

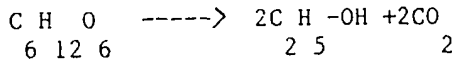
ANAEROBIC PROCESSES

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Anaerobic processes are mediated by a very heterogenous assembly of aerophobic microorganisms that can thrive only in the absence of oxygen. Molecular oxygen in appreciable amounts is found only in those areas on earth that are in direct contact with air or inhabited by organisms that carry out oxygenic photosynthesis. The rest and the greater part of the environment is the deeper water layers and most of the bottom sediment is anaerobic. For anaerobic growth it is not only the absence of oxygen that is important but also the presence of reducing conditions. The oxidising and reducing condition is conveniently expressed by the Eh scale with hydrogen at one end at -0.4 v and oxygen at the other end with $+0.8$ v. Depending on the Eh of the niches there are the strict aerobes at one end and the obligate anaerobes at the other end with microaerophilic and facultative organisms in between.

The various anaerobic processes occurring in the marine environment may be discussed under four broad heads. They are fermentation and anaerobic respiration under catabolic process and photosynthesis and anaerobic chemosynthesis under anabolic processes. Arranged along the Eh scale, the fermentative bacteria follow the aerobic microorganisms. When the Eh falls and the end products of fermentation accumulate, nitrate acts as the electron acceptor followed by sulfate and the carbonate. The sulfur-oxidising photosynthetic and chemosynthetic organisms live synergistically with sulfate-reducing bacteria forming sulfureta.

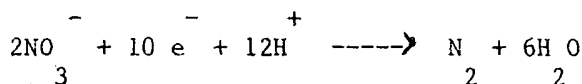
Fermentation is the process in which there is the oxidation of organic compounds occurring in the absence of external electron acceptor. Here only partial oxidation takes place and the release of energy is small. A typical example is the fermentation of glucose by yeast.



The Enterobacteriaceae are the most prominent representative of this group, and they are facultative anaerobes which grow as typical aerobes in the presence of oxygen, and in its absence they carry out fermentation. The other prominent group is the clostridia. These are however strict anaerobes requiring an Eh of -0.4 v. Most of them ferment sugars to 1) butyric acid 2) acetone and butanol 3) ferment proteins or aminoacids to acetic acid, fatty acids NH_3 , CO_2 and sometimes H_2 4) purines to acetic acid 5) cellulose to glucose or 6) even fix N_2 .

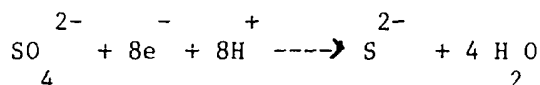
Anaerobic respiration. The pathway for oxidation of organic compounds and conservation of energy in ATP in which an electron acceptor other than oxygen is involved is referred to as anaerobic respiration.

Nitrate reduction is the process in which the electron acceptor is nitrate. In the assimilatory nitrate reduction the nitrate is reduced and incorporated into the cell. In the dissimilatory process where the turnover is much higher, the end products are NO₂, NO, and N₂O and N₂. When the end product is N₂ the process is termed denitrification. For each molecule of nitrate reduced 5 electrons can be accepted as shown:-

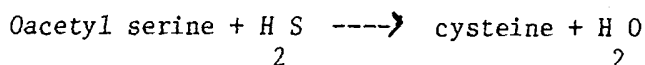


It is possible that dissimilatory nitrate reduction can be fermentative in nature when nitrate becomes an incidental electron acceptor. Nitrate is used to oxidise the reduced NAD thus sparing the substrates which would have to be wasted for the process. In general NH₃ production is indicative of fermentative pathway. The process of nitrate reduction shows variability in O₂ sensitivity in different bacterial species but can occur at quite high O₂ tension. Most bacteria that reduce nitrate are called facultative anaerobes since they will transfer electrons to O₂ if it is present or to NO₃⁻ when oxygen is absent. The main end product may be NH₃ or N₂. Representative examples for dissimilatory NH₃ formers are Enterobacteriaceae, Aeromonas, Micrococcs and those for N₂ formers are Pseudomonas, Thiobacillus, Spirillum. Although the ultimate product of denitrification is N₂, under some conditions NO and N₂O may form in major amounts. These gases are important in the regulation of the amount of ozone in the atmosphere. It has been calculated that an increase of 1% in the emission of N₂O will cause 0.25 decrease in stratosphere ozone.

