CHAPTER II

REVIEW OF LITERATURE
Large volume of literature is available on degradation of cellulosic materials by micro-organisms. Among these, fungi are largely responsible for the biodeterioration of cellulosic materials under favourable conditions of moisture and temperature. When cotton fabrics are exposed in tropical and subtropical regions, filamentous fungi, such as, Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti appear on the fabrics. Fungi Imperfecti are highly cellulolytic and constitute the overwhelming majority due to their ability to grow under adverse environmental conditions. Phycomycetes are mostly non-cellulolytic and require more moisture for growth than other groups. Ascomycetes occupy an intermediate position with respect to their cellulolytic activity and capacity to withstand unfavourable conditions. Basidiomycetes are very rare on cotton fabric in comparison to other groups of fungi.

In general, the growth of fungi begins to appear when relative humidity approaches 80% and the temperature ranges between 25°-35°C (Armstead and Harland, 1923; Galloway, 1935;
Prindle, 1937; Snow, et al., 1944 and Bonner, 1948). Different fungi utilise different cellulosic substrates for growth and enzymes production.

*Gladosporium halodes*, *Alternaria tenuis*, *Curvularia lunata* and *Helminthosporium halodes* have been reported to grow on decaying leaves of sugar-cane and utilise the major contents of cellulose therefrom. While, intermediate utilisation of cellulose from the decaying leaves have been obtained by *Myrothecium* and *Pithomyces* species (Rai, 1970). *Myrothecium verrucaria* has been found to grow best and produce cellulase enzymes near the end of the rapid growth period in a medium with glucose or glycerol as carbon source (Hulme and Stranks, 1971). A number of species of genus, *Trichoderma*, were isolated from sugar-cane bagasse (Toyama, et al., 1972). Three marine cellulolytic fungi - two *Aspergillus* species and one *Paecilomyces* specie were isolated from canvas cloth exposed to sea water in Bombay (Desai and Betrabet, 1971). They have also found that *Aspergillus niger* was the most active marine isolate. Peitersen, (1975) has reported the growth of *Trichoderma viride* on barley straw, while certain other fungi, such as, *Aspergillus niger*, *Alternaria macrospora*, *Cochliobolus specifer* and *Curvularia lunata* have been reported to utilise ground nut shells as C-source for their growth (Sethi, et al., 1977). Sethi and Sood (1977) have reported the growth of fungi on banana peels. The highest producer of cellulase was *Trichoderma viride* on dried peel and the highest protein producer was *Myrothecium*
verrucaria. Most of the fungi have been found to grow on cotton wastes, filter paper pulp, wheat bran, rice bran, sugar-cane bagasse and other cellulosic wastes, mainly farm waste products, utilising them and producing soluble sugars (De Menezes, et al., 1973, Khotyanovich, et al., 1974).

A large number of plants, including cotton, harbour fungi in their vegetative as well as floral buds. The cotton fibers may be infected by fungi right from when fiber start developing in cotton seeds in the bolls of the plants. The bolls, which remain open normally, undergo some weathering before harvestation. A number of fungi, including mostly the species of *Alternaria*, *Fusarium*, *Cladosporium*, *Diplodia*, *Rhodotorula*, *Collectrichum* and *Rhizopus* have been observed growing during these stages of cotton fibers (Prindle, 1934; Owen and Carolina, 1938; Marsh and Bollenbacher, 1949; Marsh, et al., 1949 and 1950; Amdt, 1950 and 1953). Some of these are commonly observed on deteriorated cotton textiles. Spores of some of the fungi are resistant and retain their viability even, when the cotton fibers undergo various treatments during fabrication (Siu, 1951).

Fungi, encountered in large numbers on cellulosic materials, differ in their property of destroying cellulose. Some of these liberate cellulolytic enzymes of high activity, while in case of others, the cellulolytic enzymes are not so active in deteriorating the cotton textiles. A large number of
fungi, such as species of *Aspergillus*, *Penicillium*, *Memnoniella*, *Chaetomium*, *Alternaria*, *Helminthosporium* and *Curvularia* etc. have been largely studied at DMSRDE, Kanpur (DRL(M), Kanpur, Technical Report No. Bio./47/69, 1947) and have been reported to be potent cellulose destroyer, possessing highly active cellulolytic enzymes. Certain genera, such as, *Trichoderma*, *Myrothecium* and *Aspergillus* have been extensively studied for the production of cellulolytic enzymes. Rodionova, et al. (1970) have reported the ability of *Myrothecium verrucaria*, *Trichoderma viride* and *Helix pomatia* to produce cellulases, disintegrating paper to give soluble sugars.

A doubling of cellulase production by *Trichoderma viride* was possible by increasing the concentration of cellulose in the medium (Sternberg, 1976a). Mutants, produced by diethyl sulphate treatment of *Trichoderma viride*, showed variations in sporulation and relatively high cellulase production (Morozova, 1973). Another mutant strain of the fungus *Trichoderma viride*, obtained by irradiation, secreted twice as much cellulases as the parent strain (Mandels, et al., 1971).

*Trichoderma lignorum* has been reported to hydrolyse and destroy hemicellulose in potato pulp (Veche and Levitskaya, 1972; Kozhemyakina, et al., 1974). Ghose and Haldar (1970) have also reported the capacity of *Trichoderma viride* to produce cellulases. Other fungi, such as, *Cladosporium halodes*, *Alternaria tenuis*, *Curvularia lunata*, *Helminthosporium halodes*,
species of *Trichosporium*, *Chaetomium globosum*, *Sordaria humana*, *Aspergillus niger*, *Aspergillus terreus* have been reported to utilise various substrates, such as, cotton waste, wool fiber, filter paper pulp etc. (Rai, 1970; Sankov, *et al.*, 1972; Kovasc-Szabo and Cseh, 1972; Mehta and Agrawal, 1973; Strzelec, 1973). Besides these common genera, some rare fungi have also been shown producing cellulolytic enzymes. *Gliomastix murorum*, isolated in North and Central America, India, Australia and Europe, has been implicated in rotting of cotton clothing and tent canvas in the tropics owing to its cellulolytic property (Williams and Pugh, 1971). Honey fungus, *Armillaria mellea*, has been investigated for producing cellulolytic enzymes by Fedorov and Badyai (1973). Three white rot fungi, viz., *Polyporus versicolor*, *Ganoderma applanatum* and *Ganoderma peniophora* produced an adaptive cellulase complex that would degrade both soluble cellulose and microcrystalline cellulose (Highley, 1973). *Penicillium funiculosum* (Juric, 1977), *Phialophore malorum* (Berg, 1978) have been found to produce active cellulolytic enzymes.

Various species of *Chaetomium* have been reported to be active producers of cellulolytic enzymes. These *Chaetomium* species have been preferred due to their less sensitive nature, good cultivability and well established cellulolytic properties (Novak, 1970). Among these, *Chaetomium indicum*, *Chaetomium aureum*, *Chaetomium thermophile*, *Chaetomium globosum* have been specially mentioned (Belen’kaya and Kanevskaya, 1973; Ghora
and Chaudhuri, 1975; Romanelli, et al., 1975; Ghosh and Bose, 1976; Brewer and Taylor, 1978). Khotyanovich, et al. (1974) have found that among the three fungi, viz., *Trichoderma lignorum*, *Chaetomium globosum* and *Coprinum domesticus*, the latter two fungi were most effective in degrading cotton wastes. They further reported that these two fungi may be recommended for use in composting of cotton wastes. Chahal and Howksworth (1976) and Young, et al. (1977) have reported a new thermo-tolerant fungus, viz., *Chaetomium cellulolyticum*, which was found to be a good cellulase producer, besides being an excellent protein producer. *Chaetomium thermophile* has also been reported to be a good producer of active cellulase enzymes (Eriksen and Goksysr, 1977). Sharma and Saksena (1979) have found that thermophilic fungi were more active than mesophilic fungi. The most active thermophiles were *Chaetomium thermophile*, *Humincola grisea* and *Sporotrichum thermophile*.

Cellulases were found to be present in the latex of species with articulated laticifers and were involved in the removal of end walls during the differentiation of articulated laticifers (Sheldrake, 1970). Cellulase activity was also found in the pyloric ceca of 8 species of starfish (Araki and Giese, 1970). Cellulases, along with proteases and chitinases, have been reported to be released during the excystment of *Hartmanella culbertsoni*. These enzymes may be involved in the degradation of empty cyst walls (Kaushal and Shukla, 1976). Fungal cellulases have also been reported to be stable at high
pressures existing in the deep sea (Kim and ZoBell, 1972).

Effect of nutritional and environmental factors on the growth and enzyme elaboration

Minerals and nitrogenous compounds

Fungi require certain essential nutrients for their growth, sporulation and elaboration of enzymes. Potassium, magnesium, phosphorus, calcium and sodium, in the form of inorganic compounds, are basically needed for the growth of fungi (Greathouse and Wessel, 1954). In addition, presence of vitamins, amino acids and other nitrogenous compounds and rare elements greatly influence the growth and elaboration of enzymes by the fungi. Panwar (1970, 1973) has reported that the presence of neutral amino acids, glycine, DL-valine and asparagine enhanced the growth of Curvularia lunata. Whereas, Shukla and Bias (1971) have reported a favourable effect of vitamins, viz., thiamine HCl, inositol, riboflavin, pantothenic acid, biotin and choline on the growth and sporulation of Curvularia lunata. Cellulase activity in 4 Fusarium strains was generally increased by methionine and cystaine or cystine (Szajer, 1975). When Trichoderma lignorum was cultivated in a medium containing different amino acids as nitrogen source, maximum cellulases were observed with asparagine, valine and glutamate (Tashpulatov, et al., 1977). They further reported that of various vitamins tested only thiamine and biotin stimulated fungal growth and cellulase
production. L-Asparagine was found to be the best nitrogen source for growth, sporulation and enzyme production by *Fusarium oxysporum* (Olutiola, 1978). Some inorganic compounds, such as, \((\text{NH}_4)_2\text{SO}_4\), \(\text{NaNO}_3\) and \(\text{Ca(NO}_3\)) have also been shown to be good sources of nitrogen for the growth and enzyme production in case of *Trichoderma viride*, *Sporotrichum thermophile*, *Helminthosporium apattarnae* and *Aspergillus fumigatus* (Kalunyants, *et al.*, 1976; Coutts and Smith, 1976; Ghewande and Deshpande, 1977; Trivedi and Rao, 1979).

