

# Advances in Biotechnology

## Chapter 3

### Microorganisms in environmental biotechnology application

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#### Abstract

Microorganisms are essential to our very existence. They are ubiquitous, found in common environments such as soil, water, and air as well as exotic locales as diverse as deep sea hydrothermal vents and soda lime lakes. They are abundant in natural environments associated with wide range of activities like fuel and biomass production, mineral, nutrient recycling and energy recovery etc. Concerns of microbial studies have been identified as important area where background information is essential for recovery of natural component for our use. Useful applications of microbes, study of environment balance and their correlation are valuable for living organism. Microbes are used for environmental restoration and biomediation processes which are needed to lead more sustainable lifestyles and use of resources more justifiably. Microbes are everywhere in the biosphere, and their presence invariably affecting the environment. The effects of microorganisms on environment can be beneficial or harmful. Since a good part of this text concerns with a discussion of the beneficial activities of microorganisms as they relate to human welfare. Microorganisms are very diverse and their jobs are highly specific in the environment. Recognizing the numerous invaluable functions of microbes, this book chapter give the information about the environment and role of microbes in various applications

like biomass degradation, fuel production, mineral recovery and nutrient recycling.

**Keywords:** microbes; biomass; biochemical; environmental application

## 1. Microbes in Biofuel Production

Our environment provides us natural resources that include, air, water, soil, minerals, along with the climate and solar energy, which form the non-living or abiotic component of nature. The 'biotic' or living parts of nature consist of plants, animals, and microbes (Nejat and Mantri 2017). Plants and animals survive by using abiotic component and microbial help. Microbes play a major role in the balance of environmental biotic and abiotic components. They break up/decompose dead plant/animal (biomass) and act on the detritus to reform/recycle soil nutrients. Biomass is a very good and abundant renewable source of energy. Trees, crops, garbage, and animal waste all are biomasses but most of the biomass is lignocellulosic biomass of the plants that we use as a source for energy production. The major types of biomass for ethanol production recognized to date are monoculture crops grown on fertile soils (such as sugarcane, corn, soya beans, oilseed rape, switch grass, willow, and hybrid poplar; Farrell et al., 2006), waste biomass (such as straw, corn, and waste wood; Kim and Dale, 2004), and municipal solid waste (such as processed paper and newspaper; Kuhad et al., 2010). Another type of biomass is cellulose of woods, viz. *Eichhornia crassipes*, *Lantana camara*, *Prosopis juliflora*, *Saccharum spontaneum*, *Typha latifolia*, *Crofton*, *Chromolaena odorata*, etc., which are promising and cheaper feedstocks for fuel ethanol production. Plants store the solar energy in terms of photosynthesis a form that can later be converted into fuel with the help of microorganism (Figure 1).

Fuel derived from biological origin is termed as biofuel. Biofuel is a fine alternate of conventional energy sources like Coal, Petrol, and Diesel etc which is actually derived from lignocelluloses component of the plant. The main constituents of lignocellulose are cellulose, hemicellulose and lignin (Reddy and Yang, 2005). Cellulose is the main structural component of plant cell walls. It is a long chain of glucose molecules, linked by glycosidic bonds. Hemicellulose, the second most abundant constituent of lignocellulosic biomass, is a family of polysaccharides, composed of monosaccharide units. After cellulose, hemicellulose is the most potent constituent of biomass to serve as a source of bioethanol production. Lignin is a three dimensional polymer of phenylpropanoid units (Van, 2001). The carbohydrate fractions of the plant cell wall can be converted into fermentable monomeric sugars through acidic and enzymatic (hemicellulase/cellulase) reactions, which have been exploited to produce biofuels like ethanol, butanol, isobutanol via microbial fermentation processes. Thus simple sugars are subsequently converted into fuels by microorganisms (Bacteria, cyanobacteria, yeast).