Sulfate-reduction. The ability to use sulfate as the electron acceptor is present in morphologically different groups of bacteria which have the common capacity to carry out sulfate -respiration. All these bacteria are obligate anaerobes and sulfate-reduction is therefore not an alternative to normal respiration as in denitrification but a completely respiratory process. The nature of the anaerobic electron transport system that couples substrate oxidation with the reduction of sulfate is not yet fully established. In



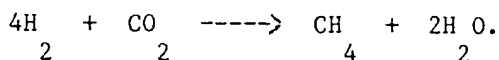
dissimilatory sulfate-reduction, sulfate is activated by ATP in the presence of ATP sulfurylase to form APS (adenylyl sulfate) and PPi (pyrophosphate). APS then gets reduced to bisulfite in the presence of APS reductase. Bisulfite reductase is then responsible for reducing bisulfite to sulfide in the presence of hydrogen. In assimilatory sulfate reduction, the reduction occurs with PAPS (phosphoadenylyl sulfate) rather than APS, and the reduced sulfide is used to form sulfur containing aminoacid viz cysteine.



Irrespective of the kind of reduced compound that might be formed in

intermediary fermentation steps, not more than 33% of the organic matter can be degraded completely if an anaerobic decomposition of acetate is impossible. If electron donating degradation products other than acetate are arranged in order of decreasing complexity, there are phenyl substituted acids, higher fatty acids or sugars at one end and hydrogen at the other. SRB thriving at the hydrogen end may have very limited capacity to degrade organic carbon compounds but they will scavenge hydrogen with high affinity and use it as electron donor or carry out incomplete oxidation of ethanol or lactate with acetate as end product. Some of the common SRB are Desulfovibrio vulgaris and Desulfotomaculum acetoxidans. These microorganisms are responsible for most of the corrosive activities on immersed structures and the sulfide produced by them is a menace to offshore oil industriars. However they are known to fix N₂ in anaerobic sediments and precipitate heavy metals. Consequently bound phosphate is released and made available to other organisms. Formation of hydrogen sulfide also helps in the detoxification of methyl mercury and methyl lead.

Methanogenesis. Methanogens are unique among the Prokaryotes in that they produce a hydrocarbon CH₄ as the major product of metabolism. Though methane is a minor compound of the global carbon cycle it is of great importance in many localised situations. Most methanogenic bacteria use CO₂ as their terminal electron acceptor in anaerobic respiration converting to methane. The donor used is generally H₂. The overall reaction is as follows:-

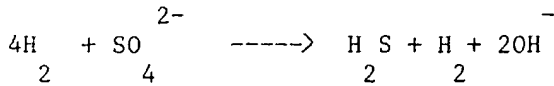


A few other substrates can be converted to CH₄ including methanol, formate, methyl mercaptan and acetate. Despite the exceedingly narrow range of organic substrates used by methanogenic bacteria, methane production is widespread during the decomposition of a wide variety of organic materials. Methane formation occurs during the decomposition of such diverse substrates as cellulose, starch, sugars, proteins and aminoacids, fats fatty acids alcohols and a variety of other substances to either acetate or H₂ or CO₂ which are then used by methanogenic bacteria. Thus methanogenesis from organic carbon is virtually always a process carried out by a mixture of bacteria none of which can complete the reactions themselves.

In most situations the rate limiting step in methane formation from organic materials is not the final reduction of CO₂ to CH₄ but the steps involved in the breakdown of complex organic material into fermentation products. Since CO₂ is very common in anaerobic environment either in the form of carbonates or derived from microbial respiration or fermentative processes, the methanogenic bacteria are generally limited by the availability of H₂. As soon as H₂ is formed, by a fermentative bacteria, it is quickly consumed by methanogen. Though intensive methanogenesis may occur in highly anaerobic environment, like the deep sediments and anaerobic water masses, they can also occur in anaerobic pockets in apparently aerobic surface sediments or water column. Biogenic production of methane exceeds

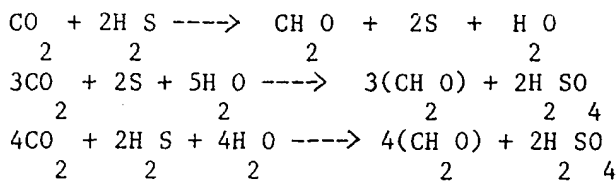
considerably the production rate from gas wells and other fossil fuel sources.

Methanogenesis is much more common in fresh water and terrestrial environments than in the sea. This is because SO_4^{2-} a major anion in the sea water strongly inhibits methanogenesis. The SRB also use H_2 as a major electron source.



