

ENVIRONMENTAL MICROBIOLOGY

Natural and Xenobiotic compounds in the environment - Transformation, bioaccumulation, biomagnification in air, water and soil

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Keywords

Atmosphere; Authigenesis; Bioconcentration; Biodegradation; Biogenic element; Biogeochemical cycle; Biomagnification; Biomobilization; Carbon cycle; Cellulose; Chitin; Cometabolism; Fixation; Hemicellulose; Hydrosphere; Immobilization; Lignin; Lithosphere; Mineralization; Nitrogen cycle; Pectin; Recalcitrance; Reservoir; Saprotroph; Transformation; Volatilization; Xenobiotics.

Basic Concepts

Earth is a dynamic structure. The elements (material) cycle, energy flows and the information builds up with the passage of time. In fact energy is the reason for whatever exists and the driving force for whatever happens in the entire cosmos. It can neither be created nor destroyed. However it may be converted to mass and vice versa as is established by Einstein ($E = mc^2$). This is how energy cycles. The process of creation from big bang to big crunch is also cyclic.

In Hindu philosophy, Rigveda states a story of Aditi in the Aditi Sukta emphasizing the cyclic nature of change. Aditi, the mother goddess of creation eats that which it creates. Later, the Greek philosophers also endorsed it by inventing the concept of Oroborous worm, according to which, the worm grows by eating over its tail. These concepts are significant as they epitomize the concept of cyclic nature of change. This chapter dealing with the fate of the material in nature is based on this concept.

The energy reaches earth in the form of electromagnetic radiations of the sun, gets absorbed, transformed, temporarily stored and eventually dissipated. Thereby creating and transforming material in the process. In search of low- energy 'stable' states, the elements undergo transformations from one form to another resulting in their cycling. But all these transformations, lead to only transient periods of stability due to interactions with several other elements and their molecular combinations along with physical and biological activities. Thus, over the period of time, each element returns back to the state from where it had begun. However one must remember that the cycles of elements cannot exist aloof of the rest of the cycles as a number of molecules exist that have combinations of different elements. The structures and organisms that we see on earth are a result of how long the elements remain logged in a particular form.

Reservoirs

Various chemical forms of a particular element are slow to transform and thus constitute the pools or reservoirs (Table 1). These global reservoirs tend to be stable in the time frame of human history but may undergo shifts during geological ages. Transfer rates of elements between reservoirs are affected mainly by microorganisms because of which the macroflora and fauna are sustained. Thus disturbing microbial populations will also indirectly affect the visible forms of life.

Biogenic Elements

Those elements that are the essential building blocks of living organisms are called - biogenic elements (Table 2). With the advent of life and its subsequent evolution the role of physical and chemical processes in the transformations and cycling of elements has considerably lowered down particularly with reference to the biogenic elements. The biogenic elements meet specific atomic weight and chemical reactivity criteria and so form definite groups within the first five periods. This allowed predicting biogenic significance of nickel even before its requirement and function were experimentally established.

Table 1: Carbon reservoirs in nature

Reservoirs	Carbon (billion metric ton)*
Actively cycled	Atmosphere-CO ₂ (0.034% or 700 bmt after 1978, 560-610 bmt before 1850). Surface sea water : Dissolved inorganics : CO ₂ , H ₂ CO ₃ , HCO ₃ ⁻ and CO ₃ ²⁻ (500 bmt) Living biomass (terrestrial and aquatic) : 600-900 bmt Dead (not fossilized) organic matter : as humus and organic sediment -1500 bmt
Slowly cycled	Deep sea (34500 bmt) through vertical sea water movement
Naturally negligibly cycled	Fossil fuels (10000 bmt) – biological degradation of oil and gas seepages (negligible turn over) and carbonaceous sedimentary rocks-limestone, dolomite and sediments (10,000,000 bmt)- slow dissolution of rocks by biologically produced acids but the turn over is negligible.

(Source: Bolin et al., 1979)

Table 2: Position of biogenic elements in the periodic table

	Period Number				
	1	2	3	4	5
Atomic Number and Element	1 H	3 Li	11 Na	19 K	37 Rb
		4 Be	12 Mg	20 Ca	38 Sr
				21 Sc	39 Y
				22 Ti	40 Zr
				23 V	41 Nb
				24 Cr	42 Mo
				25 Mn	43 Tc
				26 Fe	44 Ru
				27 Co	45 Rh
				28 Ni	46 Pd
				29 Cu	47 Ag
				30 Zn	48 Cd
		5 B	13 Al	31 Ga	49 In
		6 C	14 Si	32 Ge	50 Sn
		7 N	15 P	33 As	51 Sb
		8 O	16 S	34 Se	52 Te
		9 F	17 Cl	35 Br	53 I
	2 He	10 Ne	18 Ar	36 Kr	54 Xe

Major biogenic elements in Bold letters, Minor and trace in italics. Most biogenic elements cluster in the first four periods with only 3 falling in the fifth period. No known biogenic elements are known above atomic number 53.

Based on the requirement of elements for the living organisms the intensity of cycling may be high or low, so major elements (C, H, O, N, P, S) cycle more intensely than the minor (Mg, K, Na and halogens) and trace elements (B, Co, Cr, Cu, Mo, Ni, Se, Sn, V and Zn). However

exceptions exist for example, Fe and Mn cycle extensively in an oxidoreductive manner. Ca and Si are minor components of protoplasm and form important exo- and endoskeletal structures in all organisms and are cycled at a rate of many billions of tons per year. Others that show cycling to some extent include radioactive Strontium and Cesium (bioaccumulate), mercury (methylation), tin and arsenic. Still others cycle as a result of their cross reactivity with biogenic elements under specific environmental conditions.

Transformation

Physical, chemical and biological components interact with each other. These interactions force a molecule/an element to modify its physicochemical form. This process is called transformation. However, the change is not one way, environment too pays the cost in terms of modification of temperature, osmolarity, chemical reaction (pH), conductivity etc. Growth of a plant depletes some nutrients from soil and affects change in environment. Similarly atmosphere is affected by way of gaseous exchange.

Three major forms of transformations are observed in the cycling of elements in nature:

1. Physical transformations that include fixation, dissolution, precipitation and volatilization.
2. Chemical transformations including precipitation and solubilization of minerals and other molecules. One such important reaction is the solubilization of rock phosphate under acidic conditions.
3. Biochemical transformations, where the physical and chemical changes are brought about by the living processes. For example, the processes involved in fixation and transformations during biosynthesis or biodegradation.

Besides these, some other processes such as spatial translocations of materials (from water column to sediments or from soil to atmosphere) and changes in the physical environment such as pressure due to piling may also be involved.

Journey from atmosphere to lithosphere and hydrosphere

Many gases have certain amount of solubility in water and thus may reach the terrestrial or aquatic systems through rainfall, for example, oxides of sulfur, nitrogen and carbon (particularly carbon dioxide). When dissolved in water, these forms turn into mild acids and may then be further transformed by way of oxidoreductive processes or might enter the living systems (Fig.1). Fixation is another way to reach the surface of the earth. It might be biological, physicochemical and even an anthropogenic process. It is a term that is used to depict transformations of energy or molecules that relatively slow down mobility of constituting biogenic element usually by way of change in their physical state so as to affect availability to the saprotrophs, i.e. the organisms that absorb the nutrients from the environment such as plants and microorganisms. For example, conversion of the radiant energy (solar or geothermal) to chemical energy, carbon dioxide to sugar and nitrogen to ammonia (as ammonium in cytoplasm), or nitrite, nitrate (atmospheric electric discharge). In phosphorus fixation, orthophosphates (MH_2PO_4 , M_2HPO_4 , M_3PO_4) react with Ca^{2+} , Al^{3+} and Fe^{2+} to give low solubility hydroxyphosphates. NH_3 , H_2S , methane, hydrogen and carbon mono oxide may be directly utilized by specific microorganisms. Ammonia is the substrate for nitrosifying bacteria that convert it to nitrite and then nitrifying bacteria convert nitrite to nitrate. Both utilize the processes to generate energy. In atmosphere, H_2S is rapidly oxidized by atomic oxygen (O) or ozone to SO_2 that may dissolve in water and form sulfurous acid.

Alternatively following a slower oxidation path, it forms SO_3^{2-} that on dissolution in water forms H_2SO_4 . Hydrogen is converted to water and then sugar, CO to CO_2 and then sugar and methane to methanol and then formaldehyde. Formaldehyde is further used to generate structural material and energy. All of these gases are converted to considerably stable forms.

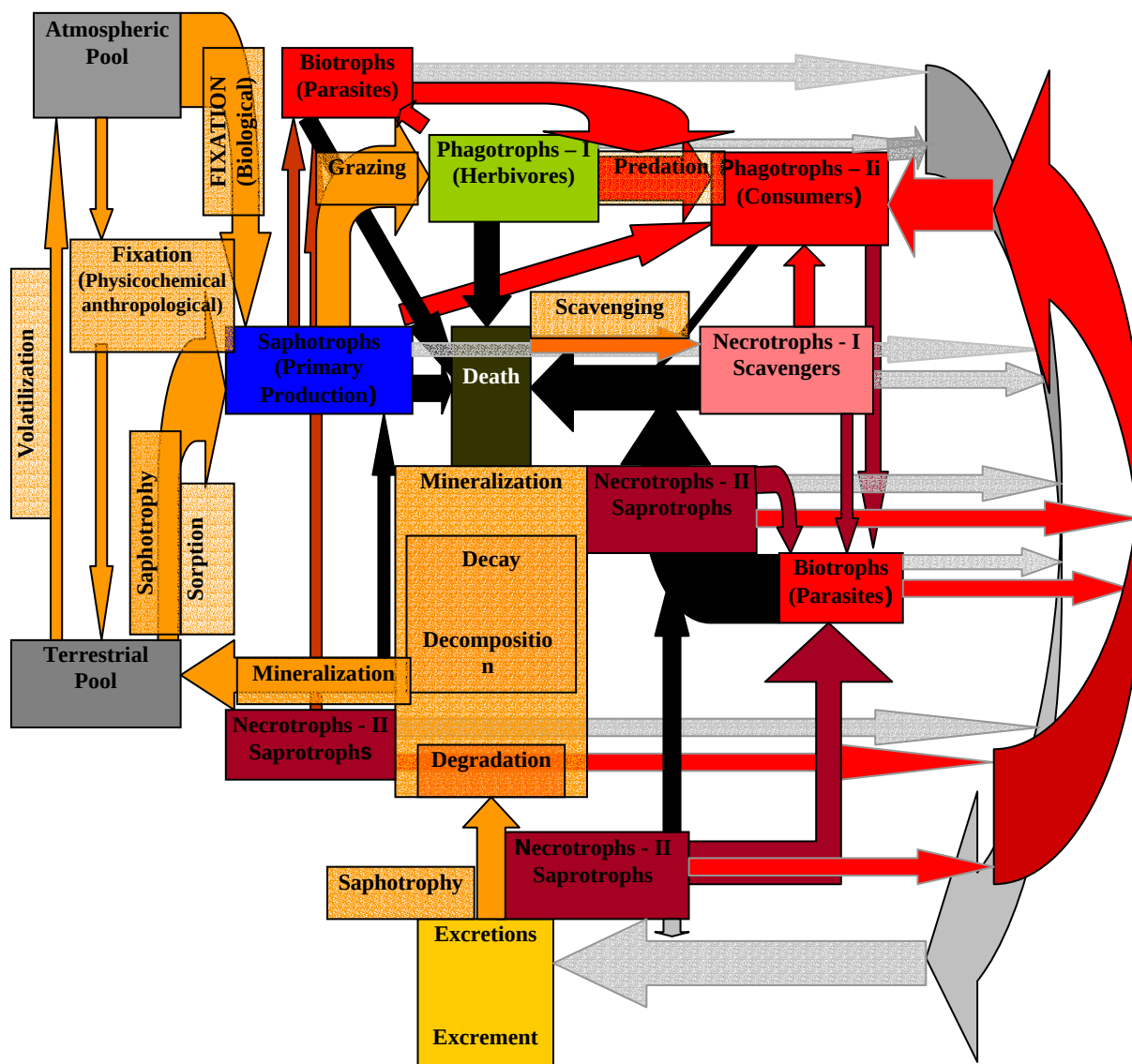


Fig. 1: Biogeochemical cycle: A general view

Light Grey arrows: Excretion and excrement, Black arrows: Death, Red arrows: Predatory paths, Brown arrows: Parasitic paths

Destination land/water

On reaching the earth, molecules add up to the total chemical reaction of soil, water or rocks. An ability to modify the pH in itself is a big change in terms of order of magnitude. A unit change in pH 7 to 6 is reduction in hydrogen ion activity from 10^7 to 10^6 , i.e. by one order of magnitude. This affects the redox state and in turn might render many biogenic elements available or unavailable for the living organisms. For example, Al and Mn become increasingly soluble with increase in acidity and become toxic to plants. Rhizobium too is quite sensitive to Al toxicity and thus is adversely affected by low soil pH. On the other hand,

pH below 5.5 and above 6.5 reduces phosphorus availability as a result of formation of Fe and Al phosphates. Increase in pH above 6.5 reduces availability of Mn also.

Transformation is also brought about by the living organisms that require the molecules to build up their structure. The molecules may either yield energy or act as building blocks for other molecules. Further they might gradually pass the journey through polymers, supramolecules, composites, organelles, tissues and organs in order of complexity of structures. (Composites are complex structures made by assembling more than one polymer together (say a bone)).

Immobilization

When an element is used by an organism for its growth then it becomes temporarily unavailable to the saprotrophs. This unavailability of an element to the saprotrophs, due to its utilization in the growth of an organism is termed immobilization in biogeochemical cycles.

From Cell to Environment

The duration of immobilization within the cell may vary. The element may be released into the environment during growth of an organism by way of metabolic processes such as respiration, excretion of wastes, liberation of 1) enzymes to transform molecules into forms that may enter the cell, 2) polymers to attach, trap moisture and nutrients and reduce toxicity of toxic molecules, 3) wastes and 4) semiochemicals i.e. molecules that communicate information viz. kairomones used to attract or repel other species, allomones- that induce a behavioural or physiological change in a member of another species so as to benefit the producer, pheromones- alarm pheromones, signaling alarm to neighbouring fellow beings, attractants as sex pheromones, repellants for competitors and allelochemicals that are essential chemical weaponry against enemies. In syntrophic and free living symbiotic associations, even essential molecules (growth factors) of life may be released to the environment to help sustenance of those friends who cannot synthesize them.

All molecules if not intercepted by the target organism(s), remain in the environment and are once again transformed into simpler molecules by degradation. Other immobilized elements might have to wait till the death of the organisms for their release. After death, the body decays, followed by decomposition of composites and then degradation of polymeric and oligomeric molecules leading to the formation of simpler inorganic molecules or functional groups - a process called mineralization. Mineralization results into forms that may either be easily sorbed by saprotrophs or if gaseous in nature, may be released into atmosphere. This process is called volatilization - returning back what was obtained from air. Other than volatilization, it may further be transformed into other comparatively stable states that may lead to mineral formation by way of physicochemical or biological processes or may get degraded incompletely thus getting logged for unusually very long periods (for example, humin from humus).

Bioaccumulation and Biomagnification

Time to secure freedom from the living system may still go longer if the organism is fed upon by another organism. Some molecules simply go on transforming from one form to other.