We need for efficient breakdown of lignocellulosic biomass for biofuel production. A variety of organisms have evolved to take advantage of this source, including the free-living

organisms and symbiotic animal–microbe consortia invariably present in biomass-rich environments. Increasing our knowledge of the biochemical machinery used by these organisms for the breakdown of biomass offers new avenues for the development of biologically based processes that could potentially accomplish biomass conversion at an industrial scale (Edward, 2008). The capture of solar energy through photosynthesis is a process that enables the storage of energy in the form of polymers (cellulose, hemicellulose and lignin). The high energy content and portability of biologically derived fuels, and their significant compatibility with existing petroleum- based transportation infrastructure, helps to explain their attractiveness as a fuel source. Despite the increasing use of biofuels such as biodiesel and sugar- or starch-based ethanol, evidence suggests that transportation fuels based on lignocellulosic biomass represent the most scalable alternative fuel source (Hill et al., 2006). Lignocellulosic biomass in the form of plant materials offers the possibility of a renewable, geographically distributed and relatively greenhouse-gas-favourable source of sugars that can be converted to ethanol and other liquid fuels. Calculations of the productivity of lignocellulosic feedstocks, in part based on their ability to grow on marginal agricultural land, indicates that they can probably have a large impact on transportation needs without significantly compromising the land needed for food crop production (Tilman et al., 2006).

Microbial strategies for degrading lignocellulose are diverse. Our current understanding of the enzymes involved in these processes of biomass degradation is limited to a handful of model organisms such as the fungus *Trichoderma reesei* and the bacterium *Clostridium thermocellum* (DelRio et al., 2007). Research is going on to know about more biomass degraders microbes. The knowledge of biomass degradation pathways is soon to be increased even more by a number of large-scale genomic studies. Thus, enzymes derived from thermophilic and acidophilic organisms known to degrade lignocellulose, hold significant promise for industrial processes (Viikari et al., 2007). Many novel enzymes and enzyme systems that have evolved to make use of cellulosic biomass are present in those microbes which is difficult-to-culture (Hugenholtz et al., 2002). The availability of a wide range of naturally occurring lignocellulose-degrading enzymes increases the chances of successful enzyme optimization for industrial processes. Optimization of the saccharification process is crucial because the cost of cellulases remains a key barrier to economical production of biofuels (Himmel et al., 2007). A more diverse set of candidate enzymes identified through a combination of conventional cultured microbial studies coupled with environmental prospecting methods will improve the likelihood of obtaining enzymes with activities and stability suited to a variety of industrial processes.

Final steps of ethanol production from cellulosic mass will require much of the same infrastructure developed for the production of sugar- and starch-based ethanol, changes will need to be made to exploit the diversity of sugars generated from the breakdown of biomass.

Whereas the conversion of starch-based biomass results primarily in hexoses, and also the pentose sugars D-xylose and L-arabinose. In contrast to the hexose sugars, the pentose sugars cannot be fermented by wild-type *Saccharomyces cerevisiae* (Van Maris et al., 2007). Now a days genetically engineered *Escherichia coli* has been used because it has capacity for the conversion of all hexose and pentose sugars both (Görke B, Stülke, 2008). *E. coli* has recently been engineered to produce isobutanol and other alcohols via a non-fermentative pathway that may be more readily adapted to large-scale production. In the future, genomics studies and pathway engineered microbes will considerably facilitate the process of biodegradation and biofuel production. This perspective has focused on the production of biofuels derived from lignocellulosic biomass with the help of microbial activity. Thus biofuels produced by microorganisms from renewable materials are promising substitutes for traditional fuels derived from fossil sources. The demand for sustainable alternative fuels based on renewable resources is already high today but will dramatically increase in the future. Today biofuel industry primarily produces ethanol from corn starch or sugar cane, and biodiesel is generated from vegetable oils and animal fats. However, these first generation biofuels, especially ethanol produced from starch, are in competition with the food and animal feed industry. In contrast, lignocellulosic biomass like crop wastes, forestry residues and municipal solid waste offers a high potential as feedstock for biofuels because it is the most abundant sustainable raw material worldwide. Fuels produced by microbes should help meeting energy- crisis world over.

