CHAPTER II

LITERATURE REVIEW

THE CULTURAL PROPERTIES

Cultural properties comprise of objects of cultural, artistic or historic significance could be characterised via material used. In term of conservation, they can be classified into three major categories (Kulpanthada Janposri, 1988).

1. Organic materials

This could be defined as objects which are made from living material such as leather, bone, ivory, horn, wood, paper, textile, palmleaf. They are prone to deterioration by environmental changes.

2. Inorganic materials

This could be defined as objects which are made from non-living material. They can be divided into metallic and non-metallic objects. The metallic objects e.g. silver, bronze, iron, brass, pewter can be deteriorated by change in humidity of the environment except gold which is high tolerance. Another group e.g. stone-carving, porcelain, glass, pottery all are rather tolerate to deterioration.

3. Paintings

This group is by itself classified as another group due to their support which made from organic or inorganic material. Thus, deterioration could occured in two ways.

The conservation of cultural properties mainly divided into two methods i.e. preservation and restoration. The preservation is better than restoration since if deterioration does occur on cultural objects, it is rather difficult to restore them back to original or in good condition. For this reason, the protection of these valuable objects from deterioration is of importance.

DETERIORATION OF CULTURAL PROPERTIES

A variety of factors, broadly classified as physical, chemical and biological, are responsible for the deterioration of cultural properties. In principle, the objects of inorganic nature are less susceptible to deterioration than organic objects. The examples of deteriorating factors are as follows:

1. Physical factors e.g. humidity, heat, light.
2. Chemical factors e.g. atmospheric pollutants.
3. Biological factors e.g. human, bat, bird, rodent, plant, insect, microbes.

The problem of deterioration of cultural properties caused by biological agents is very important in the tropical countries like Thailand where the climate is hot and humid for most part of the year. The decay and destruction of materials caused by biological agencies is termed as biodeterioration while causative agents as "biodeteriogens".

CLASSIFICATION OF BIODETERIORATION (Singh, 1994)

1. Mechanical biodeterioration

In this category, material is damaged by physical forces exerted by organisms, for example disruption of floorboards by dry and wet rot fruiting bodies, physical damage caused by rodents, insects, higher plants and birds.

2. Chemical biodeterioration

In this case, material is attacked chemically by organisms, e.g. oxidation, reduction caused by production of organic acids.

a) Chemical assimilatory biodeterioration

The material is utilised as a food for metabolism of organism, for example, the breakdown of cellulose in wood.

b) Chemical dissimilatory biodeterioration

In this case, damage is caused by excretion products from the organism, no direct concerned with uptake of nutrients, for example, production of secondary metabolites, toxins, pigments, corrosive waste products, etc.

3. Fouling

In this process, material is destructed by the presence of the organism, which causes physical obstruction, for example, clogging of pipelines by iron bacteria, fungal mycelium bridging gaps in electrical equipment.

WOOD

Wood is a natural complex substance constituted of cellulose, hemicelluloses, lignin, and extractives. The polymer substances are not uniformly distributed within the wood cell wall and their concentrations change from one morphological region to the others.

Cellulose is predominant component accounting for 40 to 55% of the total mass while hemicelluloses makes up approximately 25-40% and lignin about 18-33% (Eaton and Hale, 1993). The role of these three substances could be compared to those of constructing materials in the reinforced concrete in which cellulose, lignin, and hemicelluloses correspond respectively to iron core, cement, and buffering material to improve their binding (Fujita and Harada, 1991).

The structure and chemical composition of wood have significant impact on its degradation and the resulting patterns of decay. The wooden objects are cellulosic materials suited for biological agents, their biodeteriorating activities are accelerated under hot and humid conditions of wooden objects. Wooden objects in the collection of the museums, art galleries and the exposed monuments a sizeable part of national heritage need preservation and consolidation treatment to ensure longevity of these objects of art and architecture.

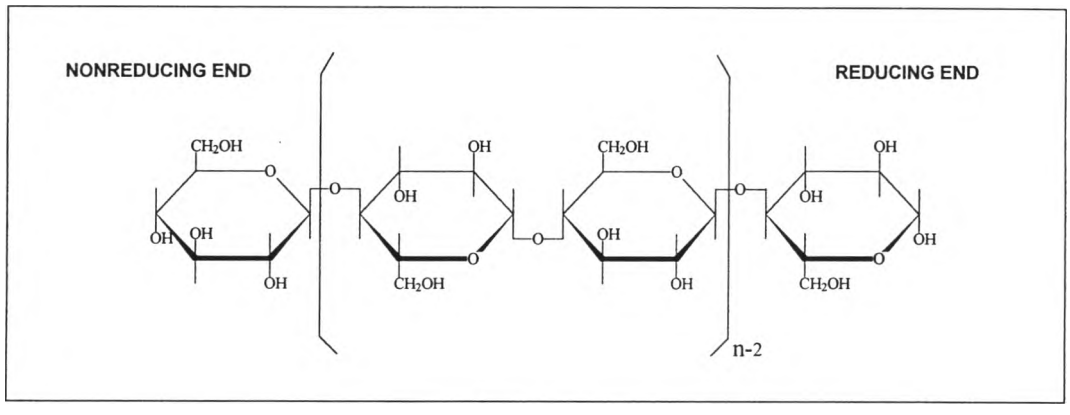

Figure 2.1: Chemical structure of cellulose. (Debye, 1944 cited in Morohoshi, 1991)

CELLULOSE

Cellulose is presented as the main structural component of wood cell wall, by nature a carbohydrate material consist of carbon (44.4%), oxygen (49.4%), and hydrogen (6.2%). The molecular formula of cellulose is expressed as $(C_6H_{10}O_5)_n$. Cellulose is a highly regular linear polymer of D–anhydroglucopyranose unit connected by β–1,4–glucosidic linkages. A Haworth formula (Figure 2.1), in which significant chemical features of cellulose are depicted, indicates that the two terminal glucose residues differ in their chemical reactivity. One contains a cyclic hemiacetal structure and is called the reducing end group, whereas the other contains an additional secondary hydroxyl group and is called the nonreducing end group.

The cellulose molecule contains three exposed hydroxyl groups per anhydroglucose unit, which control the structural properties in the wood cell wall as well as many physical and chemical properties. In addition, their properties depend on molecular weight and molecular weight distribution. The molecular weight (M) is expressed as the degree of polymerisation (DP = M/162), where 162 is the molecular weight of anhydroglucose unit. As with any polymer, the M of cellulose depends on the technique used. The average molecular weights depend on the properties of measurement methods and the chain length of cellulose. (Morohoshi, 1991)

An average DP of around 10,000 has been determined for bark and wood celluloses but the DP of plant celluloses can vary between 3,000 and 20,000 (Zabel and Morrell, 1992).

