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Biological Diversity: A Geobiological Perspective

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1.1 Introduction

Life can be understood as a living form, either cellular or acellular form, and is composed of interconnected components that are organized in a complex hierarchical structure. This hierarchy ranges from the smallest units—atoms and molecules—to cells, tissues, organs, and ultimately whole organisms. Together with its environment, life forms the biosphere, an intricate and interactive hierarchy consisting of species, populations, communities, and ecosystems (Figure 1.1). Each level in this biological hierarchy, from cells to the biosphere, operates as a self-sustaining and adaptive system that has been constantly co-evolving with physical environment since its origin. Life pervades our planet and potentially extends beyond it. Living organisms thrive in an astonishing variety of environments, from the icy poles to the scorching equator and from the depths of the oceans to several kilometers above the Earth's surface. They can be found in extreme conditions, such as freezing waters, dry valleys, undersea thermal vents, and even deep within the Earth's crust.

Over the past ~4.0 billion years, life on Earth has diversified and adapted to a myriad of environments. Despite this incredible diversity, all living organisms share common traits: they grow, adapt, reproduce, and interact with their surroundings. From microscopic organisms to expansive ecosystems, life takes many forms but is united by these shared characteristics. Every living being has the ability to replicate, driven by DNA as the molecular basis of replication. Moreover, all organisms possess mechanisms to translate the genetic information in DNA into functional products like proteins, fats, and carbohydrates, which are essential for building and maintaining cellular machinery.

Virtually every month, new discoveries are made about surprising occurrences and modes of life forms on Earth, ranging from proteorhodopsin-based phototrophy in the open ocean to methanogenesis driven by geochemical reactions in Earth's interior. Niches once considered to be uninhabitable (such as those with pH of 0 in extremely rich metal solutions) have been found to harbor thriving microbial communities; compounds once thought to be refractory (such as kerogen, or long-chain alkanes under anaerobic conditions) are now known to serve as microbial growth substrates; organisms previously believed to be unculturable (such as anaerobic benzene oxidizers or soil bacteria that produce medically relevant natural products) have been brought into culture and/or their genetic content has been expressed in recombinant strains; and enigmatic geochemical

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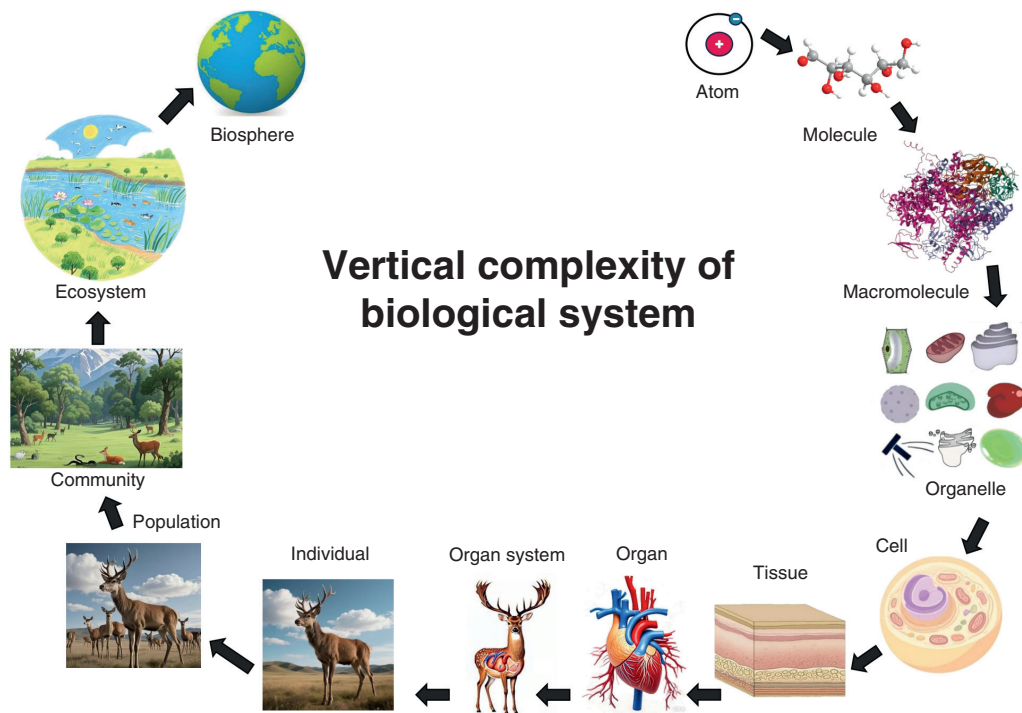


Figure 1.1 Biological hierarchy ranging from the smallest units—atoms and molecules—to cells, tissues, organs, whole organisms, and ultimately the biosphere.

transformations (such as the anaerobic oxidation of methane [AOM] or ammonium) are now attributed to the activity of consortia of bacteria and archaea. The importance and extent of biological diversity have captured the attention of life scientists, and now the community of geosciences is helping to define and explore interesting biogeochemical problems.

Up until now, we have been familiar with organisms that can be said to have a “conventional” metabolism. These are organisms that use organic compounds as energy sources, primarily via aerobic (respiratory) metabolism. In this chapter, we aim to explore the vast biological diversity, especially focusing on microbial metabolisms with geological consequences. To address gaps in biological knowledge for geology students, we begin with the current classification of acellular and cellular life forms. This is followed by an examination of metabolic diversity from a geobiological perspective, which is essential for comprehending geobiological processes.

1.2 Classification of Biological Entities

1.2.1 Acellular Life

Cellular and acellular life represent two fundamental categories of biological entities. Cellular life forms, encompassing prokaryotes and eukaryotes, are living organisms that are composed of one or more cells. These organisms are characterized by the presence of a cell membrane enclosing cytoplasm and genetic material (DNA or RNA), along with their ability to metabolize, grow, reproduce,

and respond to stimuli. In contrast, acellular life forms are biological entities that lack cellular structure and are generally not considered fully alive in a traditional sense. Acellular life forms include viruses that consist of genetic material (DNA or RNA) encased in a protein coat, viroids that are small RNA molecules infecting plants, and prions that are misfolded proteins causing diseases in animals and humans (Figure 1.2). Unlike cellular life forms that are autonomous and self-sustaining, acellular entities rely on other organisms to replicate and function. They display biological activity and functions only within living host cells, while existing as inert chemical particles in external environments, which are replicated only within a host organism (parasitic nature) and do not exhibit independent metabolism or growth.

Viruses and their allies may have influenced Earth's geology by infecting bacterial, archaeal, and eukaryotic hosts and altering their physiology. Given that bacteria and archaea have dominated Earth's biosphere for billions of years, these simple, non-self-sustaining, acellular entities—particularly viruses—may be involved in geobiological processes since the Archean Eon in multiple ways. Firstly, viruses contribute to nutrient cycling in marine environments by infecting and lysing microbial cells, such as bacteria and phytoplankton. This process releases organic matter and nutrients into the surroundings, thereby impacting sediment composition over geological time. Secondly, viruses drive microbial evolution through horizontal gene transfer (HGT), also known as lateral gene transfer, the process by which genetic material is transferred between organisms, bypassing traditional parent-to-offspring inheritance, which plays a major role in the evolution

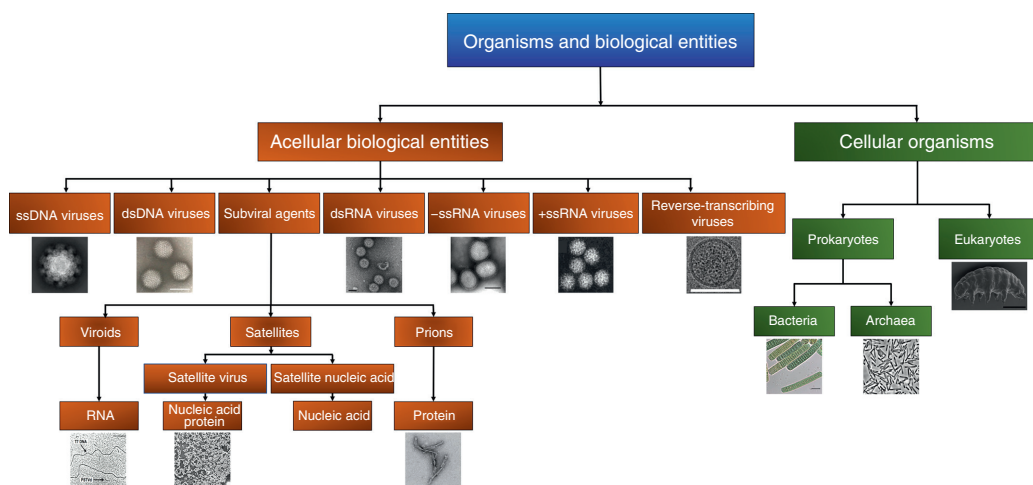


Figure 1.2 Microphotographs of acellular biological entities and cellular organisms. Source: parvovirus B19 (ssDNA viruses), Lee et al. (2024) / Springer Nature / CC BY-NC-ND 4.0; adenovirus (dsDNA viruses), Besson et al. (2020) / MDPI / CC BY 4.0; potato spindle tuber viroid (viroids), from International Committee on Taxonomy of Viruses, https://ictv.global/report_9th/subviral/Viroids; extra small virus (satellite virus), Krupovic et al. (2016) / Springer Nature; prions, Terry et al. (2019) / Springer Nature / CC BY-NC-ND 4.0; rotavirus (dsRNA viruses), Farkas et al. (2013) / Springer Science Business Media New York; influenza virus (-ssRNA viruses), Noda and Kawaoka (2010) / John Wiley & Sons; Aichi virus (+ssRNA viruses), from International Committee on Taxonomy of Viruses, <https://ictv.global/report/chapter/picornaviridae/picornaviridae>; HTLV-1 (reverse-transcribing viruses), Grigsby et al. (2010) / Springer Nature / CC BY 2.0; cyanobacteria (bacteria), reprinted with permission from Schneider et al. (2025) Schneider et al.; halobacteria (archaea), reprinted with permission from Cui and Dyll-Smith (2021) © 2021, Ocean University of China; tardigrade (eukaryotes), Hashimoto et al. (2016) / Springer Nature / CC BY-NC-SA 4.0.

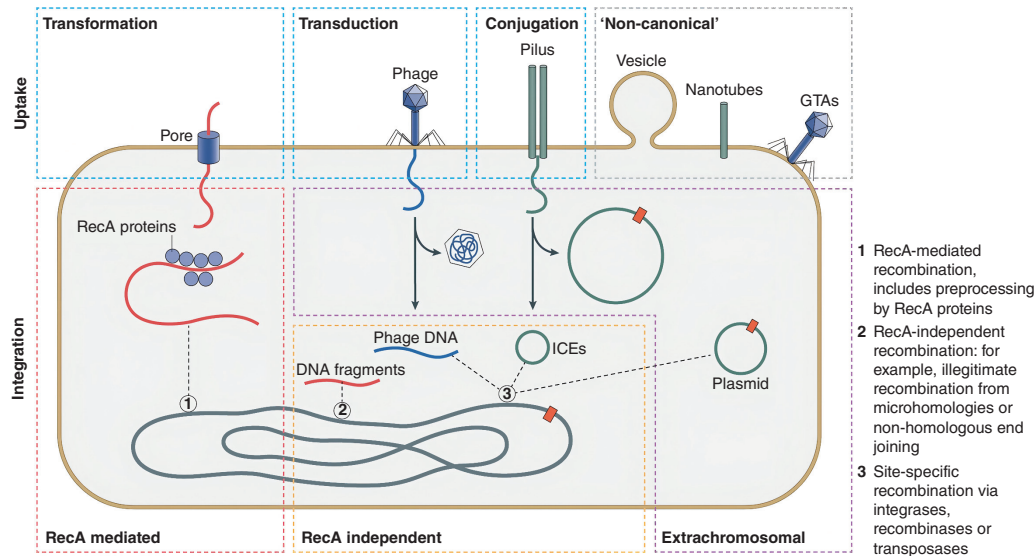


Figure 1.3 Mechanisms of horizontal gene transfer as a driving force of evolution. Transformation: direct uptake of genetic material from the environment; transduction: transfer mediated by viruses; conjugation: exchange through direct cell-to-cell contact. *Source:* Arnold et al. (2022) / with permission of Springer Nature.

of bacteria and archaea (Figure 1.3). HGT can reshape microbial communities and influence the breakdown or formation of mineral deposits by microorganisms. Thirdly, virus-like particles have been discovered in ancient sediments and rocks, offering valuable insights into Earth's early ecosystems and the evolutionary history of life. Lastly, viral activity in oceans affects phytoplankton populations, which play a role in carbon sequestration and regulate atmospheric CO₂ levels, indirectly connecting viruses to geological climate processes (Feng 2020; Wang 2022).

1.2.2 Domains of Cellular Life

The two-kingdom system of organisms, the Animalia and the Plantae, proposed by Carl Linnaeus in the 18th century, laid the groundwork for the systematic study of organisms, creating a structured approach to naming and classifying living forms. In the latter half of the 20th century, evidence began to accumulate that these were insufficient to express the diversity of life, and various schemes were proposed with three, four, or more kingdoms. This coexisted with a scheme dividing life into two main divisions: the prokaryota (bacteria, etc.) and the eukaryota (animals, plants, fungi, and protists).

However, with the advent of molecular taxonomy and phylogeny (sequence comparison of genes coding for 16S ribosomal RNA), our view of life on Earth changed radically. What were once called "prokaryotes" are far more diverse than anyone had suspected. The Prokaryota is now divided into two domains, the bacteria and the archaea. These two groups are as fundamentally different from each other as they are from Eukarya (Table 1.1). Neither of the prokaryotic domains is ancestral to the other, and each exhibits unique characteristics while sharing some common features with the others.

In the last decades, a great deal of additional work has been done to understand the nature of the last universal common ancestor (LUCA) of cellular life, the first eukaryotic common ancestor (FECA), and the last eukaryotic common ancestor (LECA), which is fundamental for

Table 1.1 A comparison of three domains of life.

Characteristics	Bacteria	Archaea	Eukarya
Nuclear envelope	Absent	Absent	Present
Membrane-enclosed organelles	Absent	Absent	Present
Peptidoglycan in cell wall	Present	Absent	Absent
Membrane lipids	Unbranched hydrocarbons	Some branched hydrocarbons	Unbranched hydrocarbons
RNA polymerase	One kind	Several kinds	Several kinds
Initiator amino acid for start of protein synthesis	Formyl-ethionine	Methionine	Methionine
Introns (non-coding parts of genes)	Rare	Present in some genes	Present
Response to the antibiotics streptomycin and chloramphenicol	Growth inhibited	Growth not inhibited	Growth not inhibited
Histones associated with DNA	Absent	Present	Present
Circular chromosome	Present	Present	Absent
Ability to grow at temperatures >100 °C	No	Some species	No

Source: Campbell and Reece (2002) / Benjamin Cummings.

reconstructing the branching pattern of life tree at the base (Figure 1.4). The three-domain system was proposed by Woese et al. (1990) based on genetic and molecular differences, especially in ribosomal RNA sequences, which highlighted the fundamental separation between archaea and bacteria. The two-domain system was proposed based on the eocyte hypothesis positing that eukaryotes evolved from a subgroup of archaea (Lake et al. 1984; Rivera and Lake 1992). Recent work enhances the view that the eukarya arose from well within the archaea and is specifically related to newly discovered archaeal species with eukaryote-like features (Doolittle 2022). Accordingly, archaea and eukaryotes were grouped into one domain due to shared genetic and evolutionary features.