## 2. Microorganism in Metal & Mineral Recovery

Since high grade ore deposits are easily accessible, so these ores become rapidly depleted. It thus becomes necessary to recover mineral resources from low grade ore deposits or extract valuable metals from industrial wastes. However, no appropriate technology is still available for recovery of metals from low- grade deposits. It is encouraging to find some microorganisms that could do it efficiently. This potential of microbes could only be realized recently and efforts are being made to use them for enhanced recovery of mineral resources from natural deposits. The process of extraction of metals from ores or concentrates, using microorganisms is called as bioleaching or microbial mining. In other words Microbial mining is the process of bioleaching recovers metals from ores that are not suitable for direct smelting due to their low metal content. Only ores containing sulfur can be bioleached because the bacteria feed on sulfur. Microbes have been used for recovery of two important natural resources - metals and petroleum. Metals are extracted economically from low grade ore by exploiting metabolic activities of bacteria *Thiobacilli*, particularly *T. ferrooxidans*, *T. thiooxidans*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Pradhan et al., 2010). They are thermoacidophilic autotrophic archaeobacteria grow in acidic and hot environments. They thrive at extremely low pH (pH 1–2) and fixes both carbon and nitrogen from the atmosphere. It solubilizes copper and other metals from rocks and plays an important role in nutrient and

metal biogeochemical cycling in environments. It has been demonstrated that these *Thiobacillus spp.* can be used for extraction of copper and uranium from insoluble minerals. This implication of microbial activity in leaching and deposition of mineral ores could develop into a recent field of biotechnology known as biohydrometallurgy.

Under optimal conditions in the laboratory, 97% of the copper in low-grade ores has been recovered by bioleaching. Laboratory experiments could show that recovery of other metals such as Ni, Zn, Co, Sn, Cd, Mb, Pb, Sb, As and Se from their low-grade sulphide-containing ores is also possible through bioleaching. The leaching process can also be used to separate the insoluble lead sulphate ( $\text{PbSO}_4$ ) from other metals that occur in the same ore. The recovery of uranium, a nuclear fuel, can also be enhanced by microbial activities, which should help overcoming global energy crisis. Insoluble tetravalent uranium oxide ( $\text{VO}_2$ ) occurs in low-grade ores.  $\text{VO}_2$  can be indirectly converted to leachable hexavalent form  $\text{VO}_2\text{SO}_4$  by *T. ferrooxidans* and release  $\text{S}^{2-}$  in presence of some growth substrate.  $\text{S}^{2-}$  is oxidized and to sulphur (S), utilized in the metabolism of *T. ferrooxidans* and completes bioleaching cycle (Figure 2).

These microorganisms actually gain energy by breaking down minerals into their constituent elements. Bioleaching uses bacterial microorganisms to extract precious metals, such as gold, from ore in which it is embedded. The bacteria feeds on nutrients in minerals, thereby separating the metal that leaves the organism's system; then the metal can be collected in a solution. The bacterium uses a chemical reaction called oxidation to turn metal sulphide crystals into sulfates and pure metals. These constituent parts of ore are separated into valuable metal and leftover sulphur and other acidic chemicals. Eventually, enough material builds up in the waste solution to filter and concentrate it into metal. In addition to metal recovery, microorganisms are also used in petroleum recovery called Microbially Enhanced Oil Recovery (MEOR) and *Xanthomonas campestris* is used in this process.

Several species of fungi can be used for bioleaching. Fungi can be grown on many different substrates, such as electronic scrap, catalytic converters, and fly ash from municipal waste incineration. Experiments have shown that two fungal strains (*Aspergillus niger*, *Penicillium simplicissimum*) were able to mobilize Cu and Sn by 65%, and Al, Ni, Pb, and Zn by more than 95%. *Aspergillus niger* can produce some organic acids such as citric acid. This form of leaching does not rely on microbial oxidation of metal, but rather uses microbial metabolism as source of acids which directly dissolve the metal. Some advantages associated with bioleaching are (i) Bioleaching is generally simpler and therefore cheaper to operate and maintain than traditional processes, since fewer specialists are needed to operate complex chemical plants. (ii) The process is more environmentally friendly than traditional extraction methods. For the company this can translate into profit, since the necessary limiting of sulfur dioxide emissions during smelting is expensive. Less landscape damage occurs, since

the bacteria involved grow naturally, and the mine and surrounding area can be left relatively untouched. As the bacteria breed in the conditions of the mine, they are easily cultivated and recycled. Yet overall, bioleaching creates less air pollution and minimal damage to geological formations, since the bacteria take place there naturally. Microorganisms play a significant role in the recovery of metal and minerals which is extensively used for our need and survival.