Others sorbed into the cells may not be used or modified further due to lack of enzymes or desired metabolic pathways and are not excreted out of it. Thus they tend to accumulate in the cells (bioaccumulation).

The uptake of nutrients in an organism may be more than its immediate need leading to their storage for emergencies (luxurious uptake). The mechanism of nutrient uptake may also sometimes mistakenly pick up and store an unwanted but analogous molecule (a pollutant) that chemically resembles an essential nutrient. If the molecule is water-soluble it may dissolve in the body fluids. Since every organism loses water to the environment, as the water is lost the pollutant would leave as well. In many cases however the pollutant is absorbed and stored in the fat bodies as is known for the pesticides- Dichloro Diphenyl trichloroethane (DDT) or Poly Chlorinated Biphenyls (PCBs). As this organism is preyed upon, the consumer may digest the fat but the pollutant accumulates in the fat bodies of the consumer. Since the consumer will feed over a number of lower trophic level organisms, the pollutant accumulates in much higher amounts than in the later. The higher the organism is placed in a food web the larger will be its accumulations.

Suppose the element we are discussing is carbon transformed into a pesticide and sprayed over the leaves of the grasses. Grasses are fed upon by the rabbits which may not die due to lesser toxicity against it and so accumulate it within their cells as it is either not degraded at all or is partially degraded. A fox may feed over a few rabbits accumulating much more amount in their body that may be toxic and lead to their death. This continuous build up of molecules from lower trophic level to the higher due to their non degradability is called biomagnification. Thus biological magnification is the tendency of non degradable molecules to become concentrated in successive trophic levels of the food web.

Influencing environment

Uptake of molecules from the environment and release of wastes as a result of the growth of the organism, manipulates the environment immediately in the vicinity. This alkalization or acidification of the environment also influences cycling of element. This may lead to authigenesis – a process in which molecules precipitate out from solutions due to change in pH. Carbonate minerals, ferromanganous concretions and desert varnish (Fig. 2) are examples of biologically driven indirect authigenesis. Sulfuric acidity in particular becomes the reason of Fe and As transformations. Similarly the minerals phosphorite and lime form from the phosphorus of the bones and the calcareous skeleton of corals respectively.

Biodegradation includes biological processes that result into degradation of complex structures of molecules to simple and/or smaller molecules. In nature, many organic nutrients exist in polymeric form. These include carbohydrates, lipids, proteins and nucleic acids. When faced with polymeric substrates, microorganisms resort to exoenzymes that are liberated out side the cell to break them down into monomers, which can then be moved inside the cell.

Some times an enzyme is released to modify a specific molecule but together with the targeted one it simultaneously modifies a non target molecule. Such an activity is known as cooxidation or cometabolic activity. This quality is harnessed by environmental microbiologists in degrading xenobiotic compounds. However, the degradation may still remain incomplete due to lack of enzymes that may degrade it further. (Xenobiotics are those man-made materials that were non existent or insufficiently available in nature and are thus

foreign to the transformational processes of nature). Hobson & Poole (1988) suggested a wider definition that is currently in use that includes all man made compounds which, although may occur naturally, are detected in parts of the environment at unnaturally high concentrations. Thus the term includes metals, phenols and herbicides along with other pollutants disturbing the natural ecosystems. However we would consider here xenobiotics in their original reference.



Fig. 2: Rock surface showing desert varnish

So what is common to all biogeochemical cycles is:

Fixation, dissolution, precipitation, oxidoreductive transformations in soil and water, formation of reservoirs or biosynthetic (immobilization) and degradative transformations (mineralization) and volatilization. However if none of the forms of an element is in gaseous state, then fixation, dissolution, precipitation and volatilization may not be part of its cycling mechanism.

Earth

Earth is the third planet from the Sun. It is the largest of the solar system's terrestrial planets and the only planetary body that modern science confirms as harboring life. The planet formed around 4.57 billion (4.57×10^9) years ago and shortly thereafter acquired its single natural satellite, the Moon.

The earth is surrounded by air containing various gases and suspended particles called atmosphere. The terrestrial portion contains huge water mass known as oceans. Besides oceans it has various other water bodies such as rivers, streams, ponds, lakes and ground water systems. All of these form what is called as hydrosphere. The rocks and the solid inner core form lithosphere.

Atmosphere

The atmosphere is a thin, gaseous layer of air that envelops the earth up to a height of more than 500 km however, 99% of its total mass is less than 80 km high. Particles of the atmosphere are found in space 1,600 km above the planet's surface.

It is generally of a uniform composition, except for a high concentration of ozone, known as the ozone layer at 19–50 km. In terms of relative volumes, the gaseous constituents of the atmosphere are nitrogen, 78.09%; oxygen, 20.95%; argon, 0.93%; carbon dioxide, 0.03%; and traces of neon, helium, methane, krypton, hydrogen, xenon and ozone. The lower atmosphere contains varying amounts of water vapor, which determine its humidity. Condensation and sublimation within the atmosphere cause clouds or fog and the resulting liquid water droplets or ice crystals may precipitate to the ground as rain, sleet, snow, hail, dew or frost. The air also carries many types of dust, of meteoric as well as terrestrial origin. It may have microorganisms, pollen, salt particles and various gaseous and solid impurities resulting from human activity (pollution). Because of the pull of gravity the density of the atmosphere and the pressure exerted by air molecules are greatest near the earth's surface (about $1 \text{ g} \cdot 10^3 \text{ cm}^{-3}$ and about $10^6 \text{ dynes} \cdot \text{cm}^{-2}$ respectively). Air pressure decreases quickly with altitude, reaching one half of its sea-level value at about 5500 m.

Layers of atmosphere

The earth's atmosphere is composed of distinct layers (Fig. 3). The troposphere extends upward from the earth to a height of about 8.1 km at the poles, to about 11.3 km in mid-latitudes and about 16.1 km at the equator. The air in the troposphere is in constant motion, with both horizontal and vertical air currents (winds). Throughout the troposphere temperature decreases with altitude at an average rate of about 2°C per 305 m, reaching about -57°C at its apex, the tropopause. Above the troposphere is an atmospheric ozone layer, which is also the lower layer of the stratosphere. Temperature changes little with altitude in the stratosphere, which extends upward to about 50 km. Above this layer is the mesosphere which extends to about 80 km above the earth; the temperature sharply decreases from around 10°C at the base of the mesosphere to -110°C before it begins to rise at the top of the mesosphere. The next layer is the thermosphere, which extends upward from the mesosphere to about 640 km; its temperature increases rapidly with altitude because of the absorption of shortwave radiation by ionization processes, although, because of the thinness of the air, little heat energy is available. The final layer is the exosphere, which gradually gets thinner as it reaches into the vacuum of space at around 700 km above the earth's surface; the atmosphere is so attenuated at this altitude that the average distance air molecules travel without colliding is equal to the radius of the earth. Although some gas molecules and particles out to about 64,400 km are trapped by the earth's gravitational and magnetic fields, the density of the atmosphere at an altitude of about 9,700 km is comparable to that of interplanetary space.

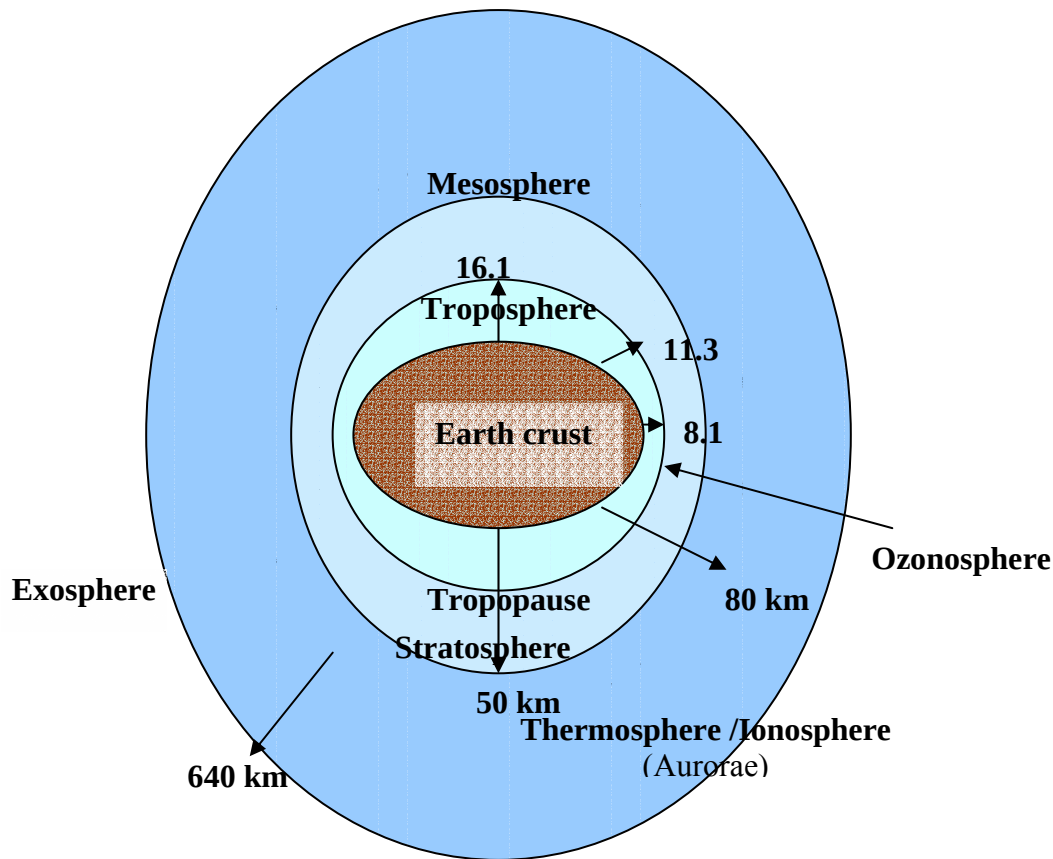


Fig. 3: Structure of Earth's atmosphere

Certain layers of the atmosphere within the main regions exhibit characteristic properties. Aurorae or northern and southern lights appear in the thermosphere. The ionosphere is in the range (80–640 km) that contains a high concentration of electrically charged particles (ions); these particles are responsible for reflecting radio signals important to telecommunications.

The lithosphere and below

The Earth is divided into several layers which have distinct chemical and seismic properties:

Layer	Depth	Features	
Crust	0- 40 km	Oceanic –basalt till Mohrovicic discontinuity; Continental crust is granitic and covered with 0 to >4 km of soil. Dominated by O, Si, Al, Fe, Mg, Na, K (98.6% w/w). Oxygen and silicon dominate	
Mohrovicic discontinuity			
Upper mantle	40- 400 km	More plastic also called asthenosphere	O, Mg and Si dominate; Lesser amounts of Fe, Al, Ca and Na are also available
Transition region	400- 650 km		
Lower mantle	650-2700 km		
Wickert-Gutenberg discontinuity	2700-2890 km		
Outer core	2890-5150 km	Molten	Rich in Fe and Ni
Inner core	5150-6378 km	Solid	

The various layers are separated by [discontinuities](#) which are evident in seismic data. At its centre earth has a solid inner core (temperature $>4300^{\circ}\text{C}$), covered by an outer molten core (temperature $>3700^{\circ}\text{C}$). The entire core is rich in Fe and Ni alloy. It is followed by Wickert-Gutenberg discontinuity and then the mantle that has dominance of O, Mg and Si with lesser amounts of Fe, Al, Ca and Na. Upper portion of mantle (temperature $>1000^{\circ}\text{C}$) is more plastic and is also called [asthenosphere](#) that in turn is surrounded by Mohorovicic discontinuity followed by the crust. The crust running is dominated by O, Si, Al, Fe, Mg, Na and K (98.6% w/w). The most abundant being oxygen followed by silicon. Crust may be divided as continental (~64%), oceanic (21%) and shelf and subcontinental (15%) crust. Under the oceans, the crust has basaltic bedrock till Mohorovicic discontinuity. The bedrock is covered with 0-4 km of sediments and sedimentary rocks. The bedrock of the continents (average 25 km) is granitic covered with 0 to >4 km of soil. In actual terms it is the crust and the asthenosphere together that are called [lithosphere](#).

Hydrosphere

Majority of the earth is covered with water giving it the typical blue colour when seen from space (Fig. 4). It is either in the form of oceans, ice caps, glaciers, flowing water (Lotic water bodies- rivers, streams), stagnant water (Lentic water bodies- ponds, lakes), ground water or vapours and clouds. Chemistry of the water (dissolved nutrients, salts, pH and toxic material) varies from place to place as also the physical and biological components of the ecosystem.



Fig. 4: View of Earth from nearby space

Biosphere

Biosphere, where life is known to exist is concerned with the upper most part of the crust and the lower most part of the atmosphere. Life in the exposed crust has been detected to a maximum of ~4 km depth (Pokrovskiy cited by Kuznetsov *et al.*, 1963 p. 26). Normal average is 10-100 m depth. The most limiting factors at 1000 m depth being temperature (-20 to $+100^{\circ}\text{C}$) and porosity of rocks that affect availability of moisture and nutrients. However

in hydrosphere, life forms a continuum at all depths upto 11 km – the depth of the Marianas trench.

For the sake of life

Life on earth is inextricably linked to climate through a variety of interacting cycles and feedback loops. The atmosphere helps maintaining relative climate stability with a self-cleansing, oxidizing environment, protects from the harmful ultraviolet rays, mediates runoff and evapotranspiration that in turn affects the quantity and quality of fresh water supplies and helps control floods and droughts and regulates nutrient cycling. It is well known that deforestation, fossil fuel burning and industrialization have directly or indirectly modified the earth's climate. Thus it is important to know how life and climate interact. The transport and transformation of a substance in the environment, through water, air, land and life is known as its **biogeochemical cycle**. One way that climate influences life is by regulating the flow of substances through these biogeochemical cycles, in part through atmospheric circulation.

The atmosphere

No life is possible in vacuum. Atmosphere is an essential component of the biosphere, allowing four of the major nutrients (C,H,N,O) to get into mobile gaseous phase. Yet it is the requirement of other essential factors that limits the growth of organisms suspended in air.

Air allows better penetrance and faster access than the other two phases or states of matter. The average time that a molecule resides in the atmosphere, before leaving the reservoir is called its lifetime. For an even mixing through out the atmosphere, gases need a lifetime that ought to be longer than their mixing time (1-2 years for the troposphere).

Living microbes have been recovered from heights 48-77 km above the earth's surface. But generally they do not grow due to lack of moisture and nutrients and continuous changes in their position in air. The later is a virtual state of non-locality despite such a huge mass of a single phase of matter. Radiations too prove lethal at higher elevations. Limited propagation though has been reported in some bacteria at high humidity and physiological temperature range. The residence time of microbes in air also varies. Those associated with solid particles in air may fallout at a rate of 10^{-3} cm sec⁻¹ if particle size is 0.5 μ m and 2cm sec⁻¹ if it is 10 μ m.

The lithosphere

Geochemical transformations

In lithosphere, rock refers to a massive, solid, inorganic matter consisting usually of two or more intergrown minerals. Igneous rocks are those that arise by cooling of magma that erupted over the surface from the interior of the earth. Fast cooling leads to rapid crystallization of minerals. The resulting rock contains tiny crystals. An example is basalt. On slow cooling, different minerals crystallize at different times depending on their melting points. In this process minerals intergrow and evolve into rocks having large visible crystals (**phenocrysts**) as may be seen in granite.