1.2.2.1 The LUCA

The concept of the last universal common ancestor (LUCA) emerged in the late 20th century, significantly enhancing our understanding of the classification of living organisms. LUCA is defined as the ancestral entity from which all cellular life—bacteria, archaea, and eukaryotes—descends, and it represents a pivotal phylogenetic clade. Its genetic composition and biological features have been inferred through comparative genomic analyses on the basis of the conservation of biochemical traits. It was estimated that the LUCA had a genome coding for approximately 355 to 2600 proteins based on various studies (NA n.d.; Moody et al. 2024). These genes likely supported essential functions such as replication, transcription, translation, and basic metabolic pathways. The exact number remains debated due to the challenges of reconstructing LUCA's genome from modern descendants. Key aspects of LUCA remain unclear, such as its membrane structure—raising the possibility of a membrane-less form—and the mechanisms of its replication process. Hydrothermal vent systems are often hypothesized as LUCA's likely habitat. However, this hypothesis is challenged by the complexity of evolution, including phenomena like non-orthologous gene displacement and extensive HGT, which obscure its precise characteristics. Despite these uncertainties,

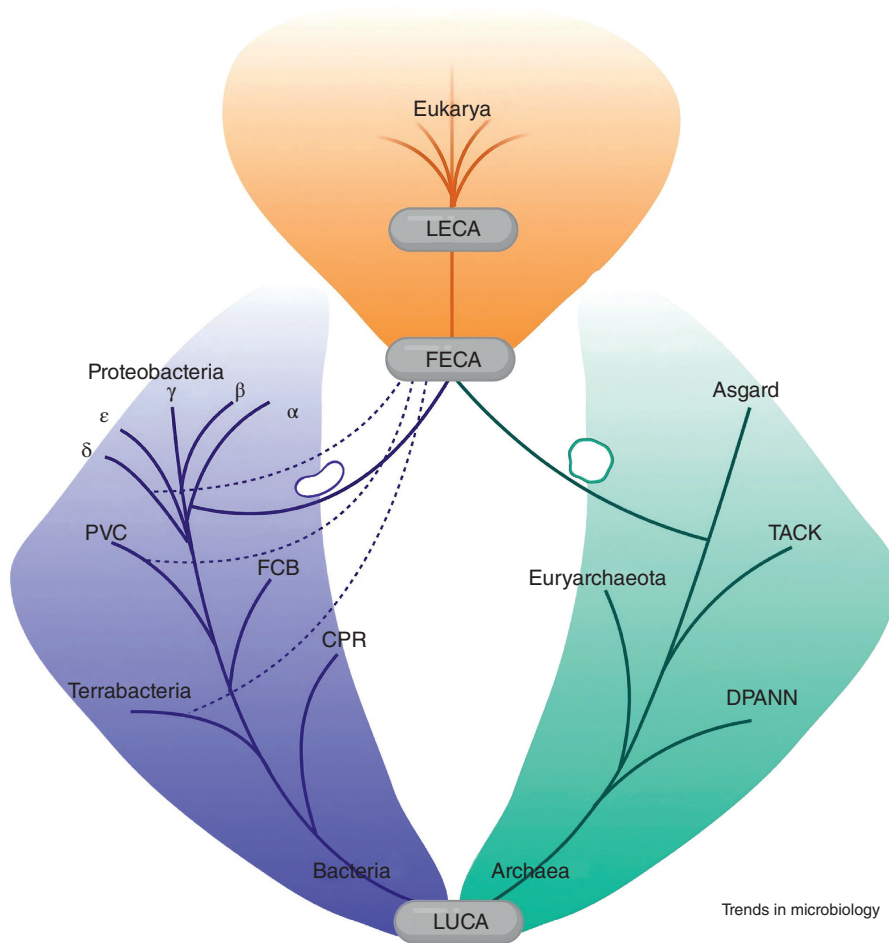


Figure 1.4 Evolution from the LUCA to LECA. A schematic based on the eocyte hypothesis that eukarya emerged from within the archaea. During symbiogenesis, the host cell was an Asgard-like archaeon, while the mitochondria originated from an ancestral alphaproteobacterial endosymbiont, both playing integral roles in shaping the eukaryotic genome. Furthermore, HGT from various bacterial lineages (represented by dotted lines) significantly enriched the genetic repertoire of the LECA, contributing to its evolutionary complexity. Abbreviations: CPR, Candidate Phyla Radiation; DPANN, Diapherotrites, Parv-, Aenigm-, Nano-, and Nanohaloarchaeota; FCB, Fibrobacteres-Chlorobi-Bacteroidetes; FECA, First Eukaryotic Common Ancestor; LUCA, Last Universal Common Ancestor; PVC, Planctomycetes-Verrucomicrobia-Chlamydiae; TACK, Thaum-, Aig-, Cren-, and Korarchaeota. *Source:* Nobs et al. (2022) / with permission of Elsevier.

current evidence suggests that the LUCA was a pre-domain organism with a streamlined core genome (a minimal set of essential genes that are crucial for the survival and basic functions of an organism), exhibiting transitional biochemical traits that preceded the evolutionary divergence of bacteria and archaea.

Despite ongoing research, certain controversial questions remain unresolved, such as whether viruses existed before the LUCA or what the nature of interactions between the LUCA and its virome might have been. Recently, a hypothesis was proposed that the LUCA served as a host to viruses, since all known life forms are closely associated with viruses or other mobile genetic elements. Efforts to reconstruct the LUCA virome have been undertaken by examining the

distribution of viruses among bacteria and archaea, as well as tracing the evolutionary history of key viral genes (Krupovic et al. 2020). The reconstruction suggested the LUCA had a complex virome that included major groups of viruses currently associated with bacteria and archaea. Furthermore, the LUCA virome was hypothesized to have split into the viromes of the last bacterial common ancestor (LBCA) and the last archaeal common ancestor (LACA). Notably, extensive viral evolution was thought to have occurred even before the existence of the LUCA, adding to the complexities of understanding its role in early life.

1.2.2.2 The FECA and LECA

The FECA is hypothesized as the earliest ancestor of all eukaryotic cells, representing the transition from simpler prokaryotic organisms to more complex eukaryotic life forms. The LECA is considered the most recent shared ancestor of all eukaryotic organisms, representing a critical juncture in evolutionary history that enabled the emergence of complex life. FECA eventually gave rise to LECA that had fully developed eukaryotic features, such as a nucleus, mitochondria, and an endomembrane system. Lynn Margulis famously popularized the hypothesis that eukaryogenesis was driven by endosymbiosis and proposed that mitochondria and chloroplasts originated from free-living bacteria (Sagan 1967). This theory explains the presence of complex internal organelles in eukaryotes, such as mitochondria, which are thought to have evolved from an ancient alphaproteobacterium. However, significant debates in evolutionary biology persist, including the relationship between archaea and eukarya and the identity of archaea as the host cell in eukaryogenesis. These unresolved issues are essential for understanding the basal pattern of the tree of life, particularly whether eukarya are a sister group to archaea or emerged from within the archaeal domain, members of the Asgard archaea or their closely related sister lineage (Nobs et al. 2022).

Eukaryogenesis is understood as an evolutionary process that begins with the microbial cell that diverged from the ancient Asgard lineage, referred to as the FECA that has no extant descendants, and culminates in the LECA, with various transitional stages in between referred to as the proto-eukaryote. Many eukaryogenic theories converge on the idea that an Asgard archaeal host cell and an alphaproteobacterial endosymbiont played fundamental roles in initiating eukaryogenesis. It is suggested that certain eukaryotic cellular features, such as the endomembrane system—comprising the endoplasmic reticulum (ER), Golgi apparatus, lysosomes, vacuoles, vesicles, and plasma membrane—along with a dynamic cytoskeleton, may have predated eukaryogenesis within the host archaeon. However, eukaryotic-specific traits, including genome compartmentalization within the nucleus and phagocytosis, represent distinct innovations that emerged within eukaryotes (Nobs et al. 2022).

The genome of LECA is thought to be a chimeric fusion of genes derived from both archaea and bacteria. Gene duplications, predominantly originating from an Asgard-related host cell, contributed to early eukaryotic complexity. Endosymbiotic gene transfer (EGT) occurred as the ancestral eukaryotic cell engulfed an alphaproteobacterial-related progenitor of the mitochondrion, leading to the integration of genetic material from both the endosymbiont and the host. Additionally, HGT from diverse bacterial groups beyond alphaproteobacterial lineages significantly enriched the proto-eukaryotic genome, further expanding its repertoire of eukaryotic genes. However, only a small fraction of eukaryotic genes appears to have originated directly from alphaproteobacteria, implying that mitochondrial acquisition was a relatively late event in eukaryotic evolution. Consequently, the LECA can be depicted as a cellular and genomic chimera, incorporating innovations from both its diverse archaeal and bacterial predecessors (Figure 1.5).

Many microbial syntrophies, forming a “microbial village,” could have nurtured the LECA. The symbiotic relationship could provide proximal space and sources for HGT. López-García and

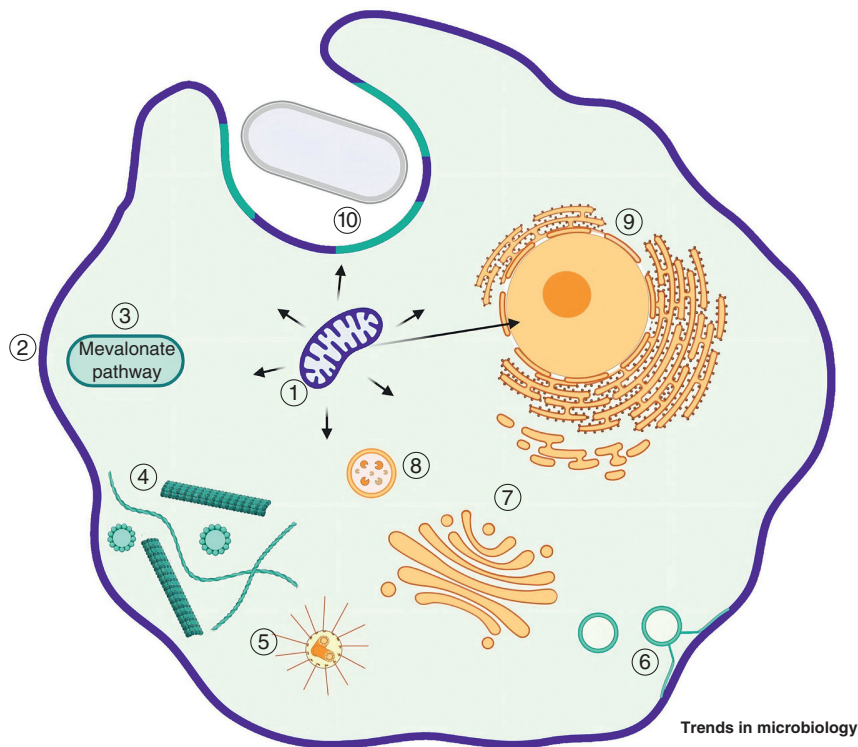


Figure 1.5 Schematic of the chimeric LECA with bacterial contributions in purple, archaeal contributions in aqua green, and eukaryotic innovations in yellow. (1) Mitochondria from an alphaproteobacterial endosymbiont which underwent gene loss and gene transfer to the host nuclear DNA; (2) bacterial-like membrane lipids with ester-bound fatty acids; (3) mevalonate pathway for isoprenoid production. (4) cytoskeletal actin and tubulin proteins; (5) centrosome regulating cytoskeletal proteins for cell shape and chromosome separation; (6) vesicle formation and membrane remodeling; (7) endomembrane system; (8) protein degradation and recycling machinery such as the lysosome; (9) compartmentalization of the nuclear genome; (10) phagocytosis. *Source:* Nobs et al. (2022) / with permission of Elsevier.

Moreira (2020) proposed the revised syntrophy hypothesis, suggesting that Paleoproterozoic microbial mats were most likely the place where eukaryogenesis occurred for dynamic metabolic interactions within the mats. The microbial mats, which are stratified microbial communities, contributed to complex biogeochemical cycling that sustained them. Furthermore, microbial mats offer an ideal ecological niche and taxonomic diversity among microbial communities, facilitating alternative sources of HGT and diverse symbiotic relationships.

1.2.3 Kingdoms of Cellular Life

Before 1970, taxonomists classified all forms of life into two kingdoms: Animalia and Plantae. Bacteria, fungi, and photosynthetic protists were considered plants, and the protozoa were classified as animals. Whittaker (1969) proposed a five-kingdom classification scheme which revolutionized biological taxonomy. This system categorized organisms into five distinct kingdoms based on their cellular structure, mode of nutrition, and ecological roles. First, Whittaker identified two kingdoms of primarily unicellular microorganisms based on whether they exhibit prokaryotic or eukaryotic cellular organization. The kingdom Monera consists of generally single prokaryotic cells, whereas

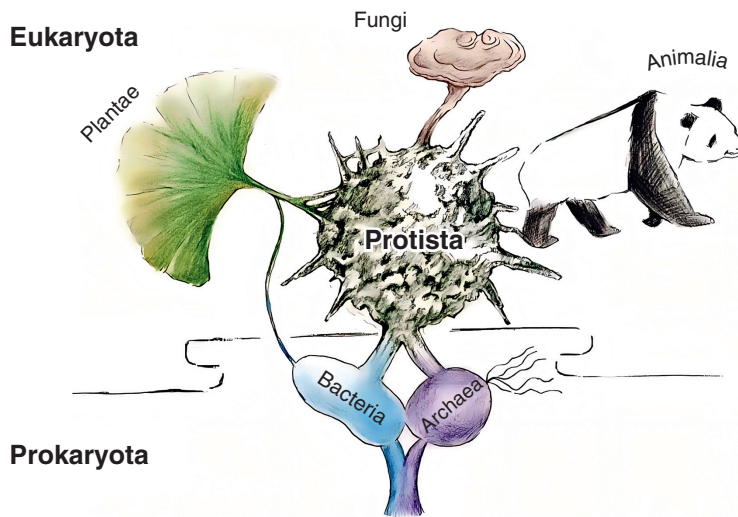


Figure 1.6 Current classification of living cellular organisms, categorized into three domains and six kingdoms.

the kingdom Protista consists of generally single eukaryotic cells. All of the organisms in the remaining three kingdoms (Plantae, Fungi, and Animalia) are eukaryotic, and most of them are multicellular. They may be classified further on the basis of their way of acquiring nutrients. Members of the kingdom Plantae photosynthesize, and members of the kingdom Fungi secrete enzymes outside their bodies and then absorb the externally digested nutrients. In contrast, members of the kingdom Animalia ingest their food and then digest it, either within an internal cavity (many animals) or within individual cells (e.g. sponge cells).

Recently, a six-kingdom scheme has been proposed, which incorporates molecular and evolutionary discoveries, and expands upon older classification methods. Many microbiologists argue that the cell structure of the archaea is so different from that of prokaryotic and eukaryotic cells that they should occupy their own kingdom (Table 1.1). Therefore, the kingdom Monera is split into the bacteria and the archaea. Following the fashion, this textbook adopts the updated six-kingdom classification system (Figure 1.6).

1.3 Diversity of Biological Metabolism

Organisms, especially microbes, show an impressive metabolic diversity in the ways in which they obtain the energy needed for growth. The process involves two basic kinds of transformations, building up or biosynthetic processes mostly via **Calvin Cycle** (the biochemical route of CO₂ fixation in many autotrophic organisms), called **anabolism**, and breaking down or degradative processes, called **catabolism**. In the process of anabolism, microbial cells use energy to convert nutrients and simple compounds into more complex structural and functional macromolecules, which include the formation of protein from amino acids, nucleic acids from nucleotides, polysaccharides from simple sugars, and lipids from glycerol and fatty acids. The opposing process, catabolism, oxidizes organic or inorganic compounds, accompanied by the release of chemical energy and excretion of waste products into the environment. Some of the energy is captured and utilized

by organisms for movements, transport of nutrients, and anabolic reactions, while the remaining energy is lost to the environment in the form of heat.

1.3.1 Metabolic Classification of Organisms

The living world can be divided into two metabolic groups: prokaryotes possess rather remarkable metabolic plasticity, and most eukaryotes are much more monolithic in their approach to metabolism (i.e. metabolizing only a few different carbon compounds for energy and respiring only oxygen). For the most part, this division also holds with regard to mineral metabolism, separating the prokaryotes, which harvest energy from minerals (and inadvertently dissolve or precipitate them) from the eukaryotes, which invest energy in biomineral synthesis (Nealson and Rye 2005).

On the basis of energy usage, we can divide metabolic life into three groups. Many microorganisms resemble animals and humans, using organic chemicals as energy sources. We call these organisms **chemoorganotrophs (heterotrophs)**. An interesting collection of microorganisms, exclusively prokaryotic (bacteria and archaea), use inorganic compounds as energy sources. These organisms are called **chemolithotrophs**. Another major class of living organisms, called **phototrophs**, use light as an energy source, which include green plants and also many microorganisms, both prokaryotic and eukaryotic. We will see that there are two distinct types of photosynthesis, oxygenic photosynthesis typified by green plants but also found in both eukaryotic and certain prokaryotic microorganisms, and anoxygenic photosynthesis found exclusively in special groups of bacteria, e.g. purple bacteria, green bacteria, and certain cyanobacteria (see Section 1.3.2.3).

Life can also be categorized according to the carbon sources utilized. Organisms that use inorganic chemicals or light as energy sources are frequently able to grow in the complete absence of organic materials, using carbon dioxide as their sole source of carbon. **Autotrophs** (meaning literally, “self-feeding”) are able to obtain all the carbon they need from inorganic sources (CO_2 or bicarbonate). Note that autotrophy does not refer to the energy source used but to the carbon source. Autotrophs are of great importance in the functioning of the biosphere because they are able to bring about the synthesis of organic matter from inorganic (non-living) sources; this process is called primary production. **Chemolithoautotrophs** (in general, chemolithotrophs) are organisms that obtain energy by degrading reduced inorganic compounds and use CO_2 as a carbon source. **Photoautotrophs** use light as the energy source and CO_2 as the major carbon source. **Heterotrophs** utilize energetically rich organic carbon as their carbon sources, breaking down and resynthesizing polymers. **Chemoheterotrophs** are organisms that use chemicals as a source of energy and organic compounds as carbon sources. **Chemoorganoheterotrophs** (in general are chemoorganotrophs) obtain both energy and carbon from organic compounds. **Photoheterotrophs** use light as the energy source and organic compounds as the carbon source.

In addition to alternate styles of energy metabolism, we also discuss in this chapter a number of specialized aspects of catabolism. As for respiratory metabolism, when O_2 is used as an electron acceptor (a substance that accepts electrons during an oxidation-reduction reaction), the process is called **aerobic respiration**. There are also a number of other electron acceptors that can participate in the electron transport process in particular groups of microorganisms. The process in which an electron acceptor other than molecular oxygen is used is called **anaerobic respiration** and will be one of the significant features of metabolic diversity considered here.

Finally, although the major thrust of this chapter is carbon metabolism, our discussion would not be complete without a consideration of one of the important metabolic reactions of microorganisms that involves nitrogen. This is the utilization of N_2 as a source of nitrogen for biosynthesis, a process called **nitrogen fixation**. This important process that is unique to prokaryotes contributes to the

recycling of nitrogen into living matter and is important not only agriculturally but also in the total function of the biosphere.