**Carbon**

Carbon compounds constitute food for fungi and are assimilated readily. Fungi prefer carbohydrates than proteins as food but glucose proved to be inhibitory towards the growth and production of enzymes by the fungi due to product inhibition or feed back inhibition (Simpson and Marsh, 1969). Cellulose is an excellent source of carbon for many fungal species (Siu, 1951). *Myrothecium verrucaria* produced active cellulolytic enzymes when grown on media containing natural cellulose. *Chaetomium globosum*, *Fusarium moniliforme*, *Rhizoctonia* species *Trichoderma* species and *Myrothecium verrucaria* grew best on raw cotton and produced active cellulase enzymes (Simpson and Marsh, 1969). Aizenberg and Musich (1975) have observed that some fungi, viz., *Fusarium oxysporum*, *Trichoderma lignorum*, *Stachybotrys alternans* and *Helminthosporium* species showed good growth and elaborated cellulolytic enzymes of high activity in mineral medium.
containing cellulose or pectin as a sole source of carbon. Chaetomium thermophile has been found to utilise cellulose or xylan or cotton as carbon source (Eriksen and Goksøyr, 1976). Cellulolytic enzymes were produced by Poronia oedipus grown on soluble and insoluble forms of cellulose (Denison and Koehn, 1977). They further observed that cellulase production is negligible when the organism is grown in presence of glucose. Curvularia verruculosa has been found to grow profusely in presence of lactose or raffinose as best carbon source (Aulakh, 1970). Mehta and Saksena (1975) have found that starch supported maximum fungal growth of Aspergillus solani.

Many fungi have been reported to grow on natural substrates. Phoma glomerata and Rhizoctonia solani produced highly active cellulases from natural substrates, such as, rice straw, wheat bran and wheat straw (El-Kersh, et al., 1973). The production of cellulases by Irpex lacteus was investigated by Lee and Koh (1975). Active cellulases were isolated with cultures growing on wheat bran or beet pulp or rice straw. These crude materials have been found to be the best C-source for growth and elaboration of enzymes by Trichoderma viride (Gupta, et al., 1972; Kalunyants, et al., 1976). Carboxymethyl cellulose was found to be utilised by Heminthosporium apattarnae (Ghewande and Deshpande, 1976a, 1977), Aspergillus niger, Memnoniella echinata (Deshpande and Kulkarni, 1979) and by some soil fungi (Ortega, 1980).
Temperature

Maximum growth and elaboration of enzymes by the fungi are greatly affected by the temperature. Different fungi require different optimum temperature for their growth. By and large, the optimum temperature for the growth of most of the fungi lies between 250 to 400C. Optimum temperature for maximum cellulose production by Aspergillus terreus and Trichoderma viride was shown to be 400C (Tashpulatov and Khakimova, 1971; Zeltins, et al., 1974; D’Aquino, et al., 1968). Thermophilic fungi, viz., Mucor pusillus, Humicola lanuginosa, Thermoascus aurantiacus, Thermoascus crustaceus and two varieties of Chaetomium thermophile were found to grow and produce cellulolytic enzymes at 450C (Oso, 1975). Coutts and Smith (1976) have also reported 450C as the optimum temperature for enzyme production by Sporotrichum thermophile. Chaetomium thermophile have also been reported by Eriksen and Goksøy (1976) to grow at 450 to 500C. Maximum growth of Culvularia verruculosa was achieved at 300C (Aulakh, 1970). The optimal temperature for growth and enzyme production of Trichoderma lignorum was 280C (Tashpulatov and Teslinova, 1974). Pass and Griffin (1974) have observed that maximum germination by Aspergillus flavus occurred at 350C. While El-Kersh, et al. (1973) have reported the optimum temperature for the growth and enzyme production by Phoma glomerata and Rhizoctonia solani as lying between 250-300C. 280C was found to be the optimum temperature for the growth of Stachybotrys atra (Samardzic, et al., 1971). Fusarium oxysporum grew and sporulated

**pH**

pH of the medium has marked influence on the growth and elaboration of enzymes by the fungi. Most of the fungi have been shown to grow in slightly acidic medium. Maximum growth of Curvularia verruculosa was achieved at pH 6.0 (Aulakh, 1970). Chaetomium globosum, Monilia sitophila, Penicillium purpurogenum, Stemphylium botryosum and Trichoderma viride grew at pH ranging from 4.6 to 6.8 (Zagulyaeva, 1972). The optimum pH for the growth of Phoma glomerata and Rhizoctonia solani was between 5.0 - 7.6 (El-Kersh, et al., 1973). Eriksen and Goksøyr (1976) have reported that the optimum pH for the growth of Chaetomium thermophile was 5.5 - 6.0. pH 4.5 has been found to be the optimum for the maximum production of cellulases by Aspergillus terreus, Myrothecium verrucaria and Trichoderma viride, whereas, pH 6.0 was most favourable for cellulase synthesis by Verticillium tenuerum and Chaetomium globosum (Nyuksha and Kossior, 1976). Fusarium oxysporum has been found to grow and sporulate best at pH 7.5 (Olutiola, 1978), while, Ghewande and Deshpande (1976a) have reported that highest cellulase production by Helminthosporium apattarnae was observed at pH 6.6. Andreotti, et al. (1977) have observed that pH 5.0 was suitable for the growth and enzyme production of Trichoderma viride.
**Moisture**

The moisture content of cotton influences significantly the rate of growth of fungi (Galloway, 1935). Growth of the micro-organisms starts when the moisture contents reach at least 10% (Urns, 1925; Brns, 1927; Thaysen and Bunker, 1927). But profuse growth of the fungi occurs when the moisture contents of the atmosphere approach 80% (Snow, et al., 1944; Bonner, 1948).

**Purification of cellulolytic enzymes**

In order to carry out proper characterisation of the structural and catalytic properties of the cellulolytic enzymes in the biodegradation of cellulosic materials, attempts have been made by many workers to scale up the purification methods for these enzymes (Reese and Gilligan, 1953; Toyama and Shibata, 1961; Ogawa and Toyama, 1967, 1968, 1972; Kanda, et al., 1970; Berghem and Pettersson, 1973, Eriksson and Pettersson, 1975). A variety of methods have been used to purify and separate the enzymes of the cellulase complex.

**Concentration of the culture filtrates**

Various methods of concentration of the culture filtrate have been used by different workers. Whitaker (1953) has reported vacuum evaporation at 35°-39°C and concentration by slow freezing in case of *Myrothecium verrucaria*. A concentration of 20 litres of culture filtrate to about 600 mL was
achieved in a climbing film vacuum evaporator, below 30°C, in case of *Stachybotrys atra* (Thomas, 1956). Hirayama, *et al.* (1978) have reported the concentration of culture filtrate, in case of *Pyricularia oryzae*, with the help of flash evaporator at 28°C.

**Deionization**

Dialysis and use of ion-exchangers are the two most widely applied processes for removal of ions from solution (Stauffer, 1950). Nitrocellulose films (Siu, 1951 and Stone, 1954), vellum and rabbit bladder membranes have successfully been used for dialysis of cellulolytic enzymes. Other dialysis membranes used for this purpose are attacked by the enzymes as they are cellulolytic in nature.

Electrodialysis procedures between cellophane membranes, against a continuous flow of distilled water, have been developed for the isolation and purification of cellulolytic enzymes of *Aspergillus oryzae* (Whitaker, 1953) and *Stachybotrys atra* (Thomas, 1956). Another protein membrane, which has been widely used is goldbeaters' skin. This membrane has been used in the preparation of cellulases from *Myrothecium verrucaria* (Whitaker, 1953).

The removal of 80-90%, by weight, of total mineral impurities present in the crude cellulolytic enzyme preparations has been achieved by dialysis for 72 hours against running tap
water (Gascoigne and Gascoigne, 1960). Rudick and Elbein (1973) have reported the use of dialysis technique, in double walled cellophane bags, against deionised water in order to remove dissolved impurities from crude enzyme preparation of *Aspergillus fumigatus*. Deshpande, et al. (1978) have also reported dialysis of crude enzyme preparation to remove soluble impurities in case of *Sporotrichum pulverulentum*.

Ion-exchange resins have satisfactorily been used for deionisation of the crude culture filtrate of micro-organisms. Cellulases from *Hydnum henningsii* (Kilroe-Smith, 1957) was desalted by passing through ion-exchange resin column. Roth (1956) successfully deionised culture filtrate from *Myrothecium verrucaria* by passing through a mixed column of Amberlite IR-120(H) and Amberlite IR A-400(OH), with 35-50% loss in total protein but no loss in cellulase activity. Crude extract from *Fusarium moniliforme* (Gascoigne and Gascoigne, 1960) was passed through columns of Amberlite resins for the removal of cations and anions. Use of buffered ion-exchange resin column has also been found satisfactory (Oncley and Dintzis, 1952). This technique readily deionised the crude enzyme extract of *Myrothecium verrucaria* and *Fusarium moniliforme*, without loss in enzyme activity. Recently, desalting has been carried out conveniently by passing through Sephadex G-25 column. Siwinska and Galas (1977) have reported that soluble impurities of inorganic anions and cations were removed by passing through
Sephadex G-25 in case of crude cellulolytic enzymes of
Trichoderma viride and Trichoderma roseum.

