APPLIED MICROBIOLOGY

Microbial degradation of grains, oil seeds, textiles, wood, corrosion of metals and bioleaching of mineral ores

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Microbial Degradation of Cereal Grains and Oil Seeds

Cereal grains and oil seeds are the major crop produce used as basic commodities to fulfill our long term requirements of food and feed. Like other plant products they harbor a variety of microorganisms which originate from soil, air and plant sources. However, mere presence of these microorganisms in or on products of human interest is not harmful. But under certain conditions they may start consuming such products for growth and reproduction and their activity is likely to cause undesirable change of varied nature in the product concerned including quality of nutrients, loss of constituent nutrients, poisoning of the products by mycotoxins, loss of germinability etc. According to Food and Agricultural Organisation (FAO) about 5% of all harvested food grains are lost before consumption. In some countries it may be upto 30% of the total agricultural produce / annual harvest. Any programme leading to reduce storage losses may result in 10-20% increase in available food, in some developing countries. This chapter deals mainly with the spoilage of grains and oil seeds caused by microorganisms during storage and the measures to minimize such losses during their storage.

Microorganisms involved

The damage to agricultural produce that occurs during storage is mainly due to rodents, insects and microorganisms. Rodents and insects consume grains and contaminate them with feces, webbing, body parts, foul odors and microorganisms. Beetles and moths are most ruinous of grain insects and are capable of destroying the stored products completely. The microorganisms associated with the stored cereal grains and oil seeds are most undesirable because of their danger to public health. Bacteria and viruses reported from various seeds of crop produce are mainly concerned with the development of diseases in plants The actinomycetes when present may grow under certain conditions and sporulate heavily thus making the atmosphere full of their spores. These spores when involved cause allergic disorders on sensitized subjects. Fungi are the major cause of deterioration during storage. They may cause total deterioration of grain mass because they elaborate secondary metabolites such as mycotoxins that render the product unsafe for human and animal consumptions. Fungi, those involved in the deterioration of cereal grains and oil seeds and other agricultural produce have been classified as field fungi, storage fungi and advanced decay fungi depending on the time of their invasion and colonization of grains and whether they occur before or after the harvest.

Field fungi

They invade grain kernel before harvest either while the crop is growing in the field or after it has been cut and swathed but before it is threshed. They require a minimum water activity (a_w) of 0.85 for their growth. The field fungi have been further divided into two groups:
(1) Specialized parasites such as smuts, bunts and ergot and
(2) Facultative Parasites such as *Alternaria, Bipolaris, Curvularia, Cladosporium, Epicoccum, Helminthosporium, Fusarium, Nigrospora* etc.

Some of the well known toxicogenic moulds like *Aspergillus flavus*, and *A. parasiticus* are also known to elaborate aflatoxins in the maize cobs and ground nut pods even in the fields.

In field seeds are known to be colonized by varied types of microorganisms among which many are plant pathogens. About 1500 types of microorganism are known that are associated with seed

borne diseases. These organisms can be grouped in to (1) obligate parasites (2) facultative saprophytes and (3) facultative parasites. In case of obligate parasites like *Sclerospora graminicola,* the causal agent of downy mildew of pearl millet and *Peronoserlerospora sorghii,* the parasitism is a part of their life cycles. These fungi transform the floral primordia into vegetative leafy stimulate or induce sterility in seeds. In facultative parasitic group, many fungi are known to cause considerable damage to seeds of food crops. Other microorganisms related deterioration that occur during anthesis are kernel smut and false smut of paddy caused by *Neovossia horrida* and *Ustilaginoidea virens*; kernel bunt of wheat *Neovossia indica* and ear cockle of wheat caused by *Anguine tritici*. The kernel smut disease of paddy is formed at the field at the time of maturity of the seeds although the infection initiates at the time of anthesis. The diseased seeds show black pustules or streaks bursting through the glumes. In green smut or false smut of rice, the fungus transforms the individual seeds of the penicle into greenish spore balls with a velvety appearance. The moderately infected seeds look dark green while heavily infected grains are completely transformed into pseudomorphs. The infection of kernel bunt of wheat occurs at the flowering stage and the disease becomes evident when seeds have developed. The fungus affect the seeds partially with some tissues of the seeds remaining normal. Normally embryo tissue except in severe cases is not damaged. The ergot fungi (*Claviceps spp.*) infect grass and cereal seeds at anthesis. After infection, a hard mass of mycelium develops in place of seed forming the ergot sclerotium.

Seed infections also occur during seed development through various ways. In cotton seed, infection by *Aspergillus flavus* results in boll penetration by Pink Boll Worms (*Pectinophora gossypiella*). The fungus enters the boll through boll worm exit holes and invades the seeds during boll rotting, finally causing seed deterioration and elaboration of aflatoxin in seeds.

Storage fungi

Storage fungi are those, which do not invade grain before harvest. Most of these fungi belong to genus *Aspergillus and Penicillium.* These are capable to grow at water activity in a range of 0.65 – 0.90 a_W and can grow at a very wide range of temperature. On the basis of temperature and a_w relationships of storage microorganisms they have been classified into following six physiological groups as follows:

(1) Psychrotolerant

They grow in the temperature range of 10^0 – 35 ^{0}C for example *P. brevicompactum, P. chrysogenum, P. cyclopium, P. expansum, P. notatum and P. viridicatum, and Chrysosporium pannorum.* Most of these fungi are common contaminants of stored products in temperate climate.

(2) Mesophilic (2^0 – 50^0C)

These fungi are extremely xerophilic and require less than 0.75 of a_W in order to colonize a substrate. Most species of this group occur in stored agricultural products in tropical climates. Species belonging to *Aspergillus restrictus* group, *Eurotium repens, E. amstelodamy, E. chevaleri, E. rubrum* and *Wallemia sebi* etc. are the major representative of this group. They can grow optimally at 25^0 – 30^0C and hence they can easily be isolated from stored products using a culture medium possessing water activity in the range of 0.65 to 0.75. In dry grain storages these species act as primary invaders of grains and oil seeds.

(3) Lower mesophilic (2^0 - 37^0)
Species belonging to this group are of diversified nature as far as their water requirements are concerned. Some species require water activity in the range of 0.75 – 0.79 and are designated as moderately xerophilic. Species belonging to *Aspergillus versicolor* group come under this category.

The other group of mould which belong to this category is slightly xerophilic for example *P. corylophilum, P. glabrum, P. palitans, P. rugulossum.* Some fungal species and some actinomycetes require more water $\geq$ 0.90 a_W. Many species of *Fusarium, Mucor, Rhizopus, Scopulariopsis, Trichoderma and Trichothecium* come under this category.

(4) Upper mesophilic (5^0 – 50^0C)
Fungi of this group differ from lower mesophilic group in the range of their growth temperature. Similar to mesophic fungi, microorganisms of this group are thermotolerant in nature but can be distinguished in their water requirement. This group includes three distinct classes of microorganisms:

(i) moderately xerophilic (0.75 – 0.79 a_W) comprising *Aspergillus candidus, A. flavus, A. ochraceus, A. terreus, A. temarii, A. niger, A. wentii* etc. Most of the species are well known for toxin production,
(ii) slightly xerophilic (0.80 – 0.89 a_W) comprising some species of *Penicillium* and *Byssochlmys* and a few of *Paecilomyces,*
(iii) hydrophilic (0.90 a_w) that include species of *Absidia, Rhizopus* and *Syncephalastrum.*

(5) Thermophilic (100 – 570C)
This group includes slightly xerophilic fungi as they grow well on the substrates having water activity equivalent to 0.80 – 0.89. Other moulds of this group show hydrophilic tendencies and require high moisture in the medium or water activity more than 0.90. Storage fungi such as *Humicola stellata, Malbranchea sulphurea, Rhizomucor pusillus* and *Sporotrichum thermophillum* etc. represent this group.

(6) Extremely thermophilic (25^0 – 70^0C)
Species belonging to this group require high moisture (more than 0.90 water activity) of the substrate. Some actinomycetes belonging to the genus *Micropolyspora, Saccharomonospora, Streptomyces, Thermomonospora* etc. are the major organisms of this category. They do not play any role in the initial colonization of the stored products but many species of these genera cause serious allergic conditions on the sensitized subjects, particularly the farm workers.

As a matter of fact, the storage microflora of the product concerned depends on the environmental and climate conditions of the area where it is grown and processed. The major source of inoculum of these microorganisms constitute the air, litter in the field, soil particles, dust and debris of the storage area. Under certain conditions when the crop is retained after harvest on farms for 10-30 days before threshing. During this period, contamination may occur from nearby compost piles, decomposing plant residues etc.

Advance decay fungi and other organisms

In stored products microbial succession leads to the development of heat and moisture which favour the growth of most thermophilic fungi and bacteria including actinomycetes. However,

the stage at which these organisms start developing the product is so deteriorated that it can not be consumed by man or even animals. And hence, it can be said that the main cause of cereal grains and oil seeds spoilage occurs in field by the field fungi and other parasitic microorganisms and in storage mainly by the storage fungi.

Storage conditions in relation to development of spoilage

The occurrence of microorganisms in or on the seeds is a natural phenomenon but mere presence of these microorganisms is not harmful until they start growing on the stored material. The moisture content and temperature are the primary factors determining the development of storage fungi in seeds.

Water activity (humidity)

Some storage fungi can start growing at moisture contents in equilibrium with relative humidites equivalent to 65 – 70%. This corresponds to 'water activity' (a_w) equivalent to 0.65 – 0.70 (water activity is the term preferred as criterion for storability of seed in place of the moisture content). Seed stored in a closed room / container produces a vapour pressure in equilibrium with the moisture content of the seed. This pressure is determined by the water activity a_W of material. The relationsship can be expressed as under

$$a_W = RH / 100$$

where a_W is the water activity, RH relative humidity of the atmosphere. The definition of a_W is

$$a_W = P / P_0$$

where P is the vapour pressure of the material at the fixed temperature, P_0 the vapour pressure of the pure water at the same temperature. Growth and development of storage fungi depends on the water activity of the product rather than on its relative moisture content. The minimum humidity that permits growth of some storage fungi is about 65% (a_W 0.65) which corresponds to about 13.5 – 14.0% moisture content for wheat, sorghum and corn, 11.5% for soyabean, 8.5 – 9.0% for sun flower and about 6.0 – 6.5% for ground nuts. At a_W lower than 0.65 the product under storage are considered safe towards microbial attack although product with a_W greater than 0.65 can be attacked by xerophilic fungus *Aspergillus halophilicus*. Fungi belonging to *Apergillus restrictus* and *A. glaucus* groups require a_W equivalent to 0.70 – 0.75 while higher water content (a_W 0.85 > 0.95) favours growth of thermotolerant fungi and *Penicillium spp.*

In addition to water activity, temperature is an equally important limiting factor. The cardinal temperatures for growth of most storage fungi are 0^0 – 5^0C minimum, 30^0 – 33^0C optimum and 50^0 – 55^0C maximum. The classification of storage microorganisms based on their temperature and water activity requirements is given in Table 1 (a). Some storage fungi such as *Aspergillus flavus* and *A. candidus* have a higher optimum temperature that is 40-50^0C and 45-50^0C, respectively. At these temperatures most thermophilic fungi can grow if moisture availability is not limiting. Most field fungi are sensitive to high temperatures and usually disappear under such conditions.