Several properties of cellulose influence its microbial enzymatic degradation: 1) the capillary structure in relation to size of cellulose; 2) the degree of crystallinity; 3) the dimensions of the crystalline portions of the microfibrils; and 4) the nature of substances with which the cellulose associated, in particular lignin. (Kirk, 1983)

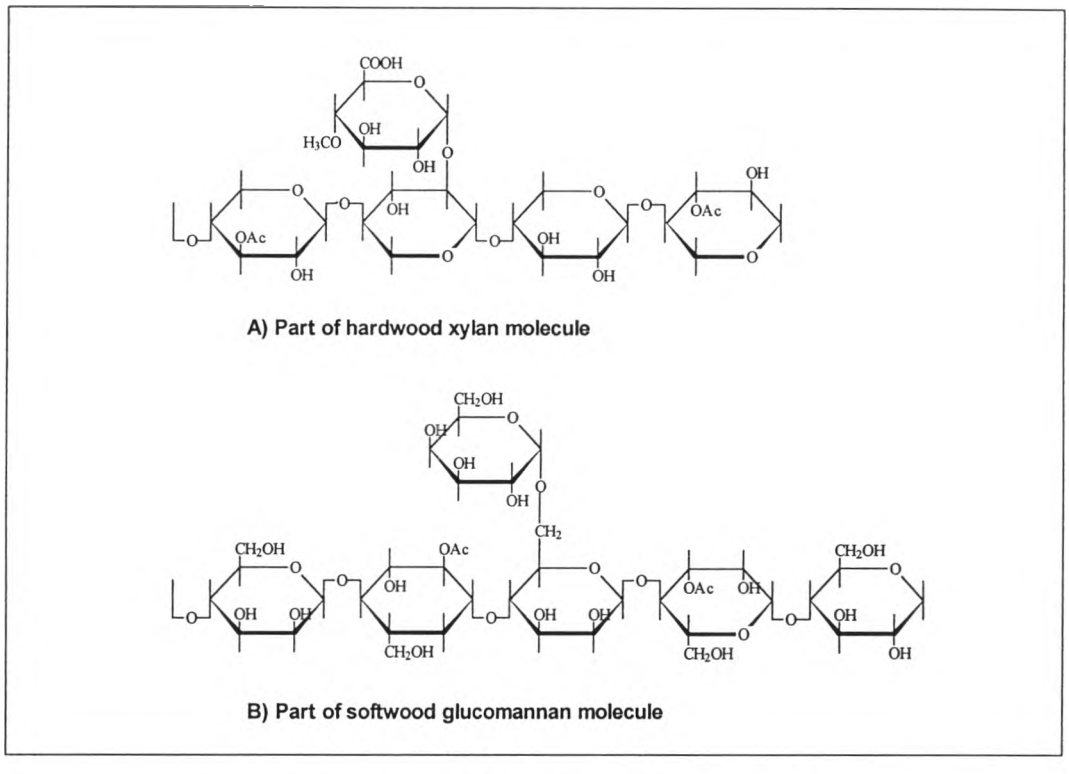

Figure 2.2: The major hemicelluloses polymer.

HEMICELLULOSES

Hemicelluloses, resemble cellulose, are relatively short, branched homopolymers and heteropolymers of anhydrosugar units linked by glycosidic bonds. Unlike cellulose molecule, however, each hemicellulose molecule is comprised of more than one kind of sugar unit. Hemicelluloses are made up of glucose and other hexose and pentose sugars and their uronic acid derivatives, the polymers possess degree of polymerisation seldomly exceeds 200 (Eaton and Hale, 1993).

The types and amounts of the hemicelluloses present in the cell walls of hardwoods and softwoods are differ. The component monosaccharides are glucose, xylose, galactose, mannose, arabinose, rhamnose, and fucose. Whereas the uronic acids of glucose galactose could be linked via 1—3, 1—6, and 1—4 glycosidic linkages.

The major hemicellulose in hardwoods is xylan (Figure 2.2 A) which exists usually as homopolymer of β–D–xylose monomers linked by 1,4–glycosidic bonds. Side chain of 4–*O*–methyl–α–D–glucuronic acid are linked via 1,2–linkages to some xylose units, and while certain hydroxyls groups could be substituted by *O*–acetyl groups. Glucomannan (Figure 2.2 B), the major hemicellulose in softwoods (up to 20% of cell wall) is a heteropolymer with backbone containing β–D–mannose units linked by 1,4–glycosidic bonds. Acetyl groups and galactose residues are attached to some monomers in the backbone. The xylan in softwoods possesses arabinose side chains rather than acetyl group. (Zabel and Morrell, 1992)

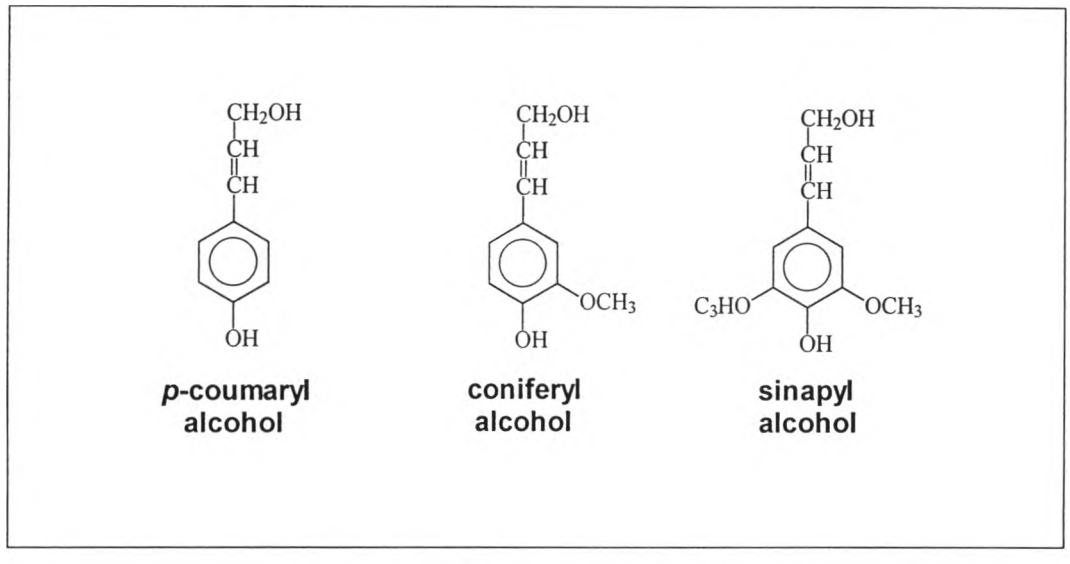

Figure 2.3: The phenylpropane monomers.

LIGNIN

Lignin, the most complex of cell-wall constituents, is a three-dimensional polymer of phenylpropane units which is completely amorphous and serves as an encrusting material surrounding microfibrils. Lignin molecules are synthesized by oxidative polymerisation of three substituted cinnamyl alcohols (Figure 2.3) namely: *p*–coumaryl alcohol (4–hydroxy cinnamyl alcohol), coniferyl alcohol (4–hydroxy–3–methoxy cinnamyl alcohol), and sinapyl alcohol (4–hydroxy–3,5–dimethoxy cinnamyl alcohol).

Lignin composes about 20 to 30% (in a few species the lignin content approachs 40%) of the wood cell wall (Zabel and Morrell, 1992). It affords considerable rigidity to the cell wall and because of its less hydrophilic properties; it also accounts for the swelling characteristics of wood.