**Fractional Precipitation**

The commonly used technique for the separation of active proteins involves salting out with ammonium sulphate. Precipitation with lead acetate and organic solvents, e.g., ethanol and acetone, have also been used for concentration and purification of cellulases from various sources. Precipitating agents, such as, Rivanol (6,9-diamino-2-ethoxyacridine lactate), polymethacrylic acid and tannin were also used. These reagents effectively precipitated the enzyme proteins by complex formation.

**Salt Precipitation**

Ammonium sulphate is commonly used for protein precipitation, because it is soluble in water and is effective. Kooiman, et al. (1953) have obtained a 25-50 fold concentration of Myrothecium verrucaria cellulases by ammonium sulphate fractionation. Stone (1954) has reported that fractional precipitation of commercial cellulase preparation from Aspergillus niger with ammonium sulphate, in the range of 45 to 60% saturation, precipitated most of the active fraction. Thomas (1956) fractionated the culture filtrate of Stachybotrys atra by gradually increasing the concentration of ammonium sulphate.
upto 80% saturation at pH 6.0 and 8.0. Lasker and Giese (1958) and Lasker (1959) have reported that the active cellulase preparation was obtained from midgut extract of *Ctenolepisma* (Silver fish) by fractionation of soluble protein with ammonium sulphate between 60 and 70% saturation. Toyama (1960) obtained a highly active cellulase preparation from extracts of *Kogi* (Wheat bran) culture of *Trichoderma koningii*.

Chang and Usami (1968, 1969) obtained active cellulase preparation from *Trichoderma viride* by precipitation with ammonium sulphate. Cellulases in culture filtrate of *Sclerotium rolfsii* were purified, 4-5 fold, by ammonium sulphate precipitation (Rogowiew, 1969). Two cellulase components were obtained from the culture filtrate of the fungus *Polyporus tulipiferae* by precipitation with ammonium sulphate (Kanda, *et al.*, 1970). Tashpulatov and Khakimova (1971) have reported that three active cellulase components, from culture filtrate of *Aspergillus terreus*, were salted out at 30%, 50% and 70% saturation with ammonium sulphate. Two cellulases extracted from wheat seedlings were purified by ammonium sulphate fractionation (Nikolov, *et al.*, 1971). Sahgal and Agarwal (1972) isolated three fractions from metabolic liquor of *Curvularia lunata* by ammonium sulphate fractionation. Ikeda, *et al.* (1973) obtained partial purification of a complex cellulase preparation from the culture filtrate of *Aspergillus niger*. Svensson (1978) has also reported purification of cellulases of fungal origin by \((\text{NH}_4)_2\text{SO}_4\) fractionation followed by other techniques. Rudick and Elbein
(1973), Berghem and Pettersson (1974), Deshpande, et al. (1978), Hirayama, et al. (1978) have reported the use of ammonium sulphate as a step towards the purification of cellulases. Soni and Bhatia (1979) obtained three fractions of cellulases from Fusarium oxysporum using different concentrations of \((NH_4)_2SO_4\). Maeng, et al. (1980) have reported precipitation of the culture filtrate from Aspergillus nidulans with 90% ammonium sulphate.

**Solvent Precipitation**

Organic solvents, such as, ethanol and acetone, have been reported as useful precipitating agents in the purification of cellulase complex. These solvents produce marked effect on the solubility when added to aqueous solution of proteins, due to change in dielectric constant of the medium.

Youatt (1958) isolated intracellular \(\beta\)-glucosidase from the mycelial extract of Stachybotrys atra grown on cellulose. Freeze dried mycelia were extracted with water and then with 20-24% aqueous ethanol. This fraction contained three active components, such as, cellulase, cellobiase and aryl-\(\beta\)-glucosidase.

Enebo (1954) has reported that highly active cellulase preparation from bacteria, Clostridium thermocellulaseum, was obtained by ethanol precipitation. Jothianandan and Shanmugasundaram (1968) have observed that cellulases of Pyricularia oxyzae were purified five fold by alcohol precipitation.
Banikova and Boshikova (1971), during isolation of cellulases from *Aspergillus niger* strain 110, have experienced that ethyl alcohol was more suitable precipitant than acetone at -3°C and at pH 3.5. Cellulolytic preparations from the culture filtrate of thermotolerant strain, *Aspergillus terreus*, were isolated by fractional precipitation with ethanol (Khokhlova and Ismailova, 1973). Sison, *et al.* (1957) used precipitation technique with acetone for the purification of the cellulolytic enzymes from the wood rotting fungus, *Poria viallantii*. Nisizawa (1955) has reported the preparation of crystalline cellulases from the dialysed concentrate of submerged culture of wood rotting fungus, *Irpex lacteus*. The method for purification involved a series of fractional precipitations with acetone at low temperature. After seven steps of fractionation with acetone, crystals were obtained, the specific activity of which remained unchanged on further recrystallisation from aqueous acetone. The overall increase in purity was three fold. Later on, Nisizawa and Hashimoto (1959) applied similar method for the separation of cellulases and β-glucosidases of this fungus. The β-glucosidase rich fraction precipitated at low concentration of acetone, while a cellulase rich fraction was obtained at 60-70% acetone concentration.

Whitaker (1951, 1952, 1953) carried out most extensive purification of cellulases from *Myrothecium verrucaria* by combination of precipitation methods. Enebo, *et al.* (1953) purified cellulases from green malt extracts by precipitation
with acetone. Elyakova, et al. (1968) purified cellulases from marine mollusc, Littorna species, by precipitation with acetone. An active precipitation of cellulase was obtained from the gastric juices of Helix lucorum by precipitation with acetone (Chomoneva, 1968-69). Extraction of cellulase from the culture filtrate of Trichoderma viride was successfully carried out by acetone precipitation (89%) at a temperature of 20°C and pH 4.5 (Chomoneva and Sofroniev, 1970-71). Ige and Wilke (1974) have reported the recovery of cellulase enzymes of Trichoderma viride by acetone precipitation. Rudick and Elbein (1973) have reported that chilled acetone (-20°C) was used in the fractionation of β-glucosidases elaborated by Aspergillus fumigatus. The crude culture filtrate of Trichoderma viride was precipitated with acetone before subjecting to further purification (Bisaria and Ghose, 1977).

**Column Chromatography**

A variety of chromatographic methods have been employed to separate and purify the enzymes of cellulase complex. Varying degrees of success has been reported with fractionation involving adsorption chromatography, either on activated alumina (Bass, et al., 1952; Nisizawa and Kobayashi, 1953) or calcium phosphate (Gilligan and Reese, 1954; Stone, 1957) or cotton gauze (Ogawa and Toyama, 1967, 1968 and 1972) or Avicel (Li, et al., 1965, Emert, et al., 1974). Ion-exchange chromatography
on Dowex, Amberlite resins or DEAE Sephadex has proved to be a valuable fractionation technique (Eriksson and Pettersson, 1975; Okada, et al., 1968). According to Wood and McCrae (1977), chromatography on weak anion-exchanger DEAE Sephadex, has found widest application and most of the investigators have used this method at one stage or other in the fractionation of cellulase complex. These workers have also mentioned the importance of molecular sieve chromatography on Sephadex gel in the purification of cellulolytic enzymes. Use of polyacrylamide gel (Berghem and Pettersson, 1973; Eriksson and Pettersson, 1975; Tomita, et al., 1974) and dextran gel (Wood, 1968; Selby and Maitland, 1967; Halliwell and Riaz, 1970) have also been reported in gel filtration technique for fractionation of cellulase enzymes.

Umezurike (1970) has reported that four cellulase components were separated from culture filtrates of Botryodiplodia theobromae by polyacrylamide gel electrophoresis and gel filtration on Sephadex. Two cellulases extracted from wheat seedlings were purified by column chromatography on Sephadex G-100 and ion-exchange chromatography on DEAE Sephadex A-25 (Nikolov, et al., 1971). Culture filtrate of Aspergillus niger was purified by gel filtration on Sephadex G-100 ion-exchanger and adsorption chromatography (Ikeda, et al., 1973). Marshall (1973) has reported the purification of cellulases from Helix pomatia to an extent of 21-fold by DEAE cellulose chromatography.
The cellulase complex of *Trichoderma viride* has been purified by gel filtration on Sephadex G-25-150 and by ion-exchange chromatography on DEAE Sephadex A-50 (Chomoneva, *et al*., 1970-71 and Hakansson, *et al*., 1978). The cellulase complex of *Rhyncosporium scaliis* was separated by gel filtration followed by ion-exchange chromatography (Olutiola and Aryes, 1973). Cellulolytic enzymes of *Geotrichum candidum* were separated into two active fractions with the help of DEAE Sephadex chromatography (Rodionova, *et al*., 1974). Yang (1973) purified cellulolytic enzymes of *Trichoderma lignorum* using Sephadex G-75, and G-100. The four principal endoglucanase components of *Trichoderma koningii* were purified by Sephadex G-75 gel filtration, DEAE and sulfoethyl-Sephadex ion-exchange chromatography (Wood and McCrae, 1978).

