallmeyer J, Ferdelman TG, Cragg BA, Parkes RJ, Jørgensen BB (2005) Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature* 433(7028): 861-864 PMID: 15729341.

Chemical analyses of the pore waters from hundreds of deep ocean sediment cores have over decades provided evidence for ongoing processes that require biological catalysis by prokaryotes. This sub-seafloor activity of microorganisms may influence the surface Earth by changing the chemistry of the ocean and by triggering the emission of methane, with consequences for the marine carbon cycle and even the global climate. Despite the fact that only about 1% of the total marine primary production of organic carbon is available for deep-sea microorganisms, sub-seafloor sediments harbour over half of all prokaryotic cells on Earth. This estimation has been calculated from numerous microscopic cell counts in sediment cores of the Ocean Drilling Program. Because these counts cannot differentiate between dead and alive cells, the population size of living microorganisms is unknown. Here, using ribosomal RNA as a target for the technique known as catalysed reporter deposition-fluorescence in situ hybridization (CARD-FISH), we provide direct quantification of live cells as defined by the presence of ribosomes. We show that a large fraction of the sub-seafloor prokaryotes is alive, even in sediments that are very old (16 million years) and deep (> 400 meters below the sea floor). All detectable living cells belong to the *Bacteria* and have turnover times of 0.25 to 22 years, comparable to surface sediments.

Remember: 1 atm = 14.6956 psi = 101,325 Pascals



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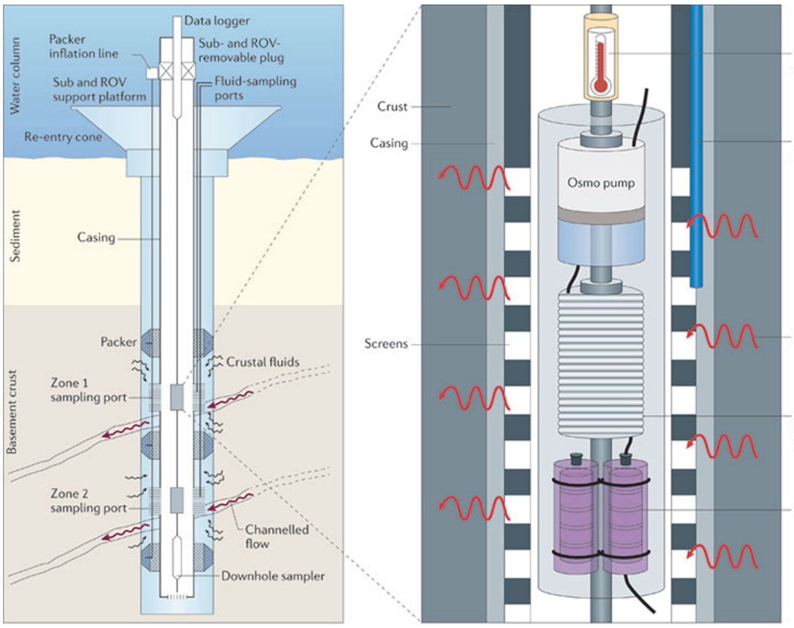
"CORK"ed borehole observatory systems for studying the seafloor subsurface microbiota



SOURCE: Edwards KJ, Wheat CG, Sylvan JB (2011) Under the sea: microbial life in volcanic oceanic crust. *Nature Rev Microbiol* 9(10): 703-712 PMID: 21894169.

Figure 4. CORK ("Circulation Obviation Retrofit Kit") observatory systems for studying the igneous oceanic crust experimentally and manipulatively. Instrument and experimental strings are deployed in situ in cored boreholes that are cased through the sedimentary layers. Discrete observatory intervals in the crust are created using packers in the hole. Experimental apparatus such as colonization devices, fluid-sampling devices and temperature recorders are coupled to seafloor instrumentation packages at the sealed well head, enabling time series studies to be conducted at the sea floor in addition to the in situ sub-seafloor borehole environment.

Abbreviations:

ROV, remote-operated vehicle

Sub, submarine.

Could H_2 oxidation be the main energy source to support microbial life in the seafloor subsurface?

Radiolysis of seawater (in seafloor sediment and crust):

$2H_2O \rightarrow O_2 + 2H_2$

Serpentinization of ultramafic rocks (in seafloor crust):

$3Fe_2SiO_4 + 2H_2O \rightarrow 2Fe_3O_4 + 3SiO_2 + 2H_2$

Microbial energy extraction by H_2 oxidation:

$H_2 + ½O_2 \rightarrow H_2O$ ($\Delta G^{o\prime} = -237$ kJ/mole)

Microbial energy extraction by NADH oxidation:

$NADH + H^+ + ½O_2 \rightarrow NAD^+ + H_2O$ ($\Delta G^{o\prime} = -228$ kJ/mole)

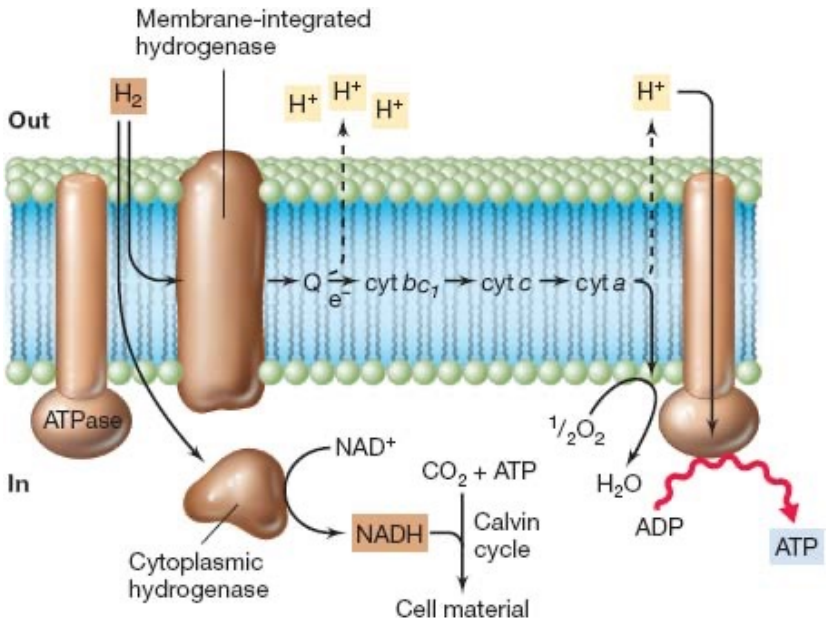
SOURCE: Edwards KJ, Wheat CG, Sylvan JB (2011) Under the sea: microbial life in volcanic oceanic crust. *Nature Rev Microbiol* 9(10): 703-712 PMID: 21894169.

SOURCE: Martin W, Baross J, Kelley D, Russell MJ (2008) Hydrothermal vents and the origin of life. *Nature Rev Microbiol* 6(11): 805-814 PMID: 18820700.

SOURCE: Martin WF, Sousa FL, Lane N (2014) Energy at life's origins – analysis of the bioenergetics of primitive organisms suggests that life began at hydrothermal vents. *Science* 344(6188): 1092-1093 PMID: 24904143.