1.3.2 Photosynthesis

1.3.2.1 Pigments, Reactions, and Photosystems

Photosynthesis is the utilization of solar energy by plants, algae, and certain bacteria for the synthesis of organic molecules. Photosynthetic organisms have pigment molecules, chlorophyll (that makes plants look green) in higher plants, algae, and cyanobacteria, or bacteriochlorophyll in green and purple bacteria. There are different types of chlorophyll, but the most common are chlorophyll a–d and f. Chlorophyll a is found in all oxygenic photosynthetic organisms and is the primary pigment in photosynthesis. Chlorophyll b is in green plants and helps broaden the spectrum of absorbed light. Chlorophylls c, d, and f are found in specific algae and cyanobacteria. Both chlorophyll and bacteriochlorophyll are able to capture light energy from the sun and use it to make ATP (adenosine triphosphate) and NADPH (abbreviations for the oxidized/reduced forms of nicotinamide adenine dinucleotide, a diffusible electron carrier). In addition, carotenoids are known to absorb blue and green light, contributing yellow, orange, and red hues, and they also protect against oxidative damage; Phycobilins found in cyanobacteria and red algae help capture light in deep-water environments.

Photosynthesis can be considered in terms of two separate sets of reactions: the light reactions, in which light energy is converted to chemical energy, and the dark reactions, in which this chemical energy is used for CO₂ fixation. Photosynthesis also spins off the energetic molecule, NADPH, which is used by green plants to convert nitrate into ammonia (in assimilative nitrate reduction). Photosynthesis contributes to big changes in the global environment, mainly as a producer of oxygen gas. Virtually all of the oxygen gas in our atmosphere came from photosynthesis. Photosynthesis also has played a minor role as a remover of carbon dioxide gas from the atmosphere (calcium carbonate precipitation greatly overshadows it). Photosynthesis occurs in two main pathways: anoxygenic and oxygenic photosynthesis (see Table 1.2 for comparison).

Photosystems are essential components of photosynthesis, responsible for capturing light energy and converting it into chemical energy (Table 1.2). They are protein-pigment complexes found in the thylakoid membranes of cyanobacteria, algae, and plants. Photosystem I (PSI) uses light energy to produce NADPH that is used for the subsequent photosynthetic dark reaction or Calvin cycle and absorbs light mainly at 700 nm (P700). PSI transfers electrons across the thylakoid membrane from plastocyanin to ferredoxin. Photosystem II (PSII) splits water molecules to release oxygen, electrons, and protons, absorbs light mainly at 680 nm (P680), and plays a key role in the oxygenic photosynthesis process. Enzymes in PSII capture photons of light to excite electrons that are transported through a range of coenzymes and cofactors to reduce plastoquinone to plastoquinol. Excited electrons are replaced by the oxidation of water, resulting in the production of molecular oxygen and hydrogen ions. Oxygenic phototrophs have both PSI and PSII while anoxygenic phototrophs carry a single photosystem, either PSI or the one similar to PSII (Blankenship 2010).

1.3.2.2 Oxygenic Photosynthesis

Oxygenic photosynthesis is the most familiar and widespread form of photosynthesis in modern biosphere. On the continents, it occurs mostly in green plants. It is also prevalent in aquatic environments, like lakes, rivers, and oceans. There, oxygenic photosynthesis is performed by algae (Figure 1.7), many protists, and cyanobacteria. Also, corals, lichens, giant clams, and at least one kind of tiny marine slug are photosynthetic because they are inhabited by symbiotic,

Table 1.2 Comparison of photosynthetic mechanisms by different organisms.

	Oxygenic photosynthesis		Anoxygenic photosynthesis	
	Plants, algae	Cyanobacteria	Purple photosynthetic bacteria	Green photosynthetic bacteria
Location of the photosystem ^a	In membranes of thylakoids, which are within the stroma of chloroplasts?	In membranes of thylakoids, located within the cell.	Within the cytoplasmic membranes; extensive invaginations in that membrane effectively increase the surface area	Primarily within the cytoplasmic membrane; chlorosomes attached to the inner surface of the membrane contain the accessory pigments
Type of photosystem ^a	Photosystem I and photosystem II		Similar to photosystem II	Similar to photosystem I
Primary light-harvesting pigment	Chlorophyll <i>a</i>		Bacteriochlorophylls	
Mechanism for generating reducing power	Non-cyclic photophosphorylation using both photosystems		Reversed electron transport	Non-cyclic use of the photosystem
Source of electrons for reducing power	H ₂ O		Varies among the organisms in the group; may include H ₂ S, H ₂ , or organic compounds	
CO ₂ fixation	Calvin-Benson cycle		Calvin-Benson cycle	Reversed TCA cycle
Accessory pigments	Carotenoids	Carotenoids, phycobilins	Carotenoids	Carotenoids

^a Photosystem: protein complexes within which chlorophyll and other light-gathering pigments are organized. Two types of photosystems are called photosystem I and photosystem II, in order of their discovery; each has a characteristic of reaction center. The reaction center of photosystem I is known as P700 because the pigment is best at absorbing light having a wavelength of 700 nm, and the reaction center of photosystem II is called P680 because its absorption spectrum has a peak at 680 nm. P700 and P680 are actually identical chlorophyll α molecules. However, their association with different proteins affects the electron distribution in chlorophyll molecules and accounts for the slight differences in light-absorbing properties.

Source: Adapted from Nester et al. (2004) / McGraw-Hill Education.



Figure 1.7 Green alga *Cladophora* at Qingdao's rocky beach in China is actively photosynthesizing, releasing oxygen gas bubbles into the water. Source: Photo by Xingliang Zhang.

photosynthetic microorganisms. These organisms use water as the electron donor (a compound that donates electrons during oxidation-reduction reaction) to fix carbon.

Oxygenic photosynthesis is a complex process that involves a cascade of biochemical reactions. In essence, however, it is fairly simple. The general reaction equation is:



This reaction equation demonstrates that the process of photosynthesis takes in carbon dioxide gas from the atmosphere, water from the soil (in the case of green plants), and light energy from the sun. Photosynthesis then uses these resources to make carbon chains. The carbon chains can later be used to make a variety of molecules including sugar. Note that water is used as an electron donor and a source of hydrogen, and oxygen gas is given off as a waste product.

Please draw your attention for a moment to the issue of hydrogen. In order to make organic molecules from carbon dioxide, photosynthesizing organisms need a source of hydrogen that plays an essential role here. During the light-dependent reaction, water molecules are split into oxygen, electrons, and hydrogen ions. The electrons help generate ATP and NADPH, which are energy carriers needed for the Calvin cycle. Meanwhile, the hydrogen ions contribute to creating a proton gradient that drives ATP synthesis.

Oxygenic photosynthesis has played a monumental role in shaping Earth's surface environment. Before oxygenic photosynthesis, Earth's atmosphere lacked free oxygen. Cyanobacteria, the earliest organisms to perform oxygenic photosynthesis, began releasing molecular oxygen perhaps early in the Mesoarchean (3.2–2.8 billion years ago), triggering the Great Oxygenation Event (Figure 1.8). The subsequent rise of eukaryotic phytoplankton and land plants further enhanced the primary production and oxygenated the Earth's surface (see Sections 8.3.4 and 8.4.4). This influx of oxygen dramatically altered atmospheric chemistry and paved the way for aerobic life. As oxygen accumulated in Earth's oceans, it reacted with dissolved iron, forming iron oxide sediments—visible today as banded iron formations (BIFs) in ancient rock records, serving as a major iron resource for global industry. These formations are vital for understanding early Earth processes and serve as valuable iron ore deposits. Oxygen from photosynthesis eventually led to the formation of the ozone (O_3) layer, which protects life from harmful UV radiation. This shielding effect together with atmospheric oxygen accumulation made it possible for aerobic, complex, multicellular organisms to thrive in the ocean and on land, and hence dramatically increased biodiversity. Oxygenic photosynthesis drives the carbon cycle, converting atmospheric CO_2 into organic matter. This process helps regulate Earth's climate, influencing carbon sequestration in sedimentary rocks and fossil fuels.

1.3.2.3 Anoxygenic Photosynthesis

Anoxygenic photosynthesis is probably the oldest form of photosynthesis, perhaps originating within 100 million years or so after the appearance of life ~4.0 billion years ago. Its biochemistry is somewhat simpler than oxygenic photosynthesis. Anoxygenic photosynthesis occurs today in purple sulfur/non-sulfur bacteria, green sulfur/non-sulfur bacteria, and heliobacteria that inhabit shallow, oxygen-free waters and mud in lakes, swamps, and oceans. Some cyanobacteria also grow via anoxygenic photosynthesis. These organisms use a wide range of electron donors (H_2S , H_2 , Fe^{2+} , S^0 , organic carbon), each of which is oxidized during the process, leading to the fixation of reduced carbon and the oxidation of the electron donors.

Experiments and ecological studies provide key insights, demonstrating that anoxygenic photosynthetic bacteria often outcompete cyanobacteria when alternative electron donors (e.g. H_2S) are

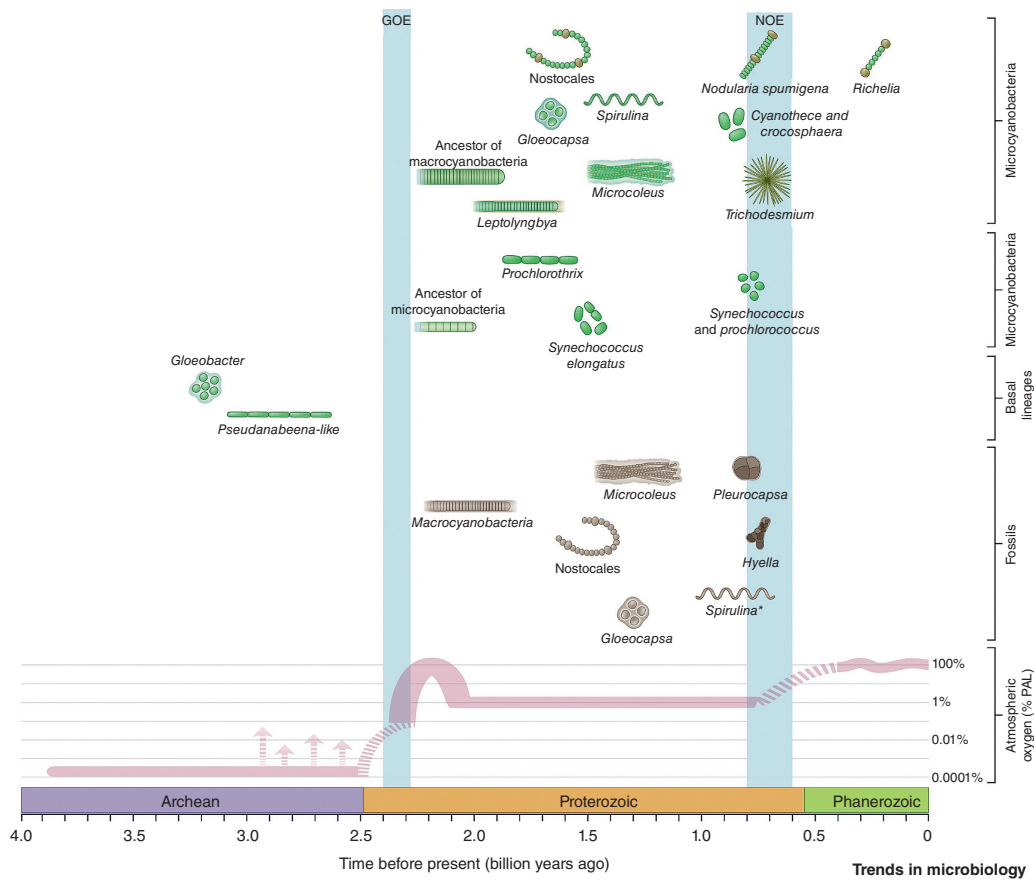
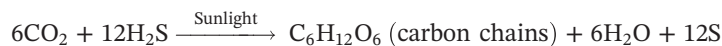


Figure 1.8 Timeline of oxygen concentration in the atmosphere, the antiquity of cyanobacterial lineages based on molecular clock estimates and fossil occurrences. *Source:* Sánchez-Baracaldo et al. (2022) / with permission of Elsevier.

present (Klatt et al. 2015; Ozaki et al. 2019). Additionally, certain modern cyanobacteria (e.g. *Leptolyngbya* sp. strain hensonii) are capable of transitioning to anoxygenic photosynthesis in environments rich in hydrogen sulfide (Hamilton et al. 2018). This metabolic adaptability is fundamentally significant for deciphering Earth's billion-year anoxic history.

Anoxygenic photosynthesis is different from oxygenic photosynthesis in two main ways: (1) a variety of reduced chemicals (e.g. H_2S) can be used instead of water as electron donors and a source of hydrogen; and (2) the bacteria, under strict anaerobic conditions, photosynthetically generate sulfur, sulfate, or iron instead of oxygen gas. The general reaction equation for anoxygenic photosynthesis is:



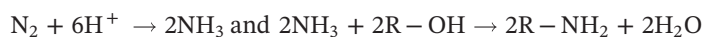
There are major limitations on the ecological distribution of anoxygenic photosynthesis. In order for it to occur, the environment must have a combination of three things: (1) it must be free of oxygen gas; (2) reducing electron donors (e.g. H_2S) must be available; and (3) sunlight must be available. Today, oxygen-less, hydrogen sulfide-rich environment (typical of deep lake waters or deep in muddy environments) is difficult to find with good exposure to sunlight. However, it might be ubiquitous in remote Earth history. During the Archean Eon (~4.031–2.5 billion years ago), the Earth's

surface was anoxic and rich in reductants such as H_2S , H_2 , and Fe^{2+} that were ready to serve as electron donors.

Anoxygenic photosynthesis may have a profound impact on Earth's early history. Anoxygenic photoautotrophs either predated or coexisted with oxygenic photoautotrophs (Sánchez-Baracaldo et al. 2022), potentially serving as key drivers of geochemical cycles in early ecosystems. They thrived in Earth's surface environments and formed extensive microbial mats that shaped sedimentation patterns. Though their photosynthesis did not release oxygen gas, anoxygenic photoautotrophs (likely cyanobacteria) involved in this process may have played a crucial role in removing excess reductants (H_2S and Fe^{2+}) from ancient oceans, thus creating favorable conditions for the eventual atmospheric accumulation of oxygen. Furthermore, when iron was used as an electron donor, anoxygenic photosynthesis may have contributed to the formation of BIFs (see Sections 1.5 and 6.2.6).

1.3.3 Nitrogen Fixation

Nitrogen fixation is a process in which N_2 is reduced to form ammonia (NH_3), which then can be incorporated into cellular material, by reactions:



Nitrogen is required by all living organisms. Most nitrogen on Earth is in the form of dinitrogen (N_2), which is not bioavailable though it makes up 78% of modern atmosphere. Abiotic sources of nitrogen produced through mechanisms such as lightning discharge or mineral-based catalysis are only able to support a small biosphere. Only a small fraction of prokaryotes including bacteria and archaea can fix nitrogen. No eukaryotic organisms have been known to reduce and fix nitrogen. Therefore, just as animals, including humans, depend on other organisms to fix carbon, most organisms rely on prokaryotes to convert atmospheric nitrogen to a form they can assimilate to create biomass.

The reduction process is catalyzed by the enzyme complex nitrogenase, which drives the production of at least half of Earth's fixed nitrogen. This mechanism plays a crucial role in alleviating fixed nitrogen limitations within natural ecosystems and is likely to exert a disproportionate influence on ecosystem functionality compared with inputs from other populations. Consequently, nitrogen-fixing organisms in natural communities are recognized as keystone species (see Boyd and Peters 2013).

Three nitrogenases have been recognized, which occur as either Mo, Fe-only, or V at the active site cofactor. All known nitrogen fixers (or diazotrophs) encode canonical Mo-nitrogenases that are more efficient than Fe-only and V-nitrogenases that are typically viewed as “backup” enzymes, used only when Mo is not available (McRose et al. 2017). These alternative nitrogenases allow microbes to adapt to different environmental conditions, ensuring nitrogen fixation continues even when molybdenum is scarce.

Nitrogenase is readily inactivated by O_2 . Therefore, nitrogen-fixing aerobes must have a mechanism to protect them from exposure to O_2 . In addition, nitrogen fixation requires a tremendous expenditure of energy, at least 16 molecules of ATP for every molecule of nitrogen fixed, because N_2 has a very stable triple bond. Diazotrophs may be free living, or they may live in symbiotic association with higher organisms, particularly certain plants (Table 1.3).

Symbiotic nitrogen fixers may be localized on the plant root tissues in the form of nodules. In effect, the plant provides nitrogen fixers with a source of energy, in the form of simple sugars, and a sheltered environment in which access to oxygen is controlled so that nitrogenase is not inactivated. Meanwhile, the nitrogen-fixer supplies the plant with a source of nitrogen. Symbiotic nitrogen-fixers are very important agriculturally because they provide a renewable nitrogen source to soils.

Table 1.3 Nitrogen fixers, including free-living and symbiotic prokaryotes (Madigan et al. 1997, 2003, 2018).