Table 1 (a): Classification of storage microorganisms based on their temperature and water relationships

Physiological classification		Growth limits		Representative species
Temperature	**Water activity**	**°C**	**Min. a_w**	
Psychrotolerant		10 –35	-	*Chrysosporium pannorum*, , *Penicillium brevicompactum, P. expansum, P. notatum, P. cyclopium, P. viridicatum*
Mesophilic	Extremely xerophilic	2 – 50	<0.75	*Eurotium spp., E. repens, E. chevalieri, E. amstelodami, E. intermedium, E. rubrum, Aspergillus restrictus, Wallemia sebi*
Lower mesophilic	Moderately xerophilic	2 – 37	0.75-0.79	*Aspergillus versicolor* group
	Slightly xerophilic	2 – 37	0.80-0.89	*Penicillium corylophilum, P. glabrum P. palitans,, P. rugulosum, P. urticae, P. luteum*
	Hydrophilic	2 – 37	>0.90	*Fusarium culmorum, Stachybotrys atra, F. tricinctum, Trichothecium roseum, Mucor hiemalis, M. racemosus, M. spinosus, Streptomyces antibioticus, S.aureofaciens, S. olivaceus, Rhizopus nigricans, Scopulariopsis brevicaulis*
Upper mesophilic	Moderately xerophilic	5 – 50	0.75-0.89	*Aspergillus candidus, A. niger, A. flavus, A. ochraceus, A. tamarii, A. wentii*
	Slightly xerophilic	5 – 50	0.80-0.89	*Byssochlamys fulva, P. funiculosum, P. nivea, P. islandicum, Paecilomyces variotii, P. piceum, P. citrinum*
	Hydrophilic	5 – 50	>0.90	*Absidia corymbifera, Rhizopus arrhizus, Syncephlastrum racemosum, Streptomyces griseus*
Thermophilic	Slightly xerophilic	10 –57	0.80-0.89	*Aspergillus fumigatus*
	Hydrophilic	10 –57	>0.90	*Humicola stellata, Malbranchea sulfurea, Rhizmucor pusillus, Sporotrichum thermophilum, Thermoascus crustaceus, Streptomyces albus*
Extremely thermophilic	Hydrophilic	25– 70	>0.90	*Humicola lanuginosa, Talaromyces thermophilus, Saccharomonospora viridis, Streptomyces thermoviolaceus, Thermoactinomyces thalpophilus, T. vulgaris, Thermomonospora curvata*

(Adapted from Lacey et. al., 1980)

Thermogenesis

Self heating and spontaneous combustion can be a serious problem of storage leading to complete spoilage of stored products. Ferdinand Cohn was the first who had shown that moulding barley seedlings in an insulated container could heat to 60^0C and that it was probably due to metabolic activity. Later H. Miehe conducted a series of experiments on hay and demonstrated the role of microorganisms in heating during storage. According to him the level of heating caused by the given organism depends upon its own maximum growth temperature. He stated that 'if there are no thermophiles present in self heating hay, the temperature should measure only to the point where mesophiles are no longer able to thrive i.e., 45^0C. If thermophilic forms are present they would take over at 40-45^0C and their metabolism may carry the heating to a higher level 60-70 ^{0}C. A comprehensive account of the water and temperature produced by the activity of different microorganisms in stored products is given in Table 1 (b). During thermogenesis a continuous accumulation of heat due to microbial activity may raise the temperature of stored products to approximated 70^0C. Non biological process may further raise the temperature to ignition under certain conditions. Microbial thermogenesis can be more rapid if thermophilous fungi receive optimum growth conditions under storage. Tropical climate favours early development of thermophilic and thermotolerant fungi on stored products.

Table 1 (b): Storage microorganisms in relation to likely water activity (a_W) and maximum temperature rise during storage

Predominant microorganisms	**Approximate a_w**	**Likely maximum temp. °C**
Aspergillus restrictus	0.7 - 0.6	Ambient
Eurotium spp.	0.8 – 0.9	35
A. versicolor group. Scopulariopsis brevicaulis, Streptomyces griseus	0.9 –0.95	40
A. candidus, Penicillium spp.	0.95	45
Absidia spp., A. nidulans, Streptomyces albus	0.98	50 – 55
A. fumigatus, Rhizomucor pusillus, Malbranchea sulfurea, Humicola lanuginose, Talaromyces thermophilus, Saccharomonospora viridis, Saccharopolyspora rectivirgula (Micropolyspora faeni), Thermoactinomyces spp.	1.0	65

Predisposing conditions

Mechanical damage in seed, cracks, breaks or scratches in the pericarp or seed coat are conditions that favour invasion of storage fungi. The level of damage of seeds may become a major determining factor for early deterioration of seeds. Under humid conditions the moisture content of harvested grain is often found to be 25% or more that require a rapid drying. It may be difficult to obtain quick drying and hence the grain is kept for some time at a too high moisture

content resulting in spontaneous heating and initial invasion of storage fungi. Such grains even after drying when arrive at the storage site may contain a lot of biomass of microorganisms.

Types of spoilage caused by microorganisms in cereal grains and oil seeds

The damage to cereal grains caused by storage fungi due to their growth and metabolic activities can be categorized in 8 categories:

(1) decrease in germinability
(2) fat acidity
(3) discolouration
(4) heating
(5) production of mycotoxins
(6) mustiness
(7) caking and
(8) total decay.

Major types of qualitative losses are discussed here.

Seeds may be attacked by microorganisms in both field and storage, and hence both field and storage fungi damage seeds by production of extra cellular enzymes and secondary metabolites like toxins. Enzymes involved in seed deterioration are primarily cellulases, pectinases, amylases, lipases, proteases and nucleases. Many field and storage fungi produce these enzymes abundantly. These fungi affect seed damage by different strategies which depend on the spectrum of extracellular enzymes produced by them. A shift in extracellular enzymes can be observed during experimentally deteriorated seeds and that the shifting correlates with the species succession which in turn characterize the nature of degradative changes brought about by each species. A rapid increase in concentration of free fatty acids occurs in damaged seeds. This indicates hydrolysis of triglycerides into glycerol and fatty acids by lipases. Similarly, protease production brings about a change in protein banding pattern and amino acid content of sound and deteriorated peanuts. Depending on the nature of seed content attacked by the microbial enzymes a variety of qualitative changes occur in the seed during storage. Major types of qualitative losses are as follows:

Mycotoxins

Many field and storage fungi produce metabolites that are poisonous, some time fatal to man and animals. The production of these toxic substances (mycotoxin) by fungi depends on species or strain of the fungus and on ecological conditions in which the fungus grows, particularly the food source, temperature and relative humidity. Important producers of different mycotoxins among storage fungi are given in Table 1(c). Storage fungus *Aspergillus flavus* and *A. parasiticus* produce a variety of aflatoxins among which aflatoxin B, is the most toxic. *Aspergillus candidus, A. fumigatus, A. ochraceus* are well known producers of ochratoxins*; Penicillium citrinum* produces hepatotoxins and nephrotoxin. All these moulds are responsible for poisoning of cereal grains and oil seeds. It must be noted that grain / oil seeds can be highly toxic without appearing at all mouldy. One of the classical example was an incidence in England in 1960. About 100,000 turkey poults died because of feeding on ground nuts invaded by *Aspergillus flavus.*

Aflotoxin is produced in the highest amounts in groundnuts, wheat and rice but may also be produced on other oil seeds such as cotton seeds, soybean and maize.

Table 1 (c): Mycotoxins and Storage fungi producing them

Mycotoxin	Storage fungi
Aflatoxin	*Aspergillus flavus, A. parasiticus*
Citrinin	*Penicillium citrinum, P. viridicatum*
Ochratoxins	*Aspergillus ochraceus, P. viridicatum, P. cyclopium*
Patulin	*Penicillium patulum, P. expansum, Byssochlamys nivea, Aspergillus clavalus, A. terreus*
Penicillic acid	*Penicillium cyclopium, Aspergillus ochraceus*
Sterigmatocystin	*A. nidulans*
Zearalenone	*Fusarium spp.*

Decrease in germinability

Invasion of the embryos by storage fungi causes decrease in germination percentage of seeds during storage. *Aspergillus flavus, A. candidus , A. repens and A. restrictus* are mainly involved in infection of embryo and causing loss in germinability of seeds. Partially damaged seeds develop as poor seedlings.

Discolouration

Storage fungi may cause discoloration of seed or the embryos (Fig. 1A and Fig. 1B). In trade of grains wheat kernels that have developed dark germs are called 'Sick Wheat'. In Fig. 1C and Fig. 1D healthy and germ damaged wheat grains have been shown. Sick seeds possess poor feeding quality because of loss of germ constituents. They do not germinate and thus considered dead seeds.

Fat acidity

Deterioration of stored grain containing fats and oils may be accompanied by increase in fatty acids (Fat acidity value, FAV). The odours and flavours of these fatty acids make the seeds rancid thus causing loss of quality and poor market value.

Rancidity is an important quality factor in cotton seed oil for human consumption. The problem of rancidity is common in most oil seeds under poor storage conditions. The action of lipase produced by storage fungi degrades oil and fats into free fatty acids. The major fungi involved in such deterioration are *Aspergillus amstelodami, A. candidus, A. flavus* and some *Penicillium* species.

Fig. 1A: Sound sorghum grains

Fig. 1B: Deteriorated sorghum seeds showing discoloration

Fig. 1C: Sound wheat grains

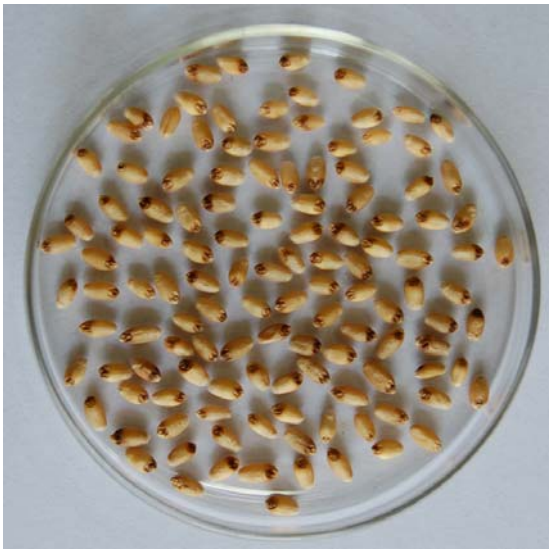

Fig. 1D: Germ damaged wheat grains

Heating and mustiness

All organic matters with moisture level heat during storage because of spontaneous heating associated with metabolic activities, respiration in a grain mass, resulting in a rise in temperature locally. Since grain is a good insulator and provides excellent conditions for development of 'hot spots' these may result in spoilage while most parts of the bulk may not be affected.

Possible control methods

Practically it seems impossible to eradicate the cause of spoilage of grains and other agricultural products but the losses during storage can be minimized by practices such as reducing the initial load of deteriogenic fungi and also through manipulation in storage conditions. The possible control methods are as follows:

Avoidance of foreign materials

The foreign materials which include broken seeds, soil particles, crop residues, animal faces, plant debris, dust etc. can be sorted out from the healthy grains before they are put into storage. The sorting can be done using sieves of different mesh size. The load of storage fungi including other microorganisms can be minimized in the grain through this step besides reducing the chances of growth of insects and opportunistic saprophytic fungi.

Seed testing

Seeds can be tested for the associated microflora and for moisture content in order to treat the seed prior to storage. To obtain complete picture of the seed associated microorganisms the test procedure should enumerate mesophilic xerotolerant and thermophilic fungi. For this, standard methods such as blotter test, Direct plating of seeds / grains on malt extract agar and malt salt agar medium are suggested. Fig. 1E indicates the growth of field fungi on some seeds when blotter test was performed. The growth of storage fungi on and around the seeds plated on malt salt (7.5%w/v) agar medium has been shown in Fig. 1F. For enumeration of bacteria, fungi and actinomyecetes causing respiratory diseases standard aerobiological methods be followed.