The porous cation-exchanger KMT-12 was used to separate and purify enzymes in the complex of *Pectawamorin* P-10x. The specific activity of Cx cellulase increased by 8-fold after ion-exchange chromatography on KMT-12 (Vorob'eva, *et al*., 1976). Eriksson and Pettersson (1975) have reported the purification of extracellular enzyme system of *Sporotrichum pulverulentum* by affinity chromatography on Concanavalin A-Sepharose and activation on Dowex 2-x8 anion-exchanger. By concentration of culture filtrate of *Chaetomium thermophilum*, followed by ion-exchange chromatography on DEAE-Sephadex A-50 and gel filtration on Bio gel P-100, three electrophoretically pure components of cellulase complex were obtained (Eriksen and Goksøy, 1977).
However, Soni and Bhatia (1979) fractionated cellulase complex of *Fusarium oxysporum* by chromatography on DEAE cellulose column. Osmundsvåg and Goksøyr (1975) have reported isolation of two extracellular cellulases from the culture supernatant of *Sporocytophaga myxococcoides* by a series of gel filtration and ion-exchange chromatography steps. Two carboxymethyl cellulases were purified from the culture of *Aspergillus aculeatus* by DEAE-Sephadex column chromatography, SP Sephadex column chromatography and gel filtration with Sephadex G-75 and Biogel P-60 (Murao, et al., 1979). Fractionation of multienzyme cellulase complex of *Trichoderma koningii* on DEAE-Sepharose column gave two types of carboxymethyl cellulases and β-glucosidases (Ghai, 1980). A procedure involving Affinity Chromatography on cross-linked cellulose was developed for separating enzymic components of a cellulase complex from *Trichoderma viride* (Weber, et al., 1980).

**Electrophoresis**

As proteins are ampholytes, they migrate to one electrode or the other in an electric field, however, at a particular hydrogen ion concentration, the isoelectric point, no such migration occurs in the protein solution. With materials of low molecular weight, e.g., amino acids, sugar phosphates, sugar borates the migration is called ionophoresis but in case of high molecular weight proteins, the term electrophoresis is
generally used. As the various proteins have different structures and the array of component amino acids present differs in overall ionic characteristics, each protein migrates at a characteristic rate at certain pH value. It is possible, therefore, to analyse a mixture of proteins by electrophoresis.

Gillespie and Woods (1953) used moving boundary electrophoresis to examine the components of the enzyme preparation from Aspergillus oryzae, using Veronal buffer at pH 8.6, for three hours with a current of 15 mA. Whitaker (1953) has studied the different cellulolytic components, present in culture filtrate of Myrothecium verrucaria, with moving boundary electrophoresis technique. Free boundary electrophoresis of one per cent solution of acetone precipitated fractions, from the culture filtrate of Poria vaillantii, in acetate buffer (0.1 M, pH 4.6 - 5.3), at 150 volts and 10 mA for 45 min, enabled Sison, et al. (1958) to show that the fractions consisted principally of two components. Pettersson (1968) has reported that, after each purification step, the enzyme preparation in case of Penicillium notatum was tested for homogeneity by polyacrylamide gel electrophoresis at pH 8.0, using a current of potential difference of 50 V. Ikeda, et al. (1973) have reported the purified enzyme preparation of Aspergillus niger as homogeneous in ultracentrifugation, disc gel electrophoresis and ampholine electrophoresis. The extracellular β-glucosidase from Aspergillus fumigatus has been purified to homogeneity as judged by polyacrylamide gel electrophoresis (Rudick and
Elbein, 1973). Two components of purified cellulases from *Sporocytophaga myxococcoides* were assayed for purity by electrophoresis in polyacrylamide gel and isoelectric focusing (Osmundsvag and Goksøyr, 1975). Siwinska and Galas (1977) have reported that the homogeneity of purified enzymes, in case of *Trichoderma viride* and *Trichoderma roseum*, has been examined by starch gel electrophoresis. Bielecki and Galas (1977) have performed electrophoresis in polyacrylamide gel at pH 4.3 in order to test the purity of cellulase preparation of Yeast. The isolated enzymes of *Trichoderma viride* appeared homogeneous upon polyacrylamide gel electrophoresis at pH 2.9 and isoelectric focusing in a polyacrylamide gel slab (Hakansson, 1978).

Isoelectric focusing (Wood and McCrae, 1972; Nisizawa, *et al.*, 1972b; Bergham and Pettersson, 1973; Svensson, 1978; Wood and McCrae, 1977, 1978) has proved to be valuable fractionation technique. Hirayama, *et al.* (1978) have used the technique of isoelectric focusing to examine the purity of cellulases of *Pyricularia oryzae*. Deshpande, *et al.* (1978) have reported the use of preparative slab gel isoelectric focusing in the purification procedure of cellulase enzymes from *Sporostrichum pulverulentum*. The purified enzymes were found to be homogeneous as judged by disc gel electrophoresis on sodium dodecyl sulphate (Murao, *et al.*, 1979).
Use of fungicides

The damage, caused by the microbial attack on the cellulosic materials, is so enormous that the use of fungicides becomes inevitable. A wide variety of fungicides are in use to protect the cellulosic materials from fungal attack. The cellulolytic enzymes, elaborated by the fungi, aid in the biodegradation of cellulosic substances, either directly, by providing cellulose break-down products for the nutrition of micro-organisms, or indirectly, by the release of nutrients from the disrupted cells. Thus, inhibition of the action of cellulolytic enzymes and control of microbial growth are the effective measures against microbial damage. Many fungicides, natural and synthetic, for combating the activities of fungi, are extensively used. Products of enzyme-cellulose reactions often inhibit the activity of cellulolytic enzymes, because of the structural analogy and competition with the substrate for the active centres, or due to feedback inhibition.

Natural fungicides of pectic enzymes have been frequently reported from plant sources (Byrde, et al., 1960; Wood, 1960; Byrde, 1963; Mandels and Reese, 1963; Williams, 1963). These naturally occurring fungicides belong chiefly to the phenolics, tannins or leuco anthocyanins. But, these fungicides are not competitive and their specificity is questionable. A water soluble fungicide for cellulolytic enzymes was extracted from the leaves of Vitis rotundifolia (Bell, et al., 1960). 4% concentration of the fungicide was required for 50% inhibition.
of cellulase activity of *Aspergillus niger* (Bell and Etchells, 1958; Etchells, et al., 1958a and 1958b; Bell, et al., 1962). Another water soluble substance, which inhibited the cellulases of *Stachybotrys* and *Coptotermes* species, has been reported from the woods of *Eucalyptus rostrata* (Youatt, 1961). Mandels, et al. (1961) have screened about 500 plants for cellulase inhibitors and found that natural inhibitors are common and are present in diverse groups of plants. Moore-Landecker and Stotzky (1972) have reported that volatile metabolites, produced by cultures of *Proteus vulgaris*, *Aerobacter aerogenes* and 6 other bacterial species effectively inhibited the growth and sporulation of fungal species (names of species not mentioned). Recently, an antifungal metabolite has been isolated, as a white needle shaped crystalline substance, from methanol extract of the rose flowers (Tripathi and Dixit, 1976). These workers further identified the metabolite as gallic acid and reported that it exhibited inhibitory properties against as many as 17 fungi. Another metabolite, Chaetomin, was isolated from *Chaetomium* species, viz., *Chaetomium cochloides* and *Chaetomium globosum*, which was found to exhibit anti-bacterial as well as antifungal activities (Brewer, et al., 1972; Brewer and Taylor, 1978).

Many inorganic and organic compounds have been reported to check the growth of the fungi, as well as, inhibit the production and activity of cellulase enzymes, elaborated by them.

Ziese (1931) has reported that *Helix* cellulases were inhibited by copper sulphate and hydrogen cyanide. Enebo (1954)
has reported that cellulolytic enzymes from *Clostridium thermocellulaseum* were inhibited by addition of silver, cupric and ferric ions. Jermyn (1952), Basu and Whitaker (1953), Reese and Mandels (1957) have studied the inhibitory effect of copper, silver, zinc, lead, cadmium, iron and mercury salts on cellulolytic activity of *Aspergillus oryzae, Trichoderma viride, Pestalotiopsis westerdijkii* and *Myrothecium verrucaria*. Miller and McCallan (1957) have studied the toxic action of silver ions on spores of representative fungi.

Mandels and Reese (1963) have found that fungal growth and enzyme activity was inhibited by metallic salts of mercury, silver, copper, chromium, lead and zinc at about $10^{-3}$ M concentration. Thiam (1968) has reported that *Aspergillus* dissolved and accumulated copper. Work at DMSRDE, Kanpur, carried out by Sehra, et al. (1970), also emphasized the efficacy of CuSO₄ and HgCl₂ as fungicides against the growth of *Memnoniella echinata*. In vitro germination of *Botryodiplodia theobromae* was also inhibited by mercuric chloride and copper sulphate (Ragab, et al., 1971). Highley (1975) has reported that cellulases of white and brown rot fungi were inhibited by mercury, silver, copper, manganese ions and mixture of copper sulphate and potassium ferricyanide. The toxic effect of salts of heavy metals on the production and activity of cellulolytic enzymes of *Helminthosporium apattarnae* has been reported by Ghewande and Deshpande (1976b). In the year 1976, Gullino and Fiussello
have found that lead salts caused complete inhibition in many fungi, the most sensitive being *Fusarium* species. Addition of 0.005% of alkyl derivatives of tin in cement successfully prevented the growth of *Penicillium expansum*, *Aspergillus flavus*, *Chaetomium globosum* and *Geotrichum candidum*. Cellulases of *Pyricularia oryzae* have been reported to be inhibited by higher concentrations of sodium chloride and potassium chloride (Sudo, *et al.*, 1977), whereas, cellulases of some fungal species were inhibited by other potassium salts (Chrapkowska, *et al.*, 1977). Inactivation of enzyme activity, by salts of heavy metals, may be due to precipitation of protein moiety or due to combination of metal ions with thio or carboxyl groups of protein.