Box 2. Serpentinization: the source of H_2 and CH_4 at Lost City Hydrothermal Field (LCHF). Hydrothermal vents, and Lost City in particular, have sparked interest in a geochemical process known as serpentinization. At off-axis vents, sea-water invades the warm (100° C) to hot (400° C) oceanic crust through cracks and crevasses where the chemical reactions of serpentinization take place. The relevant sea-water constituents for the serpentinization reaction are H_2O and CO_2 (dissolved as HCO_3^-). The relevant crustal constituents are Fe^{2+}-containing rocks. At Lost City, this rock consists mainly of the mineral olivine ($Mg_{1.6}Fe_{0.4}SiO_4$). Seismic data indicate that the fluids beneath Lost City percolate to depths of 500 meters (or deeper) beneath the sea floor at moderately high temperatures (150-200° C). The crust beneath Lost City is 1-2 million years old based on magnetic anomaly information, and it is likely that the rocks which are 500-1,000 meters beneath the sea floor reach temperatures of 300° C. Under these conditions, Fe^{2+} in the rocks reduces H_2O to produce Fe^{3+}, H_2, and hydrocarbons. Unaltered and hydrothermally altered mantle rocks contain various carbon compounds, including graphite, CH_4 and CO_2. The resulting minerals are magnetite (Fe_3O_4), which contains Fe^{3+} as a product of Fe^{2+} oxidation, brucite ($Mg(OH)_2$) and a hydroxylated Mg-Fe silicate called serpentine ($Mg_{2.85}Fe_{0.15}Si_2O_5(OH)_4$), after which the process is named. Serpentinization has probably been ongoing since there were oceans on the Earth. One cubic meter of olivine can deliver approximately 500 moles of H_2 during serpentinization. Most of the Earth's oceanic crust consists of olivine (or pyroxene, which can also participate in serpentinization reactions), and the **total volume of the Earth's ocean is estimated to circulate through hydrothermal vents every 100,000 years**. Thus, the vast amounts of Fe^{2+}, the Earth's electron reservoir for H_2 production via serpentinization, in the mantle is nowhere near exhaustion. Serpentinization delivers, and has always delivered, a substantial amount of H_2 as a source of electrons for primary production in submarine ecosystems. At LCHF, serpentinization produces H_2 that can reduce CO_2 to CH_4 geochemically. The same geochemical process might have given rise to the energy-releasing chemical reactions at the core of carbon and energy metabolism in methanogens and acetogens, reactions that were eventually augmented by cofactors and enzymes.

Energy conservation in H_2-oxidizing microbes



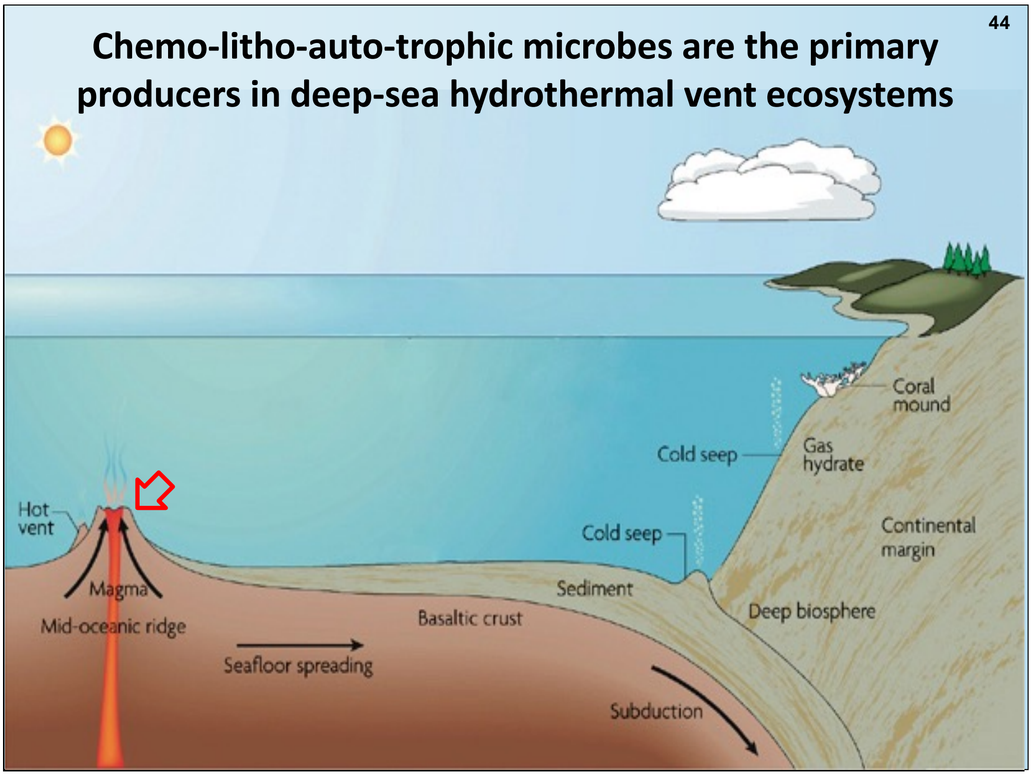
SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.26. Bioenergetics and function of the two hydrogenases of aerobic hydrogen (H_2)-oxidizing bacteria. In *Ralstonia eutropha* two hydrogenases are present. The **membrane-bound hydrogenase** participates in energetics. The **cytoplasmic hydrogenase** makes NADH for the Calvin-Benson-Bassham cycle. Some H_2-oxidizing bacteria have only the membrane-bound hydrogenase, and in these organisms reducing power is synthesized by reverse electron flow from Q back to NAD^+ to form NADH.

Abbreviations:

Cyt, cytochrome

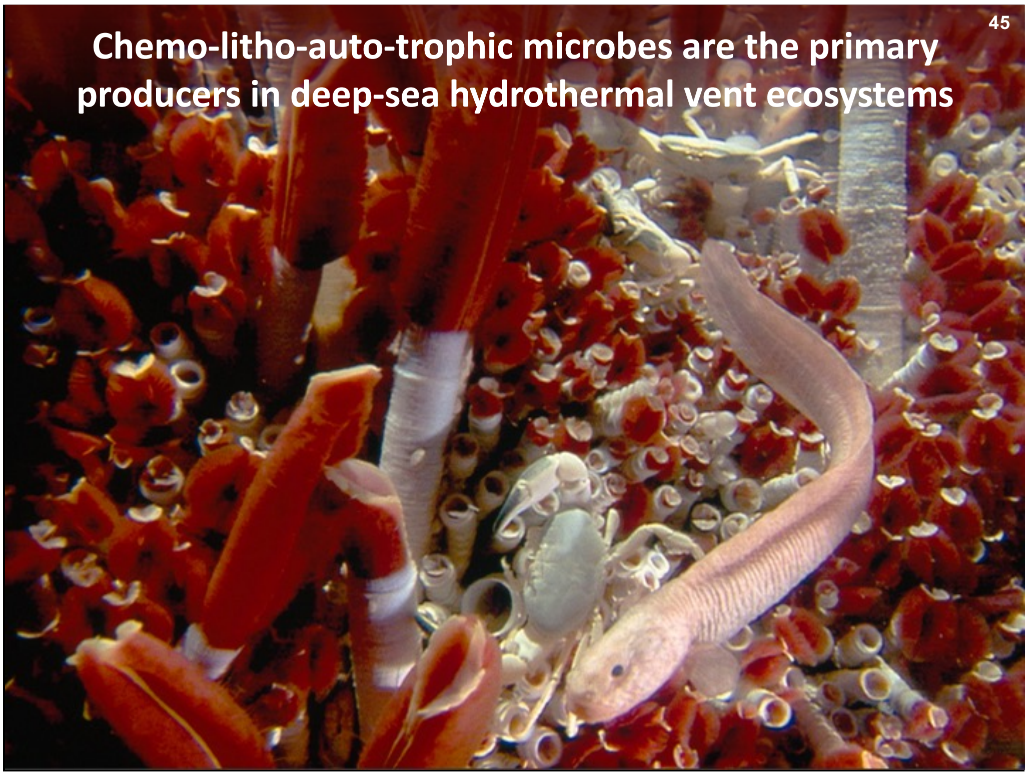
Q, quinone



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 20: Microbial Ecosystems (pp. 651-686), published by Pearson Education Inc., San Francisco © 2019.

SOURCE: Jorgensen B, Boetius A (2007) Feast and famine - microbial life in the deep sea bed. *Nature Rev Microbiol* 5(10): 770-781 PMID: 17828281.

Figure 1. Vertical section of the seabed and seafloor structures. This figure shows the plate-tectonic conveyer belt. At the mid-oceanic ridge new basaltic crust is formed continuously. The ocean plates move towards the continents where they are subducted again. Sinking particles form thick sediment piles on the ageing crust and on the continental margins. Plate tectonics and gravitational and hydrological forces cause a range of structures to form on the seabed, including hydrothermal vents at mid-oceanic ridges and subduction zones, gas-seeping mud volcanoes on continental margins and carbonate mounds inhabited by cold-water corals.



SOURCE: http://photography.nationalgeographic.com/photography/wallpaper/deep-sea-tubeworms-photography.html

SOURCE: http://environment.nationalgeographic.com/environment/habitats/deep-sea-vents/

SOURCE: http://news.nationalgeographic.com/news/2002/10/1028_021028_TVtubeworm.html

"Deep-sea hydrothermal vent giant tubeworms. With the help of high-intensity lighting, high-resolution cameras, and the deep-sea submarine *Alvin*, Kristof and his crew captured dramatic imagery in the dark, superheated depths of the Mid-Atlantic Ridge. At these nadirs, chemosynthesis - the thermal and chemical energy given off by deep-sea vents - gives life to creatures like the eelpout fish and tubeworms pictured here."

Along the Galápagos Rift is a hotbed of volcanoes and hydrothermal vents that spew boiling soups of metals, salts, and poisonous gas. These seemingly inhospitable vents are home to some of the toughest, most exotic life on Earth, including clams and mussels as big as dinner plates and bright-white tubeworms six feet long. The giant tubeworms, discovered during a path-breaking exploration of the rift in **1977**, forced a rethinking of life on the ocean floor. The rift lies 500 kilometers north of the Galápagos Islands, and the seafloor extends 2,500 meters below the waves. "The rule of thumb was that only small organisms could occupy the deep sea," says Tim Shank, a biologist at the Woods Hole Oceanographic Institution in Massachusetts and co-director of a recent expedition to the Galápagos Rift organized to mark the 25th anniversary of the hydrothermal vent discovery. "We knew there were microbial communities that could survive without sunlight or oxygen, but tubeworms are massive life-forms," he added. The recent expedition focused primarily on tubeworms, which have developed unique adaptations to their environment. These worms live in pitch-black ocean depths in water laced with acid and toxic gas – harsh conditions that may resemble those in which life first evolved.