### 3. Environmental Nutrients and Microbes

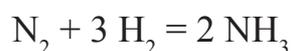
All living organism require nutrients for growth. Nutrients are the chemical elements consumed by plant and animals in the largest quantities. Nutrients are of two types (i) Organic nutrients: include carbohydrates, fats, proteins and vitamins. (ii) Inorganic chemical compounds such as carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur, water, and oxygen may also be considered nutrients (Sizer & Whitney, 2007). Carbon (C), Hydrogen (H), Oxygen (O), nitrogen (N), phosphorus (P), and Sulphur (S) are the elements considered basic for cell growth. An organism needs nutrients to live and grow because they are used in an organism's metabolism which must be taken in from its environment (Elanor & Rolfes, 2005). They are used to build and repair tissues, regulate body processes and converted to and used as energy. Methods for nutrient intake vary, with animals consuming foods that are digested by an internal digestive system, but most plants ingest nutrients directly from the soil through their roots or from the environment. Microbes (most frequently bacteria) play an important role as mineralizers of organic detritus and recyclers of essential nutrients in environment.

Bacteria constitute the foundation of ecosystem, being responsible for degradation and recycling of elements. They are often integrally involved in the chemical alteration of minerals. Minerals, or intermediate products of their decomposition, may be directly or indirectly necessary to their metabolism. The dissolution of sulphide minerals under acidic conditions, the precipitation of minerals under anaerobic conditions, the adsorption of metals by bacteria or algae, and the formation and destruction of organometallic complexes are all examples of indirect micro-organism participation. Where minerals are available as soluble trace elements, serve as specific oxidizing substrates, or are electron donors/acceptors in oxidation-reduction reactions, they may be directly involved in cell metabolic activity. There are three categories of oxidation-reduction reactions for minerals with micro-organisms: (i) Oxidation by autotrophic or mixotrophic organisms. Energy derived from the oxidation reaction is utilized in cell synthesis. (ii) Electron acceptance by minerals (reduction) for heterotrophic and mixotrophic bacteria. Chemical energy is used to create new cell material from an organic substrate. (iii) Electron donation by minerals (oxidation) for bacterial or algal photosynthesis (reaction is fuelled by photon energy).

Nutrient recycling occurs when nutrients are released into the environment. Carnivorous animals feed on herbivorous animals that live on plants. When animals defecate, this

waste material is broken down by worms and insects mostly beetles and ants. These small soil animals break the waste material into smaller bits on which microscopic bacteria and fungi can act. This material is thus broken down further into nutrients that plants can absorb and use for their growth. Similarly the bodies of dead animals and plants are broken down into nutrients so that plants can absorb the nutrients through their roots. Thus nutrients are recycled back from animals to plants and other organism by microbial consumers. If the dead material, or detritus, is not broken down by microbes, those nutrients will never become available to help sustain the life of other organisms. As we know that C, H, O, N, P and S are important for living organism. Recycling of these elements in environment is called as biogeochemical cycle or nutrient cycle. Biogeochemical cycle is a pathway by which a chemical element or molecule moves through both biotic (biosphere) and abiotic (lithosphere, atmosphere, and hydrosphere) compartments of Earth. All are recycled in environment mainly by microbial activity. Microbes like photosynthetic algae and bacteria are the most important agents of CO<sub>2</sub> fixation and recycle carbon in atmosphere. Recycling of H and O is actively involved with the other cycles like the carbon cycle, nitrogen cycle, sulfur cycle and phosphorous cycle as well. Among all biogeochemical elements recycling, most studies on recycling have focused on N, P, S because these elements often limit primary production (Vanni 2002).

Nitrogen is a substance that is essential for all life on the earth. Most nitrogen can be found in air in the gaseous form (78%), but nitrogen can also be found in water and soil in different forms. There, it will be decomposed by bacteria (decomposer) and absorbed by plants and animals. Nitrogen is a part of vital organic compounds because it is chief constituents of amino acids, proteins and DNA. Nitrogen in the gaseous form cannot be absorbed and used as a nutrient by plants and animals; it must first be converted by nitrifying bacteria, so that it can enter food chains as a part of the nitrogen cycle. During the nitrogen fixation process cyanobacteria first convert nitrogen into ammonia and ammonium (ammonium fixation). Plants use ammonia as a nitrogen source. Ammonium fixation is carried out according to the following reaction:



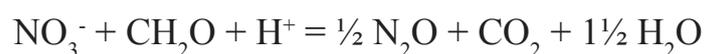
After ammonium fixation, the ammonia and ammonium is converted into nitrite (NO<sub>2</sub><sup>-</sup>) by Nitrosomonas bacteria and subsequently Nitrobacter convert nitrite to nitrate (NO<sub>3</sub><sup>-</sup>) through the nitrification process. Nitrite and nitrate are the main plant nutrients.