Many workers have reported the inhibition of cellulolytic enzymes by metal ions. Cellulases of *Pseudomonas* species were found to be inhibited by Hg\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\) (Tiwari, *et al.*, 1977). Cu\(^{2+}\) and Mn\(^{2+}\) greatly influenced the activities of cellulolytic enzymes of *Alternaria* species (Kim and Lee, 1976), *Stachybotrys* species (Kim and Kim, 1976), *Penicillium notatum*, *Trichoderma viride* and *Aspergillus niger* (Choi *et al.*, 1976). Hong, *et al.* (1976) have found that Cu\(^{2+}\), Hg\(^{2+}\) and Pb\(^{2+}\) effectively inhibited the activity of *Trichoderma koningii* cellulases. Soni and Bhatia (1979) have reported that Hg\(^{2+}\) completely inhibited the cellulase activity of *Fusarium oxysporum*. They further reported that Cu\(^{2+}\) also showed marked inhibition of cellulases, while,
$\text{Ni}^{2+}$, $\text{Zn}^{2+}$, $\text{Co}^{2+}$ and $\text{Ba}^{2+}$ were inhibitory only to a detectable extent.

Very many organic compounds, possessing fungitoxic properties are in use since long time back and have been reported in the literature. Reese and Mandels, in 1957, have tested one hundred and seventy five compounds for their ability to inhibit fungal cellulases and $\beta$-glucosidases. They have found that bis-dithiocarbamates, $\text{N}$-chloro- and $\text{N}$-bromosuccinimide, chloromelamine, chlorohydantoin, trichloroisocyanuric acid and tetraglycine-hydroperiodi dide are active inhibitors of cellulases of \textit{Pestalotiopsis westerdijkii}, \textit{Trichoderma viride} and \textit{Myrothecium verrucaria.} In the year 1959, these workers found that gluconolactone inhibited the cellulosytic enzymes of many other fungi, such as, \textit{Asperillus} species, \textit{Sclerotium rolfsii} and \textit{Penicillium funiculosum}. Cysteine, glutathione, and sodium sulphide have occasionally been reported as toxic to cellulases at $10^{-2}$ to $10^{-3}$ M concentration (Gascoigne and Gascoigne, 1960; Hanstein, 1960, Mandels and Reese, 1963). Thiocarbamate and its derivatives were used as fungicides for the first time by Tisdale and Williams (1934). Later on, many workers have reported the use of these fungicides (Hester, 1943; Hauberger and Mann, 1943; Klopping and van der Kerk, 1951; Diamond and Horsfall, 1944; Sisler, \textit{et al.}, 1951; Horsfall, 1956; Nagasaw, \textit{et al.}, 1962). Organic sulphur compounds (Horsfall and Rich, 1951; Ludwig, \textit{et al.}, 1955), quinones, quinolinol and quinolinates (McNew and
Burchfield, 1951; Block, 1956b), quaternary ammonium compounds (Domagk, 1935; Rosella and Chabert, 1955) have been reported as potent fungicides for the growth and enzyme activity of fungi. Hochstein and Cox (1956) have studied the fungicidal action of Captan on the respiration of growing and non-growing conidia of Fusarium roseum. Rich (1959) has studied the fungitoxicity of Captan against Monilinia fructicola. Richmond and Somers (1963, 1966, 1968) and Richmond, et al. (1967) have also studied the toxic action of Captan on the growth of Neurospora crassa. The systemic fungicides, dimethirimol and ethirimol, were found to be toxic to spores of Erysiphe graminis and Shaerotheca fuliginea (Bent, 1970). Dihydroxy phenylalanine inhibited the cellulase activity of many fungi (Bull, 1970). Thiol inhibitors, such as iodoacetate and p-chloromercuric benzoate inhibited the cellulase activity of Myrothecium verrucaria (Basu and Whitaker, 1953) and of Fusarium vasinfectum (Sampathnarayanam and Shanmugasundaram, 1970). Jothianandan and Shanmugasundaram (1968), Ghosh and Basu (1969) have demonstrated the inhibition of cellulases from Pyricularia oryzae and Aspergillus terreus by p-chloromercuric benzoate and iodoacetate. 2,4,5-Trichlorophenyl ethers have been reported to be effective fungicides, bactericides, as well as preservatives for plastics (Darsow and Schnell, 1969).

Horsfall and Lukens (1966-67) have reported that sporulation of Alternaria solani was inhibited by N-phenylcarbamates,
several purine analogs, 2,4-dinitrophenols, glycollic acid, sulphydryl inhibitors and phenylhydrazines. Pechmeze (1970) has reported that cellulose products, such as, cotton or linen textiles were protected, against micro-organisms, by nonachloro-biphenyl acetate. 2,4-Dichlorophenoxybutyric acid specifically inhibited cellulase activity of Aspergillus terreus (Ellgehausen, 1971). Cycloheximide and dinitrophenol prevented the cellulolytic enzymes of Chaetomium thermophile (Lusis and Becker, 1973). Kochetkova and Mičiene (1975) have found that 6-chloro-4-sec-butylphenol was strongly active against Fusarium, Botrytis cinerea and Ascochyta pisi, while 6-bromo-2-sec-hexyl-p-cresol showed only weak fungicidal activity. Diethylmalonate at 2.0% concentration completely inhibited the cellulolytic enzymes of Curvularia lunata (Sahgal, 1971) and of Chaetomium globosum (Rastogi, 1973). Spraying, the cellulosic shoe making material (inner part of foot wear), with 0.3% of 2,4,5-phenyl-acetate inhibited the growth of Aspergillus niger, Aspergillus versicola, Chaetomium globosum and Paecilomyces variotia and prevented the damage of foot wear by fungal attack (Orlita, 1976).

Hurst, et al. (1977) have found that N-bromosuccinimide completely inactivated the cellulases from Aspergillus niger. Addition of 5-fluorocystosine to Yeast or hyphal culture of Candida albicans caused an immediate inhibition of cell growth (Polák and Wain, 1977). Patwa, et al. (1978) have found that
substituted hydroxy sulphones (100 µg/ml) were active against Cryptococcus neoformans and Saccharomyces cerevisiae and at 200 µg/ml, totally inhibited Aspergillus niger growth. Phenols and their derivatives have been found to be toxic to the growth of many fungi (Chakravarty and Baruah, 1967-68; Bhaskaran and Prasad, 1973; Kaye, 1974; Gangopadhyay and Wyllie, 1978; Soni and Bhatia, 1979).

**Volatile fungicides**

In the tropical climate of India, a large variety of Service stores/equipment develop fungal growth under favourable conditions. Microbial growth also takes place on the stores and materials when they are in transit and are stored under adverse climatic conditions. In order to prevent microbial attack on stores, numerous preservatives have been developed and chemical formulations evolved. These treatments are, sometimes, incompatible with substrate materials or adversely effect the performance of stores. Many of the sophisticated optical, electrical and electronic equipments held by the Defence installations are damaged by microbial attack and application of preservatives on these equipments is not feasible/practicable due to intricate and complicated nature of the equipments.

There has been a long felt need to have a protective method, which can be used for prevention of fungal attack on
all types of Service stores and equipment, without any deleterious effect on the substrate or on its performance properties. Extensive work has been carried out in DMSRDE, Kanpur, on the use of a volatile fungicide to preserve and protect the Service stores from fungal attack. Dayal, et al. (1968) have developed Volatile Fungal Inhibitive (VFI) Papers, Tablets and Strips which have been found very effective for prevention of microbial growth on stores and materials, both under storage and transit. These VFI Tablets and Papers/Strips have been patented by DMSRDE, Kanpur. These workers have found that VFI Papers, Tablets and Strips slowly emitted toxic vapours which saturated the atmosphere and killed all viable micro-organisms. They have further reported that VFI Papers/Strips were effective for protection of finished leather goods against Aspergillus flavus, Aspergillus fumigatus and Penicillium species. These VFI Papers/Strips were specially recommended by them for packing of shoes which are exported from India.

Agarwal, et al. (1969) have reported that the following different types of stores could be protected from microbial damage by the use of VFI Papers/Tablets/Strips.

1. **Textiles**— Dosootie, cotton garments, personal clothings, canvases, threads/yarns in hanks or copes, gauges and bandages, parachutes, hoisery.

2. **Leather**— Leather accountrements, shoes, boots, belts,
harness and saddlery items and holsters, etc.

3. Rubber— GS masks, respirators, antigas materials and stores, condoms etc.

4. Paper— Records, files, forms, stationery items, maps and charts etc.

5. Miscellaneous— Tapes adhesive, tapes and other items like cork, gaskets, linings etc.

They have further stated that VFI Paper, Tablets and Strips were prepared from indigenously available chemicals and were not costly. The VFI Papers were prepared by treating the tissue/kraft paper with the chemical composition (patented) and drying out them at about 40°C. These papers have been found useful as initial wrap for stores or as packing material for stores/equipment. They have further reported that these Papers, while acting fungicidally, did not produce any adverse effect on the stores.