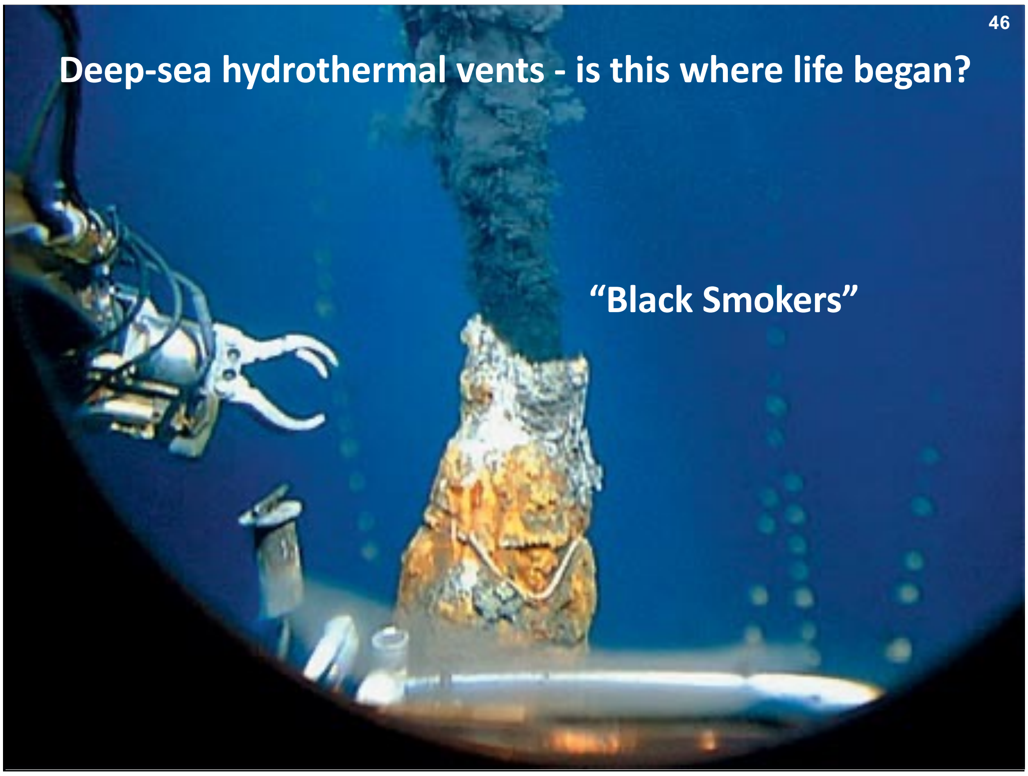


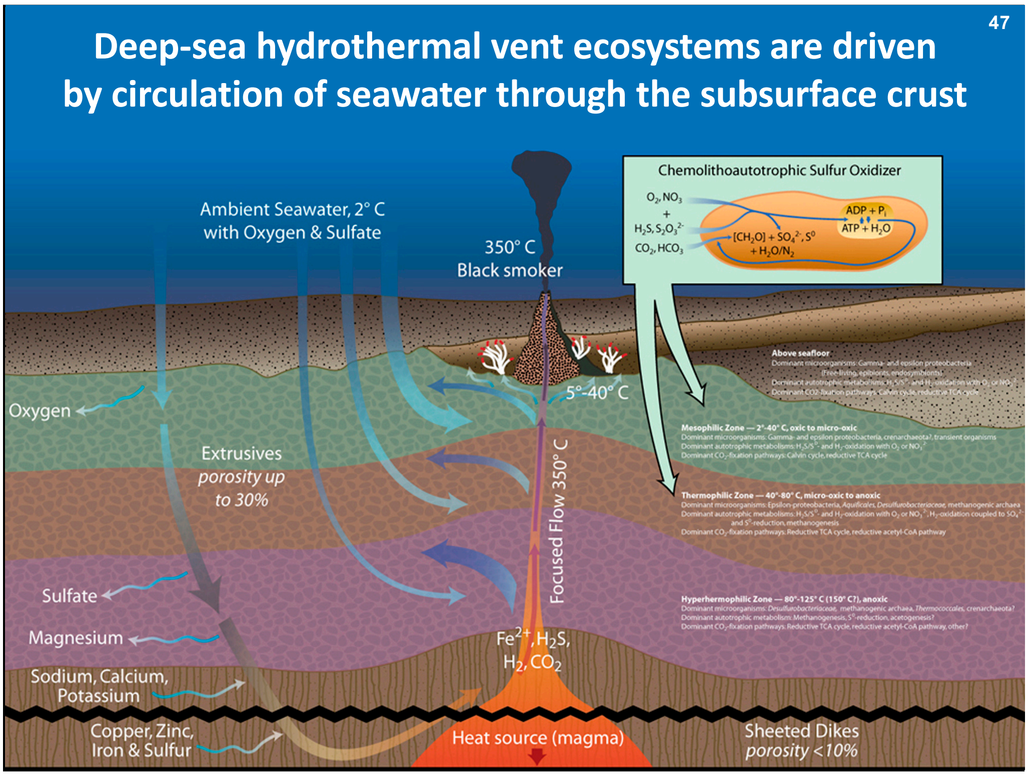
IMAGE SOURCE: http://www.whoi.edu/oceanus/viewArticle.do?id=2400

The submarine *Alvin* reaches its manipulator toward a “black smoker” chimney (hydrothermal vent), seen through the submarine's viewport, at 17° S on the East Pacific Rise. Hot hydrothermal fluids surge through the chimney at velocities of 1 to 5 meters per second. The "black smoke" consists of an abundance of dark, fine-grained, suspended particles that precipitate when the hot fluid mixes with cold seawater.

SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 20: Microbial Ecosystems (pp. 651-686), published by Pearson Education Inc., San Francisco © 2019.

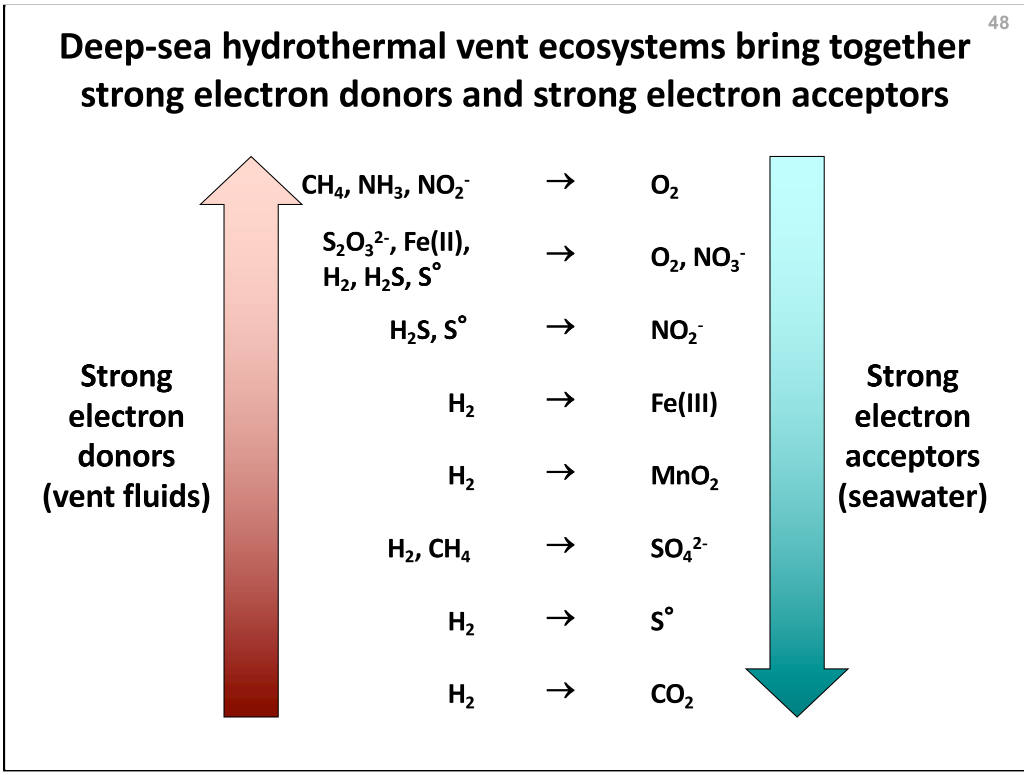
SOURCE: Martin W, Baross J, Kelley D, Russell MJ (2008) Hydrothermal vents and the origin of life. *Nature Rev Microbiol* 6(11): 805-814 PMID: 18820700.

Submarine hydrothermal vents are geochemically reactive habitats that harbor rich microbial communities. There are striking parallels between the chemistry of the H_2 / CO_2 redox couple that is present in hydrothermal systems and the core energy metabolic reactions of some modern prokaryotic autotrophs. The biochemistry of these autotrophs might, in turn, harbor clues about the kinds of reactions that initiated the chemistry of life. Hydrothermal vents thus unite microbiology and geology to breathe new life into research into one of biology's most important questions - what is the origin of life? The chemistry of life is the chemistry of reduced organic compounds, and therefore all theories for the origin of life must offer testable hypotheses to account for the source of these compounds. The best-known theories for the origin of organic compounds are based on the notion of an 'organic soup' that was generated either by lightning-driven reactions in the early atmosphere of the Earth or by delivery of organic compounds to the Earth from space. When submarine hydrothermal vents were discovered 30 years ago, hypotheses on the source of life's reduced carbon started to change. Hydrothermal vents revealed a vast and previously unknown domain of chemistry on the Earth. These vents harbour rich ecosystems, the energy source of which stems mainly from mid-ocean-ridge volcanism. The 360° C sulphide chimneys of the vent systems are primordial environments that are reminiscent of early Earth, with reactive gases, dissolved elements, and thermal and chemical gradients that operate over spatial scales of centimetres to metres. This discovery had an immediate impact on hypotheses about the origin of life, because it was recognized that the vent systems were chemically reactive environments that constituted suitable conditions for sustained prebiotic syntheses.



SOURCE IMAGE: http://www.whoi.edu/page.do?pid=18956 (Sievert Lab - Microbial Ecology & Physiology)

Schematic diagram depicting a mid-ocean ridge hydrothermal vent site and potential microbial habitats in the subseafloor. Seawater is cycling through the seafloor where it is geothermally altered. Hot, reducing fluids containing mM concentrations of reduced sulfur compounds ascend to the seafloor where they are either exiting undiluted through black smokers or are mixing in varying proportions with seawater in the subseafloor before exiting the seafloor at diffuse-flow vent sites. The latter creates a range of physicochemical conditions and energy sources that can be exploited by different types of microbes living in the subseafloor. The stylized cell depicts a chemolithoautotrophic sulfur-oxidizing bacterium that can use oxygen or nitrate as an electron acceptor. The growth of these organisms in the subseafloor is primarily expected to occur at temperatures between 4˚C and 50˚C.