Free-living aerobes		
Chemoorganotrophs	Phototrophs	Chemolithotrophs
<i>Azotobacter</i>	Cyanobacteria	<i>Alcaligenes</i>
<i>Azomonas</i>	(various, but not all)	<i>Acidithiobacillus</i> (some species)
<i>Azospirillum</i>	<i>Anabaena</i>	
<i>Klebsiella</i> ^a	<i>Nostoc</i>	
<i>Methylomonas</i>	<i>Gloeotheca</i>	
	<i>Aphanizomenon</i>	
Free-living anaerobes		
Chemoorganotrophs	Phototrophs	Chemolithotrophs
<i>Clostridium</i>	Purple bacteria:	Archaea:
<i>Desulfotomaculum</i>	<i>Chromatium</i>	<i>Methanosarcina</i>
	<i>Methanococcus</i>	<i>Methanocaldococcus</i>
	<i>Rhodobacter</i>	
	Green sulfur bacteria	
	<i>Chlorobium</i>	
	<i>Heliobacter</i>	
Symbiotic		
Root-nodule-forming bacteria		Other symbiotic diazotrophs
<i>Alphaproteobacteria</i> (e.g. <i>Mesorhizobium</i> , <i>Bradyrhizobium</i> , <i>Sinorhizobium</i>) and <i>Betaproteobacteria</i> (e.g. <i>Burkholderia</i>) with leguminous plants such as soybeans, peas, clover; <i>Actinobacteria</i> (e.g. <i>Frankia</i>) with non-leguminous plants such as alder, bayberry, and autumn olive.		<i>Teredinibacter</i> in association with shipworms
		<i>Treponema</i> in termite guts
		<i>Glomeribacter</i> in endomycorrhizal fungi
		Cyanobacteria in several fungi, algae, and plants

^a N₂ fixation occurs only under anoxic conditions.

Nitrogen fixers may also live freely in soil and water. In the oceans, planktonic cyanobacteria *Trichodesmium* and *Synechococcus* are primary nitrogen fixers, fueling up to half of the new production, despite making up only 1% of the marine biomass. In subtropical and tropical oceans, nitrogen fixation not only has a major role in the global marine N budget but also impacts the coupling of N-C-P-Fe cycles. Free-living diazotrophs require a means of protecting their nitrogenases from O₂. Some aerobic organisms accomplish this by maintaining a very high rate of O₂ utilization, thereby minimizing the O₂ diffusion into the cell. Many cyanobacteria instead possess specialized cells within their filaments, called heterocysts, which serve as O₂-free microenvironment, while others photosynthesize strictly during daylight and fix nitrogen at night (Madigan et al. 1997).

1.3.4 Chemolithotrophy: Energy from Oxidation of Inorganics

Chemolithotrophs obtain their energy from the oxidation of inorganic compounds, such as H₂, H₂S, S⁰, S₂O₃²⁻, Fe(II), Mn(II), NH₄⁺, and NO₂⁻. These microorganisms are also able to obtain all their carbon from CO₂, generally processed via the Calvin cycle. Therefore, they are also autotrophs. There are many sources of inorganic electron donors for chemolithoautotrophs, which include geological,

biological, and anthropogenic sources. Volcanic activity and hydrothermal fluids are major sources of reduced sulfur compounds, as is the microbial sulfate reduction. Agricultural and mining operations add inorganic electron donors to the environment, as does the burning of fossil fuels and input of industrial wastes. The ecological success and metabolic diversity of chemolithoautotrophs indicate that sources and supplies of inorganic electron donors in nature are abundant, which is particularly true on early Earth (see Sections 8.3 and 8.4). In most cases below, O_2 serves as the terminal electron acceptors (TEAs), but different microorganisms can substitute it for less electropositive TEAs, i.e. NO_3^- , $Mn(IV)$, $Fe(III)$, SO_4^{2-} , and S^0 when oxic conditions become diminished.

1.3.4.1 Hydrogen-Oxidation: Using the Chemical Energy in H_2 to Fix Carbon

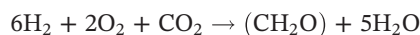
Hydrogen (H_2) is a common product of microbial metabolism, and a number of chemolithotrophs, called H_2 -oxidizing bacteria or archaea, are able to use it to generate ATP. The H_2 -oxidizers are the most widespread chemolithoautotrophs, ranging from aerobes to facultative anaerobes that can grow on NO_3^- , $Mn(IV)$, or $Fe(III)$, to obligate anaerobes that use SO_4^{2-} , and finally to methanogens and homoacetogens.

Aerobic H_2 -oxidizing bacteria are commonly termed hydrogen bacteria. The reaction:



is catalyzed by the enzyme hydrogenase and can support the synthesis of at least one ATP. The electrons from H_2 initially being transferred to a quinone acceptor. Some hydrogen bacteria have two hydrogenases. The soluble enzyme takes up H_2 and reduces NAD^+ to $NADH$, while the membrane-bound hydrogenase is involved in energetics.

Most hydrogen bacteria are facultative chemolithotrophs, e.g. *Ralstonia eutropha*, capable of growing either as chemoorganotrophs on organic compounds or chemolithotrophically by H_2 oxidation via the reaction:

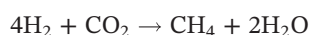


thus behaving as mixotrophs. When growing autotrophically, hydrogen bacteria fix CO_2 by the Calvin cycle. When organic compounds are present, synthesis of the Calvin cycle is repressed and enzymes involved in utilization of the organic compounds are induced. Therefore, in a natural environment, it is likely that aerobic hydrogen bacteria are able to regulate their catabolic enzymes and shift between chemoorganotrophy and chemolithotrophy (Madigan et al. 2003).

1.3.4.2 Methanogenesis and Acetogenesis from CO_2 Reduction

Carbon dioxide is common in nature and is a major product of energy metabolism of heterotrophs. Several prokaryotic groups are able to use CO_2 as an electron acceptor in anaerobic respiration. The most important CO_2 -reducing prokaryotes are methanogens including a large number of Euryarchaeota, a major group of archaea (Madigan et al. 2003). Many of them utilize H_2 as the electron donor (energy source). In addition, formate, carbon monoxide, and other organic compounds such as alcohols provide electrons for CO_2 reduction.

Methanogenesis from CO_2 involves the transfer of eight electrons, with two being added at a time. This stepwise reduction results in intermediate oxidation states of the carbon atom, transitioning from +4 in CO_2 to -4 in CH_4 . The process relies on several specialized coenzymes, which can be categorized into two groups: (1) C_1 carriers, responsible for transporting the single-carbon unit through enzymatic reduction, and (2) redox coenzymes, which serve as electron donors (Figure 1.9). The overall reaction is



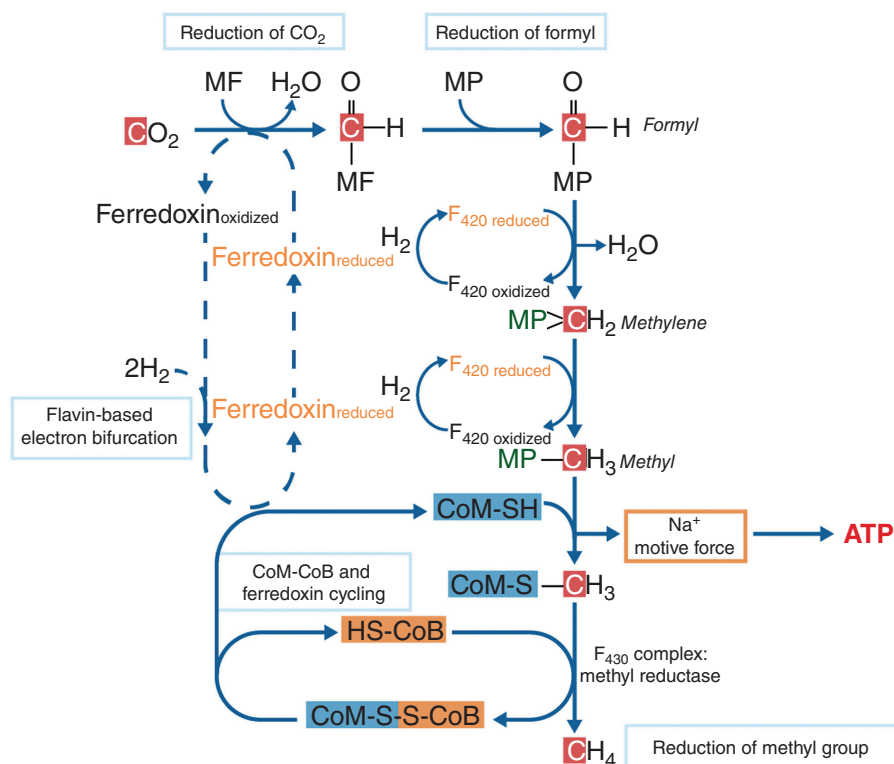
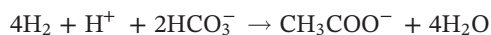


Figure 1.9 Methanogenetic steps in microbial CO₂ reduction by H₂. Carbon atom reduced is highlighted in red, and the source of electrons is highlighted in brown. F₄₂₀ reduced, a decreased form of coenzyme F₄₂₀ that is a unique, flavin-like electron carrier found in archaea (methanogens), bacteria and some fungi; F₄₃₀, coenzyme F₄₃₀ that is a unique nickel-containing tetrapyrrole cofactor essential for methanogenesis; MF, methanofuran, a coenzyme with a furan ring linked to various side chains and found exclusively in methanogenic archaea; MP, methanopterin, a pterin-based coenzyme that acts as a carrier of one-carbon (C₁) units during the stepwise reduction of CO₂ to methane. CoM (coenzyme M, 2-mercaptoethanesulfonate, HS-CH₂-CH₂-SO₃⁻) and CoB (coenzyme B, 7-mercaptoheptanoylthreonine phosphate) are central to the final step of methanogenesis.

When growing on CO₂ + H₂, the methanogens are chemolithoautotrophs. However, the carbon fixation process is not the Calvin cycle of conventional autotrophs but instead the acetyl-CoA pathway (a pathway of autotrophic CO₂ fixation widespread in obligate anaerobes including methanogens, homoacetogens, and sulfate-reducing bacteria). Quantitatively, this reaction is the most important process involving H₂ in non-sulfate environments, and it accounts for some 40% of the methane produced in sediments (Wolfe 1972).

Another group of CO₂-reducing bacteria is the homoacetogens, which produce acetate rather than CH₄ from CO₂ and H₂ through the acetyl-CoA pathway. The overall reaction is



In addition to H₂, a variety of C₁ compounds, sugars, amino acids, alcohols, and certain nitrogen bases can be electron donors, depending on the organisms involved. Many homoacetogens are also able to reduce NO₃⁻ and S₂O₃²⁻, while CO₂ reduction is the major reaction of ecological importance (Madigan et al. 2003). Homoacetogens are not a defined taxonomic group because they include

such widely differing organisms as the gram-positive spore-forming bacterium *Clostridium acetivum* and the gram-negative non-spore-forming *Acetobacterium woodii*.

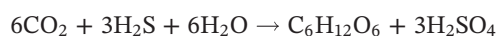
Methanogenesis and acetogenesis through CO_2 reduction play a crucial role in carbon cycling and are found in diverse environments, including soil, sediments, and the gastrointestinal tracts of herbivores. Acetogens compete with methanogens. The ability of methanogens to grow in diverse habitats and their minimal nutrient requirements make them able to survive in extreme conditions. Methanogens generally have a lower half-saturation constant for hydrogen, meaning they can effectively utilize H_2 even at very low concentrations. This gives them a competitive edge over homoacetogens, which may have a higher maximum reaction rate but require higher hydrogen availability to maintain efficient metabolism. In environments where H_2 is limited, methanogens can outcompete homoacetogens simply because they are better adapted to scavenging and using small amounts of hydrogen. This is a key factor in anaerobic processes, where methanogenesis often dominates over acetogenesis, leading to methane production rather than acetate accumulation (Karekar et al. 2022).

1.3.4.3 Oxidation of Reduced Sulfur Compounds

We have discussed sulfur oxidation by bacteriochlorophyll-containing green and purple sulfur bacteria through anoxygenic photosynthesis in Section 1.3.2.3 of this chapter. Here we talk about the colorless sulfur-oxidizing bacteria that are a diverse group of microorganisms capable of growing chemolithoautotrophically on reduced sulfur compounds, such as H_2S , S^0 , and $\text{S}_2\text{O}_3^{2-}$ (thiosulfate). Some sulfur bacteria, such as *Beggiatoa*, *Thiothrix*, *Thiovulum*, *Thiomicrospira*, and *Thioploca*, grow at neutral pH under microaerophilic conditions with O_2 concentrations around 5% atmospheric levels (PAL). Such environments exist at sulfur springs, within microbial mats, low-oxygenated marine waters, and freshwaters polluted by sewage. Similar to Fe(II) oxidation (Section 1.3.4.4), the rates of biological sulfide oxidation are considerably enhanced in comparison to chemical oxidation rates when oxygen concentrations are low. In other words, the bacteria act as catalysts, increasing the reaction kinetics. Others, e.g. *Acidithiobacillus*, *Sulfolobus*, and *Acidianus*, grow in acid environments, characteristic of metal and coal mine drainage.

Hydrogen sulfide is the most common electron donor, SO_4^{2-} and H^+ are the final oxidation products, and oxygen is typically the oxidant. When O_2 is absent, some species can utilize NO_3^- or Fe(III) as their TEAs. Many of the sulfide oxidizers can autotrophically use the Calvin cycle to fix CO_2 . The overall oxidation of H_2S to SO_4^{2-} is an $8e^-$ step, necessitating a series of $2e^-$ transfer reactions that successively lead to the intracellular formation of several different sulfur species (Figure 1.10). Less energy is released when any intermediate sulfur oxidation stage is used. There are two ways in which $\text{S}_2\text{O}_3^{2-}$ is oxidized to SO_4^{2-} . The most common pathway employs the enzyme sulfite oxidase. A few sulfur chemolithotrophs oxidize via a reversal of the activity of adenosine phosphosulfate reductase (Madigan et al. 2003).

The general reaction equation is:



This reaction equation, like all shown in this chapter, is a summation and consolidation of a series of reactions. Free O_2 is a necessary ingredient in the initial reactions leading to sulfur oxidation. Therefore, this process does best in oxic environments. Sulfur oxidation produces protons and causes acidification of the environment.

Sulfur oxidizers can tolerate high heat too. One species of *Thermotrix* lives in sulfur hot springs at temperatures up to 185°F (85°C). Sulfur-oxidizing bacteria form the ecological base of deep-sea hydrothermal vent communities. The seafloor hot springs spew out sulfur and hydrogen sulfide.

Since they occur on ocean bottoms over 6000 feet deep, there is no sunlight whatsoever. Photosynthesis is not even remotely possible. Instead, the sulfur-oxidizing bacteria perform the job normally done by photosynthesis. The sulfur-oxidizing bacteria use the chemical energy in sulfur minerals to make ATP. The ATP can then be used either to support other biosynthetic activities or to make NADPH. Then the ATP and NADPH together feed the carbon fixation cycle. Therefore, sulfur oxidizers may serve as primary producers in chemosynthesis-based hydrothermal vent ecosystems.

1.3.4.4 Iron Oxidation

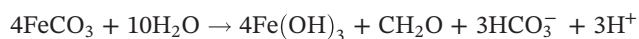
The aerobic oxidation of Fe^{2+} to Fe^{3+} is able to yield a small amount of energy, and for this reason iron oxidizers must oxidize large amounts of iron in order to grow (Madigan et al. 2003). In modern surficial environments, this reaction is generally limited by the availability of dissolved Fe because at neutral pH and under fully aerated conditions, Fe^{2+} is rapidly oxidized to Fe^{3+} abiotically, which then hydrolyzes to form insoluble ferric hydroxide and precipitate in water. However, Fe^{2+} remains stable under oxic and acidic conditions. Therefore, to overcome the stability limitations, iron oxidizers either grow under acidic conditions (as an acidophile) or under low oxygen concentrations at neutral pH (as a microaerophile). In both cases, the chemical reaction is sufficiently slow so that microbes can harness Fe^{2+} oxidation for growth. In the geological past, ferruginous water bodies may have been prevalent in the bulk ocean and there was sufficient soluble Fe^{2+} for iron oxidizers to grow (see Section 8.4.4).

There are different types of microbial iron oxidation, including aerobic oxidation under acidic conditions, neutrophilic oxidation in microaerobic environments, and nitrate-dependent oxidation under anaerobic conditions (Oshiki et al. 2013). Acidophilic iron-oxidizing bacteria that grow autotrophically on reduced iron, using O_2 as their TEA. The best-known iron-oxidizing acidophiles are *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. They grow well at mine waste disposal sites where reduced sources of iron are continuously regenerated during sulfide mineral oxidation (Liu and Zhang 2015). Another iron-oxidizing microorganism is the archaeon *Sulfolobus acidocaldarius* that lives in hot, acidic springs at temperatures up to the boiling point of water. The oxidation of Fe(II) to Fe(III) is a proton-consuming reaction: $2\text{Fe}^{2+} + 0.5\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O}$; the reaction: $0.5\text{O}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{O}$ occurs on the inner face of the cytoplasmic membrane, whereas the reaction $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ takes place near the outer face of the membrane (Figure 1.11). The overall reaction is



There are also iron-oxidizing bacteria that live near neutral pH. As mentioned above, ferrous iron is unstable at neutral conditions in the presence of O_2 . While in a neutral pH environment, Fe^{2+} is present at the interface between anoxic and oxic conditions where Fe^{2+} diffuses upward from the anoxic zone. *Gallionella ferruginea* and *Sphaerotilus natans* are known to live at such interfaces. Anammox bacteria *Candidatus Kuenina stuttgartiensis*, *Ca. Brocadia sinica*, and *Ca. Scalindua* sp. are known to oxidize Fe^{2+} using nitrate (Oshiki et al. 2013).