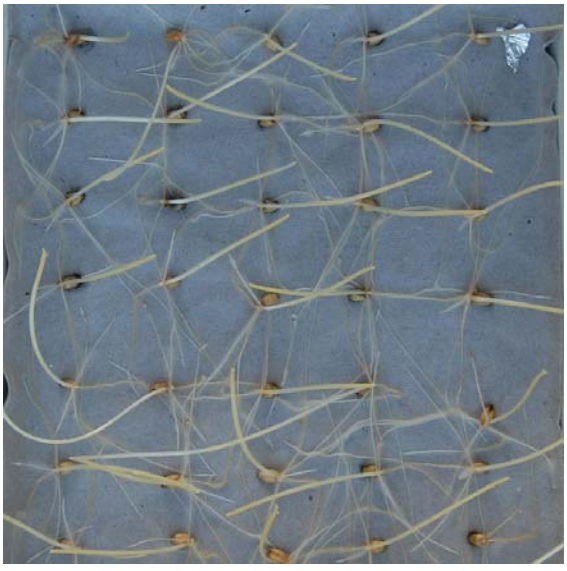

Fig. 1E: Blotter test showing growth of field fungi on some seeds

Fig. 1F: Growth of storage fungi on and around wheat grains on direct plating of seeds on malt salt (7.5%) agar medium

Drying of seeds

Bringing down the water activity of the product to be stored is the most feasible way to have a control over the storage losses. We know that fungi can not grow at lower moisture level i.e. a_w less than 0.65 the moisture content of the oilseeds and grains should be brought down to the safe levels. The lower and safe limit of water content to prevent mould growth and mycotoxin production in cereal grains and oil seeds are in given in Table 1 (d).

Table 1 (d): Safe moisture level for storage of cereal grains and oil seeds

Stored Products	Safe Moisture Level %
Cereal grains	
Wheat	12.0 – 1.25
Barley	12.0 – 12.5
Maize	12.0
Sorghum	12.0 – 12.5
Rice (rough)	11.5
Rice (polished)	13.0
Oil seeds	
Soybeans	9.5 – 10.0
Sunflower seeds	7.5
Ground nuts	6.0

Ventilation

Ventilation of grain during storage with ambient air removes heat and CO_2, introduces O_2, can reduce or introduce moisture depending on the ambient relative humidity and water activity of the seeds. It, thus upsets the equilibrium that had been reached in the grain / seed bulk and consequently prevents the sporulation of moulds although the conditions may be suitable for their growth within the seeds.

Use of sealed silos for storage of moist grain

Moist grain when stored in sealed silos accumulate CO_2 produced by respiration of both grain and its microflora. When CO_2 concentration reaches a level of more than 60% of the intergranular atmosphere or O_2 decreases to less than 1%, the conditions so formed are often found sufficient to prevent fungal growth. However, fungi may differ in their CO_2 tolerance limits.

Use of preservatives and fumigants

Organic acids and their salts have been used as effective preservatives to avoid moulding in damp grains. Amongst these, propionic acid and acetic acid are widely used throughout the world to control mould growth in moist grains. Prevention of heating and aflatoxin production have been successfully achieved by the use of calcium propionate in poultry feed. The main problem in using these chemicals as preservatives is their fungistatic nature. The propagules of some fungi may develop tolerance, which may then dominate the microflora and consequently damage the product in storage. The use of methylbromide to control pests is widely accepted; in addition, this fumigant is also known to suppress development of fungi in grain storage. Several workers have reported substantial decrease in the count of storage fungi in grain and pelleted poultry feed when fumigated with methylbromide. Phosphine is the most toxic fumigant for insects but it does not prevent the growth of microorganisms. However, current environmental concerns have to be taken into account while advocating use of such chemicals in large doses in food and feed.

Microbial Deterioration of Textiles

Biodeterioration is defined as "any undesirable change in the properties of economically important materials caused by vital activities of living organisms". Biodeterioration includes fouling, rotting, decay, loss of strength of the material and can be assimilatory or dissimilatory. In assimilatory deterioration organism utilizes a component of the material as a carbon or energy source resulting in loss of strength. Such damage are caused by microbial enzymes breaking down cellulose in cotton textiles and rotting of woods. While in dissimilatory damage mainly waste products, like pigments or acidic compounds damage or disfigure the materials.

The textile industry is generally sub-divided into three sectors, namely spinning, weaving, and composites or processing. The industry every year faces significant economic losses due to microbial deterioration of textile during processing, storage and transport. Textile material also deteriorates while using it. Deterioration of textile fibres may occur at any of the three sectors mentioned above. Exact quantification of the extent of biodeterioration of textile materials is difficult. It account for great economic loss throughout the world. Textile includes fabric or cloth, furnishing, covers and tarpaulins etc. Textile deterioration is complex phenomenon and results from action of microorganisms, ultraviolet radiations, chemicals or a combination of these factors.

Natural fibers like cotton, linen, jute and rayon are composed principally of cellulose. The fabrics and raw fibers are subjected to microbial deterioration because of their high organic content. Products such as starch, derivatives of protein, fats and oils used in finishing of textiles also promote microbial growth. Textile made from natural fibres is generally more susceptible to biodeterioration than are the synthetic man made fibres. Variety of microorganisms like bacteria, actinomycetes, yeast and moulds are found associated with deterioration of fabrics. Growth of microorganisms on textile may cause aesthetic and structural damage. Aesthetic damage includes pigment discoloration, irregular staining of gray, black and red colour and formation of a biofilm over the surface of textile. Microbial growth, thus results into permanent discoloration of textile (Fig. 2A and Fig. 2B). Whereas structural damage includes reduction of strength of fibre due to

depolymerization. The quality parameters most affected due to infestation of microorganism is its colour and strength.

Fig. 2A: Stains on cotton cloth developed due to fungal growth

Fig. 2B: Stains on synthetic cloth developed due to fungal growth

Microorganisms causing deterioration and their effect on textile

Spores of microorganisms are virtually omni-present; they are present in soil, water and air. Under suitable atmospheric conditions microorganisms infest and proliferate on various textile materials. Fibres of textile along with other auxiliaries serve as substrate for microorganisms. Microorganism may attack the entire textile fibre or may attack only single component of textile. Deterioration process is biochemical, via the action of enzymes secreted by microorganisms. The fungal enzymes such as exoglucanases, glucosidases also expedite hydrolysis and decay of polymeric fibres. Conditions which basically determine infestation of microorganisms are temperature and relative humidity. Fungi deteriorating cellulosic textile are species of *Chaetomium, Fusarium, Aspergillus, Myrothecium, Cladosporium, Alternaria, Stachybotrys, Penicillium* and *Trichoderma* etc. Bacterial deteriogen are species of *Pseudomonas, Arthrobacter, Sarcina* and *Streptomyces* etc. and certain algal species requiring less water. Mild surface growth makes fabric look unattractive because of unwanted pigments produced by fungi, actinomycetes and algae. Heavy infestation by these organisms may result in rotting and breakdown of the fibres and subsequent physical changes such as loss of strength or flexibility.

Animal fibres like silk and wool are mainly less susceptible to attack by most organisms but are susceptible to attack by proteolytic microorganisms. Proteolytic enzymes of microbes hydrolyze disulphide and peptide bonds. Peptides in turn are hydrolyzed to amino acids by peptidases. Microorganisms cause staining of fleece wool. Such damage may affect processing and dyeing of wool. The important bacteria that deteriorate wool are *Bacillus mesentericus, B. vulgatus, B. subtilis, B. cereus* and *B. putrificus*. Species of *Streptomyces, Penicillium* and *Aspergillus* are also potent deteriogens of wool. Microbial infection results in staining, musty odour and rotting that make fibre unfit for use. Use of microbial enzymes, proteases in removal of wool from skins

may also deteriorate wool. Washing of wool with detergents containing proteolytic enzymes further deteriorates wool. Microorganisms such as Bacillus spp. cause staining of wool due to pigmentation. Staining is localized and fibres become rotten and fused into pink colored mass. The tip damage by sunlight and weathering is a prerequisite for the fungal infection. The processed wool is superficially covered by microorganisms and is called 'mildew'. This results in discoloration, tendering and variation in dyeing properties. The damage remain incipient in the beginning, hence early detection of mildew is rarely possible.

Growth conditions differ from organism to organism but in general, temperatures above 24˚C (75˚ F) and RH above 65% provide optimum conditions for growth. The water activity (a_w) is the deciding factor. Water activity promotes fungal spores to germinate and grow while bacteria need more than 0.98 water activity for growth. The extent and type of deterioration is proportional to the number and kind of microorganisms present on textile material.

Factors affecting microbial deterioration

Fiber content

Plant fibres such as cotton, jute and hemp used in textiles are very susceptible to microbial deterioration. Like other organic materials, plant produced fibres also have the tendency to equilibrate the relative humidity of atmosphere which increases the moisture content of the fibres and are susceptible to microbial infestation. They are basically infested by cellulytic fungi and the extent of degradation depends upon quantity of enzyme secreted by microorganism. Rayon is readily attacked by mildew and bacteria whereas synthetic polymers like acrylic, nylon, polyester etc are resistant to microbial decay. Natural fibres obtained from plants and animals are readily deteriorated because they are made from polymerization of sugar, amino acid etc. Pure silk is less susceptible to microbial decomposition if completely degummed. Further products such as starch, oils, dyestuffs etc used in the finishing of textiles may promote microbial growth. White rot fungi and anaerobic bacteria readily degrade dyestuff.

The hydrophobic nature of synthetic fibres is probably most important factor rendering resistant to microbial attack. Synthetic fibres contain chemical bonds, which are not found in nature, and so perhaps microorganisms have not evolved to produce the enzymes necessary to initiate their breakdown. Although the substance of synthetic fibre by itself will not support microbial growth but presence of residual oils, lubricants and low molecular weight contaminants used in the finishing of the textile provide sufficient nutrient for mild growth of microorganisms on surface causing discoloration .

Animal fibres like silk and wool are more resistant to microbial decay. Wool is keratinous protein containing sulphur containing aminoacids, stabilized by intermolecular salt linkages and disulphide bonding. These cross linkage are largely responsible for their resistance to microbial decay. However, wool deterioration occurs under very adverse environmental conditions such as very high relative humidity. Mechanical damage during processing increases its susceptibility to biodeterioration. The impurities such as vegetable oils, soaps and nitrogenous materials are vulnerable to attack by mildew than clean wool.

Humidity and temperature

Factors such as atmospheric humidity and water activity of textile material are most important determinants of biological deterioration of textile. Damp conditions such as high humidity and temperatures above 25˚ C promote infestation of microorganisms on textile. Heavy deterioration occurs at relative humidity above 80% because such condition promotes rapid germination of spores lying on the surface of textile. Temperature is another critical factor for spore germination. Rate of spore germination increases at temperatures above 25˚ C upto 35˚ C. Another factor which affects biodegradation is aeration. Poor air circulation or stagnant air increases the problems. Rapid fluctuations in environmental conditions further increase the chance of microbial deterioration. Under highly moist condition fibres absorb moisture and swells while in dry conditions it dries causing cracking of fibre.

Cleanliness of the textile surface

Alongwith textile the added auxiliaries, dust, dirt, food stains, soilants etc. promote microbial growth. Due to hygroscopic nature of the above, they readily absorb moisture and help in adherence of air borne microbial spores on surface of textile material. In addition they provide nutrients and moisture for their germination. Degummed silk, pure cotton and wool etc. can be stored for a long period of time without getting deteriorated. Clean, dry and impurities free textile are more resistant to degradation.

pH

Extent and type of microbial deterioration of textile also depends on its pH. Textile material vary in their pH, which depends upon its constituents, acidic or alkaline nature of fibres used, auxiliaries added, extent of processing, dyes used in coloration etc. The microorganisms bacteria, fungi, actinomycetes, algae can survive and tolerate both alkaline and acidic conditions. The optimum pH range for number of microorganism lies between pH 4.0 to 9.0. Neutral pH supports maximum number of microorganism and thus maximum biodegradation is found at this pH.

Other factors

Other factors affecting microbial deterioration are fibre quality, duration of storage, amount and nature of foreign matter present on textile etc. Extent of processing and storage conditions are also very important. Microbial deterioration may start at any of the above stage if textile is improperly handled.