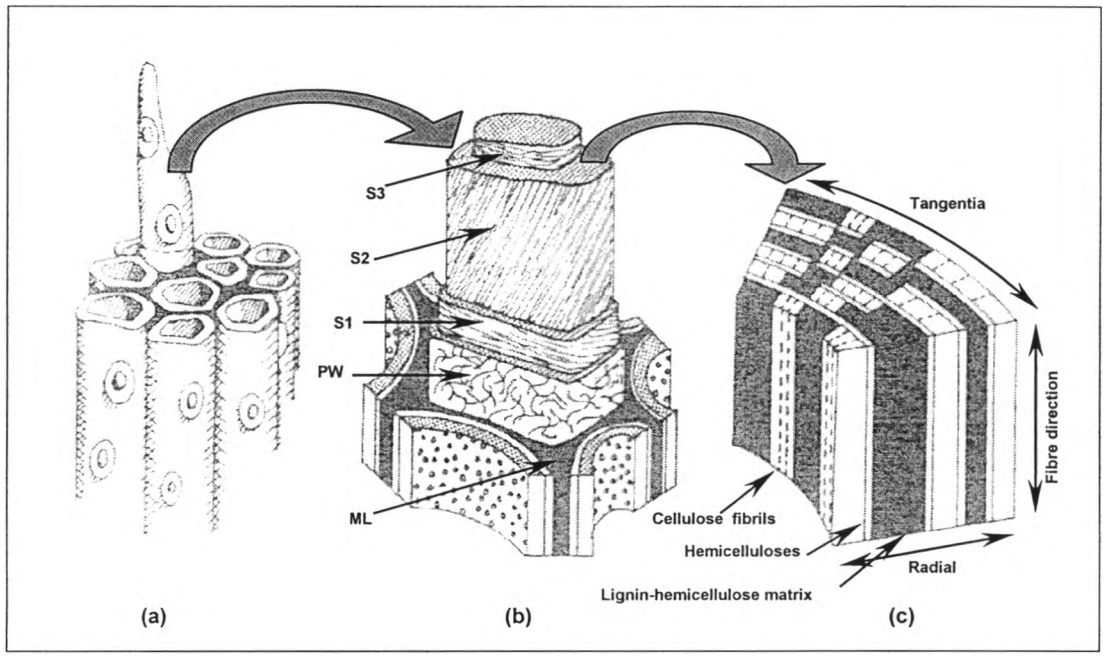

Figure 2.4: The ultrastructure of wood. (Kirk, 1983)
(a) The relationship of contiguous cells to each other
(b) A cut-away view showing wall layers
ML, middle lamella; PW, primary wall;
S1, S2, S3, layers of secondary wall.
(c) The postulated relationship of hemicelluloses and lignin to the cellulose fibrils in the secondary wall

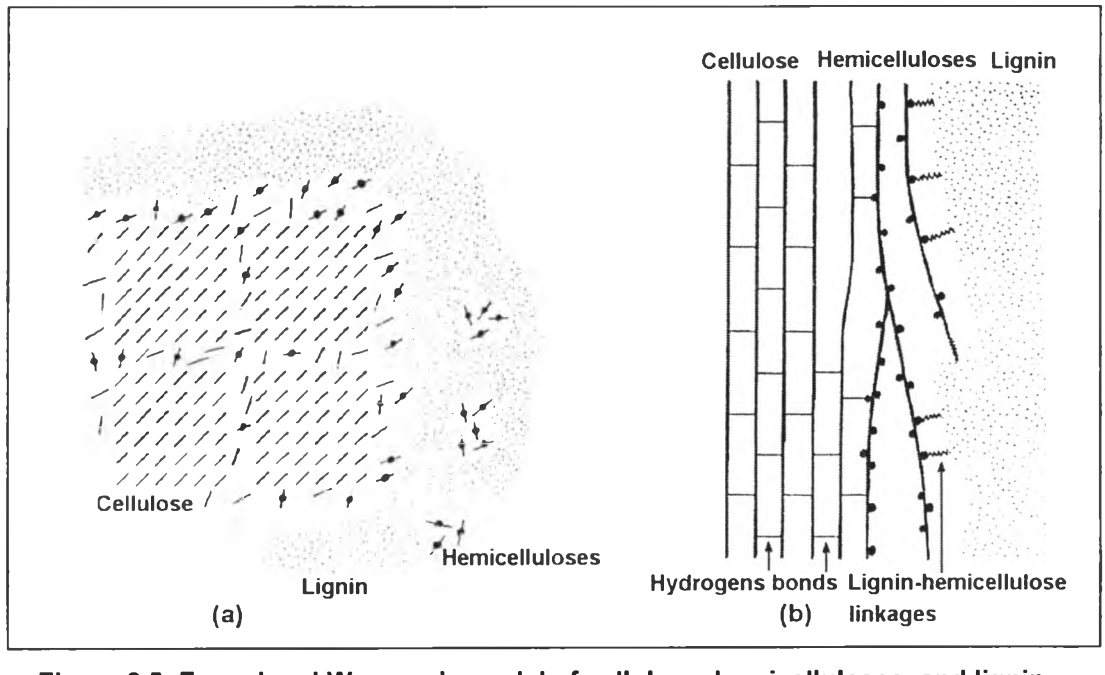

Figure 2.5: Fengel and Wegener's model of cellulose, hemicelluloses, and lignin associations in the wood cell wall. (Eaton and Hale, 1993)
(a) Transverse view (b) Longitudinal view

ULTRASTRUCTURE OF WOOD

The wood cell wall composed of various layers, each of which is significantly different in their chemical composition. Figure 2.4 illustrates the ultrastructure of wood cell, the middle lamella (ML) is a narrow zone between contiguous cells and consists primarily of pectin and lignin. It is derived from the new cell plate that forms during the mitotic division of a cambial cell into another. The primary wall (PW) is initially laid down during enlargement and maturation of the cambial initially into a tracheid in softwoods or fiber in hardwoods. The wall consists of a loose network of mostly axially oriented cellulose microfibrils. The secondary wall (SW) develops next and consists of three successively formed layers designated S1, S2, and S3. The S1 and S3 are narrow zones in which the cellulose microfibrils are arranged in a flat helix. The S2, which composes the bulk of the cell wall, consists of microfibrils arranged in a steep helix, oriented nearly parallel to the longitudinal axis of the cell. The S2 layer is the most important zone of the cell wall and is responsible for a majority of wood-strength properties, particularly its remarkable tensile strength. In some woods, the warty layer could develop on surface of S3. The lumen is the inner cell cavity of wood cell wall. The lumen volume, collectively, in most woods is large, and will be seen later as the critical cell-wall zone where most decay fungi initiate their decay processes. (Zabel and Morrell, 1992)

Several models have been put forward to explain the association between the polysaccharide and lignin components in wood cell wall. It is well accepted that cellulose microfibrils make up the backbone structure of the wall while the hemicelluloses and lignin found associated between these different component. (Eaton and Hale, 1993)

Fengel and Wegener (1989) recognised the close associations between cellulose-hemicelluloses and hemicelluloses-lignin and incorporated this into a model which demonstrates the tie at interfaces of these components. The model also highlights the association of separate hemicelluloses within the surrounding lignin matrix (Figure 2.5).

WOOD DETERIORATION AGENTS

The major agents and types of wood deterioration are grouped under abiotic and biotic categories and listed as follows:

Abiotic damage

1. Weathering – primarily photodegradation by ultraviolet light and oxidation

2. Thermal decomposition – distillation or burning
   a. Low-temperature exposure (below 200°C)
   b. High-temperature exposure in absence of oxygen (above 200°C)
   c. Combustion (above 275°C)
3. Chemical decomposition – hydrolysis and oxidation
   a. Exposure to strong acids
   b. Exposure to strong bases
   c. Exposure to strong oxidizing agents and some organic solvents
4. Mechanical wear – breakage and erosion of surface fragments

Biotic damage

1. Animal attack – mechanical disruption
   a. Boring and surface rasping by marine borers
   b. Tunneling and excavations by insects (termites, boring beetles, and carpenter ants) and marine borers (shipworms, pholads, and isopods)
2. Decays and discolouration – penetration and digestion
   a. Cell-wall etching and tunneling by bacteria
   b. Surface moulding by fungi
   c. Sapwood staining by fungi
   d. Decay by fungi (soft rots, brown rots, and white rots)

The descriptions of wood damage are summarised in Table 2.1.