The other type of rocks are sedimentary that are formed by accumulation and compaction of sediment that mainly consists of mineral matter derived from other rock(s). It may also result

from the cementation of accumulated inorganic sediment by carbonate, silicate, aluminium oxide or ferric oxide. The cementing material may be microbiogenic. The transformations from loose sediments to rocks are called **lithification**. Examples of sedimentary rocks are limestone, sandstone and shale. Rocks may also be **metamorphic**, that is produced through alteration of igneous or sedimentary rocks by the action of heat and pressure. For example, marble (from limestone), slate (from shale), quartzite (from sandstone) and gneiss (from granite).

Minerals are inorganic compounds, usually crystalline, sometimes amorphous, of specific chemical composition and structure. They may be very simple in composition as sulphur (S^0) or quartz (SiO_2) or very complex such as the igneous mineral biotite $[K(Mg,Fe,Mn)_3AlSi_3O_{10}(OH)_2]$. **Primary or igneous minerals** crystallize during the cooling of magma as feldspars, pyroxenes and amphiboles, olivines, micas and silica. Minerals that result from chemical alteration (**weathering or diagenesis**) of primary minerals are called **secondary minerals**. Examples include clay minerals kaolinites, montmorillonites, illites; hydrated iron and aluminium oxides and carbonates. Microbes play a role in this transformation of primary to secondary minerals. Minerals that form by way of precipitation from solution are called **authigenic minerals**.

Examples of geochemical processes

Authigenesis

Silicon in nature exists as silicates (Si_2O_3) and silicon dioxide. In solution, it exists as undissociated silicic acid (H_2SiO_4) till pH 2-9. At 9 and more pH it transforms to silicate ions. Monosilicic acid presents an interesting case of self polymerization at a concentration of 2×10^{-3} M forming oligomers of polysilicic acid. This however is favoured at pH near neutral. Silica can exist in partially or fully hydrated forms (metasilicate (H_2SiO_3) orthosilicate (H_4SiO_4) respectively). Each of these can polymerize. Silica polymers may exhibit colloidal properties.

The carbonates are relatively insoluble in water. They are readily precipitated from solution at relatively low carbonate- and counter-ion concentrations (ion activity).

Weathering

Weathering that may be physical, chemical or biological, is a slow but the most important process that transforms rocks to soils. Freezing and thawing of water in the cracks (ice being more voluminous than water) and the consequent crystallization of some dissolved minerals exert pressure on the rock by way of expansion. Expansion and contraction are also resulted by heating and cooling during diurnal thermal variations. Mere alternate wetting and drying may also cause break up. Winds abrade the rocks by bombarding sand particles. Similarly flowing water and the abrasives and rolling rocks collide on the surfaces and cause weathering of the rocks. Seismic activity may result into massive rock pulverization.

Chemical processes of weathering include solvent action of water, CO_2 of volcanic origin, mineral acids such as H_2SO_3 , HNO_2 , HNO_3 , H_2CO_3 formed from gases such as SO_2 , N_2O_3 , NO_2 , CO_2 respectively. It may also be caused by redox reagents such as H_2S of volcanic origin or nitrate of atmospheric origin. Such chemical activity might have both biological and nonbiological origin.

Life and Molecular dynamics of earth

Microenvironment modification

As a result of the growth of the organism, molecules are removed from the environment and wastes are released into it, resulting in the modification of the environment immediately in the vicinity. This alkalization or acidification of the environment also influences cycling of elements that may or may not be biogenic in nature. Sulfuric acidity in particular becomes the reason of Fe and As transformations.

Weathering

Biological agencies of weathering include roots and microorganisms especially algae and fungi that penetrate cracks and fissures and force it apart. The inhabitant microorganisms are called **chasmoliths** (living in fissures/cracks) or **cryptoendoliths** (living in structural cavities of porous rocks). In limestones actual boring may be performed by **calcicolous** (lime loving) algae. Dissolution is performed by reactive metabolites such as NH_3 , HNO_3 , H_2SO_4 , CO_2 (forming H_2CO_3 in water) and organic acids (oxalic, citric, gluconic and other acids).

Elemental S, sulfides and several other inorganic S compounds can be oxidized in soil by purely chemical processes. Sulfur in the soil may originate from the weathering of soil minerals and from organically bound S. It is released into the inorganic pool in a variety of oxidation states from sulfide (oxidation state -2) to sulfate (oxidation state +6).

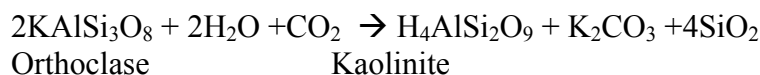
Biological diagenesis

Direct role

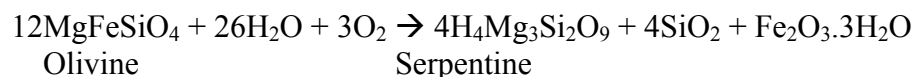
Complete oxidation of antimony sulfide ores is possible in two steps. First catalyzed by *Thiobacillus* Y or *T. ferrooxidans* converts Sb_2S_3 to Sb_2O_3 . In the second, Sb_2O_3 is converted to Sb_2O_5 by *Stibiobacter senarmontii*.

Indirect role

Waksman and Starkey (1931) suggested that orthoclase mineral is converted to kaolinite clay mineral by CO_2 produced during respiration



Similarly olivine may be transformed to serpentine by O_2 that may be a product of photosynthesis



Soils may derive from aquatic sediment or alluvium deposited by water or by progressive weathering of parent rock followed by further processing by different agencies. They undergo **eluviation** (removal of ions by leaching) and/or **alluviation** (depositions from aquatic solutions). The soils undergo further transformations through biological activity.

Authigenesis (precipitation)

Microbes may also cause authigenesis of carbonates and ferromanganese concretions. Carbonate precipitation from solutions may be driven indirectly by organisms by increasing dissolved CO_2 concentrations in soil solutions by aerobic and anaerobic oxidation of carbohydrates, organic acids and hydrocarbons (bacteria precipitate aragonite and other calcium carbonates on their surface), organic nitrogen compounds releasing both NH_3 and CO_2 (ammonia helps raising pH so as to lower down the critical concentrations of carbonates required to precipitate calcium carbonate as calcite, aragonite, vaterite) and the reduction of CaSO_4 to CaS by sulfate reducing bacteria using organic carbon as their source of reducing power. The CaS formed hydrolyzes readily to H_2S . The Ca^{2+} released reacts with CO_3^{2-} derived from the CO_2 produced in oxidation of the organic matter by sulfate reducing bacteria. They form calcite (CaCO_3) from gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and anhydrite (CaSO_4), strontianite (SrCO_3) from celestite (SrSO_4) and witherite (BaCO_3) from barite (BaSO_4). Urea hydrolysis forms ammonium carbonate and may cause precipitation of Ca^{2+} and Mg^{2+} . Removal of CO_2 from a bicarbonate containing solution increases carbonate concentration in the solution as may often happen with photosynthesis. Cyanobacterial carbonate deposition in the form of travertine, lacustrine is an example of this process. A marine pseudomonad and a marine yeast adsorb Ca^{2+} and Mg^{2+} ions on their cell surface. Respiratory CO_2 then helps precipitation of carbonates.

Changes in soil reaction by lowering down pH are the most significant mode of degradation of carbonates. Release of CO_2 during heterotrophic metabolism leads to carbonic acid formation in water, sometimes organic acids are also released corroding the carbonates. Mineral acids, HNO_3 and H_2SO_4 are generated by chemoautotrophic organisms particularly nitrifying and sulfur oxidizing bacteria that degrade limestones.

Bioconcentration

Some organisms tend to accumulate molecules, a process called **bioconcentration**. The bacterium *Proteus mirabilis* and some fungi take up little amounts of Si when furnished with silica gel, quartz or sodium silicate. Si substitutes part of P in P deficient media.

Diatoms- a group of unicellular algae are the best known silicon consumers. They remove as high as 25% of the dissolved silica from the river water. Diatoms are enclosed in a wall of silica consisting of two valves that are usually perforated plates that may have thickened ribs. The shapes may be pinnate or centric.

Diatoms take up Si in the form of orthosilicate (H_4SiO_4). The mechanism of its incorporation in walls is not known. Their walls are composed of hydrated, amorphous silica, a polymerized silicic acid. Marine diatoms may have 96.5% silica, 1.5% alumina (Al_2O_3) or Fe_2O_3 and 1.9% water. After the death of the diatom, the frustules may dehydrate to form amorphous silica, which is much less soluble in alkali and may account for the accumulation of diatomaceous earth.

On the other hand some algae accumulate arsenic 200-3000 times in excess of its concentration in the growth medium. Luxurious uptake of phosphorus by algae i.e. uptake of amounts in excess of that required for growth is another case of bioconcentration.

Carbonate deposition

Sometimes accumulation is a structural need of an organism. Much of the insoluble carbonate at the earth's surface is of biogenic origin, while some resulted through magmatic and metamorphic activities. Carbon may be fixed in the form of carbonates extracellularly (some bacteria including cyanobacteria and some fungi or intracellularly (the bacterium *Achromatium oxaliferum*), some green, brown, red and Coccolithophoride algae and foraminiferan protozoans lay it down as tests or shells. Calcium carbonate is deposited in to two crystalline forms either calcite or aragonite. A species may have one or other, but no species has both. There are more calcified species in tropical than in temperate waters.

Calcium carbonate is also incorporated into skeletal support structures of certain sponges and invertebrates such as coelenterates (corals), echinoderms, bryozoans, brachiopods, mollusks and arthropods (associated with chitinous exoskeleton). In all of these Ca and Mg are combined with carbonate ions. On the contrary mechanisms of intracellular depositions are largely unknown.

Bioconcentration of organic molecules

Lipoproteinaceous cell membrane tends to act as a solvent for hydrophobic organic molecules, wherein they get trapped with no further utilization and get immobilized in the body for long periods.

Similarly passive ports in the membrane may allow passage to many molecules as they tend to be homologous in size and/or charge of those that need to be brought in. These may be pumped back to the exterior or may get associated with some or other body structure. In the later case this also gets immobilized.

In both the cases, grazing or predation over these organisms leads to further concentration of these molecules as one grazer or predator will feed over several such preys that have concentrated these molecules. At a still higher trophic level, then there will be still more concentration. This process is called **biomagnification**. If the levels reach toxic amounts, biomagnification may harm or even lead to the death of the organisms.

Biomobilization

Microorganisms might attack the immobilized molecules in minerals such as silicate and phosphates (Ca, Al, Fe phosphates) by producing a. ligands/chelators of the cations, b. organic/inorganic acids, c. alkali (ammonia or amines) or d. extracellularly produced polysaccharides (glycocalyx). Other than this phosphorus may be solubilized by reduction of iron in ferric phosphate (strengite) to ferrous iron by dissimilatory iron reduction in sediment-water systems or through production of H₂S that may react with iron in iron phosphate to form precipitates of iron sulfide and release phosphate.

Organic acids such as 2-ketogluconic acid are liberated by *Pseudomonas* sp., *Erwinia* sp., *E. herbicola* and many others. This acid dissolves calcium/zinc/magnesium containing silica and minerals wollastonite (CaSiO₃), apophyllite (KCa₄Si₈O₂₀(F,OH).8H₂O] and olivine [(Mg,Fe)₂SiO₄]. This yields low molecular weight or colloidal polymeric silica of higher molecular weight that does not react with dilute hydrofluoric acid and an amorphous form soluble in aqueous sodium carbonate.

Bacteria- *Arthrobacter pascens*, *A. globiformis*, *A. simplex*, *Nocardia globerula*, *Pseudomonas fluorescens*, *P. putida*, *P. testosteronii*, *Thiobacillus thiooxidans*; and fungi- *Trichoderma lignorum*, *Cephalosporium atrum* and *Penicillium decumbens* generate mineral acids in their vicinity that may solubilize Li and Al. *Bacillus mucilaginosus* could solubilize Si also. The arsenic ores orpiment (As_2S_3), arsenopyrite (FeAsS) and enargite (Cu_3AsS_4) are also solubilized in this manner. *Thiobacillus ferrooxidans* leaches out 6% arsenic from carbonaceous gold ores and also accelerates leaching from arsenopyrite. Similarly antimony (Sb) minerals tetrahedrite ($4\text{Cu}_2\text{S} \cdot \text{Sb}_2\text{S}_3$) and stibnite (Sb_2S_3) are oxidized by *T. ferrooxidans* to pentavalent antimony.

Bacterial oxidation of Sb-Pb sulfides, Sb-Pb-Te or Sb-Pb-As sulfides leads to the formation of minerals as anglesite (PbSO_4) and Valentinite (Sb_2O_3). In the formation of valentinite, Sb is solubilized but not oxidized.

The Al-O and Si-O bonds are very labile under alkaline conditions. Ammonia produced by ureolytic and other microorganisms may release silicon readily from nepheline, plagioclase and quartz. Extracellular polysaccharides react with siloxanes and form organic siloxanes. This does not need any enzyme.

Mechanism for metasilicate polymer depolymerization is not known. However, *Proteus mirabilis* and *Bacillus caldolyticus* are capable of doing this.

Reductive mobilization

Some bacteria, algae (*Chlorella*) and fungi (*Aspergillus*, *Mucor*, *Fusarium*, *Paecilomyces*, *Candida humicola*, *Scopulariopsis*) are able to reduce arsenic compounds. The fungus *Scopulariopsis brevicaulis* is able to convert arsenite to trimethylarsine, wherein methionine and other methyl groups are the methyl donors. Bacteria perform it both aerobically as well as anaerobically.

The bio-transforming engine

The two basic requirements of life- energy and nutrients make the organisms act as transformers of various elements that obviously are obtained from and released to the earth.

The energy is obtained either from solar radiations ([phototrophs](#)) or through chemical reactions ([chemotrophs](#)). Phototrophic mode of energy yielding mechanism is found in higher plants, algae, cyanobacteria and purple and green bacteria and haloarchaea. While all of these utilize photosynthetic paths with minor variations (if any), haloarchaea utilize a novel path of rhodopsin based proton motive force to generate ATPs. Major variants of photosynthetic mechanisms include oxygenic (evolving oxygen) and anoxygenic (not evolving oxygen) photosynthesis. Oxygenic photosynthesis is an important phenomenon from the point of view of oxygen cycle also, as photolysis of water in this process generates oxygen. Anoxygenic photosynthesis occurs in purple and green bacteria only.

Chemotrophy may be dependent over organic substrate(s) or inorganic substrates to yield energy. Many bacteria are capable of utilizing inorganic substrates to yield energy while higher animals, most of the protozoans, fungi and many other bacteria utilize organic substrates as the source of energy as well as carbon ([Heterotrophs](#)). When an organism

utilizes inorganic source of carbon, it is called an **autotroph**. Thus most of the phototrophs and many chemotrophs are autotrophic as well. The chemotrophy is a major force in geological transformations of minerals (dealt in the section on lithosphere).

Oxidations for energy generation

Phototrophs trap energy of the photons into chemical energy and generate ATP and reducing power that in turn help synthesize organic molecules. Chemoheterotrophic energy generation depends on releasing free energy from the bonds of organic carbon substrates. Release of such bond energies almost always involves oxidation (removal of an electron(s)), however for organic molecules, oxidation frequently involves the removal of hydrogen ions (protons) in addition to electrons, since biological oxidation reactions are frequently also dehydrogenation reactions. Thus this in effect yields an equivalent of a hydrogen atom (one electron and one proton) making chemoheterotrophy an important part of carbon, hydrogen and oxygen cycle.

