CHAPTER I

REVIEW OF LITERATURE
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The microbial decomposition of cellulose was first recognized by Hoppe-Seyler in 1883. Probably deBary (1886) was the first to record that the fungus *Peziza sclerotium* secreted enzymes which "dissolved" cellulose. The presence of cellobiase in addition to glucose in arrested cultures of thermophilic organisms led Pringsheim (1912) to suggest a hydrolytic pathway of cellulose breakdown. His summarising equation is as follows: \[ \text{Cellulose} \rightarrow \text{Cellobiase} \rightarrow \text{Glucose}. \] The enzyme carrying out the first reaction was named 'cellulase' and the second as 'cellobiase'.

Whatever studies have been carried out on the decomposition of cellulosic material by a few species of *Alternaria* by Gueguen (1914), Bright et al. (1924), Galloway (1930), Jensen (1946) and Basu (1948), it is scanty, and little has been reported on the production of cellulases by this fungus. It is interesting to note, however, that there are quite a few species of *Alternaria* like *A. chartarum*, *A. cucumerina*, *A. humicola*, *A. iridis*, *A. polymorpha*, *A. solani* and *A. varians* which have been isolated from soil and found to be strong cellulose destroyers, but have escaped detection on exposed cotton textiles as has been reported by See (1919), Marsh et al. (1949), Rapu (1949) and Goldzweig (1947). Siu (1951) has reported that *Alternaria* spp. are involved in the retting of fibres and possess
cellulolytic activity. Very recently the production of cellulase by \textit{A. solani} and \textit{A. tenuis} on a few culture media has been reported by Mehta et al. (1974).

It is now an established fact that the production of cellulolytic enzymes is of great importance in the development of diseases caused by several plant pathogens. Many investigators are engaged in the study of cellulases. Some of them are Siu, Reese, Levinson, Walseth, Mandels, Saunders, King and his co-workers in the U.S.A.; Whitaker and Thomas in Canada; Norkrans and Eriksson in Sweden; Gascoigne, Selby and Wood in England; Toyama, Nisizawa and Kobayashi in Japan; Jermyn in Australia, and Basu, Ghosh and Bose in India.

The pathogens like \textit{Cladosporium cucumerianum} (Strider and Winstead, 1961), \textit{Diplodia zeae} (BeMiller et al., 1968, 1969) have been reported to produce cellulolytic enzymes in a very simple synthetic medium devoid of cellulosic substances. Therefore, they are said to be constitutive in nature for the production of cellulases. Others like \textit{Sclerotium rolfsii} (Hussain and Rich, 1958), \textit{Alternaria} sp. and \textit{Botrytis cinerea} (Van Parijs, 1961) do not have the ability to produce cellulase in the medium devoid of cellulosic substances. Such pathogens in which cellulase production is inducible are said to be adaptive in nature. Yet another group where induction by substances other than
cellulose or their derivatives, is reported in *Trichoderma viride* (Reese and Mandels, 1957 and Mandels and Weber, 1969).

The classification of cellulolytic enzymes varies with the various workers according to its mode of action and substrate specificity. Reese et al. (1950) stated that many microorganisms are able to hydrolyse modified cellulosics, while only some of them are able to attack native cotton. C₁ is said to produce linear anhydroglucose chains from native cellulose as in cotton. Cₓ subsequently hydrolyses the chains to soluble low molecular products, ranging from cellohexoses to glucose. King and Vessal (1969) studied the cellulase complex of microorganisms and classified cellulases as follows:

1. C₁ is an enzyme whose action is unspecified. It is required for the hydrolysis of highly oriented cellulose (cotton, Avicel, etc.) by β-1-4 glucanases.

2. β-1-4 glucanases (=Cₓ) are the hydrolytic enzymes. This is usually measured by action on soluble cellulose derivatives usually carboxymethyl cellulose. The "x" in Cₓ emphasizes the multi-component nature of this fraction. They are clearly of two types:
   (a) exo-β-1-4 glucanase, successively removing single glucose units from the non-reducing end of the cellulose chain.
(b) endo-β-1-4 glucanase with action of a random nature, the terminal linkages generally being less susceptible to hydrolysis than internal linkages.

3. β-glucosidases vary in their specificities. Those involved in cellulose breakdown are highly active on the β-dimers of glucose including cellobiose.

**Influence of physical factors on production:**

The conditions affecting the in-vitro production of cellulolytic enzymes are many. They have been worked out in the case of several fungi. A review of this type of work by Basu (1948), Siu (1951), Reese and Levinson (1952), Gascoigne and Gascoigne (1960), Norkrans (1963, 1967), Cowling (1958) and Mandels and Weber (1969) is particularly relevant.

Grassman et al. (1933) by their extensive work on *Aspergillus oryzae* have suggested that a cellulase was able to hydrolyse cellulose and polysaccharides down to cellohexose and a cellobiase acted in the hydrolysis of cellobiose. Reese et al. (1950) tested 30 organisms including cellulolytic and non-cellulolytic fungi on CMC and 10% hydroxymethyl cellulose and found that they produced cellulase ($C_x$) enzyme. Beckman (1956) reported that the substrate does not appear to have any effect on the
influenced by carbon source. With glucose, enzyme accumulation was low whereas, highest enzyme production occurred when CMC was used as the carbon source. Ghewande (1973) has observed the production of cellulases ($C_1$, $C_2$ and $C_x$) by *Helminthosporium apattarnae* in a medium containing 1% carboxymethylcellulose.