FUNGI AS AGENTS FOR BIODETERIORATION OF WOOD

Like all living organisms, fungi have certain requirements for growth and survival. The major growth needs of wood-inhabiting fungi are as follows: (Zabel and Morrell, 1992)

1. Water – free water on surfaces of cell lumen
2. Oxygen – atmospheric oxygen at relatively low levels for most fungi and very low levels for some facultative anaerobic fungi
3. Temperature – optimum range for most wood-inhabiting fungi from 15 to 45°C
4. Substrate (Wood) – provide energy and metabolites for synthesis via metabolism
5. pH – optimum range from 3 to 6
6. Chemical growth factors – nitrogen compound and essential element

Singh (1994) suggusted the typical decay cycle of fungi on wood (Figure 2.6). The cycle consists of colonizing hyphae that penetrate and ramify wood cells eventually lead to the decay of wood. When conditions are permitted this mycelium proceed to form fruiting bodies and sporophores on the surface in various forms and shapes. The appearance of fruit bodies usually indicates a fairly advanced stage of wood decay. Spores are produced in the fruiting bodies which, when mature, are released

into the air. Under favourable environmental conditions spores germinate and set up a new round of infection. The secondary spread is mainly by the germination of spores and formation of conducing strands.

Table 2.1: Major types of wood damage and their descriptions.

Type of damage	Causative agent(s)	General descriptions
Weathering	Ultraviolet light, oxidation, swelling and shrinkage, leaching, and fungi	Unprotected surfaces develop a gray colour and roughened texture
Thermal decomposition	High temperature	< 200°C, uniform surface brittleness > 200°C, charcoal in absence of oxygen, combustion around 275°C
Chemical decomposition	Caustic chemicals	With acids wood turns brown, chars, and becomes brittle; with bases wood bleaches and defibrillates
Mechanical damage	Mechanical forces rupturing surface tissues	Selective surface erosion in heavy friction zones
Insect damage	Termites	Localised honeycomb cavities, wood soiled and filled with frass
	Borers	Tunnels, cavities, pinholes
	Ants	Localised honeycomb cavities, wood channels clean
Marine borer damage	Shipworms	Interior tunnels with lime-coated walls
	Pholads	Large interior tunnels—near surface
	Gribbles	Surface tunneling in tidal zone
Decay	Fungi	White fibrous pockets or punky texture Brown fibrous pockets or cubical checking pattern Soft surface embrittlement and exfoliation in small fragments
Moulds	Fungi	Coloured spores or mycelium on the wood surface
Stains	Fungi	Sapwood discoloured gray, black, brown, blue and intensified in ray parenchyma
Ray cell and cell-wall damage	Bacteria	Soft surface, ray cells destroyed, microscopic tunnels in cell walls

Source: Zabel and Morrell (1992)

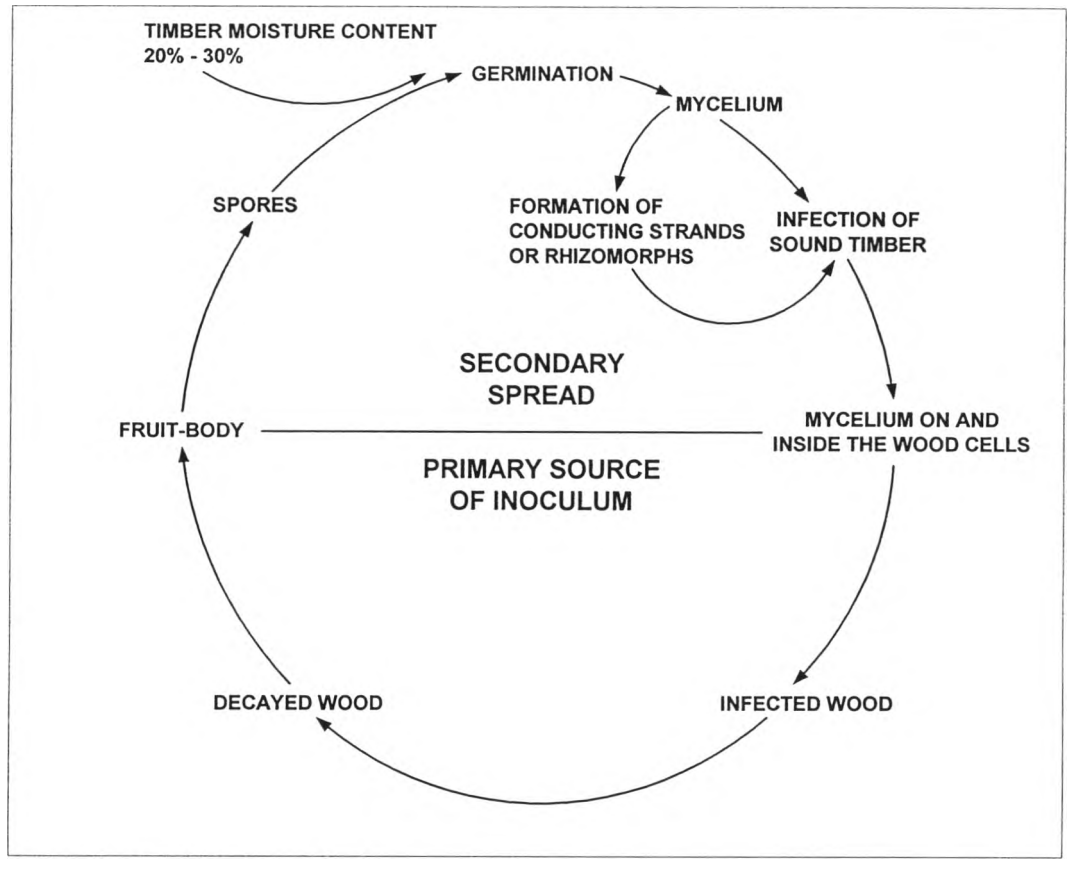

Figure 2.6: The typical decay cycle of fungi on wood. (Singh, 1994)

Two major types of wood-inhabiting fungi are decayers and nondecayers (stainers). Decay fungi destroy the cell walls of wood, resulting in the change of chemical and/or physical properties of wood via enzymatic activities, while the staining fungi are responsible for change in colour of wood. The appearance of the wood attacked by these fungi is blue to dark brown, or greenish in some case which take place only in the sapwood. A summary of types of wood-inhabiting microorganisms is depicted in Table 2.2.

Wood-rotting fungi are, by definition, those which can bring about significant weight loss and structural change in woody tissues. Three basic types of rot are recognised, each conspicuously different and taking its name from the general appearance of the decayed wood. White-rotted wood takes on a lightened bleached appearance although in the early stages of decay, darkened and brown tinges and streaks may occur. In late stages of decay the wood surface does become softened and shrinkage. Brown-rotted wood is reddish brown to dark brown in appearance and softened to some depth when wet. At late stages of decay and when dry the wood commonly shows deep cuboidal cracking due to shinkage. In soft rot, when wet the wood surface

is cheesy soft and considerably darkened. When dried the rotted surface shows fine shallow longitudinal and cross-cracking. Although wood attacked by brown rot fungi shows a similar darkening of the surface, more cracks are visible in soft-rotted wood and cracking is much shallower because of the superficial nature of the attack. (Eaton and Hale, 1993)

Table 2.2: A summary of the anatomical and chemical features of the major types of wood-inhabiting microorganisms.

Wood-inhabiting microorganisms	Cell-wall constituents used	Anatomical features	Causative agents
		Decayers	
		(Cell-wall erosion and/or large bore holes formed > 2 µm)	
Simultaneous white rots	All	Cell walls attacked progressively from lumen surface	Basidiomycotina Some Ascomycotina
Sequential white rots	All, but hemicelluloses and lignin used selectively initially	Cell walls attacked progressively from lumen surface	Basidiomycotina Some Ascomycotina
Brown rots	Carbohydrates, but lignin modified	Entire wall zone attacked rapidly	Basidiomycotina
Type 1 soft rot	Carbohydrates	Longitudinal bore holes develop in secondary wall	Ascomycotina Deuteromycotina Some bacteria
Type 2 soft rot	Carbohydrates	Secondary wall erosion from lumen surface (in conifers mainly the S2)	Ascomycotina Deuteromycotina Some bacteria
		Nondecayers	
		(No cell-wall erosion and occasional bore holes are minute < 1 µm)	
Sapstainers	Wood extractives	Invade parenchyma cells in sapwood primarily	Ascomycotina Deuteromycotina
Moulds	Wood extractives	Surface growth on wet wood	Zycomycotina Ascomycotina Deuteromycotina
Scavengers	Wood extractives and decay residues	Penetrate wood cells primarily through pits	Bacteria Zycomycotina Ascomycotina Basidiomycotina Deuteromycotina

Source: Zabel and Morrell (1992)

The micromorphological characteristics of decay resulting from white rot, brown rot, and soft rot attack are examined, it is found that fundamentally different patterns of attack of the S1, S2, and S3 layers occur (Figure 2.7).