Nitrification is carried out according to the following reactions:



During the assimilation process, plants absorb ammonium and nitrate, after which they are converted into nitrogen-containing organic molecules, such as amino acids and DNA.

Animals cannot absorb nitrates directly. They receive their nutrient supplies by consuming plants. When nitrogen nutrients have served their purpose in plants and animals, specialized decomposing bacteria will start a process called ammonification, to convert them back into ammonia and water-soluble ammonium salts. After the nutrients are converted back into ammonia, anaerobic bacteria will convert them back into nitrogen gas, during a process called denitrification. Finally, nitrogen is released into the atmosphere again (Figure 3). Denitrification is carried out according to the following reaction:



Phosphorus is found on earth in water, soil, rock and sediments. Phosphorus is taken by plants and animals in the form of phosphate ( $\text{PO}_4^{3-}$ ) and Monohydrogen phosphare ( $\text{HPO}_4^{2-}$ ) ions. It is a part of DNA, store energy molecules like ATP and ADP, and of fats of cell membranes. Phosphorus is also a building block of bones and teeth of the human and animal body. Phosphorus is usually liquid at normal temperatures and pressures. In the atmosphere phosphorus can mainly be found as very small dust particles. Phosphate salts that are released from rocks through weathering process usually dissolve in soil water and is absorbed by plants. The phosphorus cycle is the slowest one of the matter cycles that is described here. Because the quantities of phosphorus in soil are generally small, it is often the limiting factor for plant growth. That is why humans often apply phosphate fertilizers on farmland. Animals absorb phosphates by eating plants or plant-eating animals. When animals and plants die, phosphates will return to the soils or oceans (environment) again during decay of dead bodies (either plant or animal) by microbial enzymatic activity. Important organisms active in phosphate recycling are bacteria and fungi such as species of *Bacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Aspergillus*, *Penicillium*, *Fusarium*. The enzymes involved in cleaving phosphate from organic phosphorus compounds are collectively known as phosphatases. These enzymes show a broad range of substrate specificity and are grouped into two groups based on their pH optima the alkaline phosphatases and the acid phosphatases. Bacteria play the role of disintegrator in the phosphorus cycle.

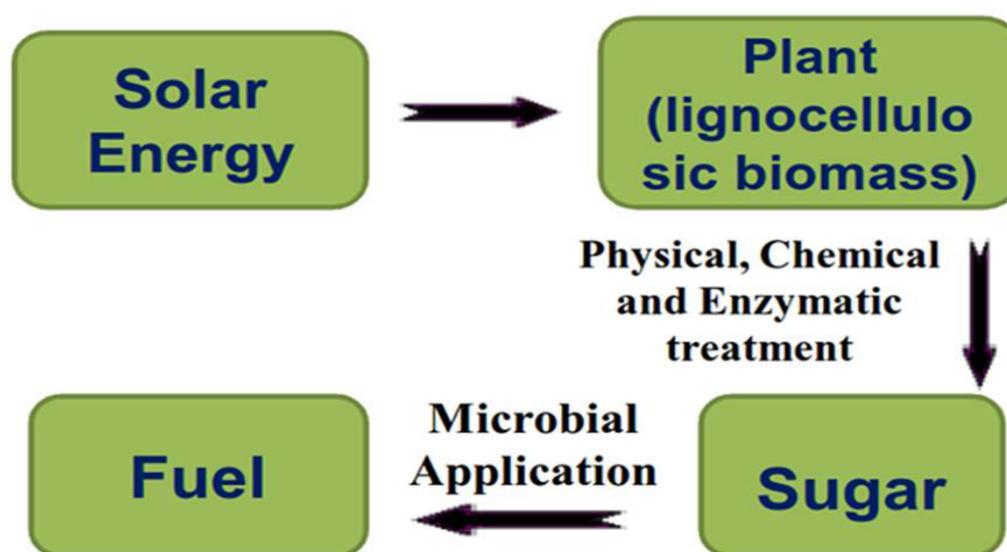
Sulphate ion ( $\text{SO}_4^{2-}$ ) is taken up from soil by plants, which incorporate it into protein, and plant protein is consumed by animals that convert plant protein to animal protein. Death of plants and animals allows bacterial decomposition of protein in remains to produce hydrogen sulphide and other products. Members of the genus *Thiobacillus* are the main organisms involved in the oxidation of elemental sulphur. The ability to oxidize sulphur is not restricted to only the genus *Thiobacillus*. Heterotrophic bacteria (*Proteus vulgaris*), actinomycetes and fungi are also reported to oxidize sulphur compounds. For example species of *Bacillus*,