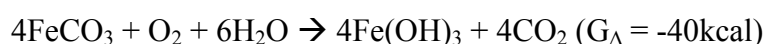
Electron acceptors in electron transport chain involve iron and iron sulfur proteins and so are part of iron and sulfur cycles.

Chemoautotrophy

Chemoautotrophy is generation of energy by chemical reactions that involves inorganic molecules. Many groups of bacteria specialize in such activities.

Iron oxidations

Thiobacillus ferrooxidans – an acidophilic Gram negative, motile rod, derives energy from oxidation of ferrous iron, reduced forms of S (H_2S , S_0 , $\text{S}_2\text{O}_3^{2-}$) and metal sulfides and carbon from carbon dioxide. Iron oxidation however yields lesser energy than from the oxidation of glucose.



There is chemiosmotic energy coupling and so ATP is generated consequent upon formation of proton motive force (pmf).

Arsenic and antimony oxidations

Pseudomonas putida, *P. arsenitoxidans* and *Alcaligenes eutrophus* oxidize arsenite viz.



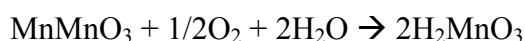
Some evidences indicate that these organisms may derive energy from this process. They pass electrons from arsenite to oxygen via an electron transport system involving cytochrome c and cytochrome oxidase. *Stibiobacter senarmontii* isolated from a Yugoslavian mine drain, oxidizes senarmontite (Sb_2O_3) or Sb_2O_4 to Sb_2O_5 in order to derive energy from the process. It possesses ribulose biphosphate carboxylase when grown on reduced antimony oxide, indicating its chemoautotrophic nature.

Manganese oxidations

Dissolved Mn^{2+} is used for energy generation by some marine Gram negative rods, *Hyphomicrobium manganoxidans* and certain *Pseudomonas* strains.

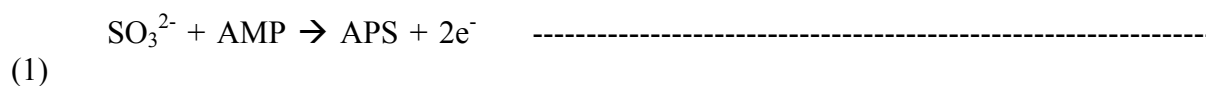


Useful energy may also be derived by attacking Mn^{2+} bound to Mn(IV) oxide, ferromanganese oxide or some clays (montmorillonite or kaolinite) enzymatically. Organisms are *Arthrobacter*, *Oceanospirillum* and *Vibrio*. Iron oxidation enzymes require very acidic pH for reaction, but Mn oxidation enzymes are not favoured at pH values much lower than neutrality.

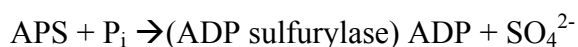


Sulfur oxidations

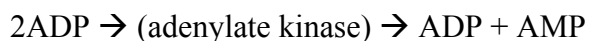
Sulfite oxidation may or may not involve substrate level phosphorylation, but in both mechanisms useful energy may be generated. In substrate level phosphorylation, sulfite oxidatively reacts with adenylic acid (AMP) to yield adenosine 5'-sulfatophosphate (APS).



The sulfate of APS is then exchanged for phosphate



ADP can then be converted to ATP:



Thus oxidation of 1mole of sulfite yields 0.5 mole of ATP. However, most energy as ATP is generated from shuttling the electrons in the equation 1 through the membrane bound electron transport system to oxygen.

Biosynthetic (anabolic) activities

Anabolic pathways usually involve an increase in atomic order and decrease in entropy. They almost always require energy (**endergonic**) and are often reductive in nature. For example, synthesis of nucleic acids and proteins.

Obtaining Nutrients- Building up trophic hierarchy

All nutrients are ultimately utilized by cells by way of sorption, but the process termed as **saphotrophy** deals with processes in which the organisms sorb nutrient directly from its physicochemical environment and thus it is different from **parasitism** (**Biotrophy**) wherein the living form is residing within a live biochemical environment, **phagotrophy** which is actually either **herbivory** (engulfing plants/ primary producers) or **predation** (feeding over either the whole organism or part of a killed animal) and **saprotrophy** (**necrotrophy**)- residing and sorbing majority of nutrients by feeding over dead organisms (by macroorganisms as insects,

mites, vultures, dogs, wolves etc) or by way of liberating extracellular enzymes (by microorganisms) to **degrade** the dead parts of organisms (**detritus**) into forms that may be sorbed by the cells (Box 1). The process of saprotrophy involves spontaneous transformation of the body by the enzymes that are lytic and continue their activity for quite some time- a process called as **decay**. This then is followed by degradation. A classic example of decay by design is that of fruits since the plant has to place the seed at its rightful place. All of these processes transform molecules to build up structure of organisms at the expense of energy.

Box 1: Decay Decomposition Degradation Deterioration

Decay

With the death of the organism some enzymes continue to act and degrade molecules. The process along with the attack by microorganisms is called decay.

Decomposition

Breakdown into components as a result of consumption of readily available sources of nutrients from the dead part(s) of the body.

Degradation

Lowering of the grade. Degradation involves breakdown of larger molecules into smaller ones.

Deterioration strictly involves loss of economic or aesthetic value of material, system or building.

Saprotrophy

Photo and chemoautotrophs are the primary producers and they obtain their nutrients from the physicochemical environment in their vicinity and are thus the true saprotrophs. Such transports across membrane are both active and passive. Many heterotrophs also utilize nutrients saprotrophically such as bacteria. But many heterotrophs are largely dependent on biotrophy or phagotrophy or necrotrophy.

Biotrophy (Parasitism)

There are some parasites that cannot live without their host called obligate parasites as chlamydia, rickettsia, viruses, virusoids and prions, others that may survive without a host are facultative parasites (many fungal pathogens of plants, bacterial pathogens of animals and opportunistic pathogens), still others require more than one host to complete their life cycle. They directly sorb the nutrients from the cell sap of the host.

Phagotrophy and saprotrophy

Those that engulf food particles have to liberate enzymes to break them down to simpler forms and then the cells of the elementary tract imbibe these simpler molecules. Similarly macroorganisms biting and chewing over the detritus depend on the enzymes of their guts to degrade it and sorb the nutrients.

It is the saprotrophy of microorganisms that leads the role of moving the element forward by way of degradative transformations. The elements logged in the polymers of the biomass will not be returned back to the atmosphere unless the saprotrophic microorganisms degrade the latter.

Biological oxidations (Catabolism/ degradations)

Catabolic processes usually liberate energy (**exergonic**) as they involve the oxidative release of chemical bond energies. They involve decrease in atomic order and increase in entropy. Mostly catabolism and anabolism share many sequences of reactions.

Biogenic elements logged in the bodies of organisms are liberated back to the atmosphere by decomposition lead by microorganisms especially bacteria and fungi. Animals are also known to degrade organic matter, but they are not known utilizing refractory materials as cellulose, lignin and chitin without the association of microorganisms. Bacteria and some yeasts and fungi can also degrade hydrocarbons (aliphatic and also aromatic) and halogenated compounds.

Carbon Cycle

Carbon dioxide (CO₂)

Carbon dioxide is the fifth most abundant gas in the atmosphere. Its volume has increased by over 25 % in the last three hundred years. This increase is primarily due to human induced burning for fossil fuels, deforestation and other forms of land-use change. This increase is believed to be causing global warming through an enhancement of the greenhouse effect. Speculative estimates claim that CO₂ levels of 0.15 atm would be sufficient to melt ice sheets covering the entire globe.

Breathing gas mixtures containing more than 0.06 atm of CO₂ for more than five to ten minutes causes respiratory muscle fatigue. Inhalation of 0.15 atm CO₂ leads to general anaesthesia and death occurs in a few hours with levels above 0.25 atm.

On earth, the carbon dioxide produced by living things is variously dissolved in the oceans (it is one of the most water soluble gases), converted by autotrophs into sugars or locked up in the form of mineral carbonates.

CO₂ is fixed photoautotrophically by green, purple and blue-green bacteria, algae and higher plants and then assimilated using Calvin-Benson cycle, except in case of green bacteria that use reductive tricarboxylic acid cycle. Chemoautotrophic fixation (source of energy chemical, carbon- inorganic carbon compounds) of carbon dioxide takes place in nitrifying bacteria, iron bacteria, hydrogen bacteria (Calvin-Benson cycle), sulfur oxidizing bacteria, methanogens (acetyl coenzyme A pathway) and acetogens (acetyl coenzyme A pathway). Many obligately autotrophic thiobacilli (sulfur oxidizers) and nitrifying bacteria possess carboxysomes that contain enzyme Ribulose Bisphosphate Carboxylase (Rubisco) that fixes carbon dioxide.

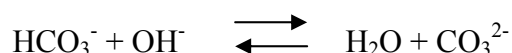
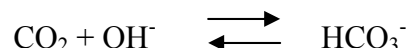
Photosynthesis is the most important means of carbon dioxide fixation. This fixation is a reductive process utilizing light energy captured by the chlorophylls and other accessory pigments as carotenoids and phycobiliproteins. The enzyme Rubisco fixes carbon dioxide to phosphoglycerate that enters the Calvin cycle through various steps and converts to glucose. The net reaction of the process is



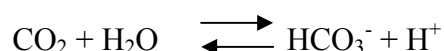
Photosynthesis in green and purple bacteria is anoxygenic and takes place under anaerobic conditions. Photosynthetic sulfur bacteria utilize H₂S/thiosulfate as the electron donor for the reduction of CO₂ transforming it to elemental sulfur. Non-sulfur photosynthetic bacteria use organic compounds as the source of electron.



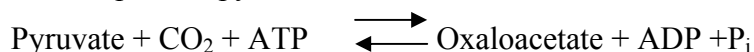
When CO₂ is dissolved in slightly alkaline water, bicarbonates (HCO₃⁻) and carbonates (H₂CO₃⁻) are formed.



In such CO₂ limited aquatic environments as oceans, bicarbonate serves as the reservoir of carbon in aquatic environments. Uptake of bicarbonate is an active transport process in phototrophic aquatic microorganisms, which utilize it by the action of carbonic anhydrase -



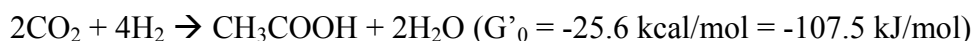
In heterotrophs, there is an exchange of CO₂ but no net fixation. The traces of carbon dioxide are fixed here through two assimilatory reactions -



The first reaction is catalysed by phosphoenolpyruvic carboxylase and the later by pyruvic carboxylase.

Acetogenesis

Some facultative chemoautotrophic anaerobes such as *Clostridium thermoaceticum*, *Acetobacterium woodii* can reduce CO₂ with H₂ to acetate.



However, the energy yield is less favorable than in methanogenesis. So enrichment on CO₂ with H₂ tends to yield methanogens and not acetogens. Still in nature, they are able to coexist as methanogens are specialists in resource utilization while acetogens being generalists are able to utilize other substrates too and are better equipped to tolerate lower pH than the methanogens.

Acetogenic bacteria form a heterogenous group in eubacteria while methanogens fall in a homogeneous group of archaea. They may even cooperate syntrophically in nature. Acetogens provide acetate for methanogens and the later utilize and prevent accumulation of acetate to toxic levels. In addition to acetogenesis from CO₂ with H₂ these microbes are able to ferment CO, formate and methanol to acetate.

Carbon monoxide (CO)

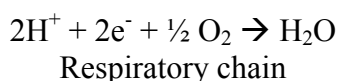
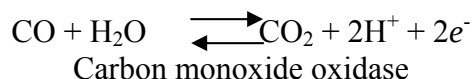
The most obvious source for CO is the incomplete combustion of organic material e.g. cigarette smoke (1%), automotive exhausts (10% CO by volume), 'coal gas' and house fires (up to 20% CO). Physiologically CO is produced by the degradation of the porphyrin ring of haemoglobin, mediated by the enzyme haem oxidase. It may also be produced by the hepatic metabolism of dichloromethane (methylene chloride, CH₂Cl₂) or the decomposition of phosgene (COCl₂) in water (e.g. the moist air in one's lung). Carbon monoxide has also been found to be a neurotransmitter substance. This may explain some of the neuropsychiatric sequelae seen in some survivors of carbon monoxide poisoning.

CO has great affinity for Fe²⁺ and binds avidly to haemoglobin (240x better than O₂) rendering it useless for oxygen carriage. On inspiration of 0.01% CO, 14% Carboxyhaemoglobin (COHb) is formed while 0.1% inspiration yields 60% COHb leading to profound reduction in the oxygen carrying capacity of blood. Firefighters not using facemasks and engaged in strenuous activity can have COHb levels of 75% in less than a minute.

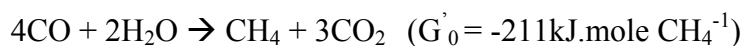
The heart and the brain, being the most metabolically active organs, are first to suffer the effects of hypoxia. Initial headache, drowsiness and confusion eventually lead to coma. Decreased oxygen supply and increased work commonly infarct heart muscles.

An obscure photochemical side reaction in photosynthetic organisms also produces CO as part of photooxidation of cellular organic carbon. Such production is proportional to light intensity but is independent of CO₂ concentration and photosynthesis rate.

Carboxydobacteria like *Azotobacter*, *Alcaligenes*, *Pseudomonas* convert carbon monoxide to carbon dioxide using a soluble carbon monoxide oxidoreductase catalyzing the reaction



All carbon monoxide oxidizing bacteria are hydrogen oxidizers too but all hydrogen oxidizers are not carbon monoxide oxidizers. All methanogenic archaea can also utilize CO however, *Methanobacterium thermoautotrophicum* grows slowly over it.



G'₀ is Gibb's Free energy, negative sign means the reaction is exothermic.

Methane

Methane is emitted from a variety of both human-related (anthropogenic) and natural sources. Human-related activities include fossil fuel production, animal husbandry (enteric fermentation in livestock and manure production), rice cultivation, biomass burning and waste management. These activities release significant quantities of methane to the atmosphere. Western policy makers claim agriculture in terms of paddy cultivation and cattle husbandry as the core culprit for rise of methane in atmosphere. More than 60 % of all rice

paddies are found in India and China. Grazing animals (ruminants) release methane to the environment as a result of herbaceous digestion. Some researchers believe the addition of methane from this source has more than quadrupled over the last century. Natural sources of methane include wetlands, gas hydrates, permafrost, termites, oceans, freshwater bodies, non-wetland soils and other sources such as wildfires.

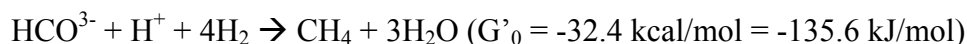
The atmosphere and more precisely the troposphere, is the largest sink for methane. Methane in the troposphere reacts with hydroxyl (OH) radicals, forming mainly water and carbon dioxide. In total this reaction accounts for about 500 million tonnes of methane each year. An indirect effect of atmospheric methane oxidation is that it can magnify the effects of other pollutants. Increased methane in the atmosphere means fewer OH radicals and so less oxidizing power in the atmosphere as a whole. Some methane, about 40 million tonnes a year, is oxidized in the same way, but in the stratosphere.