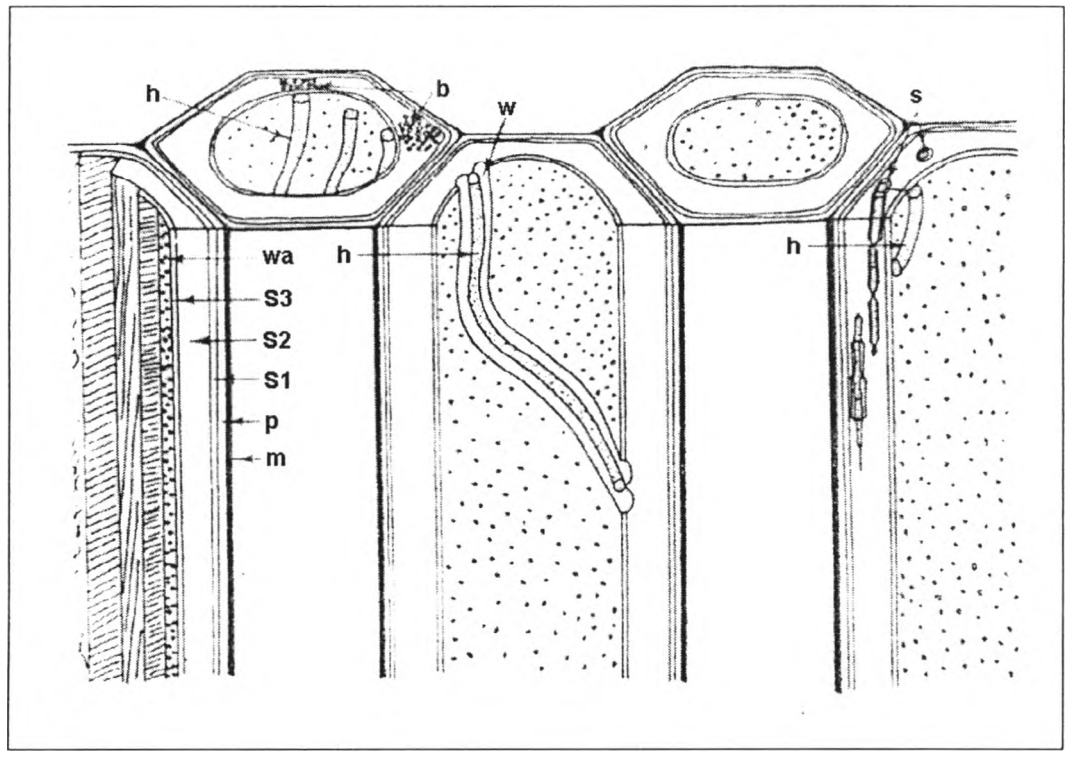

Figure 2.7: The micromorphology of different types of wood decay. h, hyphae; b, brown rot; w, white rot; s, soft rot (type 1); m, middle lamella; p, primary wall; S1, S2, S3, layers of secondary wall; wa, warty layer. (Rayner and Boddy, 1988)

Rayner and Boddy (1988) intimated these patterns in outline here. In white rots hyphae typically penetrate into the cell lumen, where they lie on the inner surface of the wood cell wall. Erosion of the cell wall may be generalised or localised to the immediate vicinity of the hyphae, forming a groove of trough with a central ridge on which the hypha lies. New troughs are formed associated with hyphal branching, which rapidly coalesce, ultimately resulting in progressive cell-wall erosion from the lumen through the S3 and S2 layers. The hyphae are capable of penetrating the S3 layer into the S2; when the resultant trough is parallel to the microfibrils its edges are smooth, but when it cuts across their orientation ragged edges appear as the ends of the cell microfibrils project. These features imply the binding of enzymes to hyphae, perhaps by an external layer of mucilage; the ridge may result from intimate contact between the hypha and the cell wall, possibly via surface-tension effects, thereby excluding the enzyme-bearing mucilage from immediately beneath the hypha.

Soft-rot fungi produce two distinct types of attack, termed 1 and 2. Type 2 attack is similar to localised white-rot attack, in that it works outward from the lumen and results in a eroded groove with the hypha lying on a central ridge. Type 1 attack is

characterised by the formation of chains of cavities with pointed ends in the S2 layer, with follow the orientation of the microfibrils.

Brown rot has entirely different morphological effects. Here hyphae lie on the surface of the S3 layer in the lumen, but not within it. The hyphae, S3 layer and compound middle lamella alter very little but the S2 and S1 layers become extensively hollowed out owing to removal of cellulose and hemicelluloses.

CELLULOLYTIC FUNGI

Whereas many fungi are able to degrade modified cellulose products, only a limited number are able to degrade cellulose in its native, highly crystalline form. These species are termed cellulolytic fungi. Fungi producing the necessary enzymes for a cell-free degradation of crystalline cellulose normally belong to the Ascomycete and Deuteromycete groups or to the white-rot Basidiomycetes also degrade crystalline cellulose. However, these fungi do not seem to produce exo–β–1,4–glucanases. (Eriksson, Blanchette, and Ander, 1990)

The cellulolytic enzymes have been defined as the enzymes hydrolysing cellulose, thereby ultimately yielding soluble sugars small enough to pass through the microbial cell walls. Today, a modification of this definition seems necessary since, in addition to the hydrolytic enzymes, oxidative enzymes also participate in cellulose degradation.

Cellulase was considered to be a multienzymatic system consisting of at least three enzymatic components that converted cellulose to glucose. A complete set of hydrolytic cellulase enzymes is minimally composed of the following enzymes (Rayner and Boddy, 1988):

1. Endo–β–1,4–glucan glucanohydrolase (endoglucanase, EC 3.2.1.4, synonymous with CMCase or C_X cellulase) which randomly hydrolyses β–1,4–glucosidic linkages.
2. Exo–β–1,4–glucan cellobiohydrolase (exoglucanase, EC 3.2.1.91, synonymous with Avicelase or C_1 cellulase) which remove glucose units from nonreducing ends of cellulose molecules, liberating cellobiose or glucose.
3. β–1,4–Glucohydrolase (β–glucosidase, EC 3.2.1.21, synonymous with cellobiase) which hydrolyses cellobiose and cello-oligosaccharides to glucose.

Eaton and Hale (1993) described the synergistic action of these enzymes that was shown in model (Figure 2.8). Endoglucanases attack by hydrolysing non-crystalline regions and regions of less-ordered crystalline cellulose thus opening up cellulose chains. The cellobiohydrolases bind to the edges of the cellulose crystallite and move along the cellulose chain towards the reducing end, cleaving off cellobiose units from the nonreducing ends. In addition, Endoglucanases open up various random points of the crystalline regions which are then

instantaneously attacked by cellobiohydrolases to prevent reclosure of the original scission. Further attack by cellobiohydrolases releases more cellobiose units. β–Glucosidase converts cellobiose and short chain cellodextrins to glucose. The exoglucohydrolase enzyme attacks soluble oligomers released cellulose to yield glucose molecules.