In addition, ferrous iron can be oxidized under anoxic conditions by anoxygenic phototrophs, in which Fe^{2+} is used as an electron donor for photoautotrophic growth through CO_2 reduction or for energy metabolism. Anoxically, Fe^{2+} is stable at neutral pH where these bacteria flourish. Some species of purple bacteria and green sulfur bacteria (e.g. *Chlorobium*) are known to oxidize Fe^{2+} through anoxygenic photosynthesis (Madigan et al. 2003). In the experiment, Fe^{2+} oxidizing anoxygenic phototrophs (purple bacteria) are supplied with FeCO_3 and the following reaction takes place:



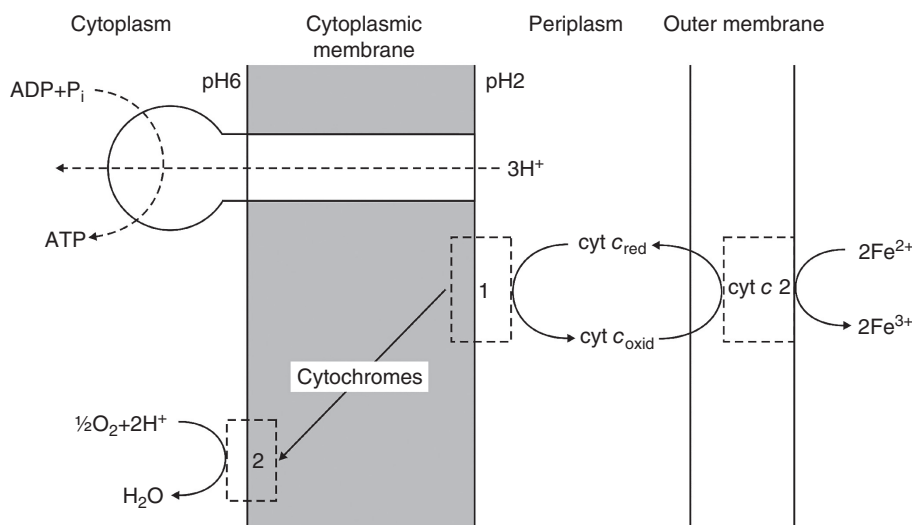


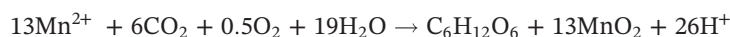
Figure 1.11 ATP synthesis coupled to oxidation of Fe(II) by *Acidithiobacillus ferrooxidans*. The reaction $0.5O_2 + 2H^+ \rightarrow H_2O$ occurs on the inner face of the cytoplasmic membrane, whereas the reaction $Fe^{2+} \rightarrow 2Fe^{3+}$ takes place near the outer face of the membrane. C-type cytochrome transfers electrons from a copper-containing blue protein, rusticyanin (1), to the terminal cytochrome oxidase (2) located at the inner face of the cytoplasmic membrane. Source: Adapted from Gottschalk (1986).

The anoxygenic purple bacteria are also able to use FeS as electron donor in which both Fe^{2+} and S^{2-} are oxidized (Ehrenreich and Widdel 1994).

The discovery of Fe(II)-oxidizing phototrophs has important implications in understanding the evolution of photosynthesis and explaining the large deposits of ferric iron found in ancient rocks (Ehrenreich and Widdel 1994; Madigan et al. 2003). Such ferric iron deposits were previously thought to have been formed from the oxidation of ferrous iron by O_2 produced by oxygenic phototrophs. However, these deposits are predominantly in Archean and Early Proterozoic in age (see Section 6.2.4). Therefore, it was proposed that anoxygenic phototrophic iron-oxidizing bacteria contributed to the deposition of BIFs in the Precambrian (Kappler et al. 2005).

1.3.4.5 Manganese Oxidation

Microbial manganese oxidation is a crucial process in biogeochemical cycles. At the Earth's surface, the activation energy of Mn(II) to Mn(III) or Mn(IV) is much larger than that of Fe(II) to Fe(III). While under oxic conditions, the non-biological oxidation reaction proceeds rather slowly when the pH is below 8. A diverse group of bacteria, fungi, and algae can catalyze the oxidation of manganese from its soluble Mn(II) form to insoluble Mn(III) and Mn(IV) oxides through enzyme or non-enzyme-mediated processes (Zhou and Fu 2020). The overall reaction is



The end products of biological Mn-oxidation, manganese oxides (e.g. manganite and hausmannite) attract much attention not only because they serve as the sink and source of bioavailable Mn but also because of their tight coupling with the geochemical cycles of other elements (e.g. Cr, S, Fe, Co, C, N, and Se). It is argued that this process is responsible for the majority of Mn(II) oxidation that occurs in natural waters and sediments (Konhauser 2007).

Even though the oxidation of Mn^{2+} to Mn^{4+} is also energetically favorable for growth, and many microorganisms catalyze Mn^{2+} oxidation, as yet no organism has been conclusively shown to derive energy from the oxidation of reduced manganese (Madigan et al. 2018). *Leptothrix* and *Sphaerotilus* are common in freshwater environments and catalyze the oxidation of both iron and manganese but do not appear to conserve energy from these reactions. Instead, they conserve energy from the oxidation of organic matter. In these habitats, their sheaths may become coated with ferric hydroxide $[\text{Fe}(\text{OH})_3]$ or manganese oxides from the oxidation of these metals.

Experiments demonstrated that the inorganic manganese oxidation was a two-step process for MnO_2 formation. Initially, Mn^{2+} is oxidized to either a metastable Mn(III) oxyhydroxide (manganite) or a mixed valence mineral hausmannite, depending on the initial Mn^{2+} concentration, pH, ionic strength, temperature, and redox conditions of the medium. Manganite subsequently transforms into MnO_2 , while hausmannite transforms very slowly to Mn^{2+} and MnO_2 by a reaction called disproportionation (the splitting of a chemical compound into two new compounds, one more oxidized and one more reduced than the original compound). Those steps become rate limiting in the abiological formation of MnO_2 . In contrast, microbial catalysis accelerates manganese oxidation by 4–5 orders of magnitude, significantly outpacing abiotic processes. Those organisms may instead utilize a completely different pathway of Mn(II) oxidation. It is possible that the bacteria first enzymatically oxidize Mn^{2+} to Mn^{3+} , and that this intermediate form remains bound to a manganese-oxidizing enzyme. The same enzyme then catalyzes the oxidation of bound Mn^{3+} to MnO_2 , rather than a disproportionation to Mn^{2+} and MnO_2 . Therefore, a single bacterium can directly oxidize Mn(II) to Mn(IV).

1.3.4.6 Ammonia and Nitrite Oxidation: Nitrification

Ammonia (NH_3) and nitrite (NO_2^-) can be used as electron donors (energy source) by nitrifying microbes to fix carbon. Aerobic ammonium oxidation is a key process in the nitrogen cycle. Ammonia-oxidizing microbes convert ammonium (NH_4^+) into nitrite (NO_2^-), which is then oxidized by the nitrite-oxidizing bacteria to nitrate (NO_3^-). The complete oxidation of ammonia to nitrate, termed nitrification, is an eight-electron transfer process consisting of two different sets of reactions (ammonia oxidation and nitrite oxidation), which are carried out by two separate groups of microbes acting in sequence (Figure 1.12). Nitrifying microbes, including bacteria and archaea, are widely distributed in terrestrial and marine environments. Most nitrifying microbes are only able to catalyze one set of these reactions. For example, *Nitrosomonas* from bacteria and *Nitrosopumilus* from archaea are only able to oxidize NH_3 to nitrite and thus are called ammonia oxidizers. The process is continued by other bacteria, called nitrite oxidizers, such as *Nitrobacter*, that oxidize NO_2^- to NO_3^- . Only certain bacteria in the genus *Nitrospira* can catalyze both sets of reactions, oxidizing NH_3 all the way to NO_3^- or directly into nitrogen gas (N_2) under aerobic or anaerobic conditions. *Nitrospira* species possess genomes encoding enzyme systems for both ammonia and nitrite oxidation, enabling a single organism to catalyze these two processes—a capability termed comammox (complete ammonia oxidation) (Madigan et al. 2018). These bacteria, nicknamed comammox bacteria, derive their name from their unique dual metabolic activity, which streamlines the conversion of ammonia to nitrate within a single species.

Ammonia oxidizers such as *Nitrosomonas* use the enzyme *ammonia monooxygenase* to produce hydroxylamine (NH_2OH) and H_2O . The enzyme, *hydroxylamine oxidoreductase*, then oxidizes NH_2OH to NO_2^- , removing four electrons in the process. In the reaction carried out by ammonia monooxygenase, two electrons and protons are needed to reduce one molecule of O_2 to H_2O . These electrons originate from the oxidation of hydroxylamine and are supplied to ammonia monooxygenase from hydroxylamine oxidoreductase via cytochrome c and ubiquinone (Figure 1.12).

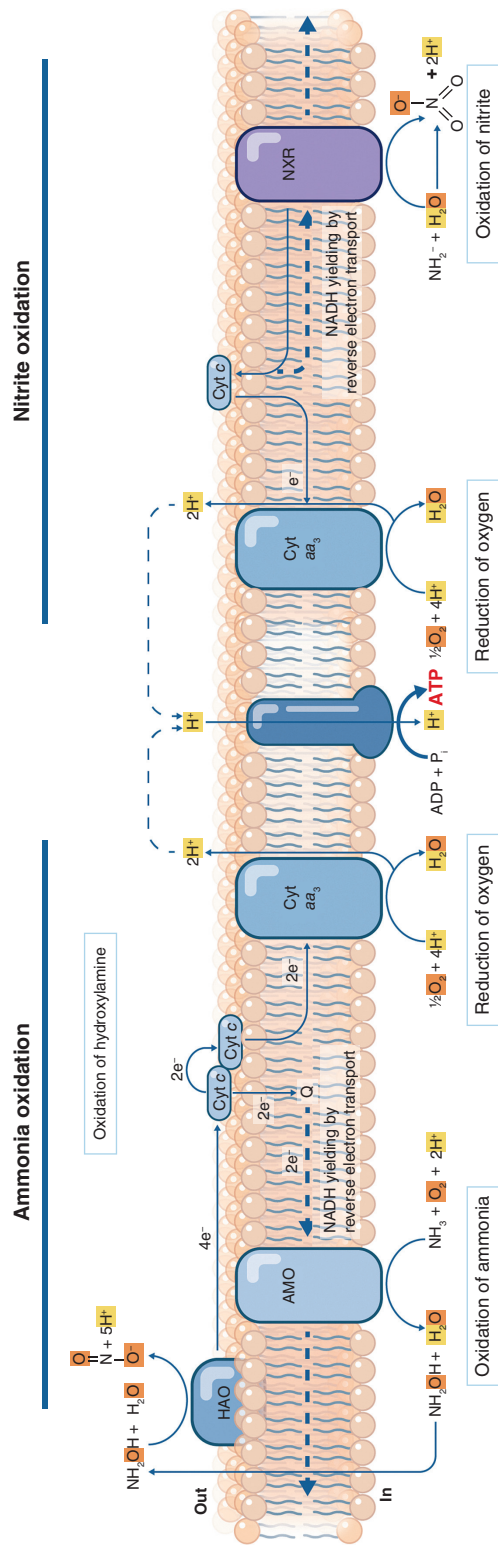


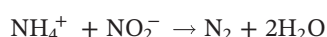
Figure 1.12 Two sets of reactions in nitrification. Left, ammonia oxidation and electron flow in ammonia-oxidizing bacteria. Right, nitrite oxidation by nitrifying bacteria. AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; Q, ubiquinone; NXR, nitrite oxidoreductase. Cyt *c*, cytochrome *c*, a small, soluble protein located in the intermembrane space of mitochondria and functions as an electron carrier in the mitochondrial electron transport chain; Cyt *aa*₃, cytochrome *aa*₃, cytochrome *c* oxidase that accept and transfer electrons. Cyt *c* and Cyt *aa*₃ work together in the final steps of oxidative phosphorylation; Cyt *c* delivers electrons to Cyt *aa*₃, which completes the chain by reducing oxygen.

Nitrite oxidizers such as *Nitrobacter* use the enzyme *nitrite oxidoreductase* to oxidize NO_2^- to NO_3^- . The electrons travel a short transport chain because of the high potential of the $\text{NO}_3^-/\text{NO}_2^-$ couple to the terminal oxidase. As is the case of iron oxidation (see Section 1.3.4.4), only small amounts of energy are available from nitrite oxidation (Figure 1.12). Hence, minimal amounts of cell material are obtained even though large amounts of nitrite have been oxidized.

Aerobic nitrifying microbes fix carbon via the Calvin cycle. Nitrification is important ecologically since it replenishes supplies of nitrate in the soil which then can be used by denitrifying bacteria or by plants in their biosynthesis. Nitrate is a more usable fixed nitrogen source for algae and cyanobacteria. Globally, nitrification is another process that requires molecular oxygen. As a result, nitrification assists in the ultimate return of nitrogen gas to the atmosphere by supplying nitrate to the denitrifying microbes and reducing the quantity of oxygen gas in the atmosphere.

1.3.4.7 Anammox

Ammonia can also be oxidized under anoxic conditions. This process is called anammox (for anaerobic ammonia oxidation) and is catalyzed by an unusual group of obligately anaerobic bacteria, using NO_2^- as the electron acceptor to yield N_2 by the reaction



Five different anammox genera have been described. *Brocadia anammoxidans* from the Planctomycetes phylum of bacteria is a well-known anammox species. Its cytoplasm has various membrane-enclosed compartments, named anammoxosome organelle (Claret Fernández et al. 2020), where the anammox reaction and energy conversion take place (Figure 1.13). Other anammox bacteria include *Kuenenia*, *Anammoxoglobus*, *Jettenia*, and *Scalindua*, all of which are related to *Brocadia* and also contain anammoxosomes (Madigan et al. 2018).

Anammox bacteria are also autotrophs, but they fix CO_2 by the reductive acetyl-CoA pathway, an autotrophic pathway widespread among some obligately anaerobic autotrophic bacteria and archaea. In the anammox reaction, NO_2^- is first reduced to NO by nitrite reductase, NO reacts with ammonium (NH_4^+) to yield N_2H_4 by the enzyme hydrazine synthase, and then N_2H_4 is

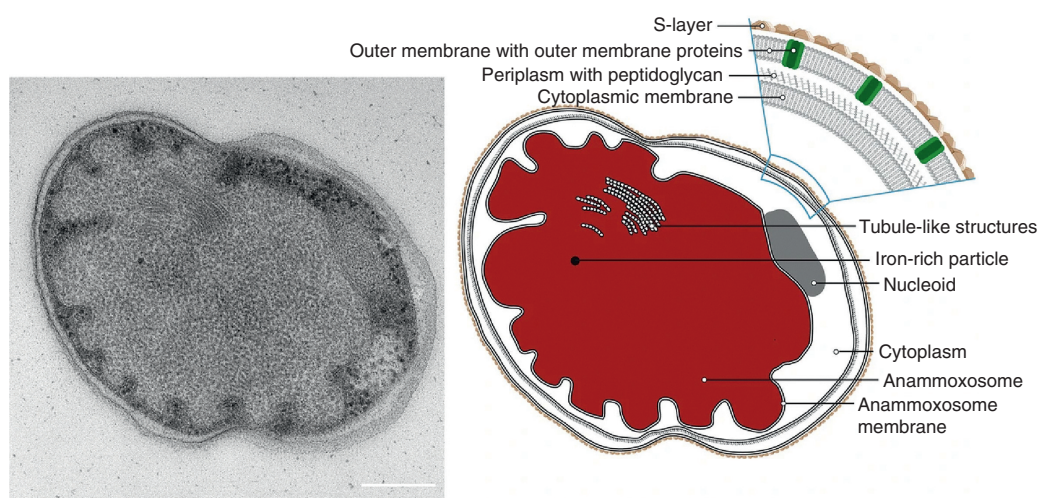


Figure 1.13 Transmission electron micrograph (left) and schematic model (right) of a cross-section of an anammox cell. Scale bar, 200 nm. Source: Claret Fernández et al. 2020 / Springer Nature.

oxidized to N_2 plus electrons by the enzyme hydrazine dehydrogenase. The electrons are funneled into the electron transport chain where they are used to reduce nitrite and nitric oxide earlier in the pathway.

The chemolithoautotrophic anammox bacteria are found naturally in anoxic or oxygen-limited terrestrial, marine, and freshwater environments. The anammox has been shown to play a crucial role in the biological nitrogen cycle where anammox bacteria are estimated to produce approximately half of N_2 present in the atmosphere. Besides their presence in nature, all genera of anammox bacteria have been detected in conventional wastewater treatment plants, where they remove ammonium from contaminated waters (Claret Fernández et al. 2020).