Detection of microbial deterioration of textile

Deterioration of textile at first observation is detected by musty odor, discolouration or staining. Textile material suspected to be deteriorated by microorganism can be directly viewed under microscope for confirmation of presence of microorganisms on the surface of textile. Under magnification, fungi look like thread like structure with conidiophores and spore. Bacterial deteriogens are difficult to identify under compound microscope but they are detected by slime produced by them.

Another means of detection is examination of textile under ultraviolet light. Microbial growth fluoresces and appear luminescent in UV light. However, chemicals such as optical brighteners

present in detergents used for washing of textiles and dyes used in coloring textiles also fluoresce in UV light thus limiting the usefulness of this technique.

These can be divided into in-use-condition test, close to the practice tests which includes soil burial test and saturated atmosphere test and *in– vitro* test on agar. The visual growth rating of mold growth, change in weight (g/m2) and tensile strength (kN) are the useful parameters to asses the degree of deterioration.

Types of damage

Microbial deterioration permanently stains or decolorizes textile by producing pigments or metabolizing dyestuff respectively, thus reducing its market as well as aesthetic value. Stains of microbial origin resist washing, alkaline and acid milling and bleaching with hydrogen peroxide. The growth of microorganisms also alters pH of textile material causing permanent change in the colour of dye. Biological activity has direct and detrimental effect on fibre strength. Loss of strength occurs due to depolymerization of fibre by microbial enzymes under humid conditions.

Prevention and control of microbiological deterioration

Textile can be prevented from deterioration by maintaining cool and dry conditions with adequate ventilation during storage and transport. Cleanliness of textile surface also prevents deterioration. Controlled environmental conditions are effective in controlling both insects and microorganisms. Relative humidity below 50 % and temperature of 18˚ C to 24˚ C is most effective in preventing textile deterioration. In order to maintain dry conditions and for removal of moisture, air dehumidifiers can be used. To avoid moisture from textile desiccating bags are commonly used. Bags absorb moisture and keeps the surface of textile dry. Chemical protection is recommended for the textiles likely to be used in adverse conditions like wet or damp conditions.

One of the best method of avoiding microbial deterioration is to use synthetic materials which are inherently resistant to attack. An alternative strategy is to apply antimicrobial chemicals known as biocides which are normally incorporated into the finished textile products. Commonly used biocides in the textile industry include organo–copper, organo–tin compounds and chlorinated phenols. The main disadvantage of such treatments is that they impart yellow - green colour to treated materials.

Mildew formation on the wool can be prevented by storing them in well-ventilated surroundings in a cool, dry atmosphere. Some products that are commonly used to prevent mildew on wool are based on dichloropen (5,5-dichloro 2,2- dihydroxy diphenylmethane), quaternary ammonium compounds [n- alkyl dimethyl (ethylbenzyl) ammonium chloride], chlorinated phenols and fatty esters of chlorinated phenols and organofin compounds.

Treatments

Method to be applied for treatment of biodeteriorating textile depends upon extent of microbial infestation. Mechanical treatment like holding the textile material under vacuum removes most of the active growth. After the removal of vacuum active growth forms decrease. Gentle air

circulation in the form of a dry, cool air flow is also effective to accomplish this. Dry cleaning is another treatment option that kills microorganism. In case of heavy deterioration, use of biocides is recommended and in extreme cases textile should be sterilized or incinerated to prevent spread of microorganisms.

Microbial Degradation of Wood

Wood is structurally and chemically complex material of living or dead trees. The major constituents of wood are polysaccharides (66-80%) and lignin (19-25%). Angiospemic wood has high content of pentosans while gymnosperm wood in characterized by its high contents of hexosans and lignins (Table 2a). Several mono, di- and oligosaccharides are also found in non-polymerized form in wood while, cellulose exists partly as crystalline and non-crystalline form making microfibrils. The space between fibrils is filled with hemicelluloses and lignin. These principal substrates of wood are susceptible to attack of microorganisms.

Table 2a: Composition of wood

	Softwoods	**Hardwoods**
Polysaccharides	66-72	74-80
Lignin	24-30	19-25
Extractives	2-9	2-5
Ash	0.2-0.6	0.2-0.6

Microbiological degradation of wood

The woods under uninjured bark of healthy trees are generally free from microorganisms. Microbial degradation of wood can cause undesirable changes in colour, lustre, texture, odor, grains and structural integrity of wood thus causing huge loss by destroying wood in forest and wood used in buildings, houses etc. The disease causing microbes alone are responsible for losses amounting more than 65% of the wood volume in forest. Microbes that degrade wood produce extracellular enzymes that breakdown woody cell wall. Different kinds of microorganisms are involved in degradation of wood. Growth characteristics of microorganisms in the wood and type of degradative system results in different decay patterns. During the process of degradation, substrate changes continuously and results in successive change in the microbial population.

Microorganisms found to colonize and degrade wood include (a) Basidiomycetes (b) Ascomycetes (c) Phycomycetes (d) Deuteromycetes and (e) Bacteria. The chemical and structural effects of the attack on wood and resulting decay patterns can be correlated with these groups of microorganisms. The greatest loss of wood, however, are due to the basidiomycetous members of families *Polysporaceae*, *Thelophoraceae* and *Agaricaceae*. Many a times interection between insect and microorganism plays a crucial role in wood decay. Mechanical destruction by insects renders wood exposed for action of microbes. Some insects are ectosymbionts with fungi and some termites prefer to colonize on wood attacked by fungi.

Many wood degrading fungi feed exclusively on intracellular contents whilst others continue to decompose components of cell wall as well. This is mainly dependent upon the hydrolytic efficiency based on enzyme secretion. Wood decaying microorganisms can therefore be grouped broadly into:

(I) Microorganisms utilizing cell contents- (but not degrading lignified cell walls)
   a. Moulds
   b. Blue stain fungi.

(II) Microorganisms that breakdown lignified cell walls:
   (i) With limited degradation ability
      (c) Bacteria (d) Soft rot fungi
   (ii) Microorganisms with high degradation ability:
      (e) Brown rot fungi (f) White rot fungi.

Wood decaying fungi

Moulds belonging to Ascomycetes and Deuteromycetes mainly feed on dead cell contents and their hyphae accumulate in ray parenchyma cells or in cell lumina after penetrating pit-tori. The infestation resembles incipient soft rot. Many members of these classes cause discoloration of wood due to their pigmented hyphae (Blue stain fungi). These are common in softwoods but hardwoods are no exception. Initially their hyphae grow in ray parenchyma cells occurring only rarely in trachieds. In hardwoods the hyphae are also found in fibres and trachieds around the rays. *Alternaria*, *Bispora, Chloridium* and *Bhialophora spp*. are some common bluing fungi. Important wood deteriorating fungi and their effects on wood is given in Table 2 (b).

Soft rot

Fungal attack on the lignified cell walls characterized by a soft decayed surface of wood in contact with excessive moisture is called as soft rot. This type of degradation is caused by fungi belonging to Ascomycetes and Deuteromycetes that can cause limited enzymatic degradation of wood. These fungi principally attack carbohydrates mainly cellulose while lignin is modified or degraded to lesser extent. Characteristically, the hyphae penetrates into the cell wall and develop within S_2 layer causing regular and rhomboidal or long cylindrical cavities with conically tapered ends. In early stages, soft rot fungi primarily penetrates through pits and often cause exhaustion of storage materials in the cells, borehole formation begins both on radial or tangential walls. In hardwood, the fungus may also attack the cell walls of the lumen, causing corrosion and subsequent lysis of S_3 and S_2 layers (Fig. 3A). In softwood S_3 layer being resistant, the principal location of soft rot cavities is the S_2 layer. Before invasion of the tracheid cell walls, the longitudinal hyphae in cell lumina branch laterally and produce fine, hyaline, perforation hyphae which grow horizontally through the S_3 layer into S_2 layer. Later, the hyphae branch into T-shape giving two branches parallel to microfibrils, which grow at the same rate in opposite directions and continue to follow spiral fribrillar structure. Cavity formation is closely related to hyphal growth due to proximity of hydrolases. Apart from fungal species, the pattern of cavities is also influenced by physiological factors e.g. temperature, water content etc. Finally, the entire secondary wall becomes perforated by confluent cavities leaving a collapsible middle lamella, thus causing severe loss in strength. About 70 species of the genera *Chaetomium, Sordaria, Peziza, Conithyrium, Cytospora, Phoma, Pestalotia, Chephalosporium, Monosporium, Penicillium, Alternaria, Bispora, Chloridium, Phialophora, Stemphylium, Torula, Graphium,*

Stilbella, Doratomyces and Fusarium are capable of causing soft rot in different kinds of wood. The attack of exclusive soft-rot fungi is evidenced under extreme moisture conditions. These being the pioneers on newly exposed wood are followed by other group of fungi. A slow attack advancing inward after destruction of outer wood layers, exclusive degradation of polysaccharides (lignin remains intact), formation of chains of cavities in the S_2 layers of tracheids and fibres are identifying features of soft-rot.

Table 2(b): Wood deteriorating fungi and their effect on wood

Basidiomycotina	
Amyloporia xantha	Brown rot
Serpula lacrimans	Brown rot 'dry rot' in interior timbers in temperate climates
Armillaria spp.	While rot, tree pathogen, decay damp felled timber, spreads by rhizomorphs
Coriolus versicolor	White rot, decay of felled hardwoods
Pleurotus ostreatus	White rot, decay of stored wood pathogen of deciduous trees
Lentinus lepideus	Brown rot, decays wood in contact with the soil
Phlebia gigantea	Brown rot, decays felled pine logs
Coniphora puteana	Brown rot, decays building timbers and wood in ground contact
Stereum sanguinolentum	Brown rot, tree pathogen, decays dead stumps, logs of conifers
Gloeophyllum trabeum	Brown rot, prevalent in exterior woodwork
Ascomycotina:	
Chaetomium globosum	Soft rot decays wood in ground contact
Ceratocystis pilifera	Sap stain, blue stain and soft rot in wood chip
Deuterromycotina:	
Phialophora fastigiata	Soft rot, decays wood chips, stain wood
Trichoderma viride	Surface mould, early coloniser of freshly cut timber and wood inground ground contact
Cladosporium spp.	Surface mould, ubiquitous, early coloniser of timber
Penicillium spp.	Surface mould, ubiquitous, early coloniser of timber
Aureobasidium pullulans	Sap stain, common blue stain fungus

Brown rot

This kind of wood degradation is caused via polysaccharides and is characterized by rapid enzymatic hydrolysis of polysaccharides (cellulose) of the cell wall while lignin remains intact. Considerable loss in the wood strength occurs very early in the decay process, often before decay is visually evident. The breakdown of cellulose occurs in a diffused manner through the entire cell wall. Fig. 3B shows severely degraded cell walls caused by a brown rot fungus. Initially the

fungal hyphae are concentrated in rays and after depletion of nutrient they are spread into tracheids by destroying the pit-tori and penetration of cell wall. The perforations between cell walls expand in the later stages, leaving large openings between cells. Another character of brown-rot degradation is random cell wall decomposition occuring in patches. The cellulose degradation is well advanced in a group of cells while adjacent cells are slightly affected. This gives a cubically cracked appearance to brown-rot group including genus *Polyporus , Coniphora, Coriolellus* and *Serpulla.* Brown-rot fungi cause decay in living trees, timber and wood used in buildings resulting in large losses of strength. This can be hazardous since wood may fail in service. Brown- rot is also referred as dry-rot.