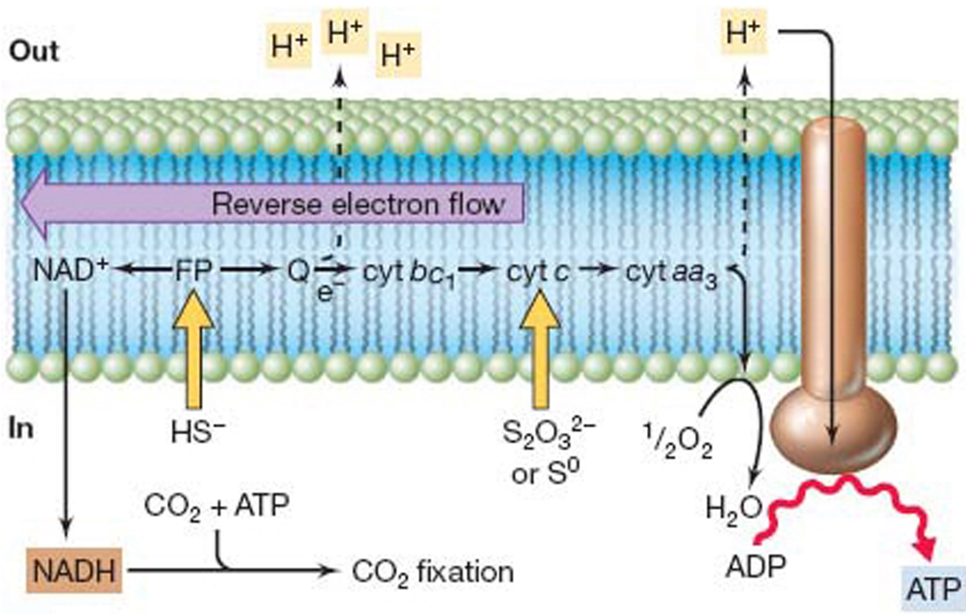
SOURCE: Martin W, Baross J, Kelley D, Russell MJ (2008) Hydrothermal vents and the origin of life. *Nature Rev Microbiol* 6(11): 805-814 PMID:18820700.

Submarine hydrothermal vents are geochemically reactive habitats that harbor rich microbial communities. There are striking parallels between the chemistry of the H_2-CO_2 redox couple that is present in hydrothermal systems and the core energy metabolic reactions of some modern prokaryotic autotrophs. The biochemistry of these autotrophs might, in turn, harbour clues about the kinds of reactions that initiated the chemistry of life. Hydrothermal vents thus unite microbiology and geology to breathe new life into research into one of biology's most important questions - what is the origin of life?

SOURCE: Jorgensen B, Boetius A (2007) Feast and famine - microbial life in the deep sea bed. *Nature Rev Microbiol* 5(10): 770-781. PMID:17828281.

Box 2. The microbial menu at hydrothermal vents. Some bacteria and archaea have the ability to produce biomass by using chemical energy to fix carbon dioxide, through a process called chemosynthesis. However, most chemosynthetic life is not independent of photosynthesis: it depends on access to oxygen and nitrate as electron acceptors, and these are ultimately derived from photosynthesis. Chemoautotrophic microorganisms gain energy by oxidizing hydrogen, methane, hydrogen sulphide, ammonia, ferrous iron Fe(II) and manganese (II), all of which occur in vent fluids. Depending on how they mix with seawater, CO_2, SO_4^{2-}, S^0, ferric iron Fe(III), NO_3^-, or O_2 can be available as oxidants. In the diagram, energy sources that are available at hydrothermal vents are shown. These comprise the redox couples of **electron donors in vent fluids** (left) and **electron acceptors in seawater** (right). Consortia of microorganisms control the rates of redox reactions and modify their environment by producing biofilms and mats or establishing symbioses with animals. This enables them to access energy more easily and reliably, as they avoid being washed away by the currents. The different microbial metabolisms that occur at vents, the distributions of bacteria and archaea in relation to physicochemical parameters such as temperature, pressure, pH, redox level, and the presence or absence of other chemicals, is not yet well understood.

Energy conservation in sulfur-oxidizing microbes



SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.28. Oxidation of reduced sulfur compounds by sulfur chemolithotrophs. Electrons from sulfur compounds feed into the electron transport chain to drive a proton motive force. Electrons from thiosulfate (S_2O3^{2-}) and elemental sulfur (S^0) enter at the level of cytochrome *c*. Electrons from hydrosulfide ion (HS^-) enter at the level of flavoprotein. NADH must be made by energy-consuming reactions of reverse electron flow because the electron donors are more electronegative than $NAD(P)^+$, i.e., the reduction potential (E_0' in V) of the electron donors is higher than the E_0' of $NAD(P)^+$.

Sulfur chemolithotrophs are acidophiles they grow optimally at pH 3 or lower! Their final oxidative waste product is $SO_4^{2-} + H^+ \rightarrow H_2SO_4$ a.k.a. sulfuric acid!

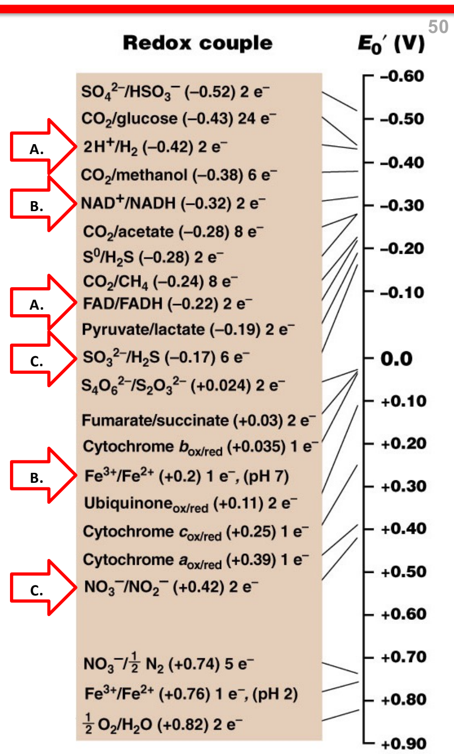
Abbreviations: **Cyt**, cytochrome; **FP**, flavoprotein; **Q**, quinone.