1.3.5 Catabolism

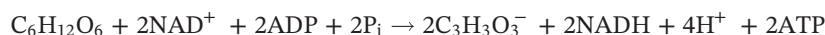
Catabolism is a combination of cellular processes that harvest the energy released during the breakdown of preexisting organic compounds such as glucose and this energy is used to synthesize ATP, the energy currency of all cells. In most heterotrophic microorganisms, the oxidation of carbohydrates provides the bulk of the cell's energy. The primary catabolism proceeds through a series of three coupled pathways: glycolysis, tricarboxylic acid cycle (TCA, or citric acid cycle), and respiration chain (electron transport and oxidative phosphorylation). To degrade sugars, cells must first secrete specific extracellular hydrolytic enzymes that break down the glycosidic bonds in polysaccharides into mono- and disaccharides that can then be transported through the plasma membrane into the cytoplasm. For optimal efficiency, organisms benefit from close proximity to the substrate, minimizing the diffusion distance for hydrolysis products to reach the cell surface. Their sugar breakdown follows two pathways: (1) partial degradation begins with glycolysis and transitions directly into fermentation; (2) complete catabolism proceeds through glycolysis, an intermediate reaction forming acetyl CoA, the citric acid cycle, and ultimately, the electron transport chain.

The pathways for the breakdown of organic compounds and energy conservation in ATP can be divided into two major groups: (1) fermentation, in which the degradation occurs in the absence of any added TEAs; and (2) respiration, subdivided into aerobic and anaerobic respirations, in which O_2 or some other oxidants serve as TEAs, respectively. Electron acceptors used in anaerobic respiration include NO_3^- , Mn^{4+} , Fe^{3+} , SO_4^{2-} , etc., each of which is discussed separately in this section. The oxidation pathways of organic matter in most anaerobic processes differ significantly from those in aerobic respiration and denitrification. While aerobes and denitrifiers can fully degrade a wide range of organic compounds into carbon dioxide on their own, anaerobes are generally restricted to a limited selection of simple organic substrates. Consequently, organic matter derived from the hydrolysis of complex polymers must first be broken down by fermentative microorganisms, which transform large polymers into smaller degradation products—such as hydrogen (H_2), pyruvate, lactate, acetate, and formate. These intermediates are then further metabolized by acetogenic bacteria and anaerobic respirers that function syntrophically to achieve the complete oxidation of organic matter into either carbon dioxide (CO_2) or methane (CH_4) within suboxic to anoxic sedimentary environments.

1.3.5.1 Glycolysis and Fermentation

Glycolysis is the primary pathway used by nearly all organisms to convert glucose to pyruvate. A six-carbon glucose molecule is partially broken down into two molecules of pyruvate, with a net gain of two ATP through substrate-level phosphorylation, and two NADH. The decomposition includes a number of complex steps, each catalyzed by its own unique enzyme. Glycolysis

does not require oxygen and thus can occur under both oxic and anoxic conditions. The overall reaction is:



Beyond glycolysis, numerous bacteria have the capability to metabolize five-carbon sugars through the pentose phosphate pathway. This pathway plays a crucial role in generating key intermediate molecules essential for the synthesis of nucleic acids and certain amino acids, while also serving as a primary source of NADPH from NADP^+ . Unlike glycolysis, the pentose phosphate pathway yields only a single ATP molecule per glucose molecule. Bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Enterococcus faecalis* utilize this metabolic route. Once pyruvate is formed, it can proceed either through fermentation—essentially the partial oxidation of glucose and other carbohydrates—or respiration.

Fermentation differs from respiration in that it lacks an electron transport chain and operates without externally supplied electron acceptors. In aerobic bacteria, protozoans, algae, fungi (including yeasts), plants, and animals, fermentation serves as a backup energy extraction process. However, for many anaerobic bacteria, it is the primary method of deriving energy from sugars, excluding methanogenic bacteria, sulfate-reducing bacteria, and acetogenic bacteria.

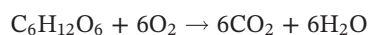
Fermentation occurs when there is a buildup of waste hydrogen at the end of respiration. Normally, oxygen or nitrate clears out these hydrogens, allowing respiration to continue smoothly. However, in environments like deep mud, stagnant water, or certain soils, where oxygen and nitrate are absent, the system gets clogged, causing respiration to halt. To keep ATP production going, cells reroute glycolysis products (pyruvate) through fermentation, which continues the breakdown of carbon chains. Fermentation generates about 20 times less ATP per sugar molecule than respiration, meaning it is far less efficient for sustaining high-energy activities.

Fermentation generates a diverse array of waste products, including lactic acid, ethanol, and acetic acid. When muscles are fatigued, fermentation produces lactic acid, which can contribute to soreness. However, in bacteria, lactic acid plays a valuable role in creating yogurt and cheese. Yeast and certain bacteria produce ethanol and carbon dioxide through fermentation—giving champagne its signature bubbles and alcoholic punch. Bakers also take advantage of fermentation, as the carbon dioxide released by yeast causes dough to rise, transforming it from a dense lump into a fluffy loaf.

Fermentation waste products sometimes serve as carbon chains that contribute to the biosynthesis of larger molecules. More often, these byproducts are released into the environment, where they can lead to contamination—such as yeast dying from ethanol buildup in a sealed bottle of grape juice or wine. Alternatively, other microorganisms may take advantage of these waste products as an energy source, turning what could be harmful into something beneficial.

1.3.5.2 Aerobic Respiration

Prokaryotic and eukaryotic aerobic respirers are ubiquitous in environments where dissolved or free oxygen is available. Aerobic respiration is able to completely oxidize a wide variety of natural and synthetic organic compounds (electron donors) to CO_2 and H_2O , with much greater energy released than during fermentation. The overall reaction is:



This capability arises because aerobes possess a suite of hydrolytic enzymes that help break down large macromolecules like proteins, carbohydrates, and fats into smaller, more easily absorbed components. For instance, proteases break down proteins into amino acids, lipases are responsible for breaking down fats into fatty acids and glycerol, amylases catalyze the breakdown of starch and

complex carbohydrates into simpler sugars, and cellulases break down cellulose into glucose for energy production. The smaller molecules are further processed by the Link Reaction, Krebs Cycle, and Electron Transport Chain, which are more efficient in energy release.

During aerobic respiration, the total possible yield of ATP is 40: 4 from glycolysis, 2 from the TCA cycle, and 34 from electron transport. However, since 2 ATPs were expended in early glycolysis, this leaves a maximum of 38 ATPs per glucose molecule, though the actual amount can vary based on the organism and specific cellular conditions. Eukaryotic cells often lose a bit of ATP when transporting NADH generated in glycolysis into the mitochondria, reducing the net yield slightly. Meanwhile, aerobic bacteria, which lack mitochondria, can maximize their ATP production since they don't need to expend energy shuttling molecules across membranes. The electron transport chain plays a huge role in ATP production, using oxidative phosphorylation to generate most of the ATP.

A potential side reaction of aerobic respiration is the incomplete reduction of oxygen to superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), both generated as byproducts of the biochemical reduction of O_2 . On the one hand, these toxic oxygen products can be very damaging to cells; aerobes have various neutralizing enzymes (e.g. superoxide dismutase and catalase). One exception is the genus *Streptococcus* (a genus of spherical gram-positive bacteria), which can grow well in oxygen, yet lacks both cytochromes (catalyzing the electron acceptance) and catalase. However, certain species of *Streptococcus* use a special peroxidase that helps them survive oxidative stress. The lack of cytochromes, catalase, and peroxidase in anaerobes as a rule limits their ability to process O_2 and contributes to its toxic effects on them. On the other hand, these highly reactive oxygen-containing radicals (H_2O_2 and O_2^-) can be used to break down refractory compounds, such as lignin.

1.3.5.3 Nitrate Reduction and Denitrification

Anaerobic respiration occurs in environments devoid of oxygen, where alternative electron acceptors replace oxygen in energy production. One of the most common substitutes is nitrate, which plays a key role in the nitrogen cycle. Nitrate reduction can be categorized into two distinct pathways: assimilative and dissimilative, each serving unique biological functions (Figure 1.14).

Both pathways are essential for maintaining nitrogen balance in natural ecosystems, influencing factors like soil fertility, wastewater treatment, and atmospheric nitrogen levels. The assimilative

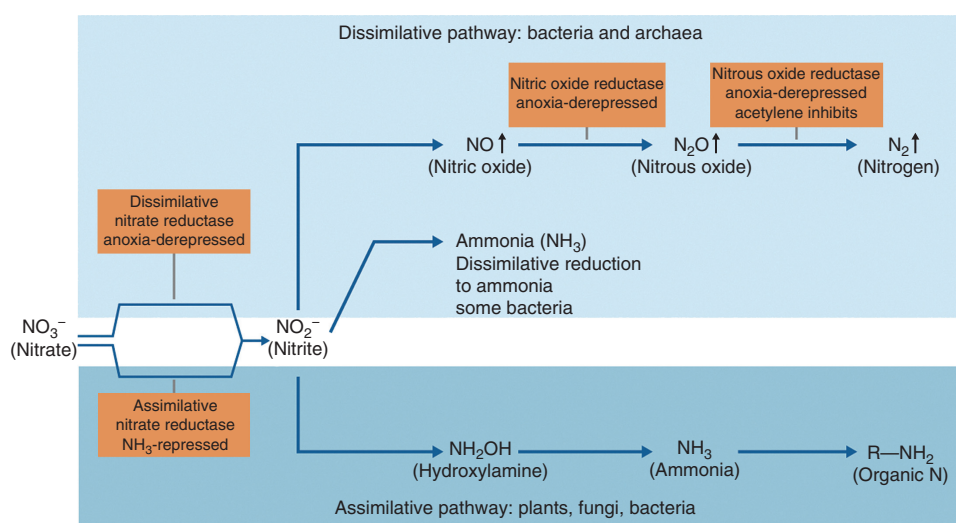


Figure 1.14 Comparison of assimilative and dissimilative processes for nitrate reduction.

pathway is prevalent in plants, fungi, and bacteria that require nitrogen for amino acid and nucleotide synthesis. In this process, nitrate is converted into ammonia (NH₃), which is then incorporated into cellular biomass. Here, nitrate is utilized as a nutrient for growth rather than as an energy source. Conversely, the dissimilative pathway involves the stepwise reduction of nitrate into gaseous nitrogen compounds—N₂O, NO, and ultimately N₂. Since these intermediate and final products are gaseous, they escape from the environment. This entire process is known as denitrification, a crucial process in nitrogen cycling. The overall reaction is:



Denitrification is a metabolic process carried out by anaerobic bacteria and archaea, where nitrate serves as an alternative electron acceptor in the absence of oxygen. Remarkably, the eukaryotic species *Globobulimina pseudospinescens*, a shelled amoeba found in anoxic marine sediments, has been identified as a denitrifier (Madigan et al. 2018). The first step of denitrification is catalyzed by nitrate reductase, a membrane-integrated enzyme containing molybdenum. Its synthesis is inhibited by oxygen, as are all subsequent enzymes involved in the pathway, which are coordinately regulated to function only under anoxic conditions. However, certain denitrifying bacteria, such as *Pseudomonas*, exhibit metabolic flexibility, switching between aerobic respiration when oxygen is available and anaerobic respiration using nitrate once oxygen is depleted. The biochemistry of denitrification has been studied in detail in *E. coli*, in which NO₃[−] is reduced only to NO₂[−] by the enzyme reductase, and *Paracoccus denitrificans* and *Pseudomonas stutzeri*, in which nitrogen oxides are formed from NO₂[−] by enzymes nitrite reductase, nitric oxide reductase, and nitrous oxide reductase.

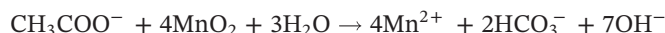
Denitrification plays a crucial role in ecological and environmental systems. Besides producing nitrogen gas (N₂), it also generates nitrous oxide (N₂O) and nitric oxide (NO), which pose environmental concerns. N₂O is a potent greenhouse gas that contributes to climate change and can be transformed into NO through sunlight exposure. NO, in turn, interacts with ozone (O₃) in the upper atmosphere, leading to the formation of nitrite (NO₂[−]), which falls to Earth as nitrous acid (HNO₂) in acid rain. Despite these negative effects, denitrification serves as a beneficial function in wastewater treatment by eliminating fixed nitrogen—a key factor in algal blooms that could otherwise occur if nitrate-rich effluent is discharged into environments like rivers or lakes (Madigan et al. 2018).

1.3.5.4 Microbial Manganese Reduction

Microbial manganese reduction is a process in which certain bacteria and archaea facilitate the conversion of manganese oxides into soluble manganese ions. This process plays a crucial role in biogeochemical cycles, influencing metal mobility, nutrient availability, and environmental remediation efforts. It occurs in various natural settings, including aquatic sediments and soil environments, where microbes utilize manganese as an electron acceptor in anaerobic respiration. There are a number of Mn(IV) oxides in soils and sediment, including those with an average composition of MnO₂, such as todorokite, vernadite, pyrolusite, birnessite, and also some mixed valence and trivalent forms, such as hausmannite (Mn₃O₄) and manganite (MnOOH). They are not equal with regard to their propensity to be reduced, amorphous phases with high surface areas being more reactive than highly crystalline oxides with low surface area, and Mn(III) oxyhydroxides being more reactive than Mn(IV) oxides.

Similar to those in Fe(III)-reduction and sulfate reduction (discussed below), the electron transport in the Mn(IV) reduction begins with the transfer of H₂ to a hydrogenase. The H₂ can come from the external environment, whereby the bacteria behave either chemolithoautotrophically as hydrogen-oxidizers or mixotrophically, whereby they assimilate organic compounds as their carbon

source. More commonly, H_2 is removed from organic electron donors such as acetate. Under these conditions, microorganisms behave heterotrophically. The sum reaction is:



Microbes involved in manganese reduction belong to various taxa, including *Pelobacter*, Colwelliaceae, Arcobacteraceae, Shewanellaceae, and Oceanospirillaceae. Many iron-reducing bacteria can also reduce manganese oxides. Microbial manganese reduction plays a significant role in carbon mineralization, contributing up to 45% in manganese oxide-rich sediments (Wunder et al. 2024). Among the most notable genera are *Pseudomonas*, *Bacillus*, *Geobacter*, and *Shewanella*. While some species function as facultative anaerobes, others primarily act as Fe(III) reducers, shifting to MnO_2 reduction when necessary. Given the high reduction potential of the Mn(IV)/Mn(II) couple, Mn-reducers demonstrate remarkable flexibility in electron donor utilization. For instance, *Geobacter metallireducens* can metabolize butyrate, propionate, lactate, succinate, and acetate, completely oxidizing them into CO_2 . Meanwhile, *Shewanella putrefaciens* primarily utilizes lactate and pyruvate, but only oxidizes them to acetate. Notably, *S. putrefaciens* is also capable of reducing Mn(III) hydroxides to Mn^{2+} (see Konhauser 2007).

1.3.5.5 Ferric Iron Reduction

Ferric iron can be reduced to a ferrous state by a variety of microorganisms. The reduction can be a major form of anaerobic respiration because Fe(III) is abundant in many microbial habitats. The reduction potential of the Fe(III)/Fe(II) couple is very electropositive, and thus Fe(III) reduction can be coupled to the oxidation of a wide variety of both organic and inorganic electron donors. Ferric iron minerals occur in nature in a wide variety of forms, ranging from amorphous to crystalline phases. The amorphous to poorly ordered iron oxyhydroxides, such as ferric hydroxide or goethite ($FeOOH$), are the preferred sources of solid-phase ferric iron for Fe(III)-reducers. More crystalline Fe(III) oxides, such as hematite (Fe_2O_3) and magnetite (Fe_3O_4) are also microbially reducible. The variations in reductive rates are related to a number of factors, including the amount of surface area exposure, crystal morphology, particle aggregation, the composition of the aqueous solution in which the microorganisms grow, and the amount of Fe^{2+} sorbed to the oxide surface.

Microbial Fe(III) reduction has been observed across a diverse array of microorganisms within the domains archaea and bacteria, spanning various chemical and physical conditions. This widespread metabolic capability highlights its ubiquity in microbial life. Extremophilic Fe(III)-reducing microorganisms, including hyperthermophilic, thermophilic, psychrophilic, acidophilic, and alkaliphilic species, have been successfully isolated and cultivated in pure cultures. Among these, one hydrothermal vent archaeal species, *Geogemma barossii* has redefined the upper temperature threshold for life, surviving above 121 °C (Kashefi and Lovley 2003). Some fermentative, sulfate-reducing, and methanogenic microorganisms are also capable of Fe(III) reduction (Weber et al. 2006). Additionally, ferric iron can serve as a TEA in fungal energy metabolism (Li et al. 2024). For instance, *Aspergillus niger* has been reported to biologically leach iron from clay minerals within a pH range of 1.7–3.0, while *Penicillium* sp. can mobilize Fe(III) from jarosite minerals by reducing it to Fe(II) (Li et al. 2024).