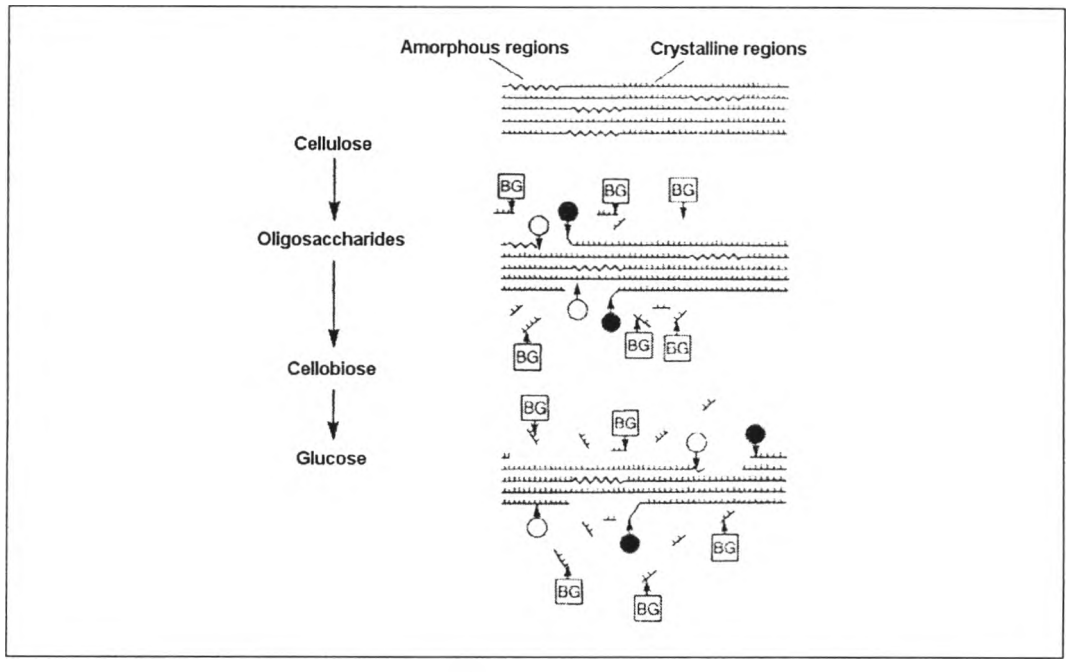

Figure 2.8: Scheme for enzymatic degradation of cellulose. (Eaton and Hale, 1993)

○, endoglucanase; ●, cellobiohydrolase; BG, β-glucosidase.

CONTROL OF BIODETERIORATION (Singh, 1994)

1. Chemical control

Many toxic chemicals are available for use as wood preservatives. The ideal wood preservative should possess the following characteristics:

1) A high toxicity towards wood-destroying organisms.
2) Permanency in treated wood, that is low volatility and high resistance to leaching.
3) Ability to penetrate deeply into the wood.
4) Non-corrosive to metals and non-injurious to the wood itself.
5) Reasonably safe to handle and without injurious effects on operatives and occupants.

2. Biological control

Microbial interactions and biological control methods have received much attention during recent years as an alternative to existing chemical control methods, which cause extensive environmental degradation, pose potential hazards to wildlife and are of one biological agent to suppress another. This involves placing a microorganism into a material which does not affect the properties but which successfully prevents invasion by species capable of damage.

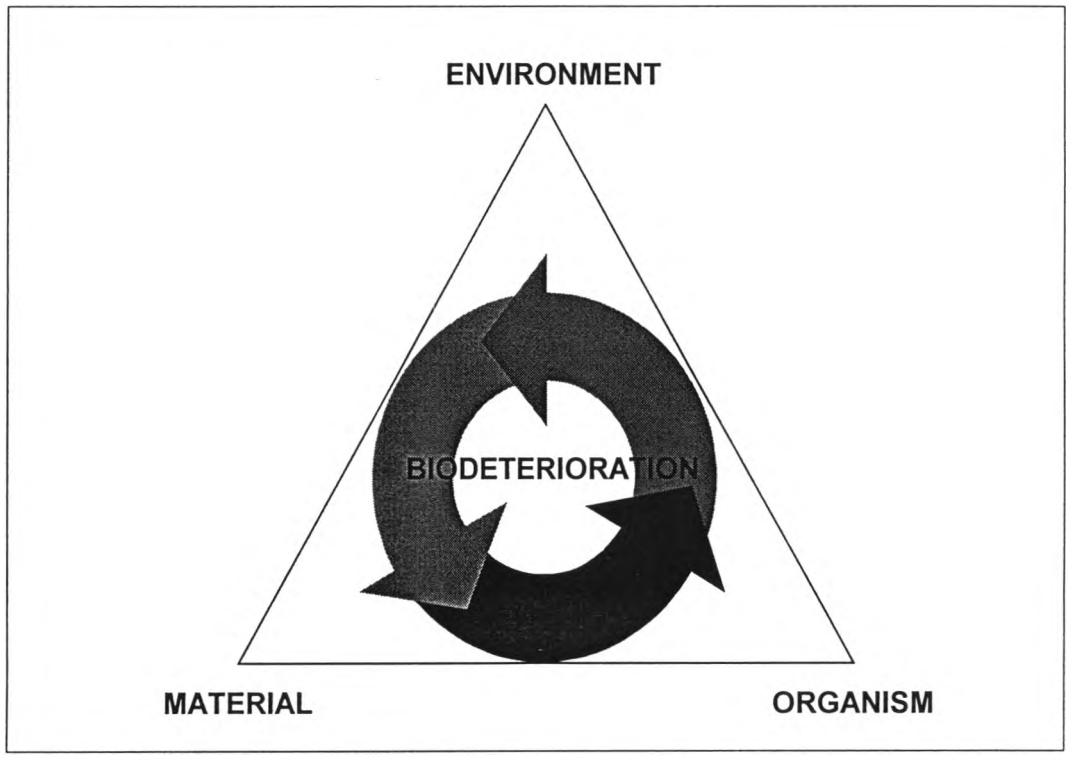

Figure 2.9: Biodeterioration cycle.

3. Environmental control

When considering the prevention of any form of biodeterioration, there are three factors which can be taken into account; the material, the environment and the organism (Figure 2.9). The removal or alteration of any one of these can prevent the growth of decay organism.

The control of the environment of a susceptible material, instead of the application of biocides, is the oldest and still the most widely used method of preventing biodeterioration. Traditionally, the control of physical conditions has been by far the most important method of preventing biodeterioration. For example, in the use of wood in construction the object has been dried to prevent its moisture content rising to levels at which wood rotting fungi can thrive.

The environment of material is complex and dynamic; all organisms live in a biological equilibrium called a biological balance, and for an organism to succeed it must be in balance within fine limits with the environment. Its balance can be disturbed to render it more hostile to the organism, less growth, and therefore less damage, will occur. The levels of moisture content and temperature necessary for the safe limits of biodeterioration are often decided much more on practical experience than on theoretical calculations and a knowledge of the physiology of the microorganism concerned. However, when a situation arises such as the need to prevent the biodeterioration of materials in building, it is absolutely necessary to predict the following:

a) How particular temperature and moisture conditions may arise and be controlled in the microenvironment of the substrate.
b) The effects of the interaction of physical condition both between themselves and with other factors on the growth and activity of decay organisms.
c) The interrelationship of building structures and materials with their environments, occupants and contents.

The basic principle in the control of fungal growth is to render the microenvironment in or around the material in building as hostile as possible to the settlement, germination and spread of microorganisms. This can be achieved in various ways:

a) To prevent or limit biological growth and proliferation by means of toxic chemicals.
b) To ensure that the material to be protected is kept, in such physical condition that growth of biological agents in severely limited or prevented entirely.