Which of the following reactions would require an input of energy to drive reverse electron flow:

A. Oxidation of H_2 by FAD

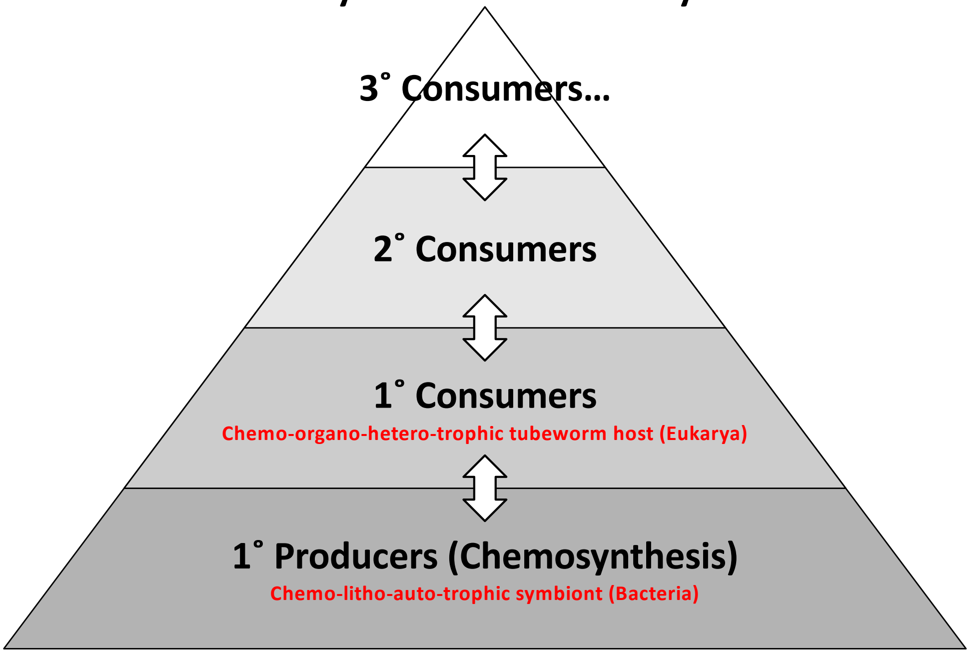
B. Oxidation of NADH by Fe^{3+}

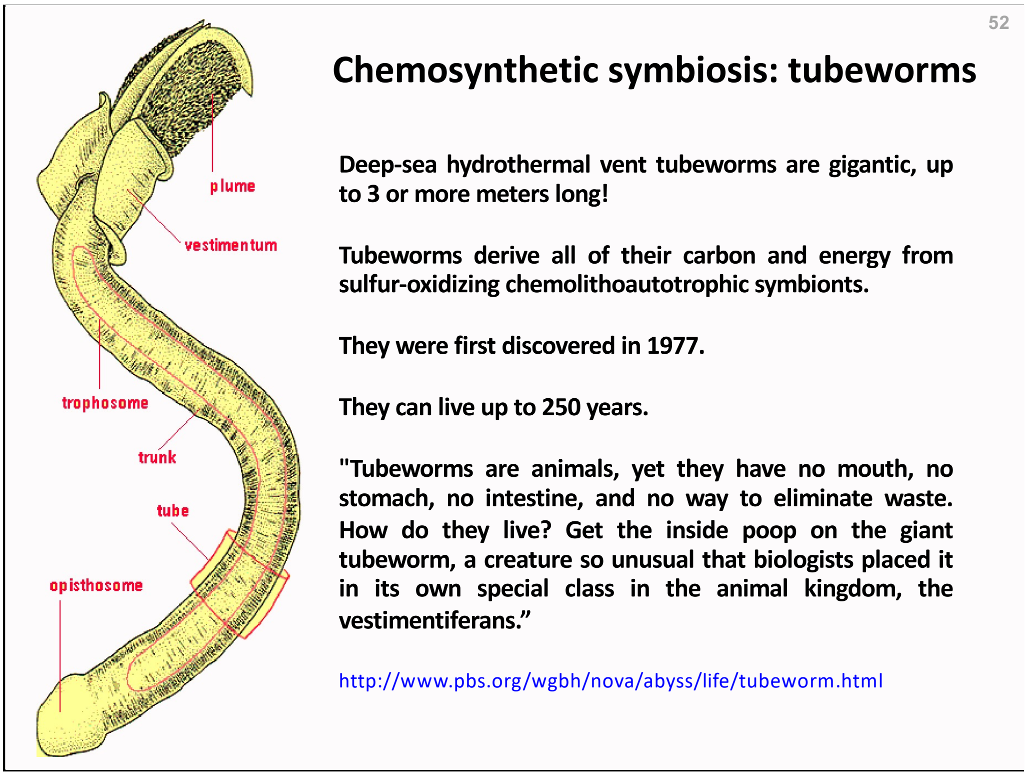
C. Oxidation of NO_2^- by SO_3^{2-}



Answer: (C)

Metabolic symbiosis: cross-feeding between a bacterial symbiont and a eukaryotic host





SOURCE: http://www.pbs.org/wgbh/nova/abyss/life/tubeworm.html

SOURCE: http://www.ocean.udel.edu/extreme2002/creatures/tubeworms/index.html#

Resembling giant lipsticks, tubeworms (*Riftia pachyptila*) live over a mile deep on the Pacific Ocean floor near hydrothermal vents. They may grow to about 3 meters long. The worms' white tube home is made of a tough, natural material called chitin.

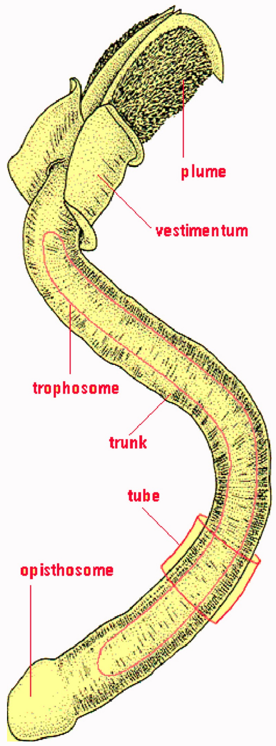
Tubeworms have no mouth, eyes, or stomach ("gut"). Their survival depends on a symbiotic relationship with billions of bacteria that live inside them. These bacteria convert the chemicals spewing out of the vents into worm food. This chemical-based food-making process is known as chemosynthesis.

Since a tubeworm has no mouth, how do bacteria enter the worm? Scientists have found that, during its earliest stages, the tubeworm does have a mouth and gut for bacteria to enter. But as the worm grows, these features disappear!

The bright-red plume is the tubeworm's breathing apparatus. The blood in it contains special forms of hemoglobin that have a super-high affinity for the oxygen in the seawater. Masses of tubeworms, with their showy plumes, inspired scientists to name one vent field "The Rose Garden" in 1979.

However, during an expedition that began in May 2002, scientists from Woods Hole Oceanographic Institution and NOAA's Ocean Exploration Program found that "The Rose Garden" may have been covered over with lava from a recent volcanic eruption. They found a thriving new site nearby that they named "Rosebud." For more details, read the story on the Woods Hole Web site.

Tubeworms were first discovered in **1977** on an expedition to the Galápagos Rift led by geologist Jack Corliss. The discovery was unexpected, as the team were studying hydrothermal vents and no biologists were included in the expedition.



Trophosome : This dark green-brown spongy tissue is where the action takes place. Here, the microbes that live symbiotically in the worm make their home in special cells. (Quite a few microbes live here: about 10^{10} bacteria per gram of tissue.) In exchange for a safe, cozy place to live, they give the worm all the food it needs. They do this by absorbing three compounds pumped down from the plume (O_2, H_2S, and CO_2) and controlling their reaction. The bacteria use the chemical energy released from O_2-mediated oxidation of H_2S into SO_4^{2-} to fix CO_2 into the organic carbon that nourishes the microbes and the worm.