Nanda, et al. (1970) and Dayal, et al. (1971, 1973) have reported that microbial deterioration of electrical and electronic components/equipment was controlled by the use of VFI Papers/Tablets/Strips. The items included voltmeters, rubber and PVC cables, rubber adapters, condensers, wireless sets, switches, hylam sheets, fuses, holders, insulating tapes, motor coils, loud speakers, paper cones, aluminium laminates and foils, mica sheets and plastic wires. The fungicidal vapours,
emitted by the VFI Tablets/Papers/Strips, entered the inaccessible and intricate parts of the equipment which could not otherwise be treated with antifungal agents.

Dayal, et al. (1970) have found that glass slides and optical lenses developed fungal growth during storage under hot and humid conditions. The slides and lenses wrapped in VFI Paper did not develop any fungal growth, discolouration, filming or fogging and were in perfect condition for microscopic work. No adverse effect of the chemical vapours could be detected on the polished surface of the glass slides and optical lenses. These workers further established that the VFI Paper, used for packing, slowly emitted fungicidal vapours which did not allow any fungal spores to germinate and thus effectively prevented fungal growth. They have also found that the treatment was effective for over a year, if the glass slides and lenses, initially wrapped in VFI Paper, were overwrapped with waxed/kraft paper to prevent undue loss of fungicidal vapours. This was also true in case of leather shoes and boots, meant for export.

Studies have been carried out at Indian Agricultural Research Institute (IARI), New Delhi, to find out the application of VFI Tablets and Papers, developed by DMSRDE, Kanpur, on deterioration of fruits and vegetables. Gaur and Raychaudhuri (1970) have found that VFI Tissue Paper effectively prevented the pathogens, viz., Alternaria tenuis,
Aspergillus awamori, Fusarium camptoceras etc. from growing on fruits, when these were wrapped in VFI Tissue Papers. Similarly, Dharam Vir and Gaur (1971) have studied the effectiveness of VFI Tablets against diseases of the fruits and vegetables, occurring in transit and storage and some seed borne pathogens. These studied clearly revealed the antifungal vapour action of the VFI Tablets/Papers against the fungi, deteriorating fruits and vegetables. Wahab and Sharma (1974) have also reported that VFI Kraft and Tissue Paper completely inhibited the growth of Pythium aphanidermatum in culture and successfully checked the development of rot and cottony-leak in cucurbit fruits when they were fully wrapped in them. They have further found that Kraft paper possessed more inhibitory influence as compared to Tissue Paper.

Tempel (1969) has reported that the addition of cysteine to the fungicide Captan, N-(trichloromethyl)thiophthalimide, N-[(dichlorofluoromethyl)thio]-N',N'-dimethyl-N-phenylsulfamide antagonised their activity and caused the formation of volatile fungitoxicants. Acetaldehyde vapours have been found to be toxic to post harvest pathogens of fruits and vegetables, such as, Erwinia carotovora, Monilinia fructicola, Pseudomonas fluorescens, Botrytis cinerea, Penicillium expansum and Rhizopus stolonifer (Aharoni and Stadelbacher, 1973). Prasad (1975) has also reported the fungitoxicity of acetaldehyde vapours to some major post harvest pathogens of citrus and subtropical fruits. Toda and Shigeno (1973) have found that
packaging materials, such as, paper, cellophane bag, polyethylene films, Al-foils for food, soap and shoes, were made mold resistant by treating them with odorless and nontoxic caproic acid monoglyceride or caprylic acid monoglyceride. They further reported that wrapping paper for fruits, after treatment, was resistant to *Aspergillus niger* at 35°C and saturated humidity. Phenyl isothiocyanate, bromthane and the light fraction of coal tar showed significant fungicidal effect when tested on *Chaetomium globosum, Aspergillus niger, Penicillium brevicompactum* (Shavlokhova, 1973). He has further found that these volatile fungicides may be used for protection of materials and manufactured articles (names not being mentioned) from fungal damage. Schluter (1977) has reported that triadimefon gave a complete protection to barley plants against powdery mildew infection. He found that the effect was due to vapour emission. Rytch-Witwicka, *et al.* (1978) have observed that a mixture of o-nitrophenol and p-chlorophenol in airtight electrical and electronic equipments in coal mines synergistically protected the equipment insulation from microbial corrosion by sublimation of o-nitrophenol and p-chlorophenol mixture and the action of their fumes. Thus, the threshold of fungicidal activity of the mixture fumes was 15-20 mg/m³ of air, whereas, o-nitrophenol and p-chlorophenol, separately, were less effective. In the year 1978, a Japanese company developed volatile antimicrobial compositions containing alkylphenols in combination with volatile aromatic compounds. It has been reported that
4-tert-butyl-3-chlorophenol and p-dichlorobenzene, placed in an enclosed space, i.e., green house, storage room etc., effectively controlled the growth of micro-organisms, such as, *Aspergillus niger* and *Botrytis cinerea*.

**Pentachlorophenol as fungicide**

Ample literature is available regarding the toxicity of pentachlorophenol against a number of fungi. As early as 1952, Wolf and Westveer have reported that pentachlorophenol and tetrachlorophenols are more active, as fungicides than tri- and dichlorophenols. Zahn and Wilhelm (1953) have reported that pentachlorophenol protects the wool from fungal damage, without entering into any combination with the wool fiber. Reese and Mandels (1957) have reported that a number of substituted phenols, such as, chlorophenols, orthophenylphenol and chlorophenylphenols are moderately active against certain fungal cellulases. Considerable work has been done in DMSRDE, Kanpur, on the fungicidal action of pentachlorophenol. Agarwal, in the year 1962, has reported the efficacy of pentachlorophenol, as a preservative, against fungal attack on paints, varnishes and lacquers. Sahgal and Agarwal (1968) have also found the use of pentachlorophenol, tetrachlorphenol and their sodium salts as fungicides in oil and emulsion paints. These fungicides were found effective against *Pullularia pullulans*, *Cladosporium herbarum*, *Phoma violacea*, *Alternaria* species and *Stemphylium*.
species which have been found to grow on paints. Dayal, et al. (1968) have observed that pentachlorophenol, in combination with other chemicals, can control the microbial damage of certain materials, such as, packing papers, cotton, jute, aluminium foils/laminates and paints. They further reported that pentachlorophenol was effective against a wide number of fungi, such as, Monilinia species, Chaetomium globosum, Chaetomium indicum, Paecilomyces species, Trichoderma species Dendrophoma species and Mennoniella echinata. Sahgal, et al., in 1970, have reported that pentachlorophenol at 5% concentration was the most promising fungicide in preventing fungal attack on paints, lacquers and varnishes. Ross, et al. (1968) have also reported that chlorinated and substituted phenols are among the oldest antimicrobial agents known and have been used extensively in the paint industry. The most frequently employed chlorinated phenols have been tetrachlorophenol and pentachlorophenol.

Chatterjee, et al. (1961) have found the inhibitory character of pentachlorophenol against the organism Aspergillus niger. They have reported that the effective concentration for 50% inhibition of growth is 0.00708 mg/100 ml. van der Plas (1966), in a survey of commercial products used to protect materials against biological deterioration, has reported the efficacy of pentachlorophenol to protect the materials, such as, wood, textiles, paper, paint and cordages against microbial damage. Mukherjee and Kundu (1973) have also reported the fungicidal action of pentachlorophenol against Helminthosporium
Oryzae, Alternaria solani and Curvularia lunata. Zamenova (1976) has reported that pentachlorophenol, at 0.5 - 1.0%, inhibited fungal growth on viscose, cotton and linen. He further found that, out of the fungicides tested, only pentachlorophenol was effective in soil burial tests.

Ionita, et al. (1977) have found that pentachlorophenol, β-naphthol and criptodin, were the most effective of several fungicides tested, in vitro, against undisclosed fungi. These fungicides showed good fungicidal activity when tested on various materials, such as, textiles and wood. Sutter (1978) has reported that wooden chips treated with pentachlorophenol resisted the growth of certain fungi, such as, Coniophora puteana, Poria placenta and Polystictus versicolor. Pentachlorophenol was found inhibitory towards Coriolus versicolor, Coniophora cerebella and Poria monticola and Coriolus trabea (Leclercq, 1977). Werner, et al. (1979) have reported that pentachlorophenol inhibited the growth of many fungi, such as, Phytophthora cactorum, Pythium debaryanum, Mucor mucedo, Botrytis cinerea, Fusarium oxysporum, Cladosporium cucumerinum, Rhizoctonia solani. Pentachlorophenol inhibited the growth of 7 fungal species to various degree. Phytophthora cactorum and Rhizoctonia solani were the most sensitive fungi (Werner, et al., 1978). These workers have further reported that electron microscopic studies revealed that the fungicide, pentachlorophenol, was highly cell damaging as compared to trichlorophenol.
Recently, Dholakia and Chhatpar (1980) have found a strain of *Aspergillus* growing on water-based poster colours and was responsible for their deterioration and spoilage. They have reported that pentachlorophenol, phenylmercuric acetate and boric acid successfully inhibited the growth of the fungus, without affecting the properties and qualities of poster colours.