The third approach will be discussed in more detail as traditionally, the control of physical conditions has been the most important method of preventing biodeterioration. The application of the general principles of the control of physical conditions and reactions of microorganisms to these conditions often results in the most effective and economical prevention of deterioration.

Microclimate and biodeterioration

The components of the microclimate contributing to the on set of infections should not only be considered individually but should also be correlated, for two reasons. Firstly because the lower thermic values, the higher the hygrometric values at which microorganisms develop, and secondly because a relationship exists between temperature, relative humidity in the air and moisture content in the materials. (Gallo, 1993)

To prevent biodeterioration of materials it is thus to keep them in rooms where temperatures and relative humidities are low.

Microclimate of rooms

There are many influencing factors, the main are as follows:

a) The geographical position of the museums.
   Obviously the climatic characteristics of the geographical area and its seasonal thermohygrometric fluctuations play a determining role in the microclimate of the museum. The influence of the climate also depends on the structural features of the building.
b) The characteristics and location of the exhibition room where the objects are kept.
   Within the same building, climatic differences are generally found between basement and ground floor rooms and those on higher floors.
c) Air-conditioning and air-heating.
   These stabilise and modify the microclimate, moderating or preventing thermic and hygrometric excesses.
d) The activities carried out in the room.
   Human activities can determine variation in temperature and humidity. For example, the hygrometric level in the room will increase on a rainy day if a large number of people are coming in the room and if their clothes are damp.

BIOAEROSOL

Bioaerosols are particles of variable biological origin, e.g., pollen, fungi, bacteria, viruses, protozoa, or other components, residues or products of organisms. The collection of bioaerosols is based on the same sampling principles as those for non-biological aerosols. However, ensuring the survival or biological activity of bioaerosols during and after collection is an important concern.

Fungal spores are ever-present bioaerosols in the natural environment. Most spores are adapted for airborne dispersal, and those present in the atmosphere will be introduced indoors along with fresh air. The size range of fungal spores, 0.5-30 µm or sometimes even larger, allows for their transport by winds to long distances. They are often resistant to various environmental stresses such as dryness, cold, heat, and ultraviolet radiation.

Most fungi are saprophytic, i.e., they utilise and grow on any nonliving organic material, provided adequate moisture is present. The moisture levels required for fungal growth are often quite low. Sufficient moisture can be absorbed from air by some organic materials, if the relative humidity exceeds 70%.

Bioaerosol concentrations have timely variations of several orders of magnitude. For example, concentrations of the order of $10-10^3$ CFU/m^3 can be found in homes or occupational environments. Lower concentrations of $\leq 10^2$ CFU/m^3 can be found in well-ventilated facilities without significant sources. High concentrations with peak concentrations from 10^4 to as high as 10^{10} CFU/m^3, can occur in the environment of seriously contaminated areas (Nevalainen *et al.*, 1993). In most of these environments, the concentrations vary considerably in time and space. This is partly because bioaerosol sources do not necessarily generate particles continuously. For example, spore production and spore release from fungal mycelium may occur in bursts under certain air humidity and velocity conditions.

SAMPLING EFFICIENCY OF BIOAEROSOL SAMPLERS.

The overall sampling efficacy of a bioaerosol sampler can be divided into three components (Nevalainen *et al.*, 1993):

1) The inlet sampling efficiency is a function of the sampler inlet's ability to extract particles from air without bias with regard to the particle size, shape, or aerodynamic behavior.
2) The removal efficiency is determined by the sampler's ability to remove the particles from airstream and deposit them into or onto the collecting medium.
3) The biological aspect of sampling efficacy is the sampling and removal of biological particles without altering their viability or biological activity, and to provide the proper conditions for the organisms to form colonies or to be otherwise detected.

PRINCIPLE OF BIOAEROSOL SAMPLING

Bioaerosol sampling involves separating the particle trajectory from the air streamline trajectory. To achieve this, different physical forces are applied as illustrated in Figure 2.10.

In Figure 2.10(a), the inertia of the particle forces its impaction onto a solid or semisolid impaction surface, usually either a culture medium, or an adhesive surface. Applications of this principle are cascade impactors with one, two, or more stages and slit samplers.

Figure 2.10(b) illustrates a virtual impactor, which is also based on the inertial behavior of particles. Size separation of particles occurs when the small low-inertia particles follow the airflow and the large high-inertia particles cross the virtual impaction surface and are collected or sensed below this interface. An experimental sampler of this kind has been used to collect outdoor air antigens.

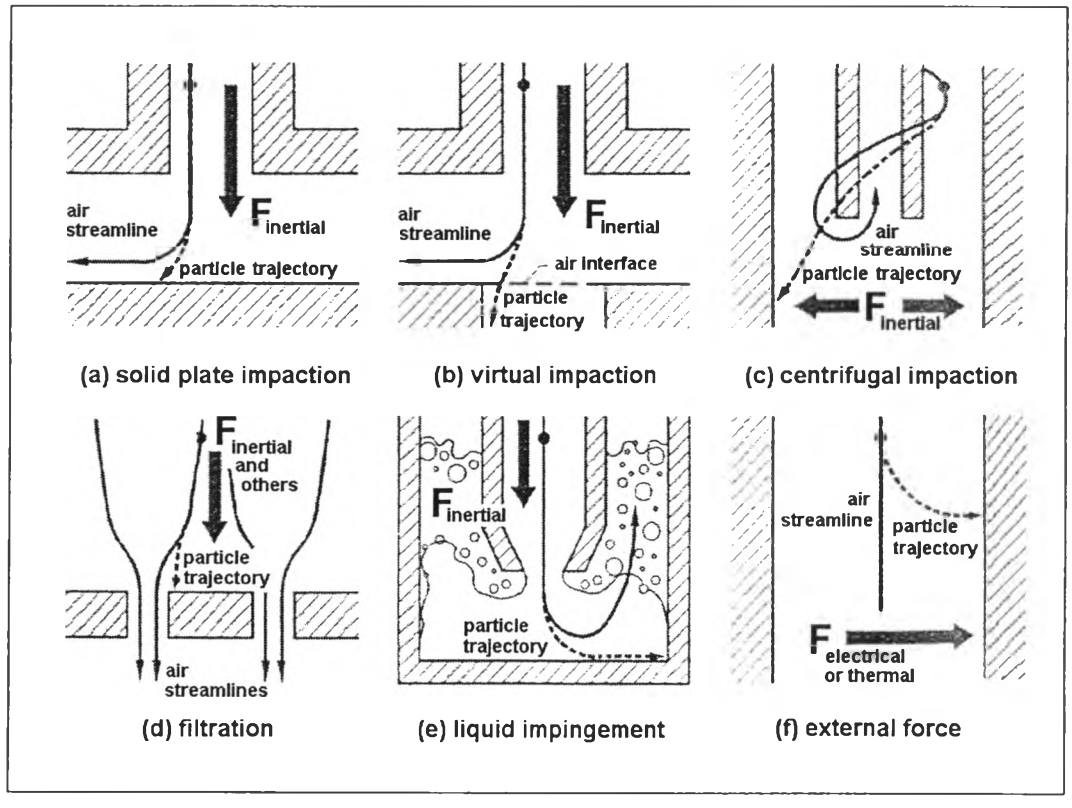

Figure 2.10: Mechanisms of particle removal from air. (Nevalainen *et al.*, 1993)

In Figure 2.10(c), The centrifugal force separates the particle from air streamline. It also uses the inertial behavior of the particle, but in a radial geometry. The Reuter centrifugal sampler is of this type.

The collection by filtration is shown in Figure 2.10(d). The inertial forces are also responsible for separating the particles from the airstream. However, in concert with inertial impaction, other mechanisms such as interception, diffusion, and electrostatic attraction contribute to the deposition of particles onto the filter material.