Methane is a potent greenhouse gas, roughly 20 times as effective as CO₂ w/w. Since 1750, methane concentrations in the atmosphere have increased by more than 150 %.

Methanogenesis

Some archaea are strictly anaerobic, active at -350 to -450mV redox potential, use CO₂ as electron acceptor and reduce it to methane using H₂ produced during fermentation. These are methanogenic archaeobacteria. Since they use CO₂ as the sole source of carbon therefore called chemolithoautotrophs.

If CO₂ is available as carbonate then:



Some forms may generate methane from carbon monoxide, acetic acid, formic acid, methylamine or methanol. Those utilizing the last three molecules are called as [methylotrophic methanogens](#).

CO₂ conversion to cell material does not follow usual ribulose phosphate path common to chemolithotrophs, instead acetyl CoA synthase pathway is used. This path involves several unusual coenzymes.



Coenzymes involved are: tetrahydromethanopterin, 2-methylthioethanesulfonic acid and 2-mercaptoethanesulfonic acid. Methyl joins the carbonyl group of acetate that is derived from the reduction of another CO₂ to CO by carbon monoxide dehydrogenase. The same enzyme catalyses synthesis of the methyl group, CO and HS-CoA to acetyl CoA and therefore is called acetyl CoA synthase. Pyruvate and other trioses are formed from acetate using reverse TCA cycle, which may later be converted to hexoses and other anabolic products.

Methanotrophs

Methanotrophs are organisms that can use methane for energy and carbon. Many methanotrophs are actually methylotrophs, i.e. they can use C₁ compounds such as methane, methanol, methylamine or formate. Other than bacteria (*Hyphomicrobium*, *Paracoccus*

denitrificans, many *Pseudomonas* and *Xanthobacter*, Obligate methylotrophs - *Methylobacterium*, *Methylococcus*, *Methylomonas*, *Methylophilus*, and *Methylosinus*), several species of yeasts (*Candida*, *Hansenula*, *Pichia* and *Torulopsis*) and molds are methylotrophic. Based on the mode of assimilation of formaldehyde, methylotrophic bacteria are classified into two groups. Type I bacteria use the ribulose monophosphate pathway:



Type II bacteria use serine pathway:



Carbon in cells

Nearly all of the solid matter in a cell is organic in nature. The building blocks are simple in structure that join together to form four major classes of macromolecules viz. proteins, nucleic acids, polysaccharides and lipids. These basic biomolecules come together in definite patterns and develop into organelles, tissues, organs and then the body as a whole. On death, they undergo the process of decomposition. Under aerobic conditions both micro and macro-organisms share the capacity to degrade simple organic components and some biopolymers as starch, pectin, proteins and lipids. however the property of carrying out fermentative decomposition in absence of oxygen lies exclusively in the microbes.

The recurring structural units in the nucleic acids are the nucleotides that join together in a phosphodiester linkage to form a chain of nucleic acid. The ribonucleic acid is single stranded while two strands of nucleic acids come together to form a helical structure of the DNA.

Proteins are composed of amino acids in amide linkages and constitute a major part of the cell. They are the direct products and effectors of gene action. Some proteins have specific catalytic activity and function as enzymes. Others serve as structural elements and some are involved in transportations of various substances across membranes. Synthesis of amino acids and then proteins is a major anabolic process, besides within the cell, proteins are degraded through catabolic activities.

Lipids are composed of fatty acids and serve as high energy rich fuel. Many lipids also contain alcohol and some have phosphoric acid. They form an important structural component of the cell membranes.

The living forms build up the structure and grow. They also store energy for contingencies. Both activities involve either structural or storage polymers. Polysaccharides composed of sugars are the common structural and storage polymers. Some like starch, glycogen and inulin serve as energy storage compounds and are easy to consume. Others like cellulose, hemicellulose, lignin (in plants), pectin (fruit skins), chitin (leaf cuticle, fungi, arthropod and mollusks), keratin (hair, nails) and murein (bacterial cell wall) form the structure. Murein or peptidoglycan is part protein and part carbohydrate. These molecules are degraded in soil (Table 3) and water after the death of the organism.

The net primary production of biomass was estimated to be 60 Gt yr⁻¹ of carbon in terrestrial and 53 Gt yr⁻¹ in marine ecosystems (1 Gt = 10¹² kg) (Cox *et al.*, 2000). About half (~45%)

dry weight) of the carbonaceous compounds in terrestrial biomass is composed of cellulose, making it the most prominent single organic compound on earth. Cellulose and hemicellulose are macromolecules from different sugars; whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors. The composition and percentages of these polymers vary from one plant species to another. Moreover, the composition within a single plant varies with age, stage of growth, and other conditions. Long cells are enveloped by a characteristic cellular wall form wood. This wall is a complex structure that acts at the same time as plant skin and backbone.

Cellulose, a linear polymer is composed of D-glucose subunits linked by β -1,4 glycosidic bonds forming cellobiose molecules. It has long chains (called **elemental fibrils**) linked together by hydrogen bonds and van der Waals forces forming microfibrils. Hemicellulose and lignin cover these microfibrils. Microfibrils group together to constitute the fiber. Cellulose can appear in crystalline form, called **crystalline cellulose**. Additionally a small percentage of non-organized cellulose chains form **amorphous cellulose**. The later form is more susceptible to enzymatic degradation.

Table 3: Composition of soil organic matter

S. No.	Organic Carbon	Plant residue	Nitrogenous substances
1	Aromatic C-50%	Cellulose (50%)	Protein and peptide N (+ a very small amount of free amino acid N) –30-40%
2	N- associated C –(20%)	Hemicellulose (20%)	Labile N forms hydrolyzed to ammonia-includes exchangeable N (25-30%)
3	Carbohydrate (15%)	Lignin (15%)	Hydrolysable, unknown N forms – 15-20%
4	Fatty acid and alkane carbon (15%)	Protein 5%	Acid insoluble N- largely associated with aromatics- 10-15%
5		Amino acids and sugars 5%	Amino sugar N (5-10%)
6		Pectin 1%,	Nucleic acid N (~ 1%)
7		Waxes and pigments –1%	

(Source: Killham, 1994)

Cellulose appears in nature associated with other plant substances and this association may affect its biodegradation. Hemicellulose is a complex carbohydrate polymer and makes up 25–30% of total wood dry weight. It is a polysaccharide with a lower molecular weight than cellulose. It consists of D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonic and D-glucuronic acids. Sugars are linked together by β -1,4- and occasionally β -1,3-glycosidic bonds.

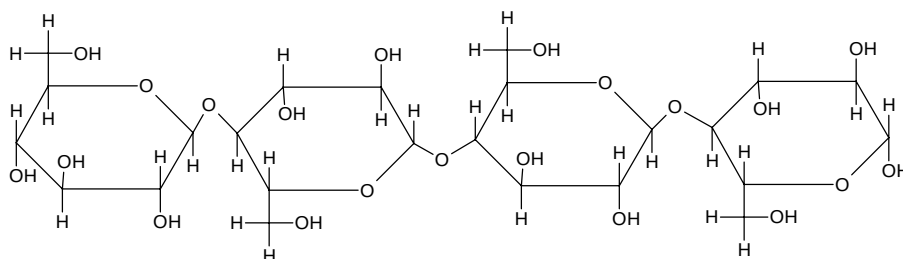
Lignin is another important polymer produced by the plants. It is the main constituent of woody plants where it makes up about 20-30% of the weight of the wood. It imparts strength and rigidity to the wood.

Since polymers are insoluble and can not cross the cell envelope, microbial degradation has to occur either in association with the outer cell envelope layer or extracellularly. Microorganisms have two types of extracellular enzymatic systems: the hydrolytic system, which produces hydrolases and is responsible for cellulose and hemicellulose degradation and a unique oxidative and extracellular ligninolytic system, which depolymerizes lignin.

Cellulose

Most of the cellulolytic microorganisms belong to eubacteria and fungi, even though some anaerobic protozoa and slime molds able to degrade cellulose have also been described. Cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions.

Variety of organisms, for example, aerobic bacteria- *Cellulomonas*, *Pseudomonas* and *Streptomyces*, anaerobic bacteria- *Clostridium thermocellum*, mesophilic fungi-*Trichoderma reesei*, *Phanerochaete chrysosporium* have been isolated from common environments including soil, compost, sewage and sludge. Other well-known anaerobic cellulolytic microorganisms are rumen bacteria, fungi and protozoa, which degrade vast amounts of cellulose.



Structure of cellulose

Microorganisms capable of degrading cellulose produce a group of enzymes with different specificities (Table 4). Cellulases hydrolyze the β -1,4-glycosidic linkages of cellulose. They are divided into two classes namely endoglucanases and cellobiohydrolases. Endoglucanases (endo-1,4- β -glucanases) hydrolyze internal bonds releasing new terminal ends. Cellobiohydrolases (exo-1,4- β -glucanases) act on the existing or endoglucanase-generated chain ends. Both enzymes can degrade amorphous cellulose but usually cellobiohydrolases are the only enzymes that efficiently degrade crystalline cellulose. Cellobiohydrolases and endoglucanases release cellobiose molecules. An effective hydrolysis of cellulose also requires β -glucosidases that break down cellobiose and release two glucose molecules. Products of cellulose hydrolysis are available as carbon and energy sources for cellulolytic microorganisms or other microbes living in the environment where cellulose is being degraded leading to build up of interactive and interdependent communities in such environments.

Table 4: Some cellulase systems and their optimum requirements

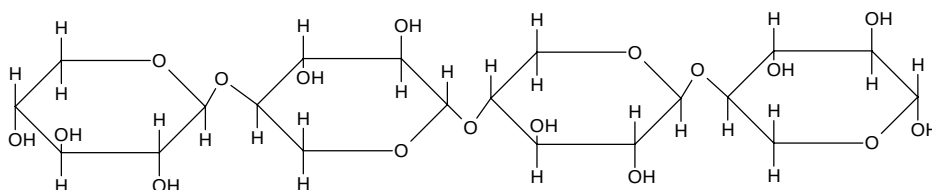
S. No.	Organism	Enzyme	Mol weight (kDa)	Temperature range	pH optima
1	Mesophilic fungi <i>Trichoderma reesei</i> <i>Phanerochaete chrysosporium</i>	Endoglucanase	25-50	Up to 45°C	Acidic
		β -glucosidases	165-182		Acidic
2	Thermophilic fungi	Endoglucanase	30-100	55-85°C	5.0-5.5
		Exoglucanases	40-70	50-75 °C	
		β -glucosidases	45-250	35-71 °C	4.1-8.1

The cellulosome

Many cellulolytic microorganisms produce intricate multi-enzyme complexes called **cellulosomes** that efficiently degrade cellulose. It is a conglomerate of subunits, each comprising a set of interacting functional modules. A multi-functional integrating subunit (called **scaffoldin**) is responsible for organizing the cellulolytic subunits into the multi-enzyme complex. This is accomplished by the interaction of two complementary classes of domain, located on the two separate types of interacting subunits, i.e., a **cohesin** domain on scaffoldin and a **dockerin** domain on each enzymatic subunit. The high-affinity cohesin-dockerin interaction defines the cellulosome structure. The scaffoldin subunit also bears a **cellulose-binding domain** that mediates attachment of the cellulosome to its substrate.

Hemicellulose

Hemicellulases are frequently classified according to their action on distinct substrates. Xylan is the main carbohydrate found in hemicellulose. Its complete degradation requires the cooperative action of a variety of hydrolytic enzymes. Endo-1,4- β -xylanase generates oligosaccharides from the cleavage of xylan and xylan 1,4- β -xylosidase works on xylan oligosaccharides, producing xylose. Additionally degradation requires accessory enzymes such as xylan esterases, ferulic and *p*-coumaric esterases, α -1-arabinofuranosidases and α -4-O-methyl glucuronosidases acting synergistically to efficiently hydrolyze wood xylans and mannans.

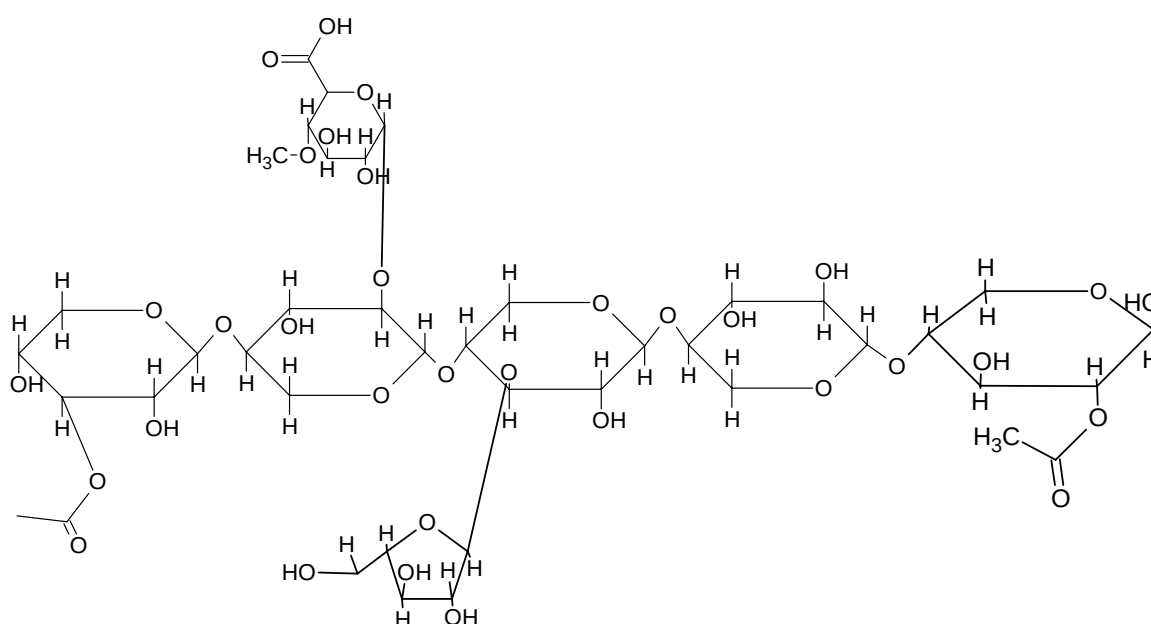


A β -1, 4- linked neutral xylan type hemicellulose

Glucuronoxylans are the principal component of hardwood hemicellulose, whereas glucomannan is predominant in softwood. The main difference with cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. In contrast

to cellulose, it is an easily hydrolyzable polymer. It does not form aggregates, even when co-crystallized with cellulose chains.

One of the most common hemicellulose is the O-acetyl-4-O-methylglucuronoxylan. It needs four different enzymes for degradation: endo-1,4- β -xylanase (endoxylanase), acetyl esterase, α -glucuronidase and β -xylosidase. The degradation of O-acetyl- α -lactoglucomannan starts with rupture of the polymer by endomannanases. Acetylglucomannan esterases remove acetyl groups and α -galactosidases eliminate galactose residues. Finally, the endomannanase-generated oligomers are broken down at β -1,4 bonds by β -mannosidase and β -glycosidase. Xylanases are hydrolytic enzymes that randomly cleave the β -1,4 backbone of the complex plant cell wall polysaccharide xylan.