**Nitrogenous compounds as fungicides**

Many nitrogenous compounds, mostly containing nitro and amino groups, have been reported in the literature as fungicides. However, very few of them have been found to be volatile in nature. The chlorinated nitrobenzenes were introduced as fungicides, in 1935, by Brown. The initial experimentation of Brown was concerned with the control of *Botrytis cinerea* on lettuce by trichlorodinitrobenzene. Last (1952) has found that penta- and tetrachloronitrobenzenes were also effective in controlling *Botrytis* disease and *Rhizoctonia* attack on lettuce. Tetrachloronitrobenzene was also used by Foister and Wilson (1951) to control *Fusarium* dry rot of potatoes. These workers have found that chlorinated nitrobenzenes exert their effect in the vapour phase. Finger, *et al.* (1955) have found that halogenated nitrophenols, such as, fluoro-3-bromo-4,6-dinitrophenol was a potent fungicide.
van der Plas (1966) has reported the use of p-nitrophenol to protect leather articles from fungal attack. However, o-nitrophenol has been reported to act as volatile fungicide when used in combination with other volatile aromatics (Rytty-Witwicka, *et al.*, 1978). 2-Nitropropenyl benzene had fungistatic activity against *Trichophyton gypseum, Trichophyton mentagrophytes, Blastomyces dermatitides* and *Cryptococcus neoformans*. If, however, a methoxy group was added in para-position to give β-nitroanethol, the effectiveness was considerably increased (Davis, *et al.*, 1959). Nitrofuraldoxime and nitromethylfurufuryl ether have been reported as very good antifungal agents for treating infections with *Saccharomyces cerevisiae* and *Candida albicans* (Gurney, *et al.*, 1965).

Weil (1969) has reported that aliphatic amine salts of nitrilotriacetic acid inhibited the growth of bacteria and fungi, such as, *Aspergillus niger, Penicillium* species and *Escherichia coli*. Charifi and Chancogne (1970) have reported that nitro and amino substitution in the benzene ring was the principal cause of fungicide efficacy. They have found that these fungicides controlled the development of *Botrytis cinerea*. Dinitrophenol has got inhibitory effect on the synthesis of cellulases by *Trichoderma lignorum* (Lobanok and Ivlicheva, 1971). These workers have further found that the inhibitory effect of dinitrophenol was dependent on its concentration. At 10⁻⁴ M concentration, dinitrophenol inhibited the cellulase synthesis.
Brown, et al. (1972) have studied the activities of nitrophenols as fungicides and found that the hydrogen bonding characteristics affected the fungicidal activities of 6-methyl-2,4-dinitrophenol, 4-bromo-6-nitrophenol and 2-cyano-6-nitrophenol. Out of 37 nitroalcohols assessed for the fungistatic activity, it has been reported (Osumi, 1972) that only quinolyl-nitroalcohols were fungistatic against *Candida albicans*. 4,6-Dinitro-o-cresol has been reported to have fungicidal properties (Chambon and Chambon, 1973). Winkelmann, et al. (1975) have found that basic disubstituted nitroanilines were active in vitro and in vivo against fungi, bacteria, protozoa etc. Garg and Manaval (1976) have tested some halopolynitrophenyl, tolyl and naphthyl thiocyanates against *Aspergillus niger* and *Alternaria solani* and found that 3-methyl-2,4,6-trinitrophenyl thiocyanate was most effective, suppressing the growth of both the fungi. They further stated that methyl group at meta-position and nitro group at ortho- and para-positions increased fungicidal activity of the thiocyanates.

Recently Albert, et al. (1980) have reported that 5-chloro-3-methyl-4-nitroisothiazole exhibited significant antifungal activity against a wide spectrum of fungi. These workers have found that nitro group is essential for antifungal activity. Kawada, et al. (1981) have reported that dinitro and diamino derivatives of ethoxylated HCHO-\(\beta\)-nonylphenol condensates inhibited the gram-positive bacilli and fungi. Cellulases of
Fusarium avenaceum were inactivated by 2-hydroxy-5-nitrobenzyl bromide and tetranitromethane (Zalewska-Sobczak and Urbanek, 1981).

**Mechanism of action of fungicide**

To facilitate selection of suitable fungicide, for controlling microbial action on cellulosic materials, the knowledge regarding mechanism of action of fungicide is of prime importance. Many workers propounded different theories for mechanism of action of fungicides, depending on their nature.

The use of elemental sulphur as a fungicide and the introduction of organic sulphur compounds stimulated research on mechanism of toxicity. Earlier studies were primarily directed in obtaining relationship between chemical structure of the fungicides and biological response of the fungus and included observations on mitosis, morphology, growth and metabolism (Bliss, 1935; McCallan and Wilcoxon, 1939; Litchfield and Fertig, 1941).

The introduction of Bordeaux mixture in the nineteenth century and its successful and extensive use as fungicide has focused considerable attention on copper and other metal salts. McCallan and Wilcoxon (1936) have found that fungal spores exuded acids, among them malic acid, capable of bringing copper
from Bordeaux mixture into solution, presumably by formation and dissociation of the copper salt. The studies of Wain and Wilkinson (1946) supported this concept. They have considered that dissociation of copper salts enabled the toxic copper cation to become available to fungal spores. Martin, et al. (1942) compared the toxicity of copper in cationic form and complex anionic form. They have found that copper was more toxic in anionic complex form than as cation. Several workers have studied the site of activity of metallic ions as fungicides. As early as 1896, Kahlenberg and True have found that the toxicity of metal poisons was correlated with ionisation and that the active agents were cation rather than the molecules. Since ions penetrate fungal spores less readily than molecules, this suggests that copper toxicity was due to surface activity of cations. However, Goldsworthy and Green (1936) showed that spores, soaked in copper sulphate, revealed the presence of copper within the spores and implied that copper exerted its toxicity at some internal site. Thus, during the years 1938-1946, many workers indicated that both adsorption and penetration inside the spores, probably, contribute to the toxicity of any cationic species. Somers (1963a) has reported that uptake of copper was initially an ion-exchange reaction followed by permeation throughout the cell; there was no evidence of an adsorption process.

Metals may bind or inactivate the anionic sites, essential for the existence of the cell or exert some deleterious
effects on cell permeability. The way, in which metals may exert their toxicity at some internal sites, was investigated by Janke, et al. (1953). They have reported that heavy metals reacted with sulphydryl groups, present in living cells in compounds, such as, cysteine and glutathione. They further stated that the reaction was either concerned with a catalytic autoxidation of the sulphydryl group which led to the formation of mercaptides and disturbed protein metabolism or enzyme action. They have also found that cysteine and thioglycollic acid reversed the toxicity of mercuric chloride, phenylmercuric acetate and copper sulphate to spores of Tilletia tritici.

Zentmyer (1943 and 1944) has suggested that fungicides owe their toxicity to their ability to rob the fungus of its metals. Hence chelation should be a potent mechanism of fungitoxicity. This view was later confirmed by Horsfall and Rich (1951). Hochstein and Cox (1952) affirmed that metal inhibited respiration by forming chelates with amino and carboxyl groups. Horsfall (1956) has suggested that the action of the dithiocarbamates was associated with chelation, i.e., the production of a highly stable complex between the molecule of the fungicides and essential metals. Sijpesteijn and van der Kerk (1952) have found that L-Histidine inhibited the toxicity of tetramethylthiuram disulphide and tetramethylthiuram monosulphide to spore germination of Botrytis cinerea, but not to mycelial growth.
Horsfall and Rich (1951) have found that Captan was very effective against *Stemphylium saricinaeformae* and they considered that some specific structural features could be involved in its fungitoxicity, e.g., the CCl₃ group, the sulphur bridge, the diketone and the carbon atoms alpha to the ketone. Hochstein and Cox (1956) have studied the fungicidal action of Captan on the respiration of growing and non-growing conidia of *Fusarium roseum*. Their studies suggested that Captan inhibited growth of fungi by interfering with decarboxylation reactions, requiring thiamine pyrophosphate as co-enzyme. Rich (1959) also studied the chemistry of the fungitoxicity of Captan. Captan, at 3 x 10⁻⁵ M, completely inhibited the growth of *Monilinia fructicola*; this toxicity was reversed by 10⁻² M L-histidine and L-cysteine. Rich concluded, from these observations, that the test organism could bypass every system, poisoned by Captan, except those needed for synthesis or utilisation of histidine.

Lukens and Sisler (1958) emphasized the function of -SCCl₃ group in Captan as a toxophore. This group reacted intracellularly to give thiophosgene which then combined with free -SH, -NH₂ and -COOH groups and thus inhibited vital metabolic processes.

Richmond and Somers (1963, 1966, 1968) have observed that toxicity of Captan owed to its reaction with sulphhydryl groups of cell proteins and inactivated enzymes and co-enzymes with functional -SH groups. From electron microscopic studies, Richmond, et al. (1967) have proposed that captan interfered with the SH oxidation-reduction cycle in the nucleus by attacking protein thiols.
The investigation of Block (1955) showed that the fungitoxicity of copper oxinate could be reversed by excess of copper or oxines, whereas, Manten, et al. (1951) have found that trace elements, such as Zn, Cu, Mn were unable to reverse the fungitoxicity of oxine to *Aspergillus niger* and considered that oxine did not exert its effect by chelation. Zentmyer and Rich (1956) have showed that L-histidine and L-cysteine were able to reverse the toxicity of oxine and copper oxinate to mycelium of *Aspergillus niger*, growing on solid media. This reversal of toxicity was due to the fact that these amino acids compete for the copper and liberate oxine. Thus, it can be concluded that chelated complex is able to penetrate the cell or cell site, but that toxicity is due to the liberation of copper which acts as a metal poison. According to Block (1956b) the fungitoxicity of copper oxinate is progressively reversed with increasing concentrations of copper, nickel and iron ions. He has further stated that the reversal with excess metals is due to the suppression of the cell-penetrating 2 to 1 chelates, whereas, the reversal with excess oxine is due to the suppression of the toxic 1 to 1 chelate within the cell, according to the requirement of the equilibrium equation. Oxine is considered fungitoxic in the form of the charged half chelate of copper but permeates in the form of nonionic full chelate (Block, 1955; Zentmyer, et al., 1968).