*Pseudomonas*, *Arthrobacter* and *Flavobacterium* are known to oxidize elemental sulphur or thiosulphate to sulphate. Some bacteria can function in the transition zone between aerobic and anaerobic environments. Hydrogen sulphide may be oxidized to sulphur by such bacteria which deposit elemental sulphur in their cells while using oxygen as the terminal electron acceptor. Hydrogen sulphide may also be oxidized to sulphate photosynthetically by the bacteria, *Chromtiaceae* and *Chlorobiaceae*. Sulhur is first converted enzymatically to sulphite which is then oxidized to sulphate.

It is believed that some of the sulphite from the first reaction reacts with sulphur to yield thiosulphate which is then either cleaved to sulphur and sulphite or converted into tetrathionite. The latter is then metabolized to sulphur or sulphite which is then oxidized to sulphate. Under anaerobic conditions, sulphate is first reduced to  $H_2S$  by sulphate reducing microorganisms, mostly the bacteria. Many bacteria including species of *Bacillus* and *Pseudomonas* are known to reduce sulphur or sulphate to  $H_2S$  but among these, *Desulfovibrio desulfuricans* seems to be the most important.

Various studies demonstrate that there are a variety of bacteria (chemo- and phototrophs, auto and heterotrophs) involved in mineralization and in the cycling of C, H, O, N, S, and P. Microbes actively preserve and protect the environment. Microbes make a balance in environment by performing a lot of activity like production, degradation, recovery, recycling (as discussed above).