SRB are able to compete with methanogens for available H_2 . Only after sulfate is depleted and SRB can no longer develop can the methanogens proliferate. Sulfate also inhibits methanogenesis in fresh water; because sulfate concentration of fresh water is so low, sulfate is readily depleted at the surface of anoxic sediments. The removal of H_2 allows the otherwise thermodynamically unfavourable reaction to occur. Therefore the anaerobic degradation tends to shift from the formation of H_2 , CO_2 and mixed organic acids to the formation of acetate, CH_4 and CO_2 . Acetate is further converted to CH_4 by other methanogens. Some common genera are Methanobacterium, Methanococcus and Methanosarcina. Residence time of methane in atmosphere is about 3.6 years. The main process consuming methane is photochemical oxidation to CO_2 . Some methane formed in soils and anaerobic sediments never reaches the atmosphere because it is oxidised by another group of bacteria.

Photosynthesis. Bacterial photosynthesis is an anaerobic process carried out by coloured sulfur bacteria (Chromatococcales) and non sulfur bacteria (Rhodospirillaceae). There is no production of oxygen during the process and instead of water photosynthetic bacteria use sulfur or organic compounds from their environment as electron donors. Photosynthetic electron flow in purple and green bacteria involves a simpler scheme than that of green plant photosynthesis. Only a single light reaction is involved which is analogous to the photosystem I of green plants. Reaction centre chlorophyll absorbs light waves in infra red region of light spectrum and is designated P 870. The electron is raised to an acceptor designated X and then passes down through an electron transport system involving ubiquinone, an iron sulfur protein and cytochromes. In this scheme which is similar to cyclic phosphorylation in photosystem I of green plants, no reducing power is formed. Reducing agents such as H_2 or H_2S are used from the environment. The overall photosynthetic reaction can be written as:

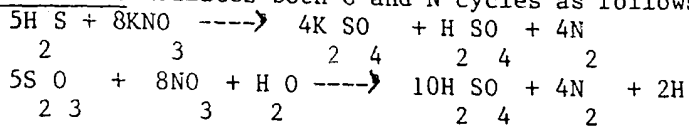


In the sulfide oxidation purple bacteria generally accumulate sulfur granules within whereas the green bacteria do so externally. Non sulfur purple bacteria have primarily a photoheterotrophic mode of growth i.e. they use organic compounds as carbon source for growth instead of CO_2 and use light

energy only to generate ATP. If the organic compounds are sufficiently reduced they can be assimilated without the need for photosynthetically generated reducing power.

These microorganisms are usually restricted to regions where light and sulfide are available. They are responsible for converting the toxic sulfide back to sulfate and make the regions inhabitable by other microorganisms. Photosynthetic bacteria also contribute to nitrogen fixation.

Chemosynthesis. When the process of CO₂ fixation takes place in the absence of light it is referred to as chemosynthesis. The process can be aerobic or anaerobic. Under anaerobic conditions, NO₃⁻ is usually the electron acceptor and reduced sulfur compounds the donors. Thiobacillus denitrificans mediates both C and N cycles as follows



With the discovery of the Galapagos rift vent site in 1979, it has been shown that microbial chemosynthesis can indeed provide the primary support for entire ecosystems. In the vents three groups of microorganisms are involved 1) aerobic chemolithotrophic 2) anaerobic chemolithotrophic bacteria active in anoxic zones prior to mixing or after mixing where free oxygen has been consumed by spontaneous chemical oxidation 3) chemolithotrophic prokaryotes in symbiotic association with certain vent invertebrates.

Though H₂Fe, Mn, NH₃ and NO₂ are some of the potential sources of electrons present, reduced sulfur compounds appear to be the most effective, not only from the bacterial point of view but also because of its abundance. The anaerobic chemolithotrophs occur most frequently as base producing (ie sulfide or thiosulfate are oxidised to polythionates and not sulfate) facultatively autotrophic or mixotrophic microorganisms.

Sulfide and other reduced inorganic sulfur compounds appear to be the energy source of the bacteria in many of the symbioses. Activities of CO₂ fixing enzyme of the Calvin-Benson cycle RuBP case in specific tissues and APS reductase all point out to the functioning of the symbiotic relationship.

Thus it can be seen that these anaerobic processes contribute substantially to the simultaneous turnover of C, S and N through oxidative and/or reductive cycles.