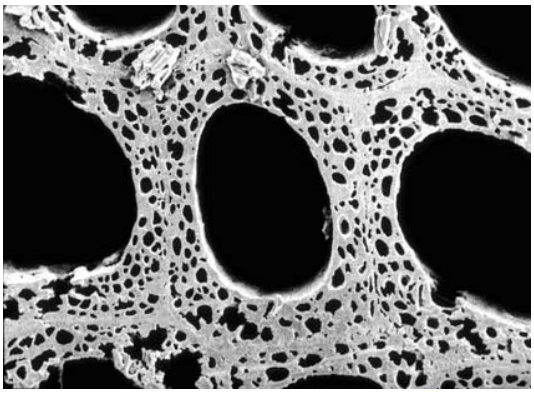

Fig. 3A: Scanning electron micrograph of a section from the wood showing soft rot. Fungus caused cavities in cell wall of wood cells

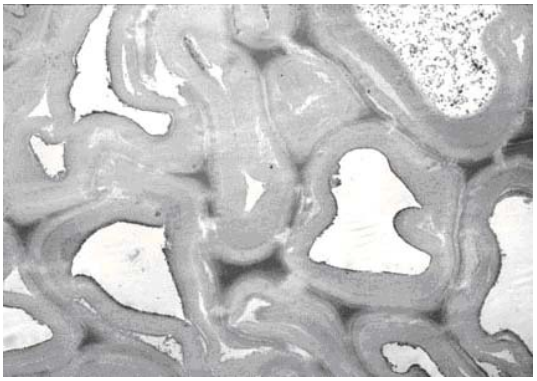

Fig. 3B: Transmission electron micrograph showing degraded cell walls caused by a brown rot fungus

White rot

White-rot causing fungi degrade all cell wall components including lignin and belong mainly to Basidiomycetes. They have exceptional ability to degrade and utilize lignin. In hardwoods, the hyphae of white-rot fungi first colonize the rays and vessels extensively and enter the fibres in later stages. The fungus attacks lignified tissues from the ray cells and vessels or by horizontal penetration of the cell walls. Cell wall penetration is supported by hydrolases liberated at the hyphal tips and lateral surfaces resulting in to wide perforation at later stages. Deepening and coalescing, lysis furrows are produced along the hyphae. In advanced stages, cavities inside the secondary cell walls are also seen. Characteristically, white-rot attack results in gradual thinning of the cell walls both in hardwoods and softwoods. Fig. 3C shows a cross section of an Oak tree with white rot. The fungus has attacked all cell wall components and decayed the wood. Chemical and micromorphological analyses suggest extensive degradation of cell wall component and subsequent utilization of products by white-rot fungi. They are common parasites of heartwood in living trees and are aggressive decomposers of woody debris in forest.

Bacteria

Wood degradation by bacteria is a slow process but it makes a significant part in continuous decomposition process of the wood. Bacteria mainly attack parenchyma cell of the rays and accumulate in resin ducts and parenchymatous tissues. The walls of parenchyma cells may also be attacked and destroyed, however, tracheids and fibres are usually not affected. Bacterial attack has been found to establish on wood after its long and constant exposure to high moisture. It appears as random patches on the surface and inside the wood it develops very slowly and often found as mixed infection with fungi.

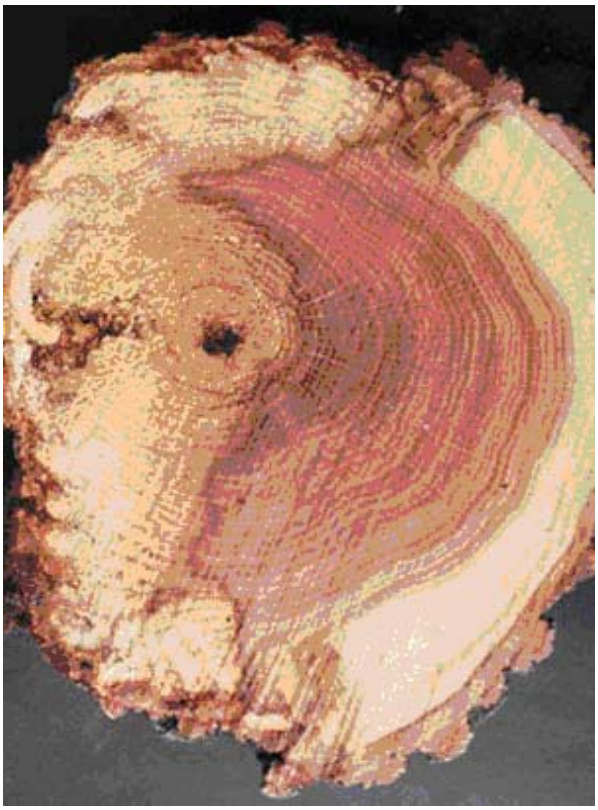

Fig. 3C: Cross section of an Oak tree showing white rot

Mechanism of wood deterioration

Microbial susceptibility of wood depends on various characteristics of the lignocellulosic materials. About 40-50% of the dry matter of woody cell walls consist of cellulose, and remaining consists of hemicellulose and lignin, whose types and amount vary within the timber groups. In hardwoods hemicelluloses are mainly xylans, lignin accounts for 21% (European beach). In soft woods mannans dominate, lignin amounts to about 27% (Scot pine). With respect to microbial nutrients, other wood components such as pectin, starch, sugar, proteins, minerals and accessory compounds are of lesser importance. Among these, carbohydrates and proteins generally enhance microbial activity whereas accessory compounds mainly inhibit. Among the 3 main cell wall components cellulose, hemicellulose and lignin, the hemicellulose are most susceptible to microbial attack.

Degradation of hemicelluloses

As an example, European beech wood consists of O-acetyl-4 -O-methylglucuronoxylan. It is a linear chain of about 200 xylopyranose units linked by β (1-4) glycosidic bonds. About 60 - 70 % xylose units have acetyl groups and on an average about 1/10 of its xylanopyranose units have a side chains of 4-O-methylglucuronic acid. Four groups of xylanolytic enzymes are involved in the hydrolysis of 4-O-methlyglucuronoxylan. Endo-β-1,4-xylanases split the xylan polymer randomly into monomeric, dimeric and trimeric xylan fragments of which some are 4-O-methylglucuronoxylotriose. Another enzyme named as exo-β-1,4-xylanosidases cut xylose from the non-reducing end of oligomers. Enzyme α-glucuronidase splits off 4-O-methylglucuronic acid and acetylxylanesterase cuts the acetyl groups and accordingly total enzymic hydrolysis can be achieved. Enzymic hydrolysis of hardwood xylan has been given in Fig. 3D.

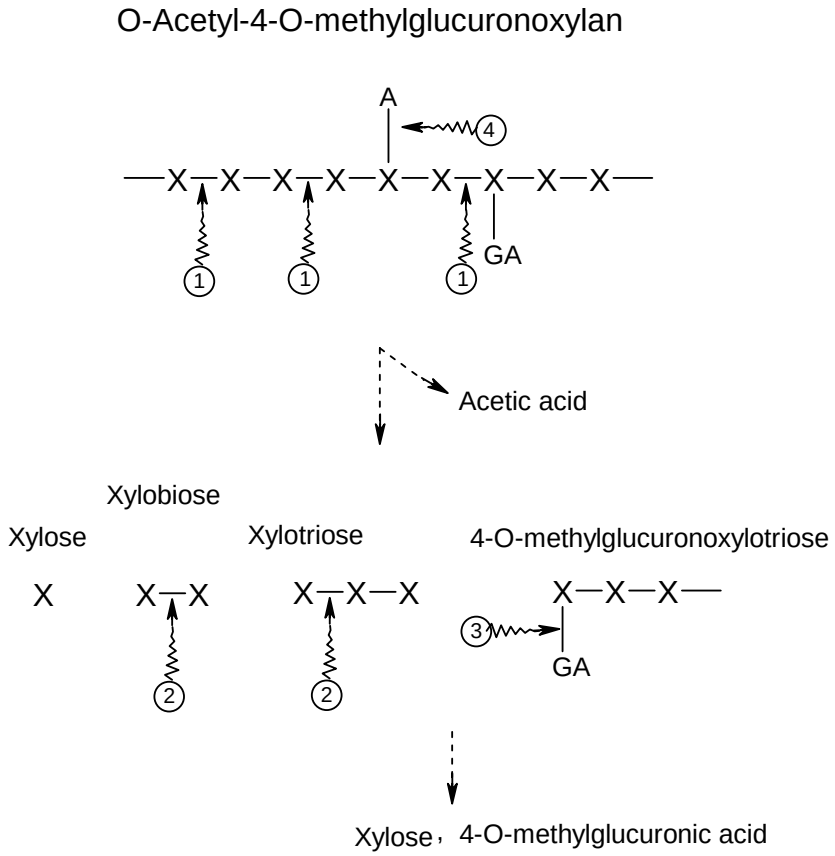

Fig. 3D: Enzymic hydrolysis of hardwood xylan. – X, xylose unit; A, acetyl group; GA, 4-O-methylglucuronic acid; — , β-1-4-glycosidic linkage; ①, xylanase; ②, β-xylosidase; ③, α-glucuronidase; ④, acetyl-xylanesterase; ↟ , site of enzymic attack

Degradation of lignin

Lignin degradation has been investigated mainly with white rot basidiomycetous fungus, *Phanerochaete* c*hrysosporium* (Imperfect state - *Sporotrichum pulverulentum*). The lignin substrates are bound to fungal mycelium and enzymes act on the surface of the polymeric lignin. The important enzyme reaction comprise C_α-oxidation and cleavage between C_α and C_β by an

extracellularly acting H_2O_2 requiring oxigenase. Demethylations are caused by mono-oxigenases and ortho-ring fission by dioxigenases. Further enzymes, such as laccase and cello,iose, quinone oxidoreductase are involved indirectly but necessarily.

Cellulose degradation

Degradation of cellulose is caused by various kinds of microorganisms including fungi, bacteria and actinomycetes which attack cellulose derivatives. Only a few are known to attack cotton and crystalline cellulose. Like hemicelluloses, cellulose in woody cell walls is generally degraded only by wood rotters and by other microorganisms only after substrate modification / pretreatment.

In wood, cellulose depolymerization by brown rot fungi requires a pre-celluloytic phase which makes cellulose fibers accessible to cellulases, this agent may be Fe^{2+} / H_2O_2. Removing hemicelluloses also increases cellulose degradation. A scheme of enzymic hydrolysis of cellulose is given in Fig. 3E which indicates the involvement of numerous cellulolytic enzymes in cellulose degradation. These enzymes act synergistically and depend on the primary attack by endo-cellulase of carboxymethylcellulase (CMCase) type on the native cellulose molecule producing crystalline cellulose. These are further attacked by *exo*- and *endo*-cellulases of avicelase type. Accordingly a number of cellobiose and cellodextrins are produced which are further attacked by *endo*-cellulase and *exo*- cellulase (CMCase type) and cellobiase (β-glucosidase) enzymes resulting in formation of glucose (complete hydrolysis of native cellulose).