#### 4. Figures



**Figure 1:** Microbial conversion of solar energy into fuels

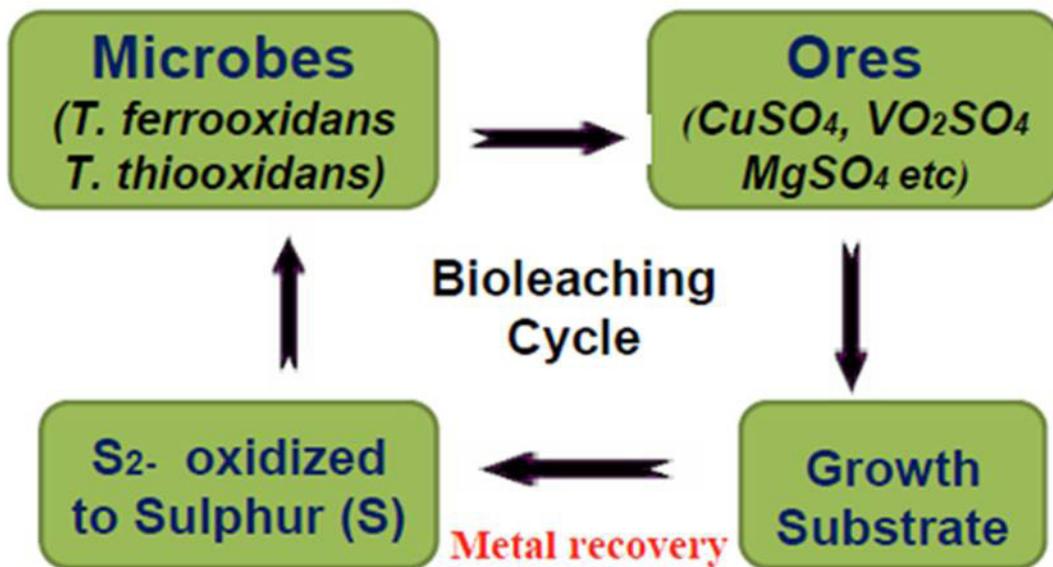


Figure 2: Bioleaching cycle

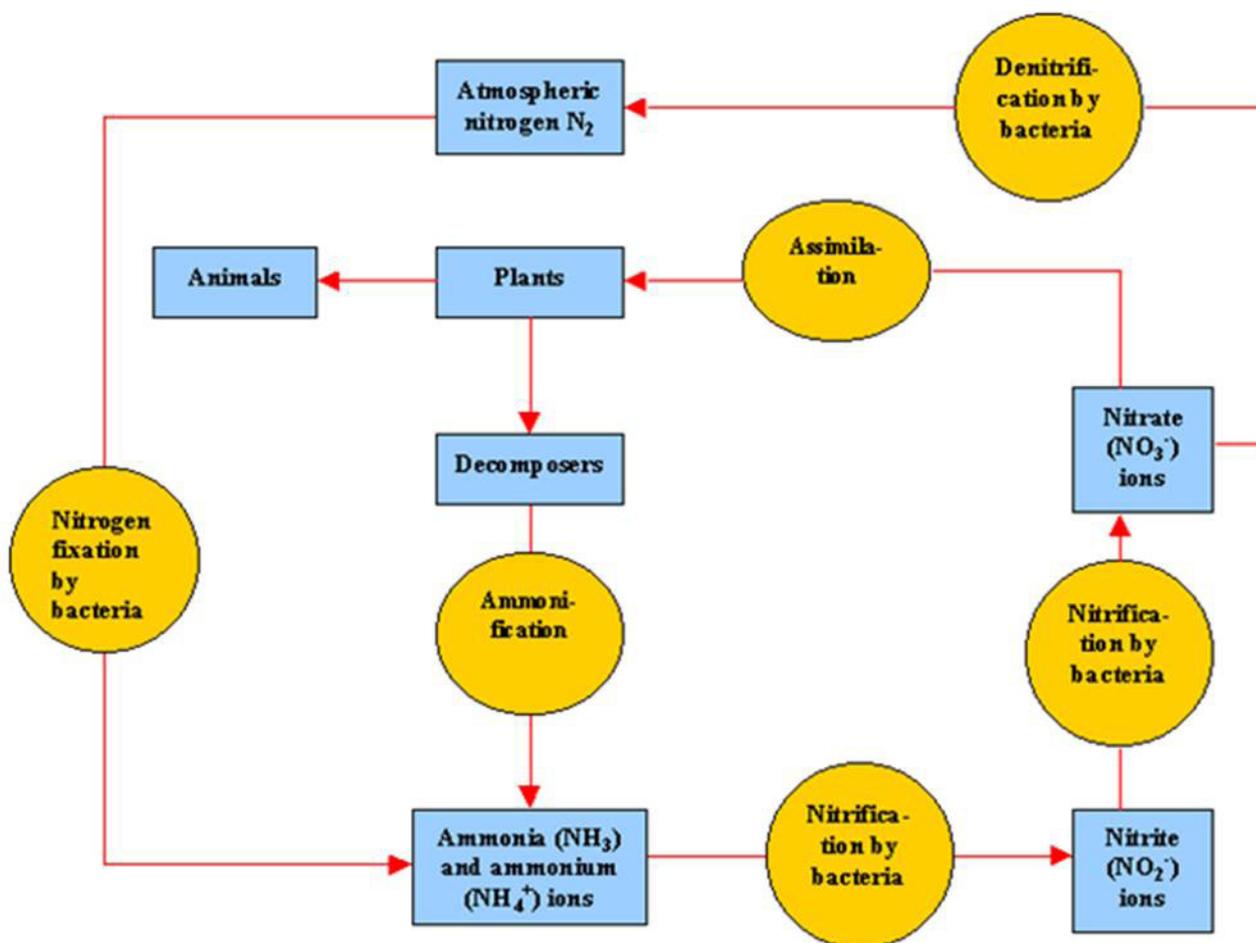


Figure 3: Nitrogen cycle

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