Fe(III) oxide minerals are insoluble, preventing their diffusion into microbial cells. As a result, electron transport to the solid-phase TEA cannot take place within the periplasm, as it does with soluble electron acceptors like nitrate or fumarate. Instead, Fe(III) reduction must occur externally to the cell, necessitating close microbial contact with the mineral surface. Notable iron-reducing bacteria include *Geobacter* and *Shewanella* (Figure 1.15). *G. metallireducens* is known to produce surface appendages, such as pili, which facilitate adherence to oxide surfaces and act as nanowires for electron transfer from the cell to the surface of Fe(III) oxides (Figure 1.16). In contrast,

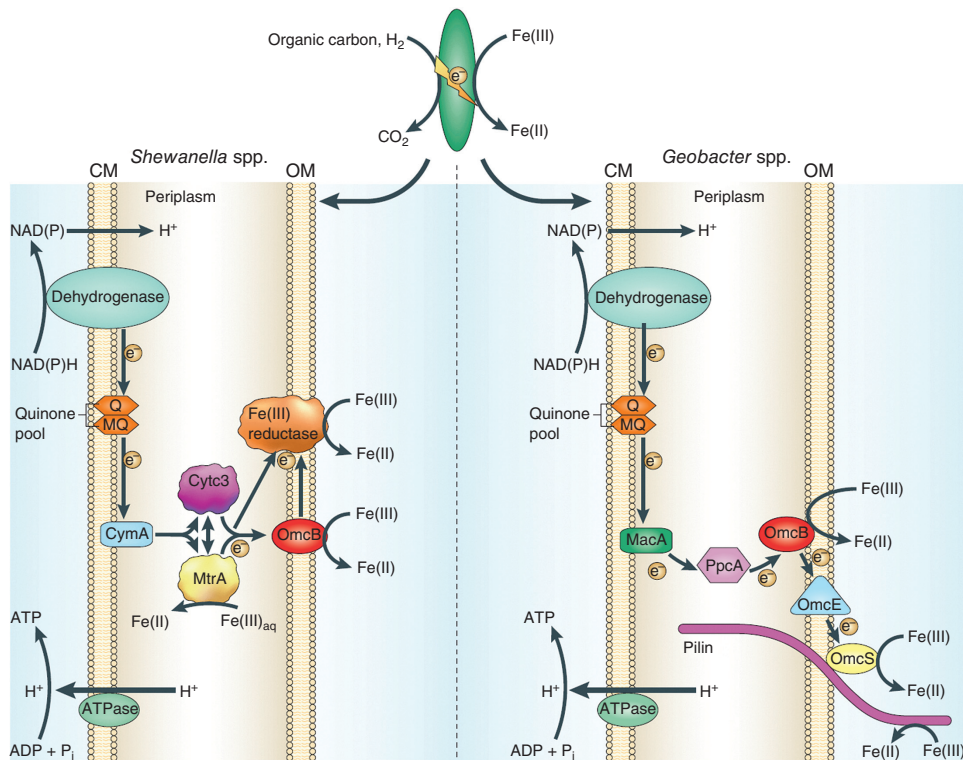


Figure 1.15 Physiological model of the biochemistry in microbial Fe(III) reduction by *Shewanella* and *Geobacter* spp. CM, cytoplasmic membrane; CymA, cytoplasmic membrane-bound tetraheme c-type cytochrome; Cytc3, periplasmic c-type cytochrome; e^- , electrons; MacA, cytoplasmic membrane-bound cytochrome; MQ, menaquinone; MtrA, periplasmic decaheme c-type cytochrome; OM, outer membrane; Omc, outer-membrane-bound cytochromes B, E, and S partially exposed on the cell surface; PpcA, a tri-heme periplasmic c-type cytochrome; Q, quinone. *Source:* Weber et al. (2006) / with permission of Springer Nature.

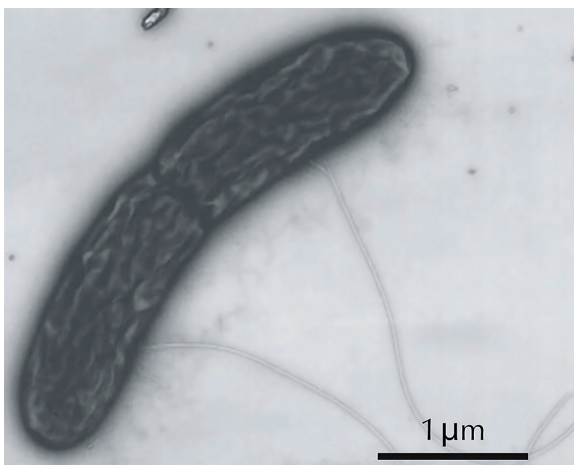


Figure 1.16 A species of *Geobacter* developing pili that helps the bacterium adhere to the oxide surfaces and serves as biological “nanowire” to transfer electrons from the cell to the surface of Fe(III) oxides. *Source:* Photo by Lucy Yin / The Regents of the University of California / <http://genome.jgi.doe.gov/geome/geome.home.html> / last accessed on July 15, 2025.

Shewanella algae employs hydrophobic surface proteins to enhance cell adhesion. Fe(III)-reducing bacteria can accumulate nanometer-scale iron oxide particles on their surfaces for later reduction. Upon attachment to the mineral surface, they initiate electron transfer from a reduced source within the cytoplasm, moving across the plasma membrane and periplasm to the outer membrane. Here, iron reductase enzymes facilitate direct electron transfer to the Fe(III) mineral surface, weakening Fe—O bonds and leading to reductive dissolution. Additionally, some bacteria use organic compounds, such as humics, as electron shuttles between the cell surface and Fe(III) oxides (Konhauser 2007).

The ubiquity of Fe(III)-reducers and their phylogenetic diversity make this microbial metabolism globally significant. The microbially mediated reduction of Fe(III) oxide minerals can generate both aqueous and solid-phase Fe(II)-bearing minerals such as siderite.

Ferric iron is among the most prevalent metals found in sediments and rocks, and its reduction produces ferrous iron, a more soluble form of iron. As a result, microbial iron reduction contributes to iron solubilization. When exposed to oxidizing conditions, ferrous iron undergoes oxidation either spontaneously or through the action of Fe(II)-oxidizing bacteria, leading to the formation of insoluble ferric hydroxide deposits. One specific type of iron deposit, known as bog iron, is believed to originate from microbially driven iron cycling in bogs. Fe(III) reduction plays a crucial role in organic matter oxidation and the transformation of Fe(III) minerals within deeply buried sediments. Furthermore, it may be responsible for post-depositional processes such as the development of variegated red beds and the release of elevated concentrations of dissolved Fe^{2+} in anoxic groundwater environments.

1.3.5.6 Sulfate Reduction

In anaerobic respiration, several inorganic sulfur compounds serve as vital electron acceptors. The Earth's crust primarily contains sulfur in the form of sulfate minerals, such as gypsum, and sulfide minerals like pyrite. In marine environments, sulfur is predominantly found as dissolved sulfate. The sulfate ion (SO_4^{2-}), which represents sulfur in its most oxidized state, is a major anion in seawater. It can be transformed into hydrogen sulfide (H_2S) through the activity of sulfate-reducing microbes, which thrive exclusively in oxygen-depleted conditions. A common sign of their presence is the distinct odor of rotten eggs—often noticeable when disturbing black mud from a pond or stagnant water. This characteristic smell arises from the H_2S released by sulfate reducers inhabiting the sediment.

Since sulfate-reducing microbes were first reported by Beijerinck (1895), more than 150 species from at least 50 genera belonging to a number of bacterial phyla (e.g. Desulfobacterota, Firmicutes, Thermodesulfobacteria and Nitrospirota) have been cultured and described, along with five species of two genera (*Archaeoglobus* and *Vulcanisaeta*) from two archaeal phyla, Halobacteriota and Thermoproteota (Figure 1.17). Sulfate-reducers are highly adaptable, thriving across a wide spectrum of environmental conditions. They have been found in shallow marine and freshwater sediments, as well as deep subsurface habitats, including oil wells, hydrothermal vents, and mud volcanoes. Some are able to survive in extreme settings—whether highly acidic or alkaline, intensely hot or cold, and in environments with varying salinity levels. Additionally, sulfate-reducers are present within living organisms, such as ruminants and the human intestinal tract. In marine worms, they establish a close association with aerobic sulfide-oxidizing bacteria, forming a symbiotic relationship essential to their survival (Muyzer and Stams 2008). Besides sulfate, most sulfate-reducing bacteria can also conserve energy from the reduction of elemental sulfur to sulfide ($\text{S}^0 + 2\text{H} \rightarrow \text{H}_2\text{S}$).

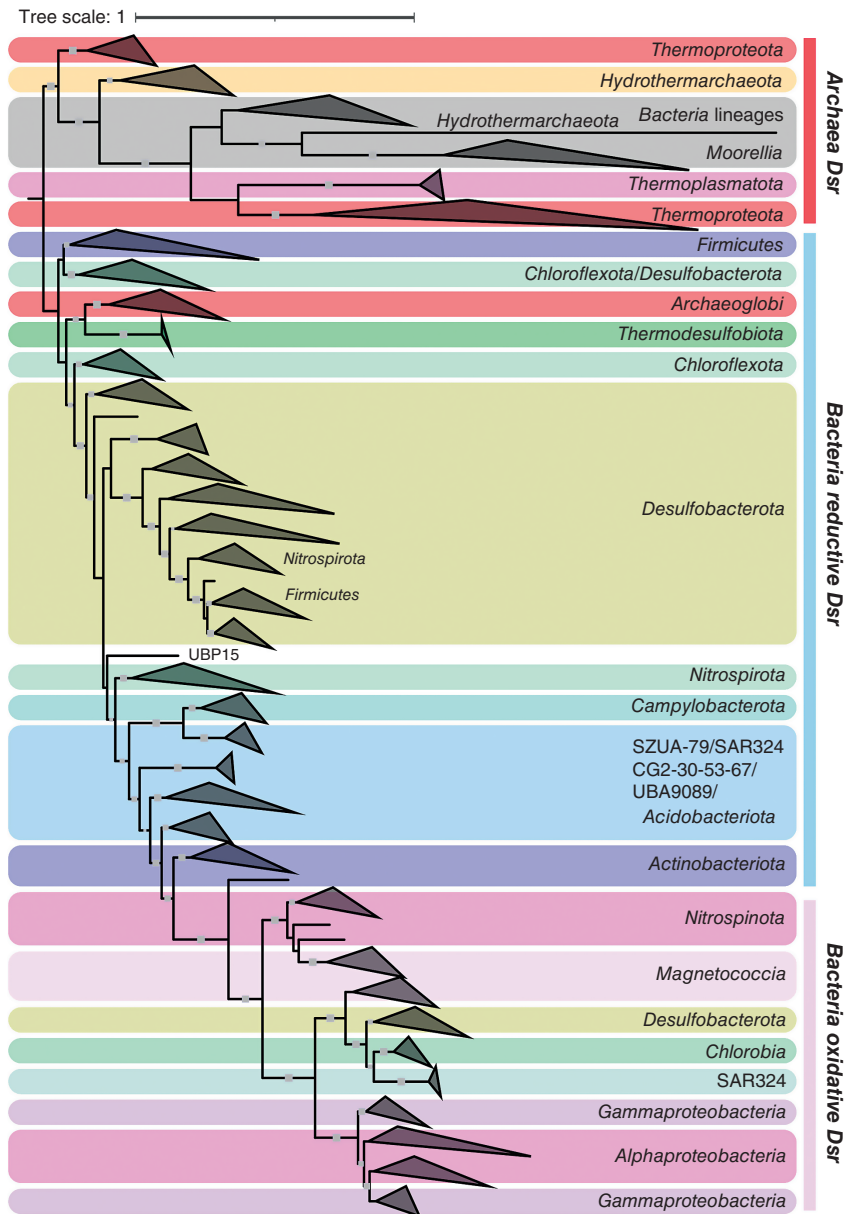


Figure 1.17 Phylogenetic tree for the dissimilatory sulfate reductase DsrAB phylogenetic tree, indicating that sulfate reducers are widely distributed in bacteria and archaea and that microbial sulfate reduction represents an ancient metabolism. *Source:* Cui et al. 2024 / with permission of Elsevier.

Similar to nitrate reduction, microbial sulfate reduction also has assimilative and dissimilative pathways (Figure 1.18). In the assimilative sulfate metabolism, many organisms, including higher plants, algae, fungi, and most prokaryotes, use sulfate as a sulfur source for biosynthetic purposes to make cysteine, methionine, and many other organosulfur compounds. In the dissimilative process, sulfate is used as an electron acceptor for energy conservation, which requires a large-scale reduction of SO_4^{2-} and is restricted to sulfate-reducing bacteria and archaea. In assimilative sulfate

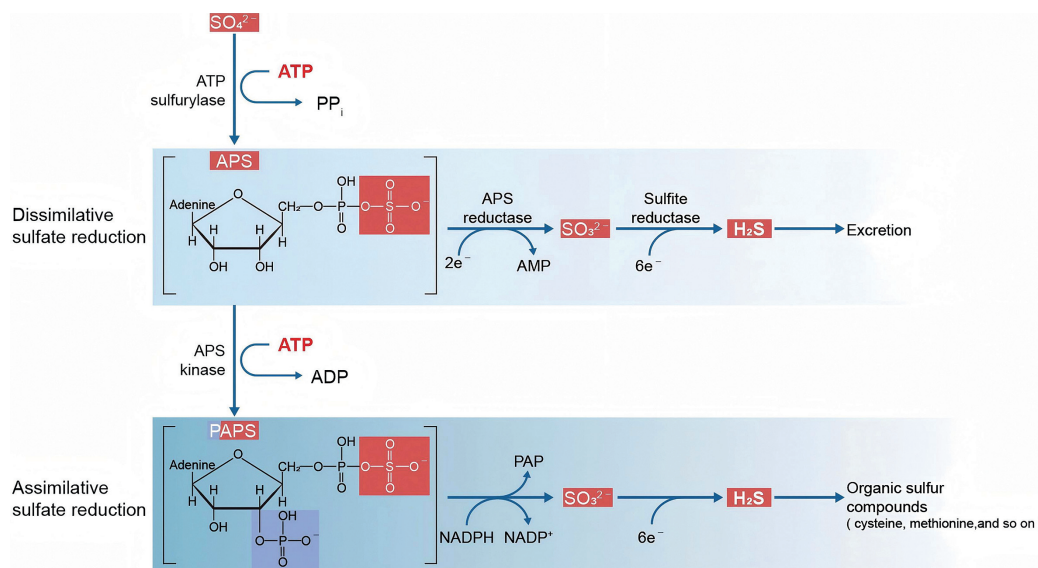


Figure 1.18 Comparison between assimilative and dissimilative sulfate reductions. In dissimilative sulfate reduction, SO_4^{2-} in adenosine 5'-phosphosulfate (APS) is reduced directly to sulfite (SO_3^{2-}) by the enzyme APS reductase with the release of AMP. In assimilative reduction, a second phosphate is added to APS to form 3'-phosphoadenosine 5'-phosphosulfate (PAPS) and only then is SO_4^{2-} reduced. In both cases, the product of sulfate reduction is SO_3^{2-} ; once formed, SO_3^{2-} is reduced to H_2S by activity of the enzyme sulfite reductase.

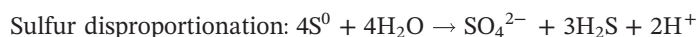
reduction, the H_2S formed is immediately converted into organic sulfur in the form of amino acids, proteins, and so on, but in dissimilative sulfate reduction, the H_2S is produced on a very large scale by sulfate reducers and is excreted from the cell, free to be oxidized by air, used by other organisms, or combined with metals to form metal sulfides.

There are two physiological types of dissimilative sulfate reducers. The complete oxidizers (e.g. *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, and *Desulfonema*) can oxidize acetate and other fatty acids completely to CO_2 , and the incomplete oxidizers (e.g. *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*, and *Desulfobulbus*) are unable to oxidize acetate to CO_2 . These two physiological groups are not phylogenetically coherent but instead are distributed widely across the phylogeny of sulfate-reducing bacteria (Madigan et al. 2018).

The reduction of SO_4^{2-} to H_2S requires eight electrons and proceeds through a number of intermediate stages (Figure 1.17). It is first activated by the enzyme ATP sulfurylase that catalyzes the attachment of SO_4^{2-} to a phosphate of ATP, forming adenosine phosphosulfate (APS). In dissimilative sulfate reduction, the SO_4^{2-} in APS is reduced directly to sulfite (SO_3^{2-}) by the enzyme APS reductase with the release of adenosine monophosphate (AMP). In assimilative reduction, by contrast, a second phosphate is added to APS to form phosphoadenosine phosphosulfate (PAPS), and only then is the SO_4^{2-} reduced. However, in both cases, the product of sulfate reduction is sulfite, and once SO_3^{2-} is formed, it is reduced to H_2S by activity of the enzyme sulfite reductase.

Microbial sulfate reduction includes specialized variants. Some sulfate-reducing bacteria can generate energy through disproportionation metabolism, a unique process in which a single compound undergoes simultaneous oxidation and reduction. As a result, one fraction becomes more oxidized, while the other is reduced, allowing these microbes to efficiently harness energy in

anaerobic environments. For example, *Desulfovibrio sulfodismutans* can disproportionate sulfite, thiosulfate, and sulfur into both sulfide and sulfate by the following reactions:



In addition, the autotrophic, strict anaerobic sulfate-reducing bacterium *Desulfotignum phosphitoxidans* can couple phosphite (HPO_3^-) oxidation to SO_4^{2-} reduction by the reaction:

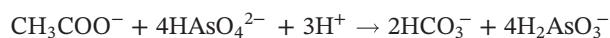
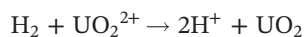
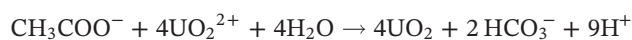


The natural sources of phosphite are likely to be organophosphorus compounds called phosphonates that are generated from the anoxic degradation of nucleic acids, phospholipids, and other cellular sources (Madigan et al. 2018).