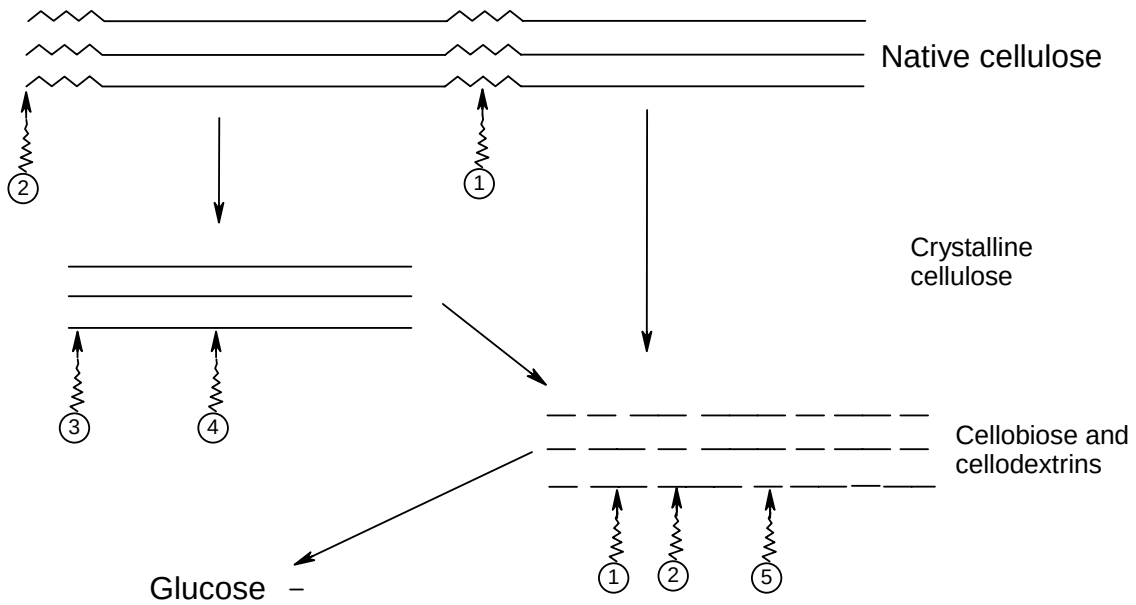

Fig. 3E: Enzymic hydrolysis of native cellulose. ①, *endo*-cellulase of carboxymethyl-cellulase type;②, *exo*-cellulase of carboxymethylcellulase type; ③, *exo*-cellulase of avicelase type; ④, *endo*-cellulase of avicelase type; ⑤, β-glucosidase (cellobiase); , site of enzymic attack

Control of wood deterioration

Several factors can be manipulated artificially for control and prevention of wood decay. Reduction in moisture content of wood (below 20%) and storing wood at relative humidity levels below 60% or storage under high osmotic pressure (using high concentrations of salt or sugar) and significant reduction in pH by allowing natural acid fermentation can be used as preventive measures to control microbial spoilage of wood. Further, the wood can be sterilized by heat or radiation and can be coated with impervious or biocidal coating. Logs are often stored in ponds to protect against fungal attack, prevent drying and splitting of ends. During shipment the moisture content should be reduced to safe moisture level. An ideal wood preservative (fixed or leachable) should be sufficiently toxic at low concentrations, capable of penetrating timber, non-deteriorating for wood, non-flammable and cost effective. These can be water based, tar oils, organic solvent based or emulsions of organic preservatives. A fixed preservative reacts with cell walls of wood and converts the active ingredient to an insoluble compound which is resistant to leaching. For example chromate, copper and arsenic (CCA) treated wood leads to their impregnation, which provides prolonged protection. Leachable preservatives (eg. sodium octoborate) do not become fixed and are applied by diffusion process. Woods under prolonged exposure of weather (telephone poles, railway sleepers, fence posts etc.) can be preserved by creosote distillate from coal tar.

Some commonly used preservatives are copper and zinc naphthamates, pentachlorophenol, tri-n-butyl tin oxide and methyl bisthiocyanate. In addition gamma - BHC, an insectiside, is also used to provide protection against insects. Preservatives can be applied for *in-situ* protection by brushing, spraying or drenching. On the other hand, wood is immersed in preservatives for periods ranging upto one week depending upon thickness of material and desired penetration. For impregnation, timber is pushed into steel cylinder containing preservative and closed with a pressure door. The cylinder is evacuated and the preservative is forced into the pores of wood by pressure.

Corrosion of Metals

Corrosion is deterioration of useful properties in metals /materials due to reactions with its environment. It may be caused due to natural tendency of refined metals to return to their natural state, mainly oxides. Microbial corrosion is the deterioration of metals where microorganisms (algae, fungi & bacteria) are involved directly or indirectly. It can be applied to both metals and non metals (such as organic paint coating, plastic fittings, linings etc.) in both the presence and absence of oxygen. Metallic corrosion is a common phenomenon causing a heavy loss to ships and marine structures; water supply and distribution systems; air craft fuel systems, waste water facilities; cooling water systems and power generation; petrochemical and process industries, and paper mills. Thus, the corrosion of material may result in great economic impact. The US Federal Highway Administration, in its release “Corrosion cost and Preventive Strategies in the United States,” (2002) worked out direct costs associated with metallic corrosion in nearly every U.S. industrial sector. The study showed that annual estimated direct cost of corrosion in the US is approximately 276 billion dollars.

Microbial assisted corrosion (MAC) in a well recognized problem in many industries. Microorganism such as bacteria, fungi and algae under certain conditions can thrive and

accelerate the corrosion of many metals / alloys, for example iron, copper, aluminium, lead and stainless steel even in otherwise benign environments.

Microorganism causing corrosion

Microorganism such as bacteria, fungi and algae under certain condition can thrive and enhance the corrosion of metals and products made of them by their mere physical presence and direct involvement in the corrosion reaction or by accelerating the corrosion process by the production of certain extracellularly produced metabolic products resulting in the formation of acids (e.g. sulphuric, carbonic or other organic acids), hydrogen sulfide and ammonia. The microorganisms may impart corrosion process singularly or combinedly with other mutualistic organism in both aerobic and anaerobic conditions.

Aerobic conditions

Corrosion caused by microorganism in aerobic conditions in often the result of the production of a corrosive metabolites, usually an acid, either mineral or organic. A wide variety of microorganism are involved in aerobic corrosion process but the sulfur oxidizing bacteria (SOB) are the commonest and most important.

Sulfur oxidizing bacteria (SOB) are aerobic microorganisms, slime formers and can produce sulphuric acid from the oxidation of sulphur or sulphide. Acidity may increase (resulting in a pH of 1.0 or lower) as a consequence of rapid attack on steel as shown below:

$$2FeS_2 + 7O_2 + 2H_2O \longrightarrow FeSO_4 + 2H_2SO_4$$

The oxidation of sulfur, thiosulfate, sulfite and several polythionates to sulfate with the simultaneous production of strong acid is carried out by certain species of the genus *Thiobacillus*. The most common species which are involved in corrosion process are *Thiobacillus thiooxidans, T. thioparus* and *T. concretivorus*. All these species are widely distributed in nature and are found in soils, muds and water.

Ferrobacillus ferrooxidans directly oxidizes iron into its oxides or hydroxides or oxidizes ferrous iron in solution to the ferric state and effect the precipitation of ferric hydroxides (e.g. *Gallionella ferruginea*). Precipitated ferric hydroxide deposits on the surface of the metal forming hard excrescences known as tubercles' which are firmly adherent to the metal surface and set up an oxygen concentration cell, leading to accelerated corrosion at the bottom of the 'tubercle' by the growth of sulfate reducing bacteria (SRB). These bacteria proliferate well in anaerobic region at the base of tubercle and add their own contribution to the total corrosion.

Anaerobic conditions

Anaerobic conditions favour the growth of sulfate reducing bacteria (SRB). These bacteria are mostly obligate anaerobe i.e. they fail to grow in the presence of even traces of oxygen. However, they can survive under aerobic conditions and are reactivated when conditions become

favourable. The best known examples of SRB are *Desulfovibrio spp*. and *Desulfotomaculum spp.* Generally these bacteria derive their carbon from low molecular weight compounds such as lactate and formate by lactic acid fermentation. They also posses enzyme dehydrogenase and can obtain their energy from the oxidation of molecular hydrogen.

Four species of the genus *Desulfovibrio* viz. *D. salixigen, D. vulgaris, D. desulfuricans* and *D. africanus* influence corrosion. The first one has on absolute requirement of about 2.5 % NaCl while *D. vulgaris* and *D. desulfuricans* may grow in both fresh and salt water conditions. Two species of *Desulfotomaculum* which cause corrosion are *Desulfotomaculum orientis* and *Desulfotomaculum nigrificans* (*Clostridium nigrificans*). The functional pH range of *Desulfotomaculum* is between 5.5 – 9.0 (optimum 7.2). They are thermophilic with an optimum temperature of 55^0 C or even up to 70 ^{0}C but are also adapted to a lower temperature range from 30°-37^0C. The *Desulfovibrio* grows well at temperature between 25^o – 45 ^{0}C with the same range of pH i.e. 5.5 - 9.0.

The corrosion process under anaerobic condition is much more complex than in conventional cases. The vigorous growth of sulfate reducers demands reducing conditions and a redox potential of around 100 mV is necessary if the bacteria are to thrive. In the absence of any interfering influences, however, even a marginal growth will produce sufficient H_2S (Hydrogen Sulphide) to reduce the redox potential to a more favourable value, so that growth once begun accelerates with time.

Mechanism of Microbiologically Influenced Corrosion (MIC)

Living organism influence anodic and cathodic reactions and create corrosive conditions. Using the term in its broadest sense, the microbes may cause corrosion by:

a) Chemical attack on metals, concrete and other materials through the by products of microbial life namely, acids, hydrogen sulfide and ammonia.
b) Microbial attack on organic materials (e.g. Organic paint coatings, plastic fillings and linings) conversion of some natural inorganic materials (e.g. sulphur) or degradation of inhibitors.
c) Depassivation of metal surfaces and induction of corrosion cells.
d) Depolarization of cathodic reaction (Hydrogen reaction), whereby the cathodic rate limiting step is accelerated by microbiological action.
e) Attack on metal by a process in which microbes and metal cooperate to sustain the corrosion reactions.
f) Attack due to combination of bacteria, which are synergizing the process of corrosion that relates to under deposit acid attack, whereby corrosive attack is accelerated by final acidic products of the MIC community metabolism principally short chain fatty acids.
g) Fixing of anodic reaction sites – In this process microbial colonies on the surface of metals lead to the formation of corrosion pits driven by the activity of microorganisms.
h) Formation of occluded surface cells, whereby microorganisms form 'patchy' surface colonies. Sticky polymers attract and aggregate biological species to

produce crevices (narrow openings, crack) and concentration cells, the basis of accelerated attack.

Types of corrosion

There are three major types of corrosion on metals which are caused or accelerated by the microorganisms.

1. Sulfide stress cracking (SSC)

Sulphide stress cracking (SSC) or sulphide stress corrosion cracking (SSCC) is a special state of corrosion, a form of stress corrosion cracking susceptible alloys where steel reacts with hydrogen sulfide, forming metal sulfides and elementary hydrogen which gets absorbed in metal and leads to hydrogen embrittlement. This type of corrosion occurs heavily at temperatures around 800C. SSC has special importance in gas and oil industries. Anaerobic corrosion is evident as layers of metal sulfides and hydrogen sulfide smell.

2. Pitting corrosion

Pitting is a form of extremely localized corrosion that leads to the creation of small pits or holes on the surface of metals. Pitting can be initiated by a small surface defect, scratch or a local change in composition or a damage to protective coating. Alloys such as stainless steel, nickel and aluminium alloys where corrosion resistance is caused by a passivation layer, are most susceptible to pitting. The presence of chlorides e.g., sea water, significantly aggravates the conditions for formation and growth of pits through an autocatalytic process.

The driving power for pitting corrosion is the lack of oxygen around a small area. This area becomes anodic while the area with excess of oxygen becomes cathodic leading to localized galvanic corrosion. The corrosion area buried in metal mass causes localized lack of oxygen. The associated anaerobic microorganisms lead the acidic conditions which accelerates the process further. A single pit in a critical point can cause a great deal of damage (e.g. explosion in Guadajara in Maxico, April 22, 1992)

3. Graphitic corrosion

The cast iron is damaged when bacteria consume iron. In graphitization iron is converted to its sulfite, leaving a matrix of low mechanical strength.

Recognition of corrosion

Microbial corrosion may appear like pitting corrosion. Anaerobic corrosion is evident as layers of metal sulfide and hydrogen sulfide smell. While corrosion of cast iron can be recognized as a graphite matrix with low mechanical strength.