Structure of a branched hemicellulose molecule

Bacterial xylanases have been described in several aerobic species and some ruminal genera. Xylanases from mesophiles act between 40-60°C. Thermostable xylanases are produced by actinobacteria (formerly actinomycetes)- *Thermomonospora* and *Actinomadura*. A highly thermostable xylanase has been obtained from a hyperthermophilic archaeobacterium *Thermotoga*. Xylanases of thermophilic fungi working at 60 to 80°C are also receiving considerable attention exhibiting a multiplicity of xylanases differing in stability, catalytic efficiency and activity on substrates.

β -xylosidases are less common than endoxylanases. Most of them are cell-bound and larger than xylanases and have been described in several fungi including *T. reesei* and *P. chrysosporium*. Their molecular masses range from 90 to 122 kDa and most have acidic pH optima. β -xylosidases are reported in *B. stearothermophilus* and the rumen bacterium *Butyrivibrio fibrisolvens*.

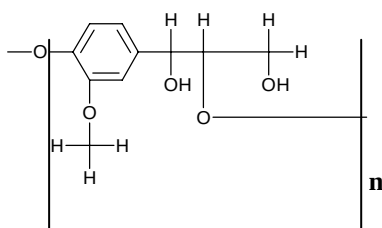
Glucomannan-degrading enzymes have been described less frequently than xylanases. Several mannanases are known from Gram-positive and Gram-negative bacteria. Endomannanases are known in white-rot fungi and ascomycetes, α -galactosidases and

acetylglucomanan esterases in *Aspergillus* and β -mannonidases in *Polyporus sulfureus* and *A. niger*.

Biobleaching and biopulping require xylanases to act at alkaline pH optima. Most xylanases from fungi have pH optima between 4.5 and 5.5. Xylanases from actinobacteria are active at pH 6.0–7.0. However, xylanases active at alkaline pH have been described from *Bacillus* sp. and *Streptomyces viridosporus*.

Lignin

Structurally lignin is an amorphous, heterogeneous, complex and 3-dimensional polymer, which is highly insoluble in water and most other organic solvents. However it is slightly soluble in organic hydrophilic solvents like dioxane. Formed of phenylpropanoid units arranged irregularly, lignin has an average molecular weight of 1-10 kD.

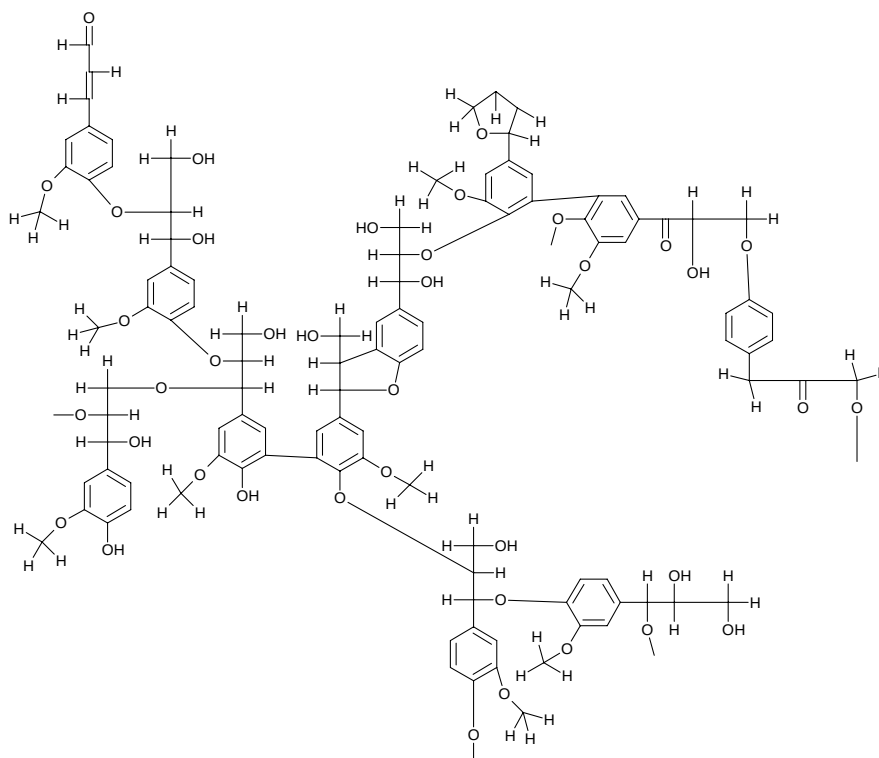


Phenyl propane subunit of lignin links by C—C and C—O—C bonds to a complex subunit that is relatively resistant to biodegradation

Special bonds link lignin to hemicelluloses. Lignin is more hydrophobic than the hemicelluloses and cellulose. Its structure, along with that of the hemicelluloses, contributes to the strength of the plant cell wall. The resistance of lignin and the crystal-like structure of cellulose fibrils are the two major barriers to the degradation of lignocelluloses. The main features that impart it recalcitrance are 1) lack of repetitive structure, 2) hydrophobic nature and 3) release of toxic products (radicals, phenolics) on degradation. Thus it acts as an ideal protective material that also imparts strength.

Lignin degradation is an aerobic process. Since bogs, marshlands and waterlogged areas lack oxygen, they tend to accumulate lignin. Over thousands of years, this lignin gets converted to peat. The white rot basidiomycetes are the most important lignin degraders as they can mineralize it to carbon dioxide and water. *Ganoderma applanatum*, *Marasmius scorodonius*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Polyporus abietinus*, *P. adustus*, *P. versicolor*, *Polystictus abietinus*, *P. hirsutus*, *Stereum hirsutum*, *S. rugosum* and *Trametes pini* are some examples of ligninolytic fungi. Many filamentous bacteria (actinobacteria) also degrade lignin however none is known to mineralize it.

The primary precursors (units) forming lignin are the monolignols, which are aryl propanols made up of *p*-coumaryl, coniferyl and sinapyl alcohols. The dimers formed by their coupling reaction are called lignans. The lignols are structurally close to the amino acids phenylalanine and tyrosine from which they are ultimately derived.



Complex three dimensional subunit of lignin

During lignification, phenolic monomer radicals couple to other monomeric radicals or cross couple with growing lignin polymer/oligomer to build up a phenylpropanoid polymer. This process produces a polymer with no extended sequences of regularly repeating units, its composition is generally characterized by the relative abundance of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units (derived from each of the 3 primary monolignols) and by the distribution of inter unit linkages in the polymer (e.g. aryl ether or phenylcoumaran or resinol or biphenyl or diphenyl ether). Although lignin structure includes bonds between aryl propane units of great variety, there are large numbers of carbon-carbon bonds and ether linkages, neither of which are easily susceptible to hydrolytic cleavage mechanisms.

White-rot fungi involve three kinds of phenol oxidases to degrade lignin. Lignin peroxidases and manganese peroxidases play a central role in carrying out H_2O_2 based oxidations of lignin and lignin like compounds. These enzymes utilize low-molecular-weight mediators to carry out lignin degradation. Some fungi produce all of the major enzymes, others only two of them and some even one. Reductive enzymes viz. cellobiose oxidizing enzymes, aryl alcohol oxidases and aryl alcohol dehydrogenases additionally assist ligninolysis in a major way.

The heme peroxidases carry out one electron transfers and attack $\text{C } \alpha$ -, $\text{C } \beta$ - cleavage by the hydroxylation of benzylic methylene groups, oxidize benzyl alcohols to the corresponding aldehydes or ketones, phenol, propyl side chains and even aromatic rings of nonphenolic molecules. Laccase - a copper phenol oxidase, catalyses reduction of O_2 to H_2O during its oxidation of phenolics, aromatic amines, ascorbate and metal cyanides in the presence of mediators. The phenolic nucleus is oxidized by removal of one electron, generating phenoxy-free-radical products that may lead to polymer cleavage. Glyoxal oxidases act on intermediary compounds as simple aldehydes-, α -hydroxycarbonyls, and α -dicarbonyls.

Cellulose oxidase and cellobiose: quinone oxidoreductase may also be involved in cellulose as well as lignin degradation. *Bjerkandera* sp. has been found to generate peroxide by way of an aryl alcohol oxidase.

In most fungi, Lignin peroxidase is present as a series of isoenzymes encoded by different genes. It is a glycoprotein with a heme group in its active center having 38 to 43 kDa molecular mass. So far, it is the most effective peroxidase and can oxidize phenolic and non-phenolic compounds, amines, aromatic ethers and polycyclic aromatics with appropriate ionization potential. Since lignin peroxidase is too large to enter the plant cell, its degradation process is carried out only in exposed regions of lumen. This kind of degradation is found in simultaneous wood decays. Manganese peroxidases are molecularly very similar to lignin peroxidases and are also glycosylated proteins, but have slightly higher molecular mass (45 to 60 kDa).

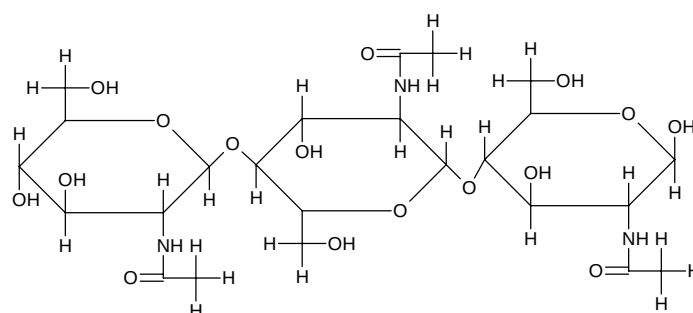
Manganese peroxidases oxidize Mn^{2+} to Mn^{3+} . They have a conventional peroxidase catalytic cycle, but with Mn^{2+} as substrate. This Mn^{2+} must be chelated by organic acid chelators, which stabilize the product Mn^{3+} . Later being a strong oxidant, can leave the active center and oxidize phenolic compounds, but cannot attack non-phenolic units of lignin. Manganese peroxidase generates phenoxy-radicals, which in turn undergo a variety of reactions, resulting in depolymerization. In addition, it oxidizes non-phenolic lignin model compounds in the presence of Mn^{2+} via peroxidation of unsaturated lipids. A novel versatile peroxidase, which has both manganese peroxidase and lignin peroxidase activities and which is involved in the natural degradation of lignin can oxidize hydroquinone in the absence of exogenous H_2O_2 , when Mn^{2+} is present in the reaction. It has been suggested that chemical oxidation of hydroquinones promoted by Mn^{2+} could be important during the initial steps of wood biodegradation because ligninolytic enzymes are too large to penetrate into non-modified wood cell walls.

The most promising applications of white-rot fungi or their ligninolytic enzymes include biopulping, bleaching of chemical pulps and *in situ* bioremediation of contaminated soils. They may also be employed to degrade toxic xenobiotics including polycyclic aromatic hydrocarbons, chlorophenols, nitrotoluenes, dyes and polychlorines and biphenyls. They might also be used in the remediation of dye industry, bleach plant and oil mill wastewater. Another alternate use is to convert lignocellulosic biomass into ethanol. This process is completed in two steps: 1) hydrolysis of the polymer, delignification to liberate cellulose and hemicellulose from their complex with lignin and depolymerization of carbohydrate polymer to produce free sugars; and 2) fermentation to ethanol using pentoses and hexoses liberated in the first step.

Chitin

Chitin is yet another of the most abundant biopolymers in nature and perhaps the most abundant in the marine environment. Billions of tons of chitin are produced annually by the invertebrates living in the water and sediments of the oceans and estuaries of the world. It consists of β -1,4-linked *N*-acetylglucosamine residues that are arranged in antiparallel (α), parallel (β) or mixed (γ) strands with the α configuration being the most abundant. The degree of deacetylation varies from 0 to 100% (chitosan). Except for the β -chitin of diatoms (chitan), chitin is always found cross-linked to other structural components such as proteins and glucans.

Bacteria produce more than one type of chitinases. This diversity may be explained in view of the vast variety of possible chitin structures. *Bacillus circulans* secretes six major chitinases differing in enzyme activity, *Serratia marcescens* secretes five and three are known from *Streptomyces lividans*. Chitinase diversity is a result of presence of separate genes (*Streptomyces lividans*) or the posttranslational proteolytic activity (*Alteromonas* sp. and *Streptomyces olivaceoviridis*). Presumably, a bacterium produces different chitinases to efficiently hydrolyze the different forms of chitin found in nature, although this has not been examined. Perhaps because it is more difficult for chitinases to gain access and hydrolyze the tightly packed, antiparallel strands of α -chitin. This may also explain why most organisms have α - rather than β -chitin in exoskeletons or cell walls. The hydrolysis of these two forms of chitin by bacteria has not been examined.



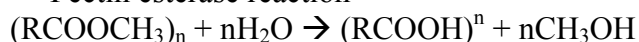
Structure of chitin

Pectin

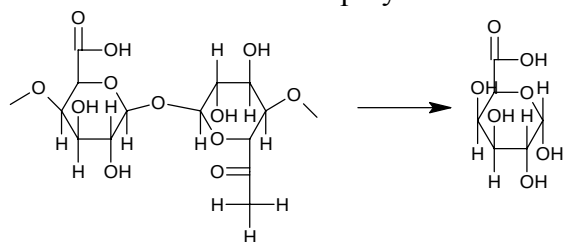
Pectin a polymer of galacturonic acid, is a type of hemicellulose. Pectic substances consist of pectin and pectic acid. Pectin is partially methyl-esterified -1,4-D-galacturonan. Demethylated pectin is known as pectic acid (or polygalacturonic acid). Pectic substances are commonly amorphous, with chains being 200 to 400 C long. Either non-sugar (acetyl) or sugar (D-galactose, D-xylose, L-arabinose & L-rhamnose) substituents can be found at the C-2 or C-3 positions of the main chain. The degree and type of branching depends on the source of pectic substance. Pectolytic enzymes are produced by bacteria, fungi, yeasts, insects, nematodes as well as protozoans. Microbial attack is important in plant pathogenesis, symbiosis and decomposition.

Pectin is degraded by extracellular de-esterifying enzymes called pectin methyl esterases (PME) and the depolymerizing (chain splitting) enzymes like hydrolases and lyases ($\square$ -eliminases). PME converts pectin specifically to pectic acid and methanol. Activity is optimal when degree of polymerization is more than 10 and decreases with increase in esterification and decrease in chain length. It can be competitively inhibited by pectate (the end product).

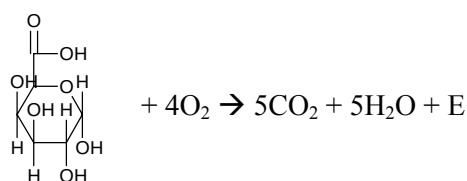
I. Pectin esterase reaction



II. Exo- and endo- depolymerises



III. Galacturonate oxidase



Hydrolases like endopolygalacturonase cleave randomly along the chain and release oligosaccharides while exopolygalacturonases cleave galacturonic acid one at a time from the non-reducing end of poly or oligogalacturonans.

Endopectate lyase introduces a unique double bond between C-4 and C-5 carbon of the sugar situated at the non-reducing end of the susceptible bond while exopectate lyase removes one monomer at a time from the non-reducing end of the polymer chain. Methanol, D-galacturonic acid, 4,5-dehydrogalacturonic acid, oligogalacturonate, 4,5-dehydrooligogalacturonic acid and methyl esters of the oligomers are some of these products formed by the action of these enzymes. Monomers released enter the cell metabolism either by diffusion or via the active mechanisms. Galacturonic acid and 4,5-dehydrogalacturonic acid are utilized for metabolism through glycolysis.