Somers (1963b) has reported that fungicides must be able to penetrate and perhaps accumulate at a site of cellular
activity on the fungal spore and there inhibit at least one vital process. He has further suggested that the fungicides act by selective toxicity, the possible mechanism of selective toxicity involve either preferential accumulation of the fungicides by the spores or selective interference with fungal cell metabolism. Somers observations have been supported by Lukens (1968). According to him, the selective action of fungicides is based upon rapid uptake and accumulation of fungicides by the cells of the fungi. He has further reported that a few fungicides react with particular cellular constituents and inhibit specific systems. The reaction of fungicides with cellular constituents may involve either oxidation-reduction or chelation or nucleophilic displacement. According to Lukens (1968) the redox fungicides destroy functional groups of fungal cells. If the excessive change in electron density cannot be neutralised by the redox buffering systems within the cells, the fungus becomes disorganised and ultimately dies.

Agarwal, et al. (1970) have shown that heavy metals like copper and mercury are absorbed by the fungal spores and bring about inhibition of germination of spores. With the increase in concentration of copper or mercury compounds, the uptake of metals increases and this accounts for the fungitoxicity of these compounds. Ko, et al. (1976) have reported that both agar and nutrients decreased the inhibitory effect of copper sulphate on fungal spore germination. The agar effect was more
marked on cationic inhibition than that due to anions or weakly
dissociated compounds. They further reported that binding of
fungicides by agar may slow their rate of diffusion and may
account for the reduced activity of fungicides against spore
germination.

With conidia of several fungi, the binding sites are consi­
dered by Miller, et al. (1953) and Somers (1963a) to be located
on the cell and possibly on intracellular membrane as well as
on the cell-wall. Miller (1962) has found that the fungicides
first bind to the cell membrane, which can accommodate more
toxicants, as it is considered to have numerous overlapping
folds, extending deep in the protoplast. The binding to the
cell membrane and passage through the same is considered to
occur by hydrophobic bonding (Horsfall, 1956; Hansch, et al.,
1965). Iwata, et al. (1972) have reported that Clotrimazole,
a synthetic antifungal agent, when added to Candida albicans
culture, was rapidly adsorbed by the cell membrane, causing
damage to it. The fungicidal activity of Clotrimazole was thus
correlated with membrane damage resulting in alterations of cell
permeability and lysis of protoplast. Similarly, Econazole
inhibited Saccharomyces cerevisiae and Candida albicans growth
and metabolism by damaging various membrane systems within the
cell (Kern and Zimmermann, 1977). Elkhouly (1978) has found
that Nystatin influenced the function of cell membrane of
Candida albicans in such a way as to induce distortions and
deformation in the cell surface.
The introduction of a lipophillic substituent, i.e., one that increases the fat solubility, is directly associated with fungitoxicity of the compounds, making them more soluble in the fungal protoplasmic membrane. Quastel (1967) has reported that free fatty acids may also serve as carriers of toxic cations across the membranes. Hoppe, et al. (1976) have found that 3-phenylindole inhibited the growth of Aspergillus niger at 5 µg/ml. This fungicide markedly affected the lipid composition of the fungus, inducing a decrease in phospholipid composition and increase in free fatty acids. Yoshikawa and Eckert (1976) have also reported the mechanism of fungistatic action of sec-butylamine against Penicillium digitatum. They have found that due to toxic action of the fungicide, synthesis of cell wall and total lipids were inhibited by 20-30%, while nucleic acid synthesis was reduced to about 50%.

Many fungicides act by inhibiting the cell multiplication and DNA and RNA syntheses. Thiabendazol prevented cell multiplication in Ustilago maydis (Sisler, 1972) and completely inhibited mitosis in hyphae and partially inhibited DNA and RNA syntheses in Aspergillus niger (Davidse and Flach, 1978). Sisler (1972) have further found that benomyl interfered with DNA synthesis or the post DNA synthesis aspect of the cell replication process in Ustilago maydis and Neurospora crassa. Glutaraldehyde caused cellular agglutination and increased settling rate in Candida lipolytica (Navarro and Monsan, 1976). Polak and Wain (1977) have found the mode of action of
5-fluorocystosine on Yeast and Candida albicans. They reported that addition of fungicide caused an immediate inhibition of syntheses of both DNA and RNA followed by inhibition of cell number. Pentachlorophenol has been reported to be fungitoxic by actively damaging the cells of Mucor mucedo (Werner, et al., 1978).

Reversal of toxicity of systemic and non-systemic fungitoxicants by chemicals was studied by many workers to establish mechanism of action of fungicides. β-Alanine has been found to inhibit the growth of Neurospora crassa and this inhibition was reversed by ammonium carbamate. These results implicated carbamyl group metabolism at the site of β-alanine inhibition (Herrmann and White, 1966). Bent (1970) has reported that the toxic actions of dimethirimol and ethirimol were markedly reversed by riboflavin and folip acid. He further suggested that folic acid overcomes a metabolic block, induced in powdery mildew fungi, by the fungicides. Srivastava, et al. (1972) have found that 8-azaademine, DL-ethionine and p-fluorophenyl inhibited the germination of Cicer arietinum seeds. They have further reported that inhibition by 8-azaademine was reversed by either adenosine, cyclic-3,5-AMP or indole-3-acetic acid.

Yamaguchi (1978) has reported that the preventive effect of egg lecithin on imidazole inhibition may be a consequence of preferential, in vitro, interaction of the inhibition with unsaturated phospholipid to form a hydrophobic complex.
Hata, et al. (1972) have found that antifungal activity of cerulenin was markedly reversed by ergocalciferol and slightly by retinol with *Candida stellatoidea* and by thiamine and pantothenic acid with *Saccharomyces cerevisiae*. These workers, thus, concluded that cerulenin acts on the biosynthesis of sterols and fatty acids. Subramanyam and Venkateswarlu (1979) have reported that the growth inhibition of *Neurospora crassa* by Cu$^{2+}$ is counteracted by histidine, histidine methyl ester, histidinol and Mn$^{2+}$. The data suggests that copper toxicity in the mould is due to the suppression of histidine biosynthesis. Widholm and Slife (1980) have found that 4-aminobenzene sulfonylcarbamate inhibited the growth of carrot cell suspension cultures. The growth inhibition was reversed by adding p-amino benzoic acid or folic acid, suggesting the structural relationship between the fungicide and reactivator.

Roberts (1959) has reported that phenols and related compounds react readily with such groups in proteins as are capable of undergoing electron interaction, e.g., -NH$_2$, -OH or -SH groups; they inactivate the enzyme by acting as non-specific reactants. According to Somers (1963b) also, fungicides act as non-specific enzyme poisons rather than inhibitors of a particular enzyme group. He has reported that although a large number of enzyme systems have been found to be inhibited by fungicides, in no instance has the specific biochemical reaction been established in vivo. He has further stated that a number of hypotheses have implicated -SH dependent enzymes as the
ultimate site of action, but the -SH groups are so widely distributed in living cells, that once again the unspecific nature of fungicide reactions is evident.

Eriksson and Pettersson (1968) have reported that the cellulase enzymes of *Penicillium notatum* are completely inhibited by mercuric chloride. The Hg$^{2+}$ inhibited enzymes have been found to be reactivated by cysteine and sodium chloride. They have further stated that the difference spectrophotometric studies, showed that Hg$^{2+}$ interact with trytophenyl groups in the enzyme. They have suggested that the inhibition might be due to a change in gross configuration of the enzyme, for instance, as a result of Hg$^{2+}$ reaction with tryptophenyl groups embedded in the hydrophobic regions of the protein molecule.

Anand and Gandhi (1971) have found that cellulase activity was inhibited by Mg$^{2+}$ and stimulated by a mercaptoethanol. This suggested that the fungicide reacted and blocked the thiol groups of enzyme protein. Bull (1970) has reported the effect of pH on the inhibition of cellulases by melanin and on the reactivation of inhibited enzymes. These studies indicated that electrostatic attraction is importantly involved in enzyme-inhibitor complex formation. Cellulase activity in four *Fusarium* strains was inhibited by alanine, serine, hydroquinone or thiourea and stimulated by methionine, cysteine or cystine (Szajer, 1975). Changes in cellulolytic activity were accompanied by changes in redox potential. Feed-back inhibition by glucose or cellobiose has been reported by Nisizawa, et al.
Hurst, *et al.* (1977) studied the site of action of a number of fungicides in inhibiting the cellulase activity of *Aspergillus niger*. They have found that bromo-succinimide completely inactivated cellulase enzymes of the micro-organism, oxidising tryptophan molecule per cellulase molecule. Reaction with 2-hydroxy-5-nitrobenzyl bromide resulted in the incorporation of 2,3-hydroxy-5-nitrobenzyl groups per cellulase molecule. Thus, 1-tryptophan residue is essential for cellulase activity. They further stated that diazocarbonyl compounds in the presence of Cu\(^2+\) inhibited cellulases, apparently, by reacting with a protonated carboxyl group. Treatment of cellulases with diethyl pyrocarbonate modified 2- of the 4-residues of histidine present in the enzyme, resulting in 60% decrease in cellulase activity. They have further suggested that inhibition of cellulase activity by Ag\(^+\) and Hg\(^{2+}\) appeared to be due to interaction with tryptophan residues rather than -SH groups.

Soni and Bhatia (1979) have reported that the inactivation of cellulase enzymes of *Fusarium oxysporum* by phenols and related compounds and the reversal of inactivation by cysteine HCl, sodium thioglycollate and hydroxylamine hydrochloride indicated the involvement of \(-\text{NH}_2\), \(-\text{OH}\) and \(-\text{SH}\) groups in fungicide-enzyme interaction. No literature could be available on the mechanism of action of meta-dinitrobenzene.