Microbial sulfate reduction is a fundamental geobiological process, significantly shaping Earth's sulfur and carbon cycles. Sulfate-reducing bacteria and archaea transform sulfate into hydrogen sulfide (H_2S), which can interact with metal ions to form intracellular or extracellular minerals such as pyrite (Cui et al. 2024). Alternatively, H_2S is released into the environment, where it becomes available for anoxygenic photosynthetic and sulfur-oxidizing bacteria, sustaining a continuous sulfur cycle. Additionally, sulfate-reducing microorganisms facilitate the anoxic oxidation of methane, inducing the precipitation of seep carbonate. In oxygen-deprived settings, these microbes contribute to the decomposition of organic matter, playing a key role in fossil fuel formation and petroleum reservoir dynamics. On a global scale, sulfate reduction accounts for the oxidation of 12–29% of the total carbon flux reaching the ocean floor (Bowles et al. 2014). Furthermore, sulfate reduction competes with methanogenesis, ultimately affecting methane emissions from marine sediments and contributing to greenhouse gas regulation.

1.3.5.7 Other Electron Acceptors

Beyond the above-discussed electron acceptors for anaerobic respiration, various metals, metalloids, and both halogenated and unhalogenated organic compounds are important electron acceptors for microbial anaerobic respiration in nature (Figure 1.19). Additionally, a few strict anaerobes can even utilize protons as electron acceptors. Among these are compounds containing arsenic (As), selenium (Se), chromium (Cr), uranium (U), technetium (Tc), and even plutonium (Pu). Although typically found in low concentrations in natural environments, these substances occasionally appear as pollutants and can sustain the anoxic growth of certain microorganisms. Their microbial reduction serves as a vital mechanism for pollutant removal, contributing to environmental cleanup efforts. Examples are U(VI) reduction and As (V) reduction that are often utilized to clean up the contaminated groundwater.



1.3.5.8 Methanogenesis

Methanogens are microorganisms that produce methane (CH_4) as an integral part of their energy metabolism. The process of methane production is called methanogenesis. Methanogens are strict

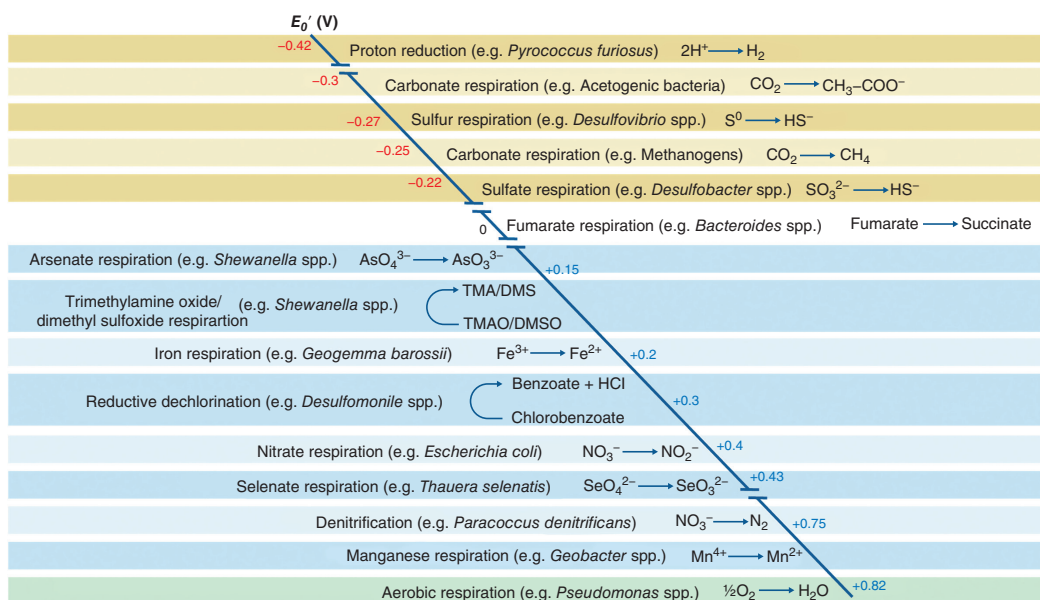


Figure 1.19 Major electron acceptors of microbial respirations. The redox couples are arranged in order from the most electronegative E'_0 (top left) to most electropositive E'_0 (bottom right) assuming neutral pH.

anaerobic archaea and occur in at least seven orders, including Methanobacteriales, Methanococcales, Methanopyrales, Methanomassiliicoccales, Methanomicrobiales, Methanocellales, and Methanosarcinales. They are spread widely within the Euryarchaeota and do not represent a single coherent phylogenetic group (Madigan et al. 2018). All methanogens use NH_4^+ as nitrogen source, and a few species are known to fix nitrogen. The trace metal nickel is required by all methanogens because it is a component of an important methanogenic coenzyme, *Factor*₄₃₀. Iron and cobalt are also important trace metals for methanogens and, along with nickel, have been the only trace metals shown to be absolutely required for growth of these organisms (Madigan et al. 1997).

Methanogens are important in a wide range of terrestrial and marine environments. They commonly occupy an important niche in anoxic, sulfate-deficient environments such as swamps, wetlands, water-logged soils, rice paddies, tundra, marine sediment, geothermal systems, the subsurface of the Earth's crust, landfill sites, wastewater treatment plants, and within the digestive tracts of many animals. Some can even be found in aerated environments that contain anoxic microenvironments, such as the interior of soil particles.

Methanogenesis occurs through three distinct pathways: CO_2 reduction, methylotrophic methanogenesis, and acetoclastic methanogenesis (Table 1.4). All these pathways depend on coenzyme M, with methane ultimately generated by the reduction of methyl-CoM. We described the methanogenesis by CO_2 reduction in Section 1.3.4.2, a major pathway of methanogenesis, in which microbes grow on $H_2 + CO_2$. This process is viewed as an autotrophy, with CO_2 serving as both carbon source and electron acceptor. In addition to CO_2 , however, certain methylated compounds such as methanol and acetate can be converted to methane by methanogens in the absence of O_2 .

Methanogenesis represents the final stage in the anaerobic breakdown of organic matter in many cases. Estimates suggest that nearly half of the organic carbon degraded globally by anaerobically respiring microbes ultimately becomes methane. Despite its widespread formation, only a few organic compounds serve as direct precursors to methanogenesis (Table 1.4). Methanogens cannot use long-chain fatty acids (with more than two carbons) or aromatic compounds, so they depend on

Table 1.4 Reactions and substrates in three methanogenic pathways.

Pathways	Reactions	Substrates
CO ₂ reduction pathway	$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	Carbon dioxide, CO ₂ (with electrons derived from H ₂ , certain alcohols, or pyruvate) Formate, HCOO ⁻ Carbon monoxide, CO
Methylotrophic pathway	$\text{CH}_3\text{OH} + \text{H}_2 \rightarrow \text{CH}_4 + \text{CO}_2$	Methanol, CH ₃ OH Methylamine, CH ₃ NH ₃ ⁺ Dimethylamine, (CH ₃) ₂ NH ₂ ⁺ Trimethylamine, (CH ₃) ₃ NH ⁺ Methyl mercaptan, CH ₃ SH Dimethyl sulfide, (CH ₃) ₂ S
Acetoclastic pathway	$\text{CH}_3\text{COO}^- + \text{H}_2 \rightarrow \text{CH}_4 + \text{CO}_2$	Acetate, CH ₃ COO ⁻ Pyruvate, CH ₃ COCOO ⁻

Table 1.5 Reactions in fermentation and acetogenesis.

Reaction types	Reactions
Fermentation of glucose to acetate, H ₂ , and CO ₂	Glucose + 4H ₂ O → 2 acetate ⁻ + 2HCO ₃ ⁻ + 4H ⁺ + 4H ₂
Fermentation of glucose to butyrate, CO ₂ , and H ₂	Glucose + 2H ₂ O → butyrate ⁻ + 2HCO ₃ ⁻ + 2H ₂ + 3H ⁺
Fermentation of butyrate to acetate and H ₂	Butyrate ⁻ + 2H ₂ O → 2 acetate ⁻ + H ⁺ + 2H ₂
Fermentation of propionate to acetate, CO ₂ , and H ₂	Propionate ⁻ + 3H ₂ O → acetate ⁻ + HCO ₃ ⁻ + H ⁺ + H ₂
Fermentation of ethanol to acetate and H ₂	2 Ethanol + 2H ₂ O → 2 acetate ⁻ + 4H ₂ + 2H ⁺
Fermentation of benzoate to acetate, CO ₂ , and H ₂	Benzoate ⁻ + 7H ₂ O → 3 acetate ⁻ + 3H ⁺ + HCO ₃ ⁻ + 3H ₂
Acetogenesis from CO ₂ + H ₂	4H ₂ + 2HCO ₃ ⁻ + H ⁺ → acetate ⁻ + 4H ₂ O

Source: Adapted from Madigan et al. (2018).

the activity of other microorganisms to break down complex organic compounds into more suitable and simple substrates. To access a broader array of organic carbon molecules, methanogens often establish syntrophic relationships with fermentative anaerobes, enabling the breakdown of diverse compounds that eventually contribute to methane production (Table 1.5; Figure 1.20). The process is named syntrophy, in which two or more organisms cooperate in the anaerobic degradation of organic compounds. Syntrophs function to supply them with precursors for methanogenesis.

High-molecular-weight substances such as polysaccharides, proteins, and fats, are converted to CH₄ by the cooperative interaction of several physiological groups of prokaryotes. In many anoxic environments, the immediate precursors of CH₄ are H₂ and CO₂, which are generated by the activities of fermentative anaerobes. For conversion of a typical polysaccharide such as cellulose into methane, as many as five major physiological groups of prokaryotes may be involved in the overall process (Figure 1.20). Cellulolytic bacteria cleave the high-molecular-weight cellulose molecule into cellobiose and into free glucose. Glucose is then fermented by primary fermenters to a variety of fermentation products, acetate, propionate, butyrate, succinate, alcohols, H₂, and CO₂ (Table 1.5). These substrates are then consumed by methanogens. Any H₂ produced in primary

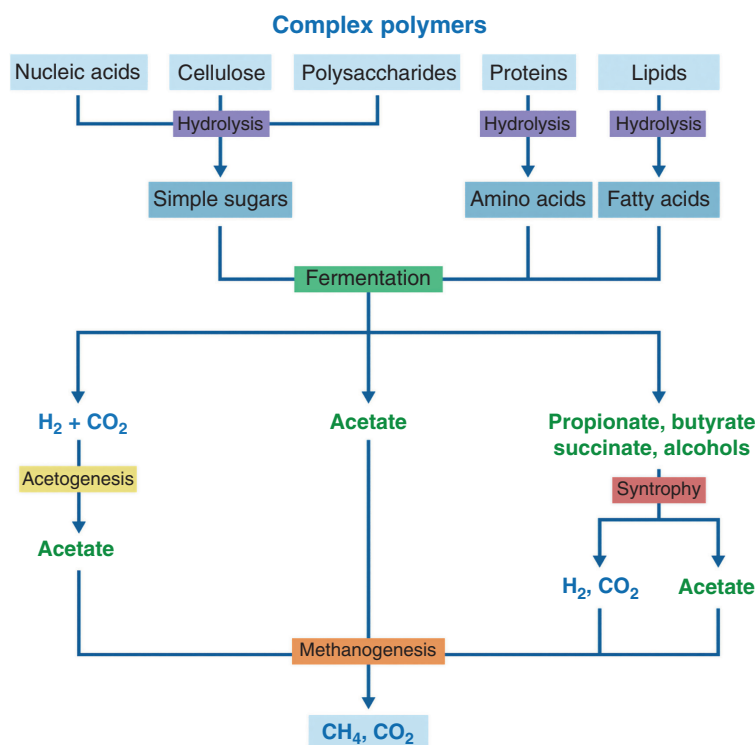


Figure 1.20 The decomposition of complex organic matter in absence of terminal electron acceptors. The breakdown involves a series of microbially mediated steps that differ significantly from the oxidizing decomposition. Hydrolysis breaks down complex polymers into simpler monomers; fermentation converts monomers into volatile fatty acids (VFAs, like propionate and butyrate), alcohols, acetate, hydrogen (H_2), and carbon dioxide (CO_2); Syntrophic oxidation of fermentative products supplies precursors for methanogenesis. Methanogenesis converts acetate or $H_2 + CO_2$ into methane, an end-product of organic matter degradation in the absence of electron acceptors.

fermentative processes is immediately consumed by methanogens, homoacetogens, or sulfate-reducing microorganisms (in environments where sulfate content is significant).

Since methane is an efficient greenhouse gas, methanogenesis has often been linked to early Earth conditions, potentially influencing atmospheric evolution and climate dynamics. In deep-sea sediments, methanogenesis is a key process in methane seeps, supporting unique ecosystems that rely on methane as an energy source (Section 3.3.1). Additionally, the interaction between methanogenesis and anoxic oxidation of methane leads to the formation of specific buildups that serve as geological markers of past methane emissions (Section 6.2.1).

1.3.5.9 Methanotrophs and Methylotrophs

Methylotrophs are microorganisms that can grow only on one-carbon compounds without a carbon–carbon bond, from methane (C^{-4}) to progressively more oxidized forms like methanol (C^{-2}) and methylamine (C^{-2}), through to formaldehyde (C^0) and formate (C^{+2}). Methane (CH_4) and many other C_1 organic compounds can be catabolized both aerobically and anaerobically. Many, but not all, methylotrophs are also methanotrophs (microbes that can grow on CH_4). Methanotrophs are also able to grow on more oxidized one-carbon compounds and some can even switch to ammonia oxidation if the supply of methane is temporarily diminished.

Methane can be used by methanotrophs in two ways: the dissimilative manner of oxidation of methane to CO_2 and the assimilative manner in which one-carbon compounds are assimilated into cell material. Two distinct assimilative pathways have been known in methanotrophs. Some methanotrophs (e.g. *Methylosinus* and *Methlocystis*) use the serine pathway, in which acetyl-CoA is synthesized from one molecule of CH_2O (produced from the oxidation of CH_3OH) and one molecule of CO_2 . Other methanotrophs (e.g. *Methylomonas*, *Methylomicrobium*, *Methylobacter*, and *Methylococcus*) utilize the ribulose monophosphate pathway for carbon fixation. The ribulose monophosphate pathway is more energy efficient than the serine pathway because all of the carbon for cell material is derived from formaldehyde (CH_2O) at the same oxidation state as cell material, no reducing power is needed (Madigan et al. 1997, 2018).

Methanotrophs are phylogenetically diverse and include bacteria and archaea. Aerobic methane oxidation is catalyzed by particulate and soluble methane monooxygenases (pMMO and sMMO, respectively). These enzymes oxidize methane to methanol, and subsequently, methanol dehydrogenases (MDHs) further oxidize methanol to formaldehyde. After formaldehyde, two more oxidation steps are involved and the intermediates are used for carbon assimilation. Aerobic methanotrophs belong to the gammaproteobacteria (Type I, with families Methylococcaceae and Methylothermaeaceae), alphaproteobacteria (Type II, with families Methylocystaceae and Beijerinckiaceae), and the Verrucomicrobia phyla (family Methylacidiphilaceae) (Guerrero-Cruz et al. 2021).

Anaerobic methanotrophs (ANME) oxidize methane in the absence of oxygen, using alternative electron acceptors such as sulfate, nitrate, nitrite, iron, manganese, and organic compounds like humic substances. They play a crucial role in mitigating methane emissions, particularly in environments where oxygen is scarce, such as deep-sea sediments, wetlands, and wastewater treatment systems. These methanotrophs include both bacteria and archaea, with archaeal ANME being particularly significant in marine ecosystems. ANME archaea are often found in symbiotic relationships with sulfate-reducing bacteria, facilitating methane oxidation coupled with sulfate reduction (Bhattarai et al. 2019). Meanwhile, bacterial ANME, such as those from the NC10 phylum, can oxidize methane using nitrite, producing oxygen intracellularly to fuel methane oxidation. Interestingly, it was proposed that some methanogens have limited capacity to reverse their normal metabolism, thereby oxidizing methane under anoxic conditions (changing to methanotrophs). Evidence for such syntrophy comes from methane-rich marine sediments, where methanotrophs have been found growing in tight clusters, closely surrounded by their sulfate-reducing bacterial partners (see Konhauser 2007).

AOM has received much attention from geobiologists for a number of reasons: (1) AOM serves as a major methane sink in marine sediments, preventing large-scale methane release into the atmosphere, which could otherwise contribute to climate change; (2) the process leads to the precipitation of carbonate minerals, forming methane-derived authigenic carbonates (see Section 6.2.1), which are often found in cold seep environments and serve as geological records of past methane fluxes; (3) in sulfate-poor environments, AOM can be coupled with the reduction of metal oxides such as manganese and iron, influencing sediment geochemistry and contributing to the global carbon cycle (Xiao et al. 2023); (4) AOM helps maintain the stability of methane hydrates in deep-sea sediments by consuming methane before it escapes, thereby influencing the dynamics of gas hydrate reservoirs.

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