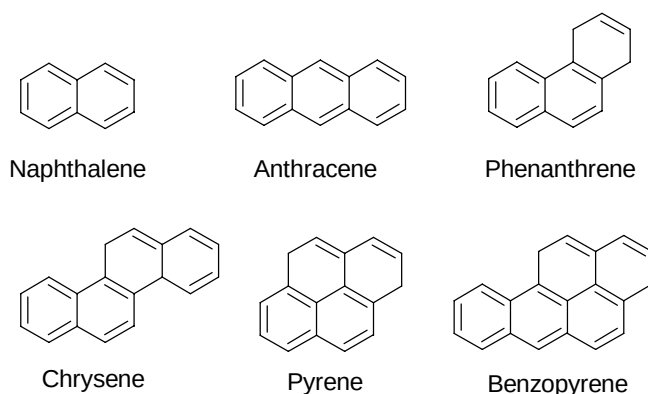
Recalcitrant molecules

Compounds that resist degradation remain in the nature for very long periods are called **recalcitrant molecules**. Natural recalcitrant molecules include peat, humus, hydrocarbons and man-made (anthropogenic also called xenobiotic) molecules include petroleum products, pesticides, detergents, solvents, fire retardants, plastics, nitrogenous compounds and transformation products such as trichloroethylene.

Most of the degradable organic compounds have hydroxyl and carboxyl groups, which are readily oxidized. The degradability of molecules however is affected adversely by lack of oxygen in molecule (hydrocarbons), saturation, chain length, polymerization, hydrophobicity, ring structure and number of rings. Branching, presence of non alkyl groups (halogens, nitro, amino, sulfonates) and the position of these substitutions, volatilization, solubility and cross-linking with other molecules also tend to affect degradability.

Unsaturated aliphatic and aromatic molecules are attacked more readily than the saturated ones. Similarly short chain aliphatic hydrocarbons are degraded faster than the long chained ones. Cross-linking with other molecules leads to enzyme inaccessibility.

Extreme environment in terms of temperature, water availability, pH and salt concentration show limited diversity of organisms and thereby affect the degradability. An organism is incapable of degradation as a result of nonexistence of required enzymes, transport of substance into the cell and lack of required nutrients. Another important concern in degradation is bioavailability that is whether the substrate is available to the organism for degradation. Availability might be dependent on the solubility of the substrate or on the capability of the organism to utilize a sorbed substrate. Some other factors that affect the accessibility of the substrate for degradation include number of organisms, binding to the clays (that affects accessibility of the molecule –imagine, 1g clay has 600 m² surface area), enzyme inactivation by the clay/humus, coating or encrustation with non degradable organic molecules or insoluble carbonates or inorganic oxides or movement of substance into zones of limited biological activity (ground water or anaerobic sediments).



Polynuclear aromatic hydrocarbons (PAHs)

Xenobiotics

Man has synthesized a number of novel chemicals and material that are either very rare to exist in nature or have not been reported existing till date. If they have any natural counterpart in existence then it may be degraded, but for others it is obvious that pathways for degradation may not have evolved, as a result they are either left non-degraded or partially degraded and persist in nature (i.e., show recalcitrance). Being ‘foreign’ to the nature they are called **xenobiotic** (*Xenos* (Greek)= foreign). Most of the xenobiotics are highly substituted high molecular weight aliphatic or aromatic compounds. They include molecules such as herbicides (phenoxy as 2,4-D or 2,4-dichlorophenoxy acetic acid; Diphenylether as NIP; Benzoic acids as Dibenon; Phenols as PCP or pentachlorophenyl; Carbamates as Benthocarb; Acid amides as propachlor; Phenylurea as CMU or chlorophenyl methyl urea; nitroaromates -Dinitroaniline as trifluraline and Nitrile as DBN), Insecticides (Organic P as Parthion; Thioether as Horate; Carbamate as Carbaryl; Organic Cl as BHC or benzene hexachloride, DDT or dichlorodiphenyl trichloroethane), Fungicides (Organic P as IBP; Dithiocarbamate as DDC or dimethyl dithiocarbamate; organometals as dimethyl mercury or dimethyl arsine or trimethyl arsine), polychlorinated biphenyls and dioxins, synthetic polymers and alkyl benzyl sulphonates.

Some classes of herbicides (used to kill weeds) that show very slow degradation include acid amide (as propachlor), benzoic acid, carbamate, dinitroaniline, diphenyl ether, nitrile, phenol, phenoxy and phenyl urea. Amongst insecticides it includes carbamates (as carbaryl), organic chlorides, organic P and thioether and fungicides include dithiocarbamate and

organic phosphorus. All of these molecules are recalcitrants. The term **recalcitrant** includes those molecules that show residual activity even after a very long period of their application in the environment.

Compounds such as polychlorinated biphenyls and phthalic acid esters are especially resistant to microbial attack. On the other hand organomercurials and organoarsenics remain toxic due to their metallic components despite degradation of their organic moieties. Amongst detergents, alkyl benzyl sulfonates are hardly decomposed due to their long alkane chain and heavy branching. The most widely known pollutants escaping degradation are the plastics.

In case of oil (petroleum) pollution though individual components may be degraded by microorganisms, it is the extreme complexity and heterogeneity of petroleum that in turn either needs presence of diverse organisms and/or absorbants, surfactants and nutrients in the environment. The recalcitrant asphaltene residues of petroleum form 'Tar Balls'.

Since these compounds show lipophilic character, they get partitioned into lipids of prokaryotes and eukaryotes. The cells can accumulate them up to three orders of magnitude, (i.e., $\times 10^3$) of the concentration of the compound in the environment and in the food chain the concentration may increase by a factor of 10^4 to 10^6 at the topmost trophic levels. This process of progressive concentration of a chemical compound as it travels up the food chain is termed **biomagnification**. In case the xenobiotic has some biological activity it may disrupt the physiological processes leading to diseases, debilitations or even death.

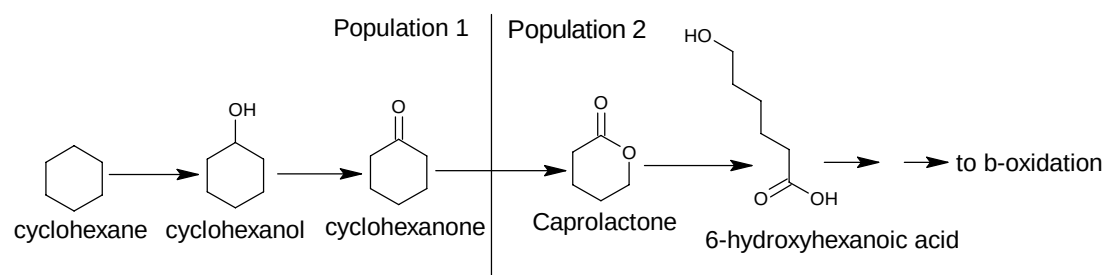
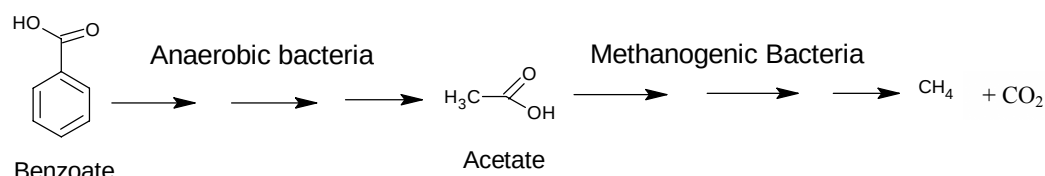
Fate of the xenobiotics

Besides a possible transformation or degradation by the living processes, the xenobiotics may show volatilization, may be transported through gas (advection) or may be dispersed via diffusion and mixing. They might get absorbed or adsorbed over other molecules or structures, may show photochemical oxidation and/or form sediment.

Microbial transformation of xenobiotics

In case of microbial degradation, the xenobiotic may be consumed as a source of carbon and/or nitrogen and/ or energy. For example, *Desulphomonile tiedjei*, a bacterium derives energy from dechlorination of chlorobenzoates. Alternatively it may be transformed/degraded as a part of cometabolic activity where a non-growth substrate is utilized in the obligate presence of another growth substrate. For example, lipid enrichment is required to degrade hydrocarbons such as hexane. Cometabolism does not lead to energetic or nutritional gain. Cometabolic degradation may be gratuitous (unprovoked or unjustifiable) or fortuitous (accidental or chance) as the degradation takes place due to similarity of the xenobiotic to a natural substrate or as a result of non-specificity of the enzyme. Here it may be difficult to induce the enzyme in absence of a natural substrate. Cometabolic degradation can still be adaptive, as it may reduce local toxicity. Methanotrophs (organisms that consume methane and other single carbon organic substrates as a source of carbon) express an enzyme called methane monooxygenase (MMO), which is quite unspecific. It is used by microorganisms to degrade a number of hydrocarbons. In general xenobiotics can not be degraded to CO_2 and H_2O by one type of organism alone and thus it requires cooperation of many other organisms.

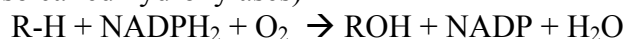
Example of Cooperative degradation:



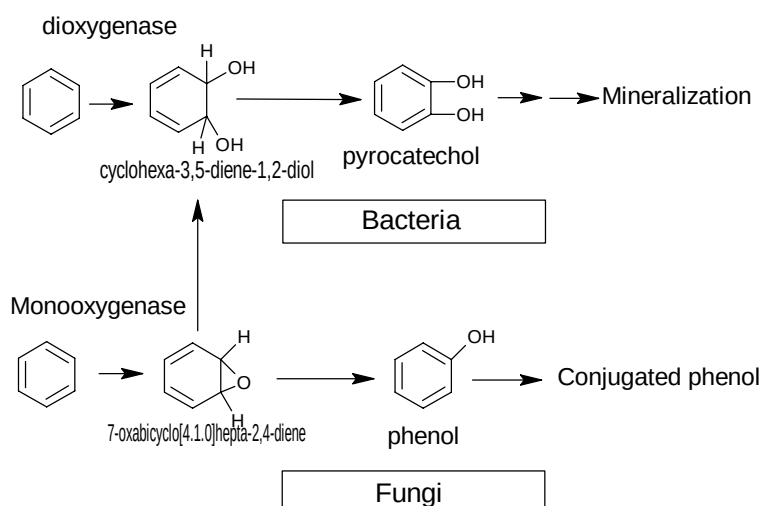
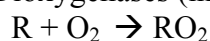
The degradation may involve cellular uptake, manipulation of substrate for ring fission, ring cleavage, conversion of cleaved product into standard metabolites and utilization of metabolites. Ability of microorganisms to degrade aromatic compounds depends on the type of radicals on the ring. It involves:

1. Oxygenases that catalyze direct incorporation of O_2 into organic compounds. These enzymes contain a variety of cofactors such as hemes, flavins, pterins, copper and manganese that "activate" O_2 to the singlet state.

-Monooxygenases (incorporate only one O as OH and the other goes off as H_2O thus also called hydroxylases)

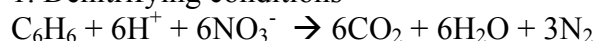


-Dioxygenases (incorporate both O)

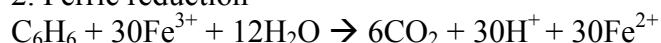


2. Reductive Ring Cleavage: Anaerobic-ring reduction is followed by ring cleavage in presence of O_2 . Several reductive processes are involved viz.

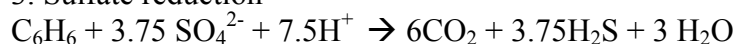
1. Denitrifying conditions



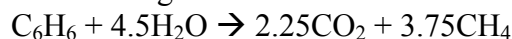
2. Ferric reduction



3. Sulfate reduction



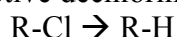
4. Methanogenic fermentation



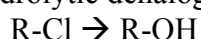
Rates of reduction for a compound however reduce from oxidative (aerobic) to reducing conditions. Up to three ring polyaromatic hydrocarbons are degraded easily by bacteria and fungi. However with the increase in ring numbers, solubility is affected adversely, the structure gets complicated and substitutions further complicate the process of degradation as a result of which the rate of degradation slows down. Mostly white rot fungi are involved in these degradations.

3. Dehalogenation: Chlorinated hydrocarbons are typically stable or only slowly degradable under aerobic conditions. Dehalogenation can be of either

- reductive dechlorination (anaerobic)



- or hydrolytic dehalogenation



- or dehalogenation (oxygenation followed by spontaneous halide loss)

Transformation does not always yield a simpler molecule. Some times an intermediate product might be more toxic than the original xenobiotic. For example, Trichloroethylene>Poly Chloroethylene>Vinyl chloride.

If a xenobiotic contains no oxygen then it will be degraded only aerobically (for example, hexane), however if it contains oxygen then it may also be degraded anaerobically (for example, hexanol). Hydrocarbons (petroleum) are quite stable under anaerobic conditions. The more chlorinated an organic molecule is the more readily it will be degraded anaerobically. The rate slows down with the loss of chlorine.

Nitrogen Cycle

Nitrogen is an essential component of amino acids and nucleotides, which in turn form proteins and nucleic acids respectively. Many fishes inhabiting saline water accumulate large amounts of trimethylamine oxide as a protectant against osmotic shock. In animals nitric oxide (NO) as a natural free radical, signals for short-term control of smooth muscles in circulation. It is derived from amino acids.

Nitrogenous fertilization improves vegetative growth of plants. However excess fertilizer may contaminate water bodies and lead to its [eutrophication](#) (excessive nutrients).

Nitrogen and Nitrogenous gases

Nitrogen (N₂) and nitrous oxide (N₂O) are the chief atmospheric nitrogen compounds. Nitrous oxide, a fat-soluble nonpolar molecule is often called laughing gas. It has anesthetic

properties. Both N_2 and N_2O (dinitrogen monoxide- nitrous oxide) are non reactive gases with long atmospheric lifetimes ($\sim 10^7$ yrs for dinitrogen). The most prominent amongst nitrogen oxides is nitrogen monoxide (nitric oxide- NO). Another common oxide is NO_2 (nitrogen dioxide)- a reddish poisonous gas responsible for smog in air. Dinitrogen trioxide (N_2O_3) and dinitrogen pentoxide (N_2O_5) are fairly unstable and explosive.

Various nitrogen oxides produced during combustion are generally called as NO_x . (In atmospheric chemistry NO_x stands for the total concentration of NO plus NO_2). When dissolved in atmospheric moisture they may form HNO_2 and HNO_3 and cause acid rain damaging the plants. In urban environments, the atmospheric NO_x contributes $\sim 25-33\%$ to the acid rains (Fig. 5).