Protection possibilities

The microbial corrosion can be prevented to minimize large scale losses. To combat microbial corrosion following steps may be considered:

(i) Prevent entry of microorganisms.
(ii) Choose materials, which are possessing resistance to microbial corrosion.
(iii) Application of heat and radiation wherever appropriate to kill or retard the growth of microorganisms.
(iv) Use biocides suitable for cooling waters and oil - water systems.
(v) For buried structures coatings and cathodic protection is applied.

A number of corrosion inhibitors (chemical compound that, when added in small concentration, stops or slows down the corrosion of metals and alloys) are in use to prevent microbial corrosion of metals. A good corrosion inhibitor may give ≥ 95% inhibition at concentration of 80 ppm and 90% inhibition of 40 ppm. These inhibitors form a passivation layer (a thin film on the surface of the material that stops access of the corrosive substance to the metal). These inhibitors are inhibiting either the oxidation or reduction part of the redox corrosion system (anodic or cathodic inhibitors) or scavenging the dissolved oxygen.

Formation of passivation layer

A thin film of corrosion product (metal oxides) forms on a metal surface spontaneously which acts as a barrier for further oxidation. When this layer stops growing at less than a micrometer thick under the conditions in which the material is being in use, the phenomenon is known as Passivation. This effect in some sense is a property of the material to form a kinetic barrier that stops access of the corrosive substance to the metal. The passivation reaction is often found to be rapid unless and until an impermeable layer forms. Passivation in air and water at moderate pH can be seen in materials like aluminium, stainless steel, titanium and silicon. However, the rust form on the surface of iron usually grows much thicker and hence it is not considered passivation and the oxide layer is not protective, any way.

Applied coatings

Applied coatings such as plating, painting and the application of enamel are the most common anti-corrosion treatments. They work by providing a barrier of corrosion resistance material between the damaging environment and the structural material. For the best results the plating should be done with a more active metal such as zinc or cadmium on steel.

Reactive coatings

In a controlled environment such a recirculating systems, corrosion inhibitors can be added to it. These inhibitors form an electrically insulating and / or chemically impermeable coating on exposed metal surface to suppress electrochemical reactions. Chemical that inhibits corrosion include some of the salts in hard water, chromate, phosphates and a wide variety of chemicals resembling surfactants (i.e., long chain organic molecules with ionic end group).

Anodic and cathodic inhibitors

The corrosion can also be prevented by inhibiting the oxidation or reduction part of the redox corrosion system by use of anodic and cathodic inhibitors. An example of an anodic inhibitor is chromate which forms a passivation film on aluminium and steel surfaces. Since chromates are toxic to humans (carcinogenic) the use of chromate to protect metal is limited. Nitrite is another anodic inhibitor, but at low concentration they can aggregate pitting corrosion.

An example of cathodic inhibitor is zinc oxide, retarding the corrosion by inhibiting the reduction of water to hydrogen gas. In the absence of oxidants such as oxygen, the rate of corrosion can be controlled by the rate of reduction of water such as in closed circulating domestic central heating system. One example of cathodic inhibitor is presence of volatile amine in streams, these are used in the boilers used to drive turbines to protect the pipe work in which condensed water passes. The amine increases the pH so making proton reduction less favourable. An inhibitor may also act as both cathodic and anodic mixed inhibitor. For instance an amine beside its cathodic inhibition properties and forming a protective film on the steel surface will be acting as an anodic inhibitor.

Antiseptics and other corrosion inhibitors

Antiseptics such as benzalkonium chloride is commonly used to inhibit microbial corrosion. Corrosion inhibitors are commonly added to coolants, fuel, hydraulic fluids, boiler water and many other fluids used in the industries. Some corrosion inhibitors are hexamine, phenylenediamine, dimethylethanolamine, sodium nitrite, cinnamaldehyde, condensation products of aldehydes and amines, chromates, phosphates, hydrazine, ascorbic acids.

The suitability of the inhibitor for the task depends on the material of system, temperature and nature of inhibitor.

Bioleaching of Metals

Lattice locked metal atoms are extracted by process of leaching of mineral ores by way of using bacteria is called bioleaching or biomining. On the other hand it is an eco- friendly way to get rid of ion which keep forming acids in the form of acid mine water. Bacterial leaching is used to recover various metals from a variety of ores. Metals whose recovery can be enhanced by bioleaching are copper, uranium, cobalt, nickel, zinc, lead and gold. Bioleaching technology is useful in minimizing some problems currently faced by the mining industries. One major problem is the continuing depletion of high-grade ores (mineral deposits) and the consequent need to mine at greater depth. The depletion of high grade ores are also forcing the mining industries to make use of low grade ores for metal recovery which by way of traditional methods is non profitable and requires bringing vast bulk of waste rock to the surface. The scope of bioleaching is gaining strength owing to depletion of high-grade ore minerals or increasing cost of their exploitation from deeper levels in the earth. The existing technology is based on cut off grades of metallurgical units. Therefore, a need is felt to enhance the percentage of metals by bacterial leaching which otherwise goes in waste due to its "lattice locked" character. In this way microbial leaching from low-grade ores require less energy and has significant environmental benefits. Another, major problem in some mining industries is of uncontrolled release of metals and acids, that is allowed to act on the waste in mine dumps and tailing dams. Such controlled leaching can result in the recovery of metal from the sites and prevent environmental pollution.

Microorganisms involved in metal leaching

Mine dumps and mine refuse piles provide a highly acidic and high temperature conditions which are not likely to favor the growth of most microorganisms. However, several chemolithotrophs that are adopted for living in high temperature and acidic conditions and can obtain energy by oxidizing inorganic substances are playing very important role in leaching of metals from ores and formation of acid mine drainage. Some of the important bioleaching microorganisms are *Thiobacillus ferrooxidans, T. thioxidans, Leptospirillum ferroxidans* and some species of *Sulfolobus.*

Thiobacillus ferrooxidans – It is a rod shaped bacterium. High acidic solutions favour its best growth. It obtains energy from the oxidation of ferrous (Fe^{2++}) to ferric ions (Fe^{3+}) and oxidizes reduced forms of sulphur to sulphuric acid. Oxygen is the preferred acceptor of the electrons removed during the oxidation reactions, but in absence of oxygen the organism can use ferric ions as an alternative electron acceptor for the oxidation of reduced sulphur. This bacterium acts as an obligate autotroph by using carbon dioxide from the atmosphere as a sole source of carbon but can not grow on organic carbon sources.

Leptospirillum ferrooxidans - It lives in environment in which sulphuric acid is dominating with excess of sulphates. Where the temperature remains static between 30 – 50 0C along with highly acidic pH (1-2). On the other hand T. ferrooxidans dominate at 20-30 0C in moderate acidic pH (2-4).

Ferroplasma acidophillum – It is an extremely iron oxidizing and strong acidophilic archaeon. It is found to be responsible for severe acid mine drainage. It is a cell wall deficient archaeon morphologically and phylogenetically related to Thermoplasma, F. acidophillum is an aerobic prokaryote and is capable of growth at extremely acidic pH and at temperatures up to 50 0C.

Leaching process

Some examples of the leaching reactions that result from direct bacterial attack are given below:

$$4CuFeS_2 + 17\ O_2 + 2H_2SO_4 \rightarrow 4CuSO_4 + 2Fe_2\ (SO_4)_3 + 2\ H_2O \quad (1)$$

(Chalcopyrite)

$$CuS + 2\ O_2 \rightarrow CuSO_4 \quad (2)$$

(Covellite)

$$FeS_2 + 14Fe^{3+} + 8\ H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (3)$$

(Pyrite)

$$2FeAsS + 7O_2 + 2H_2O \rightarrow FeAsO_4 + 2H_2SO_4 \quad (4)$$

(Arsenopyrite)

Indirect bacterial leaching is represented in the reactions (a) – (d). In these reactions ferric (iron) and sulphuric acid is generated by the direct oxidation of metal sulphides. They themselves are capable of oxidizing certain ores to form oxides and sulphates that are soluble in acidic solutions. When iron is present, both indirect and direct bacterial attack are contributing to the leaching of metal ores.

a. $2FeS_2 + 2Fe_2(SO_4)_3 \rightarrow 6FeSO_4 + 4S$
b. $CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S$
c. $UO_2 + Fe_2(SO_4)_3 \rightarrow UO_2SO_4 + 2Fe_2SO_4$
d. $UO_3 + H_2SO_4 \rightarrow UO_2SO_4 + H_2O$

Acid mine drainage

Acid coal mine drainage forms when FeS2 (Pyrite, Marcasite) in coal seams become exposed to moisture and air. Initially FeS2 oxidizes according to the following reaction.

$$FeS_2 + 3½O_2 + H_2O \rightarrow FeSO_4 + H_2SO_4 \quad (5)$$

Above reaction occurs under sterile condition, it is accelerated by the direct catalysis at the mineral surface by T. *ferroxidans*. The oxidation of the pyrite is further accelerated by chemical interaction with ferric ion which is derived initially from ferrous ion oxidation catalyzed possibly by *Metallogenium* until acidity reaches a pH of 3.5 and the ferrous ion concentration rises to 0.1 gl^{-1}. The reactions can be written as follows:

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (6)$$

$$2Fe_{2+} + ½O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (7)$$

The acid in drainage results from the hydrolysis of ferric iron produced in the oxidation in reaction (7).

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (8)$$

The Ferric oxide thus formed reacts further with some H2SO4 in the drainage resulting in insoluble ferric sulphate.

$$2Fe(OH)_3 + 3H_2SO_4 \rightarrow Fe_2(SO_4) + 6H_2O \quad (9)$$

Considering above reactions (5) – (9) it is observed that for every 2 molecules of FeS_2 transformed to $Fe(SO_4)_3$, one molecule of sulphuric acid is formed.

Acid mine drainage from copper sulphide containing ore deposits results from reactions similar to those responsible for such drainage from bituminous coal mines.

Chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), covellite (CuS) etc. are oxidized as already discussed in reaction (5) - (9) and may be the major source of iron and acid in the drainage. Chalcopyrite crystals may be oxidized on exposure to moist air and water according to the following reaction:

$$CuFeS_2 + H_2O + 8.5O_2 \rightarrow 2Cu^{2+} + Fe(OH)SO_4 + 2SO_4^{2-} \quad (10)$$

Its rate of oxidation is significantly accelerated by *T. ferrooxidans.* Chalcocite and covellite crystals in ore body on exposure to moist air or may be oxidized according to the following series of reaction.

$$Cu_2S + ½O_2 + 2H^+ \rightarrow Cu_2^+ + CuS + H_2O \quad (11)$$

$$CuS + ½\ O_2 + 2H^+ \rightarrow Cu^{2+} + S^0 + H_2O \quad (12)$$

$$S^0 + 3/2O_2 + H_2O \rightarrow H_2SO_4 \quad (13)$$

These reactions are catalysed by *T. ferrooxidans* (reaction - 13) or may be catalysed by *T. thiooxidans besides T. ferrooxidans*. Covellite (CuS) crystals in an ore body may be oxidized according to reaction (12) and (13).

Chalcocite and covellite may be oxidized by ferric ions in acid solution. The reaction will be as follows:

$$Cu_2S + 2Fe^{3+} \rightarrow Cu^{2+} + CuS + 2Fe^{2+} \quad (14)$$

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + S^o + 2Fe^{2+} \quad (15)$$

Acid mine drainage is posing a common environmental problem in coal mining regions. Mixing of acid mine waters with natural water bodies such as lakes, rivers causes irreversible water pollution problem because acid and heavy metals are toxic to aquatic life and also unsuitable for human consumption. However, the properties of ore leaching bacteria indicate their potentiality in metal solubilization which is now utilized in a controlled way to obtain metals from low grade ores.