Figure 2.10(e) illustrates a liquid impinger which mainly uses inertial forces to collect particles, but also uses diffusion within the bubbles to enhance particle collection. Several liquid impengers are currently available including a preseparating units and a multistage impinger.

Particles may also be removed from the airstream by externally applied forces, such as, electrical forces on charged particles, or thermal forces on an aerosol flow which has a thermal gradient perpendicular to its flow. This principle is illustrated in Figure 2.10(f). An electrostatic sampler has been used for virus sampling.

Numerous air samplers have been designed for use in aerobiology. These samplers can be categorised as either passive or active depending on their use of forced air flow. Passive techniques rely on the settling out of particles from the air onto a sampling platform such as open sampling dishes with culture medium or glass slides covered with a sticky film. This method assumes that the organisms of interest will settle onto the surfaces under gravity and efficiency is dependent on particle size, wind velocity, and turbulance. (Stetzenbach, Hern, and Seidler, 1992)

Inertial impaction is the most widely used mechanism of particle removal in bioaerosol samplers. As this study, the Burkard portable air sampler for agar plates was used for collection of atmospheric fungi. The impaction process depends on the particles' inertial properties, such as size, density, and velocity, and on the impactor's physical parameters, such as inlet-nozzle dimension and airflow paths.

PREVIOUS WORKS IN THE PRESENT TOPIC

Singh and Tandon (1991) conducted the mycological analysis of the air in the Bharat Kala Bhavan located in the Banaras Hindu University, Varanasi, India and reported that twenty—three species of fungi could be isolated from the air in the museum. The fungal species were *Absidia glauca* Hagem, *A. lichtheimii* (Lucet and Costantin) Lendner, *A. scabra* Cocconi, *A. spinosa* Lendner, *Alternaria humicola* Oudenmans, *A. tenuis* Nees, *Aspergillus candidus* Link, *A. clavatus* Desmazieres, *A. fumigatus* Fresenium, *A. flavus* Link, *A. nidulans* (Eidam) winter, *A. niger* Van Tieghem, *A. sulphureus* (Fresenius) Thom and Church, *A. terreus* Thom, *Circinella simplex* Van Tieghem, *Cladosporium herbarum* (Persoon) Link, *Fusarium chlamydosporum* Link, *Mortierella* Coemans, *Mucor mucedo* Linne Brefeld, *Penicillium daleae* Zaleski, *P. humuli* Van Beyma, *P. sclerotiorum* Van Beyma and *Trichoderma lignorum* (Tode) Harz. The study indicated that most common fungal species e.g. *Aspergillus flavus, A. fumigatus,* and *A. niger* could be isolated from museum objects containing cellulose.

Jain (1995) studied some biodeteriorating components in the air of central India and their impact on cultural properties. The study was made at and around Gwalior, a cultural and historical city of central India. Outdoor aerobiological studies were made by exposing glycerine jelly coated slides in the Air Sampler daily and replacing with fresh slides after 24 hours. For indoor studies Petri dishes with potato dextrose agar medium were exposed at different sites. The author has shown that concentration of these biocomponents varies from season to season and mainly depends upon climatic conditions. A total of 23 fungal spore types were observed from environment surrounding historical monuments. The predominant colonies belong to Deuteromycetes, Ascomycetes, and Phycomycetes by which

Alternaria, Aspergillus, and *Penicillium* were found to be predominant whereas *Botrytis* and *Clodosporium* were also frequently found. Other common types were those of *Candida, Cercospora, Drechlera, Fusarium, Phama,* and *Pleospora.* The same report also indicated that climatic factors such as rainfall, temperature, and humidity played an important role in growth of these biocomponents.

Makerji, Garg, and Mishra (1995) investigated the fungi in deterioration of museum objects. They concluded that the control of fungal infection was very important especially for museum objects of organic origin. A wide variety of fungal species has been found growing over the museum materials, the most common ones were *Alternaria, Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Curvularia, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis, Stachybotrys, Stemphylium,* and *Trichoderma.*

Pandey and Srivastava (1995) isolated and identified the fungi causing biodeterioration of wood, small pieces of wood were kept at 95% humidity and 30°C temperature in a humidity and temperature–controlled chamber for 60 days. Fungi occuring on wood were isolated at weekly intervals, purified and identified. Twenty six species of fungi were found infest on teak (*Tectona grandis*) namely *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. versicolor, Chaetomium globasum, C. indicum, Cladosporium cladosporioides, C. herbaceum, Curvularia lunata, Emericella nidulans, Fusarium solani, Humicola fuscoatra, Paecilomycetes lilacinus, P. varioti, Penicillium citrinum, P. funiculosum, P. javanicum, P. pinophillum, P. notatum, Scopulariopsis brevicaulis, Trichoderma harzianum, T. koningii, T. roseum,* and *T. viridae.* Species of *Aspergillus, Penicillium,* and *Paecilomyces* were found to be dominant.

Pharuhas Luksamanapha, Uraporn Sardsud, and Morakot Sukchotiratana (1995) isolated cellulose-decomposing moulds from Ton Gwen Wooden Temple located in Amphur Hang Dong, Chiang Mai province. Chip of wood from three different areas of the temple i.e. the left, the right, and the back and from the *Sala Chaturamuk* were taken for the isolation. Czapek's agar was used as an isolating medium and cellulose powder as a carbon source. Moulds from the surface of wood in the same areas were isolated by scraping the surface with sterile blades, allowing the wood particles to fall down on the agar medium in Petri dishes. Moulds from the air were also isolated by exposing the agar medium to the air near by for 10 minutes. All isolates were tested for cellulolytic activities by the detection of clear zones produced when grown on the carboxymethyl cellulose (CMC) agar medium using Congo red as a staining indicator. The activities of cellulase were also detected specific activities using crude enzyme harvested from supernatant of Czapek's broth (CMC was used as a carbon source). Twenty isolates were obtained from wood chips, the remaining 16 isolates were obtained in equal numbers from wood surfaces and from the air. They were identified to be 9 genera of imperfect fungi i.e. *Fusarium, Curvularia, Cladosporium, Chalara, Lasiodiplodia, Aspergillus,*

Alternaria, Cylindrocarpon, and one unidentified genus. All isolates were able to hydrolyse carboxymethyl cellulose. Some of them showed distinct clear zones and high specific activity of cellulolytic enzymes. Six genera i.e. *Fusarium, Curvularia, Cladosporium, Lasiodiplodia, Cylindrocarpon,* and unidentified genus were the possible causative agents of wood decay in this temple.

Chulee Chaisrisook, Poonpilai Suwanarit and Chiraporn Aranyanak (1995) surveyed a number of fungal species distributed in the air and on wooden objects of the Bangkok National Museum in the last decade. An air-trapping technique using potato dextrose agar as a culture medium was used to investigate the distribution of fungal species in the air. The major fungal species obtained in the air of 17 exhibition rooms were *Curvularia, Aspergillus, Trichoderma, Penicillium, Fusarium* and one specie of yeast, *Candida* sp. Fungal growth on wooden objects, e.g. musical instruments, furniture, baskets and weapons, was examined by swabbing technique and sticky tape technique. The dominant species were *Curvularia, Helminthosporium, Trichoderma, Aspergillus, Alternaria, Chaetomium, Nigrospora* and *Penicillium.*

Sakamoto, Kurozumi and Kenjo (1995) reported the study of effects of air flow for preventing fungal growth. Since in Japan, deterioration of art objects caused by fungi is a serious problem, biosensors were employed to measure the degree of fungal growth under certain circumstances. It was observed that a light air flow could restrained fungal growth. Thus, the combination of air flow with temperature and humidity control makes it possible to prevent fungal growth in circumstances where art objects are conserved.