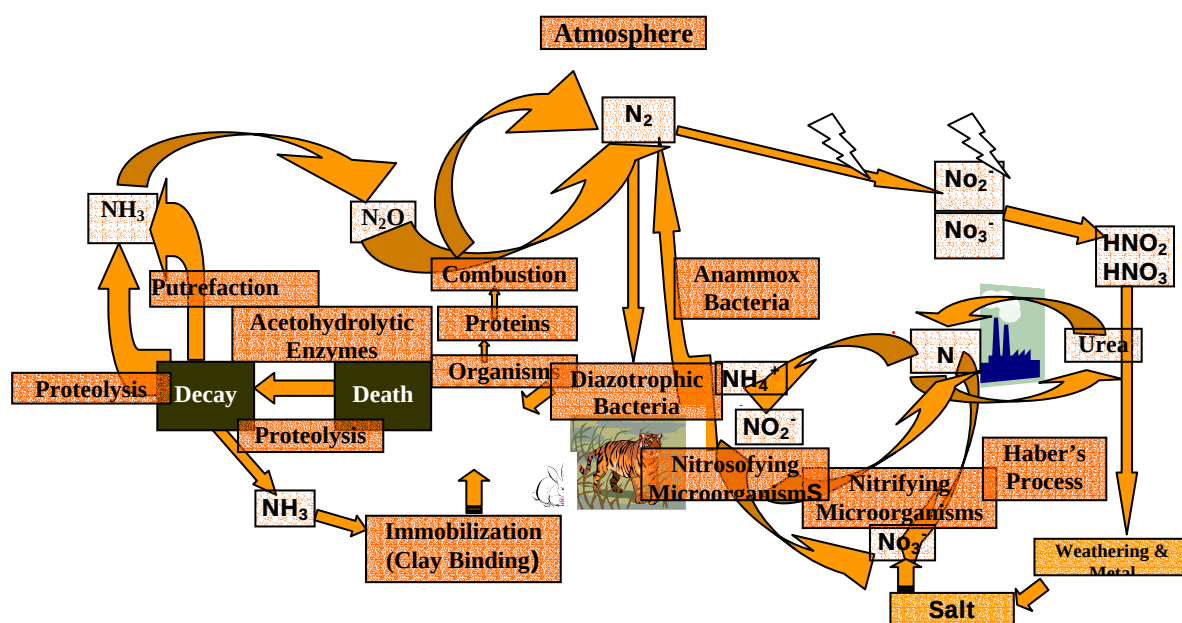
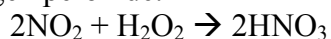


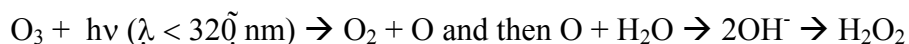
Fig. 5: Nitrogen cycle

Unlike the other nitrogen oxides, nitrous oxide is a major greenhouse gas, that causes 296 times more global warming (per unit weight) as compared to carbon dioxide (CO_2). The average concentration of the greenhouse gas nitrous oxide is now increasing at a rate of 0.2 to 0.3 % per year. Yet its contribution to the enhancement of the greenhouse effect is considered minor relative to the other greenhouse gases. Reactive gases (NO_x), have short atmospheric lifetimes ($< 1-8$ days) and have highly variable concentrations up to < 50 ppbv of the atmosphere due to their rapid removal in water vapours as HNO_2 and HNO_3 . Thus it has an important role in the artificial fertilization of ecosystems. In extreme cases, this fertilization can lead to destruction of the forests, eutrophication of aquatic habitats and species exclusion or elimination. Sources for the increase of nitrous oxide in the atmosphere include land-use conversion, fossil fuel combustion, biomass burning and soil fertilization.

The removal of NO_x requires additional oxidation by some molecular species such as hydrogen peroxide.



Hydrogen peroxide for this reaction is an important oxidant in the cloud chemistry of the troposphere. It may form by the photolysis of tropospheric O_3 .



Before the NO_x compounds are removed as HNO₃, they participate in a sequence of reactions that form ozone.

Because of the low reactivity of the strong triple bond (941 kJ mole⁻¹) in N₂, only the nitrogen fixing organisms and lightning or combustion (which promotes its reaction with oxygen to yield NO) can remove it from the atmosphere. Nitrogen is also present as ammonia in the atmosphere probably as a result of fixation by lightning. There are three processes of fixation of nitrogen: atmospheric, biological and anthropogenic (industrial).

Atmospheric Fixation

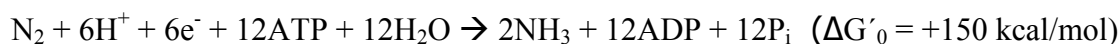
The enormous energy of lightning (30,000 °K) breaks nitrogen molecules and enables their atoms to combine with oxygen in the air forming nitrogen oxides. These oxides dissolve in rain and form nitrates that are carried to the earth. Atmospheric nitrogen fixation probably contributes ~5– 8% of the total nitrogen fixed.

Industrial Fixation

Under great pressure (100-250 atmospheres), at a temperature of 300-550°C and with the use of a catalyst (metallic iron), atmospheric nitrogen and hydrogen (usually derived from natural gas or petroleum) can be combined to form ammonia (NH₃) (Haber-Bosch process). Ammonia can be used directly as fertilizer, but most of it is further processed to urea and ammonium nitrate. Industrial fixation of nitrogen contributes lesser than the biological fixation.

Biological Fixation

The majority of the atmospheric nitrogen (Dinitrogen-N₂) on this earth is fixed as ammonia through the metabolic activities of certain groups of prokaryotes. This is made possible by an enzyme called nitrogenase. The organisms capable of fixing nitrogen are known as diazotrophs (Di-2, Azo-nitrogen).



Though the process requires high energy inputs as indicated by the positive ΔG', it takes place at ambient temperature and pressure and is highly sensitive to oxygen.

The diazotrophs may be either free-living or symbiotic (Table 5). Symbiotic nitrogen fixing rhizobia form nodules on the plant roots and fix ~44 million metric tons of nitrogen per year which is two to three order of magnitudes higher than the free living bacteria. In deserts, free living diazotrophs and in aquatic environments cyanobacteria are the major nitrogen fixers. Since nitrogen fixation takes place in absence of oxygen, many bacteria fix nitrogen only under anaerobic conditions while still others have developed special ways to circumvent the problem. Such mechanisms include - spatial or temporal compartmentalization (cyanobacteria), heavy mucilage layers over cell (*Derxia gummosa*), lamellated cell wall layers (*Gloeocapsa aeruginosa*) and high respiratory rate, thick wall and a nitrogenase cap (*Azotobacter*).

Table 5: Examples of organisms fixing nitrogen

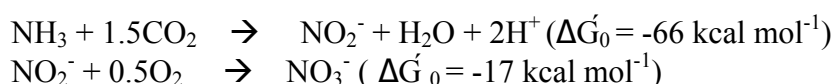
$N_2 \rightarrow NH_3$
Free Living
Phototrophs
Anoxygenic Photosynthetic: <i>Rhodobacter</i> Oxygenic photosynthetic: Cyanobacteria- Heterocystous, Non-heterocystous- <i>Chlorogloea</i> , <i>Synechocystis</i> , <i>Gloeocapsa</i> , <i>Oscillatoria</i> , <i>Lyngbya</i> , <i>Plectonema</i> , <i>Phormidium</i>
Heterotrophs
Aerobic Free living: <i>Azotobacter</i> , <i>Derxia gummosa</i> , <i>Beijerinckia</i> , <i>Klebsiella</i>
Microaerophilic: <i>Azospirillum</i> , <i>Herbaspirillum</i>
Anaerobic: <i>Clostridium pasteurianum</i>
Symbiotic
ROOT NODULES: Rhizobia (<i>Rhizobium</i> , <i>Bradyrhizobium</i> , <i>Allorhizobium</i> , <i>Sinorhizobium</i> , <i>Mesorhizobium</i> , <i>Methylobacter</i> , <i>Devosia</i>) - legumes , <i>Bradyrhizobium</i> - nonlegume (<i>Parasponia</i>), <i>Frankia</i> - <i>Casuarina</i>, <i>Myrica</i>, <i>Alder</i>, <i>Alnus</i> etc., STEM NODULES : <i>Sesbania</i> – <i>Azorhizobium caulinodans</i> OTHERS: <i>Anabaena</i> - <i>Cycas</i>, <i>Azolla</i> etc., <i>Nostoc</i> - <i>Gunnera</i> , lichens carrying diazotrophic cyanobiont such as <i>Lobaria</i> and <i>Peltigera</i> .

Letters in bold and pink depict plant names

Ammonia in atmosphere might reach the earth dissolved in water and then may be utilized subsequently or may be directly removed by the plant foliage. The rate however depends on the type of plant, density of foliage and amount of ammonia in the air. Cereal plants can fulfill as much as 10% of their N-requirement from ammonia present at normal atmospheric concentrations. On the other hand, ammonia may also lead to direct damage to the organisms since cell membranes are highly permeable to ammonia and the local pH rise in the cell is so rapid that pH compensation mechanisms might not cope up with it.

Nitrification

Alternatively nitrogen fixed as ammonia may enter the soil environment as ammonium and either gets immobilized with the clay particles or is converted to nitrate by nitrification. Ammonia is converted to nitrate in two steps catalyzed by two different groups of chemoautotrophic bacteria.



Nitrates absorbed are converted to nitrites by the enzyme nitrate reductase and then to ammonia by another enzyme called nitrite reductase. Animal metabolism of NO also results in the production of nitrite. Finally nitrogenous wastes are excreted by the animals as urea and uric acid.

Nitrite bacteria (Table 6) carry out the conversion of ammonia to nitrite while the nitrate bacteria convert nitrite to nitrate. Some oxidize ammonia directly to nitrate. The nitrification process proceeds optimally at pH 7-8 and drops sharply as the pH drops becoming negligible at pH <5. But at high concentrations in drinking water (>50 ppm NO₃⁻) nitrate may temporarily transform to nitrite.

Excess nitrite in water causes blue-baby disease or methaemoglobinaemia. In this disease, the oxygen supply in blood stream is restricted by the competitive attachment to the hemoglobin with nitrite in place of oxygen. Another potential hazard is the possibility of nitrite reacting with secondary amines of the soil to produce nitrosamines that may be assimilated by plants and are thought to be associated with cancer in humans.

Nitrate (NO₃⁻) is highly mobile and moves easily to the plant root by mass flow or can readily leach through the soil into drainage waters. Nitrate leaching (some times up to 100 kg ha⁻¹) from arable land leads to economic loss as well as eutrophication of water bodies.

Inside the cells of most of the organisms, nitrate is again converted to ammonia (nitrate assimilation), while in some prokaryotes, nitrate is reduced as part of anaerobic respiration. Many aerobic bacteria use nitrate as an electron acceptor in place of oxygen. Some can reduce nitrate to nitrite only, for example, *Escherichia coli*.

Table 6: Organisms performing various activities in nitrogen cycle

Nitrification		Proteolysis	Volatilization	
Nitrosification	Nitrification	Ammonification	Anammox	Denitrification
NH ₃ → NO ₂	NO ₂ → NO ₃	Amino acids → ammonia	NH ₃ + NO ₂ → N ₂ + H ₂ O	
<i>Nitrosomonas</i> <i>Nitrosovibrio</i> <i>Nitrosococcus</i> <i>Nitrospira</i> <i>Nitrosolobus</i>	<i>Nitrospina</i> <i>Nitrococcus rubrum</i> <i>Nitrobacter</i> <i>Nitrospira</i> <i>Nitrococcus</i>	Heterotrophs- <i>Aspergillus flavus</i> <i>Streptomyces</i> sp. <i>Mycobacterium rubrum</i> <i>Bacillus</i> sp. <i>Vibrio</i> sp. NO ₂ ⁻ → NH ₄ ⁺ <i>Clostridium</i> sp.	Planctomycetes : <i>Planctomyces</i> <i>Pirellula</i> Fresh water – <i>Brocadia</i> <i>Kuenenia</i> Marine- <i>Scalindua</i>	<i>Alcaligenes</i> <i>Bacillus</i> <i>Branhamella</i> <i>Corynebacterium</i> <i>Cytophaga</i> <i>Flavobacterium</i> <i>Hyphomicrobium</i> <i>Propionibacterium</i> <i>Pseudomonas</i> <i>Rhizobium</i> <i>Rhodobacter</i> <i>Spirillum</i> <i>Thermothrix</i> <i>Thiobacillus</i>

Mineralization (Ammonification)

Biomass generated utilizing fixed nitrogen is either fed upon by higher trophic level organisms or degraded by heterotrophic organisms. The characteristic odour of animal flesh decay is caused by nitrogen-containing long-chain amines such as putrescine and cadaverine.

Decay generates small amounts of nitrate, but most of the nitrogen ultimately returns to atmosphere as molecular nitrogen.

Degradation of amino acids generates ammonia. Such ammonification is also performed by certain clostridia that reduce nitrite to ammonium. This reduction is not linked to electron transfer and so is not a respiratory process, however it is quantitatively important since majority of nitrate added to most soils is converted to ammonia by fermentative bacteria rather than to N₂ by denitrifiers.

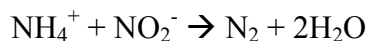
Although mineralized ammonium may often be the preferred N-source to plants (and to soil microbes and animals), nitrate tends to be the form most taken up because the cation ammonium is attracted to anionic sites on clay and organic matter particles, whereas the anion nitrate being highly mobile moves easily to the cells by mass flow.

Ammonium transforms to ammonia and volatilizes when the pH is alkaline, temperature is high and soil is dry allowing considerable air movement. At acid or near neutral pH, the equilibrium between ammonium and ammonia in the soil solution is strongly towards ammonium. Thus loss of ammonia tends to be considerably higher from arable lands compared with forest soils, where the pH is generally much lower.

Volatilization

Anaerobic ammonium oxidation (Anammox)

Anammox is the latest addition to the knowledge on the nitrogen cycle. In this biological process, nitrite and ammonium are converted directly into dinitrogen gas. The overall catabolic reaction is:



Nitrate can also be reduced to nitrite and is subsequently combined with ammonium in the anammox process, which also results in the production of dinitrogen gas. The bacteria that perform the anammox process belong to the rare order of the planctomycetes. As an intermediate to this process, hydrazine is produced which is normally known as rocket fuel and is poisonous to most of the organisms. Anammox is an important process for the waste water remediation but the organisms are very slow to grow. Their doubling takes place nearly in two weeks.

Denitrification

Nitrogen returns to the atmosphere primarily through biomass combustion and degradation. Fixed nitrogen products in dead plants, animal bodies and animal excreta encounter denitrifying bacteria that undo the work done by the nitrogen-fixing bacteria. However, many bacteria form nitrite and then follow it up with conversion to nitrous oxide (N₂O) and finally to nitrogen (N₂).

Denitrification occurs optimally at pH slightly above neutral. Under acidic conditions nitrate reduction ceases at the second step and nitrous oxide gas is produced. If nitrate reduction is delayed then nitrite accumulates in soil that causes phytotoxicity.

The late Carl Sagan and associates suggested that N_2O could be used as a signature for life on other planets, since it is a biogenic gas with no significant geochemical sources. Due to its stable electronic configuration, nitrous oxide has a long lifetime (120 - 150 yrs). Although decomposition of N_2O to N_2 and $1/2\text{O}_2$ is thermodynamically favorable ($\Delta G_0 = -103.59 \text{ kJ/mole}$), the kinetics is unfavorable below 600°C .

Unlike CO_2 , nitrous oxide has no significant acid-base properties and this blocks aqueous ionic assimilation pathways that are available for cycling environmental carbon compounds. The only sink for nitrous oxide is photochemical destruction in the stratosphere or its reaction with energetic oxygen atoms produced by photodissociation of stratospheric ozone as had been described earlier.

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