Symbionts of giant tubeworms are H_2S-oxidizing bacteria. They reduce CO_2 using two carbon-fixation pathways: the Calvin-Benson-Bassham Cycle and the Reductive Citric Acid Cycle.

SOURCE: http://www.pbs.org/wgbh/nova/abyss/life/tubeworm.html

SOURCE: http://www.ocean.udel.edu/extreme2002/creatures/tubeworms/index.html#

Plume - This soft, bright-red structure serves the same purpose as a mouth would if the tubeworm had one. It sucks in the ingredients that the microbes living in the worm's body will use to generate its food. These three ingredients, O_2 and CO_2 in seawater and H_2S in the superheated water erupting from the vent, tend to react violently when they come into contact. Yet using special hemoglobins in its blood-rich plume (hence the red color), the tubeworm has found a way to transport these compounds in its blood without this reaction taking place - and without the toxic H_2S poisoning it.

Vestimentum - This muscular structure helps to anchor the upper portion of the worm in the tube. It provides safe passage for the blood heading from the plume to the trophosome. It generates new tube material. It holds the reproductive pores from which the worm releases sperm or eggs during spawning; these combine in the water to make baby tubeworms. Finally, along with various glands, this structure harbors simplified versions of the two organs that most closely bind this primitive creature to its fellow animals: the heart and the brain.

Trophosome - This organ of dark green-brown spongy tissue is where the real action takes place. Here, the microbes that live symbiotically in the worm make their home in special cells. (Quite a few microbes live here: an estimated 10 billion bacteria per gram of tissue.) In exchange for a safe, cozy place to live, they give the worm all the food it needs. They do this by absorbing those three compounds pumped down from the plume (O_2, H_2S, CO_2) and controlling their reaction. The microbes use the chemical energy released from O_2-mediated oxidation of H_2S into SO_4^{2-} to fix CO_2 into the organic carbon that nourishes both the microbes and the worm.

Trunk - Imagine having no anus. Waste would have nowhere to go, right? Well, that's the case with the tubeworm. It has no anus, and so the sulfate left over after the microbes have done their business is simply stored in the animal's body. Since giant tubeworms can live for many decades, you can imagine quite a heap of this stuff building up in their tissues. Yet it is not this waste material but sulfide in the worm's bloodstream that gives the animal its powerful rotten-egg stench.

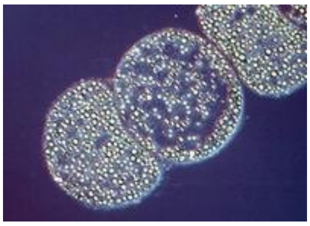
Tube - This hard cylinder, which varies in thickness between and even within species of tubeworm, is a proteoglycan-protein-chitin complex, basically like the shell of a lobster or crab. It grows as the worm grows, providing a safe home for the animal. The delicate plume, which is the tubeworm's only exposed part, can be retracted into the tube at a moment's notice, such as when a hungry predator happens by...

Opisthosome - Like the vestimentum, the opisthosome produces new tube material and helps anchor the worm in its tube, which is often planted deep within the crevices of a black smoker. Giant tubeworms can reach 3+ meters in length, and the temperatures they have to cope with over that length boggle the mind. Imagine having your head in near-freezing water and your foot planted in scalding rock. That's what tubeworms have to deal with: biologists have measured temperatures at a worm's plume of 2° C while that at its base is 70° C.

Oxidation of sulfur compounds by chemo-litho-auto-trophs

Electron donor	*Chemolithotrophic Reaction*	$\Delta G^{o\prime}$ *kJ/reaction*	$\Delta G^{o\prime}$ *kJ/e⁻*
Hydrogen Sulfide	$H_2S + 2\,O_2 \rightarrow SO_4^{2-} + 2\,H^+$	–798.2	–99.75
Hydrosulfide Ion	$HS^- + 2\,O_2 + H^+ \rightarrow SO_4^{2-} + 2\,H^+$	–796.5	–99.56
Hydrosulfide Ion	$HS^- + {}^1/_2\,O_2 + H^+ \rightarrow S^0 + H_2O$	–209.4	–104.7
Sulfur	$S^0 + H_2O + 1{}^1/_2\,O_2 \rightarrow SO_4^{2-} + 2\,H^+$	–587.1	–97.85
Thiosulfate	$S_2O_3^{2-} + H_2O + 2\,O_2 \rightarrow 2\,SO_4^{2-} + 2\,H^+$	–818.3	–102.0

$$H_2S \underset{pH < 7}{\overset{pH > 7}{\rightleftharpoons}} HS^- + H^+$$



Thiomargarita namibiensis. The bright yellow spots are storage granules of inorganic sulfur (S^0). Each cell is up to 1000 µm in diameter.

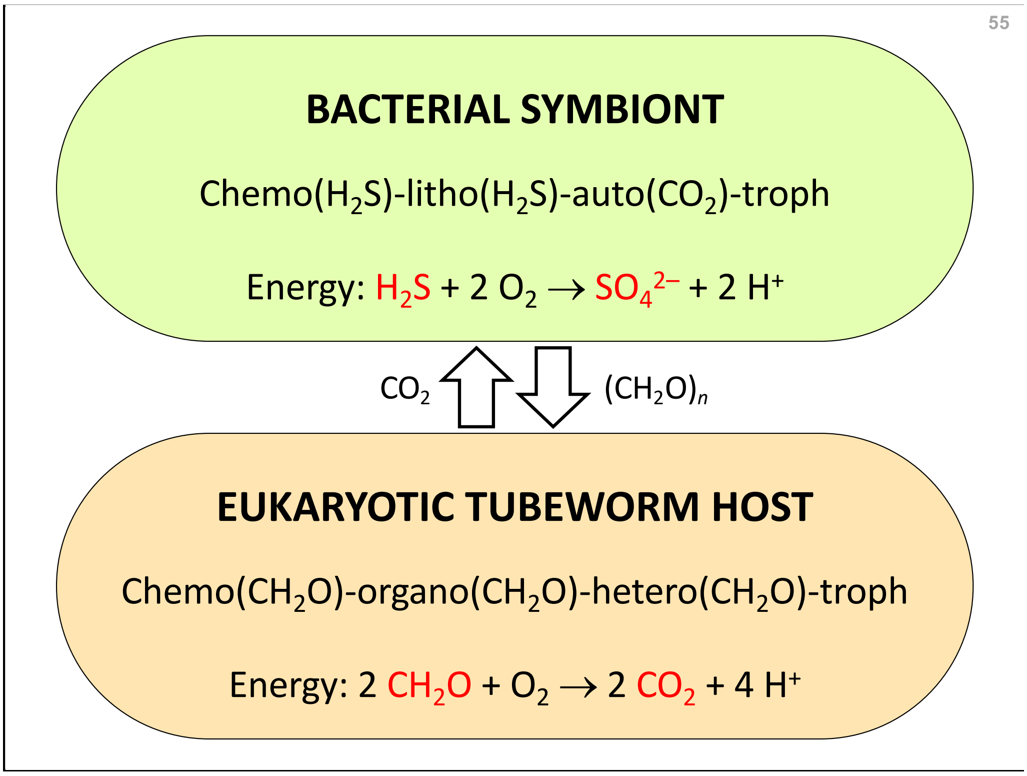
SOURCE: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 14: Metabolic Diversity of Microorganisms (pp. 428-486), published by Pearson Education Inc., San Francisco © 2019.