Bioleaching of metals

Copper leaching

Approximately one fourth of the all copper mined worldwide is obtained from leaching process. Microbial leaching is especially useful for copper ores because copper sulfate formed during oxidation of the copper sulfide ores is water soluble. Chalcocite (Cu_2S), Chalcopyrite ($CuFeS_2$) and Covellite (CuS) are typical ores that are used for the production. Low grade copper ore contains 0.1 to 0.4 % copper and the pregnant leaching solution from ores generally found to have 1-3 % of Cu per liter. Because of these facts various industries began to utilize bioleaching process for extracting copper from low grade ores. This can be achieved by two methods

(i) the heap bioleaching process,

(ii) A continuous reactor leaching process.

In the heap bioleaching process the low-grade copper ore is dumped in a large pile and a dilute sulphuric acid solution having pH about 2.0 is percolated down through the pile (Fig.4A). The leached liquid (rich in minerals) coming out from the bottom of the pile is collected and transported to a precipitation plant where the metal is reprecipitated and purified. After extraction of metal the liquid is then pumped back to the pile and cycle is repeated.

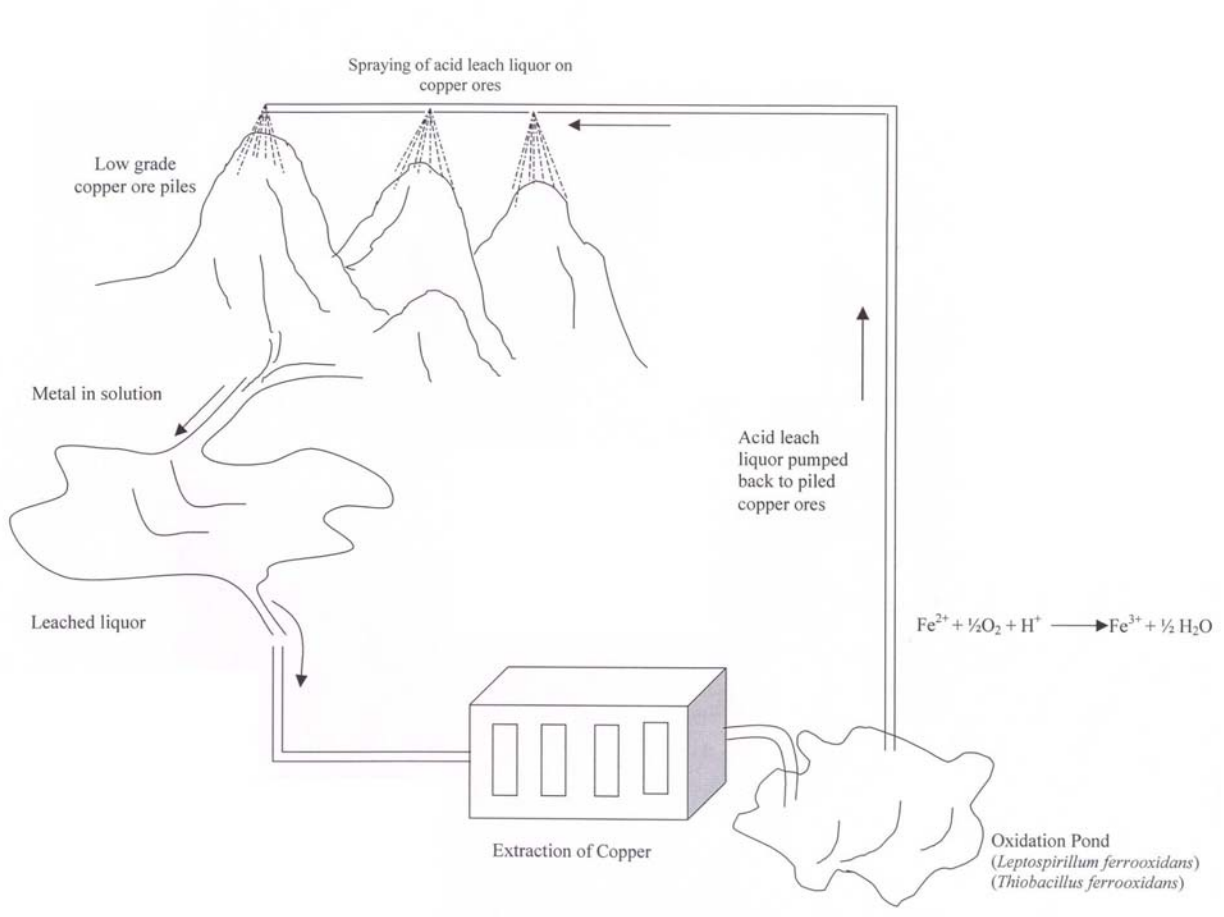

Fig. 4A: Heap leaching process for extraction of copper from low grade ore

Another process is known as a continuous reactor leaching operation for recovery of copper from its low grade sulphide ore. The processes is shown in Fig. 4B. The leaching water and ore usually supply enough dissolved mineral nutrient required for the growth of Thiobacillus ferrooxidans but in some cases NH_3 and PO_4 may be added. The leached metal is extracted with an organic solvent and then removed from solvent by stripping. In this process both the leaching liquor and the solvent are recycled.

The mechanism by which the bacteria can catalyse oxidation of the sulfide minerals is illustrated in following examples:

$$Cu_2S + ½O_2 + 2H^+ \rightarrow CuS + Cu^{2+} + H_2O \quad (16)$$

(Chalcolite)

$$CuS + 2O_2 \rightarrow Cu^{2+} + SO_4^{2-} \quad (17)$$

(Cavellite)

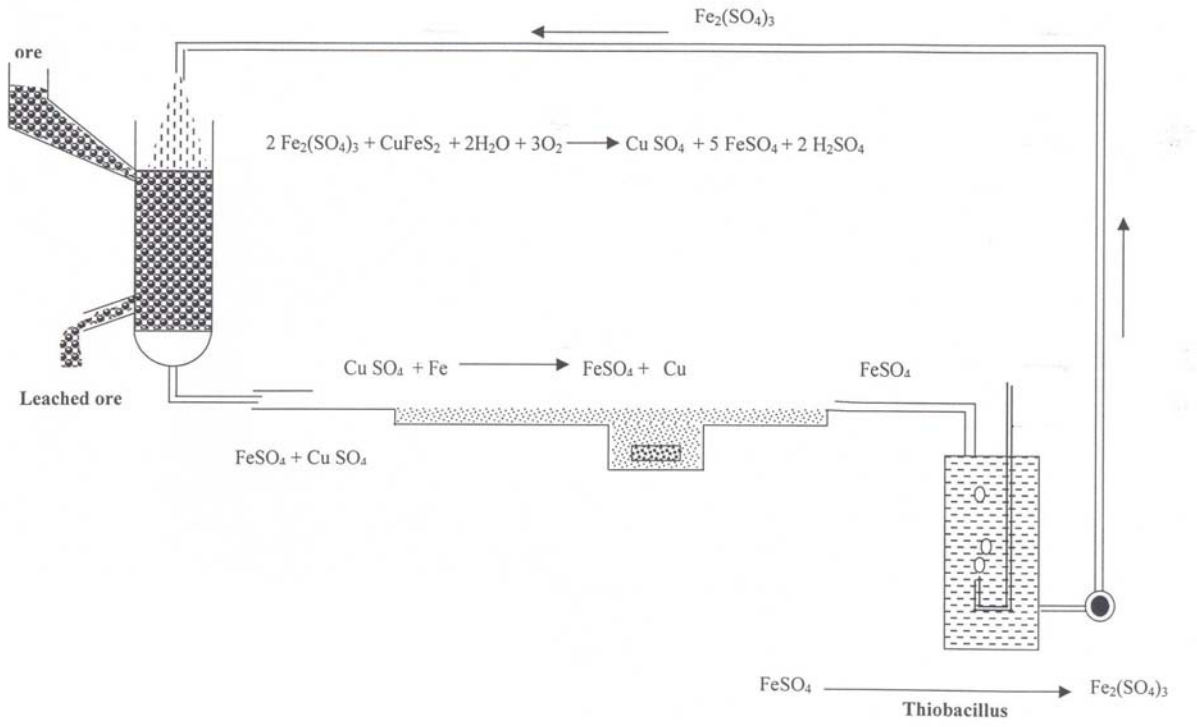

Fig. 4B: Continuous reactor leaching operation for extraction of copper from low grade ore

Thiobacillus ferrooxidans is able to oxidize Cu^+ in chalcocite (Cu_2S) to Cu^{2+} thus removing some of the copper in soluble form and forming the mineral covellite. The covellite may then be oxidized releasing sulphate and soluble Cu^{2+} as products.

Another mechanism involves chemical oxidation of copper ore with ferric ions formed by the bacterial oxidation of ferrous ions (reactions - 18)

$$CuS + 8Fe^{3+} + 4H_2O \rightarrow Cu^{2+} + 8Fe^{2+} + SO_4^{2-} + 8H^+ \quad (18)$$

In any ore if pyrite in present its oxidation leads to the formation of ferric ion, which is an excellent electron acceptor for sulphide minerals.

Reactions of CuS with ferric iron results in solubilization of copper and formation of ferrous iron. In the presence of O_2 *Thiobacillus ferrooxidans* (at acid pH value) re oxidizes the ferrous iron back to ferric iron thus the process is maintained by the oxidation of Fe^{2+} to Fe^{3+} by the bacterium.

Recovery of copper and generation of ferric iron

In precipitation plants the recovery of copper metal from leaching solution is carried out by using scrap iron Fe^0. The reaction is as follows:

$$Fe^0 + Cu^{2+} \rightarrow Cu^0 + Fe^{2+} \quad (19)$$

This results in the formation of Fe^{2+}. In leaching operation, Fe^{2+} rich liquid remaining after copper extraction is transferred to an oxidation pond where *Thiobacillus ferrooxidans* and / or *Leptospirillum ferrooxidans* transformation of ferrous to ferric is carried out. The oxidized Fe^{3+} rich acid solution is sprayed on the piled dumped of ore again. The required pH may be maintained by adding H_2SO_4 solution.

Uranium leaching

Uranium leaching is an indirect leaching process because the microbial action is not directly on the uranium ore. Although *T. ferrooxidans* can oxidize U^{4+} to U^{6+} with oxygen as electron acceptor, it is likely that the uranium leaching process depends more on the chemical oxidation of uranium by Fe^{3+}, with *T. ferrooxidans* contributing mainly through the reoxidation of Fe^{2+} to Fe^{3+} as observed in the leaching of copper ores, i.e.:

$$UO_2 + Fe(SO_4)_3 \rightarrow UO_2SO_4 + 2FeSO_4 \quad (20)$$

(U^{4+}) (Fe^{3+}) (U^{6+}) (Fe^{2+})

In this process, insoluble tetravalent uranium is oxidized by using hot H_2SO_4 / Fe^{3+} solution to soluble hexavalent uranium sulphate. The oxidized uranium mineral is soluble and can be extracted from the leach liquor with organic solvent like tributyl phosphate.

Gold leaching (Bioreactor gold leaching process)

Gold is frequently found in nature associated with minerals containing arsenic and pyrite. In the process of microbial leaching of gold *Thiobacillus ferroxidans* and other related organisms such as *T. thiooxidans and Leptospirillum ferrooxidans* organisms which are used to solubilize the arseno pyrite minerals containing gold lattice locked. In this process the locked gold is released in leach liquor.

$$2FeAsS[Au] + 7O_2 + 2H_2O + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 2H_3AsO_4 + [Au] \quad (21)$$

The gold is then complexed with cyanide by traditional metallurgical methods. The gold leaching usually takes place in small tanks. Bioleaching in this fashion is more beneficial as it helps winning more than 95% of the 'locked gold'. Since, toxic arsenic (As) and cyanides result from mining hence should be removed during the process of leaching in tanks. Arsenic is removed as a ferric precipitate and cyanide by its microbial oxidation to CO_2 and urea in later stages of gold recovery process. The conventional gold mining techniques are more costly and environment damaging and hence are applied in small scale. Microbial gold leaching is gaining popularity over the traditional methods.

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