Figure 14.28. Oxidation of reduced sulfur compounds by sulfur chemolithotrophs.

SOURCE: http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art_id=58

Figure 2-34 depicts another visible structure, termed a sulfur globule, which is found in a variety of bacteria capable of oxidizing reduced sulfur compounds such as hydrogen sulfide and thiosulfate. Oxidation of these compounds is linked either to energy metabolism or photosynthesis. Oxidation of sulfide initially yields elemental sulfur, which accumulates in globules inside or outside the cell. If the sulfide is exhausted the sulfur may be further oxidized to sulfate.

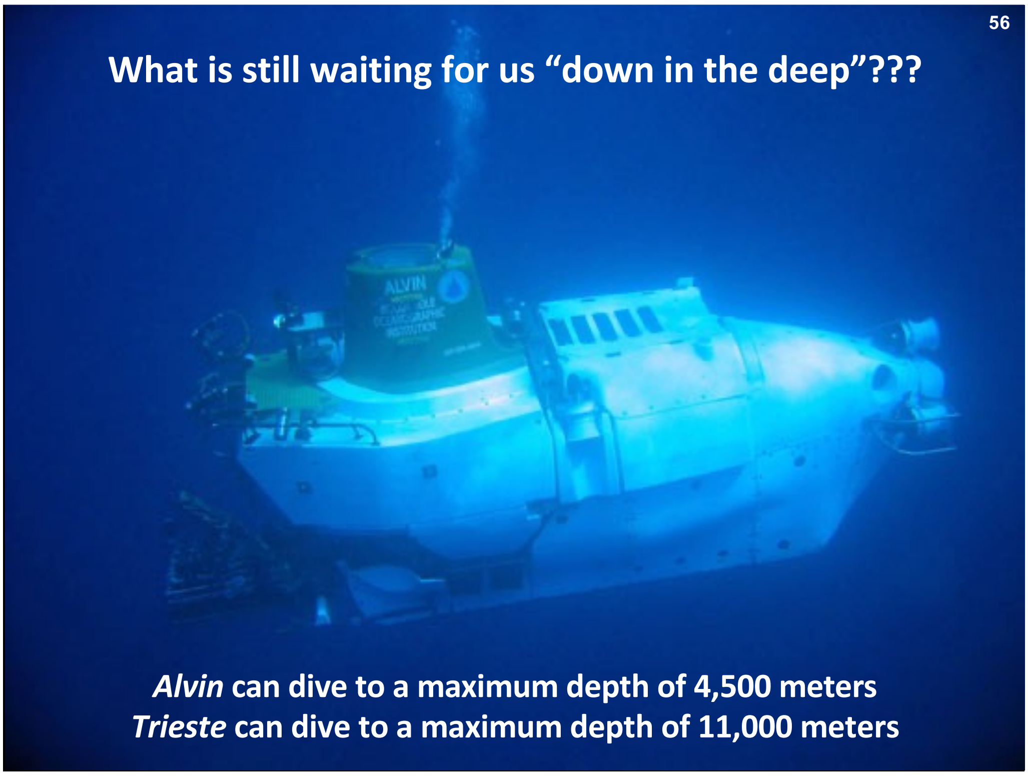
Abbreviations: $\mathbf{H_2S}$, hydrogen sulfide; $\mathbf{HS^-}$, hydrosulfide anion; $\mathbf{S^0}$, elemental sulfur; $\mathbf{SO_4^{2-}}$, sulfate; $\mathbf{S_2O_3^{2-}}$, thiosulfate.



SOURCE: Dubilier N, Bergin C, Lott C (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Rev Microbiol* 6(10): 725-740 PMID: 18794911.

The bacterial symbiont is a **chemolithoautotroph**. Its **energy source** comes from a chemical reaction (chemo-): oxidation of H_2S (electron donor) by O_2 (electron acceptor). Its **electron source** is an inorganic molecule, H_2S (-litho-). Its **carbon source** is an inorganic molecule, CO_2 (-auto-).

The tubeworm host is a **chemoorganoheterotroph**. Its **energy source** comes from a chemical reaction (chemo-): oxidation of organic compounds (electron donor) by O_2 (electron acceptor). Its **electron source** is organic compounds (-organo-). Its **carbon source** is organic compounds (-hetero-).



SOURCE: Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, Van Dover CL, Martinson TA, Plumley FG (2005) An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. *Proc Natl Acad Sci USA* 102 (26): 9306-9310 PMID: 15967984.

Abstract: The abundance of life on Earth is almost entirely due to biological photosynthesis, which depends on light energy. The source of light in natural habitats has heretofore been thought to be the sun, thus restricting photosynthesis to solar photic environments on the surface of the Earth. If photosynthesis could take place in geothermally illuminated environments, it would increase the diversity of photosynthetic habitats both on Earth and on other worlds that have been proposed to possibly harbor life. Green sulfur bacteria are anaerobes that require light for growth by the oxidation of sulfur compounds to reduce CO_2 to organic carbon, and are capable of photosynthetic growth at extremely low light intensities. We describe the isolation and cultivation of a previously unknown green sulfur bacterial species from a deep-sea hydrothermal vent at 2,500 meters below the ocean surface, where the only source of light is geothermal radiation that includes wavelengths absorbed by photosynthetic pigments of this organism.

SOURCE: http://www.wisegeek.com/what-is-the-deepest-depth-a-submarine-can-go.htm

A small submarine, the bathyscape *Trieste*, made it to 10,916 meters below sea level in the deepest point in the ocean, the Challenger Deep in the Marianas Trench, a few hundred miles east of the Philippines. This part of the ocean is 11,034 meters deep, so it seems that a submarine can make it as deep as it's theoretically possible to go. The water pressure at this depth is over 1,000 atmospheres (as a rule of thumb, the pressure increases by one atmosphere for every 10 meters of descent). Life does exist here, as well as a carpet of diatomaceous material that covers all the ocean floors of the world. *Trieste* was manned by two people and funded by the US Navy. The pressure sphere used was 2.16 meters across, with steel walls 12.7 cm thick, able to withstand 1.25 metric tons per cm^2 (110 MPa) of pressure. The pressure sphere of *Trieste*, which weighed 8 metric tons in water, was not neutrally-buoyant because the steel had to be so thick for a 2 meter-sized sphere at that depth to withstand the pressure that it would have sunk like a rock on its own. Therefore *Trieste*'s pressure sphere had to be attached to a series of gasoline floats, accompanied by iron pellets for weight. Initially slightly denser than water, the craft descended 10.9 km below sea level. At the bottom, the pellets were ejected, and the buoyant gasoline floats carried *Trieste* back to the top. This feat has never been replicated by a manned craft, although several unmanned submersibles have since explored the Challenger Deep.