Tashpulatov (1965) reported that cellulase and cellobiase activity was greatest in the thermophilic strain of *Aspergillus fumigatus* at the end of the first week, but appeared in the mesophilic strain at the end of the second week. Hence, he concluded that age of culture has an important role to play in the formation of enzymes. Earlier, the maximum cellulase production by *Ceratostomella ulmi* was obtained on 7 day culture (Beckman, 1956). Prasad and Bilgrami (1973) have found 6 day incubation to be best for cellulase production by *Aspergillus quadrilineatus*, *A. variecolor*, *A. nidulans*, *A. flavus* and *A. niger*.

With regard to the effect of light on cellulase production very few reports have appeared. BeMiller et al. (1966) studied the effect of light on the production of cellulase secreted by *Diplodia zeae* and observed more cellulolytic activity in cultures grown in continuous light than in those grown in darkness. Similarly Ghewande (1973) has observed a slight increase in production of cellulases in *H. apattarnae* cultures. But growth was very much reduced
in the latter. Cellulase production was maximum under normal day and night conditions. However, Jensen (1971) concluded that cellulase activity and dry weight production were greatest in cultures of Stereum gausapatum in continuous darkness.

As regards the influence of temperature, Saunders et al. (1948) reported 40°C as optimum by Myrothecium verrucaria. Siu and Sinden (1951) recorded an optimum of 29°C for cellulose decomposition by M. verrucaria, Curvularia lunata, Aspergillus flavipes and Gliomastix convoluta. Ishimaru and Toyama (1952) have reported 25°C for Trichoderma koningi. Hussain and Kich (1958) obtained maximum cellulase production by Cladosporium cucumerianum at 23°C but growth was maximum at 28°C. In the case of Fusarium lateritium f. cajani maximum cellulase production was at 20°C (Singh, 1968). In the case of several Aspergillus spp. Prasad and Bilgrami (1973) have recorded 25°C as optimum for cellulase production. In general, the optimum temperature range for cellulase production by various fungi lies at 20 to 50°C, hence two groups i.e. 20 to 30°C and 30 to 50°C.

Effect of nutritional factors on production:

Very little work has been done on the influence of glucose on cellulase production. It was, however, Fahraeus (1947) who reported that when glucose was added, vigorous decomposition of cellulose by Cytophaga cultures occurred,
but above 0.5% concentration, its inhibitory action was noted. Garber et al. (1965) showed that in the presence of glucose, a second carbon source influences the enzyme activity in the culture filtrate of three pathogenic penicillia (*P. italicum*, *P. digitatum* and *P. expansum*). Gupta and Heale (1970) reported that 1% glucose in the presence of CMC completely repressed the Cx production by *Verticillium albo-astrum* and even at 0.1% it decreased markedly the enzyme production.

The production of cellulase by *Coniophora puteana* has been reported by Bryan (1963) to reduce by the addition of simple sugars. Mandels and Reese (1957) studied the effect of different carbon sources on cellulase production by *Trichoderma viride* and found that cellulase production occurred on glucose, cellobiose, lactose and cellulose. Wood (1960) reported that cellulases would not be secreted in the presence of readily available carbon sources. When such sources are absent or limited and consequently growth is poor, enzyme secretion is often high. Jensen (1971) found that dry weight production was greatest in the case of *Stereum gausapatum* grown in a medium containing either a mono or disaccharide. Conversely, cellulase activity was highest in cultures containing a polysaccharide, CMC or cellulose, whereas low cellulase activity was found in cultures containing cellobiose. Eriksson and Goodell (1974)
have reported that cellulase production was repressed by glucose in the case of the wood-rotting fungus *Polyporus adustus*.

With regard to the effect of nitrogen source on cellulase production, Reese and Levinson (1952) studied many microorganisms. They observed more increase of both β-glucosidase and Cₓ concentration when NH₄NO₃ was used than when NaNO₃ in the basal medium. BeMiller et al. (1968) showed that ammonium nitrate was found to be a standard component of the mineral solution and the addition of casein hydrolysate increased the cellulase yield in cultures of *Diplodia zeae*. In cultures of *Stereum gausapatum* containing NH₄H₂PO₄, asparagine or glutamic acid as the nitrogen source, the dry weight and cellulase activity were highest, whereas, no activity and little dry weight were found in cultures containing KNO₃ as the nitrogen source (Jensen, 1971). Gupta et al. (1972) have obtained better yield of cellulase by *Trichoderma viride* when a combination of peptone, urea and (NH₄)₂SO₄ was used than when a single nitrogen source was used. Evaluating the effect of (NH₄)₂HPO₄ concentration on *Stachybotrys atra*, Thomas (1956) observed that the value of a high ammonium phosphate concentration resides in the increasing buffering capacity of the medium. The low yield of enzyme was clearly not due to exhaustion of the inorganic nitrogen supply and apparently resulted from inadequate buffering. In the case of *Schizophyllum commune* cultures,
Varadi and Jurasek (1969) have shown that enzyme activity increased by the addition of 0.2% of ammonium sulphate to the medium.

As regards the effect of C/N ratio on the production of cellulase very few reports have appeared. Quite recently, Gupta et al. (1972) have reported that a combination of peptone, urea and (NH₄)₂SO₄ as nitrogen sources and acetate and ascorbic acids as carbon sources increased cellulase production by *Trichoderma viride*. When added together, C/N had a cumulative effect and the yield doubled. Grewande (1973), in the case of *Helminthosporium apattarnae* has reported that 0.1% of D-xylose as the carbon source and 0.25% Ca(NO₃)₂ as the nitrogen source stimulated cellulase production. 4:1 ratio of these C/N was found to yield maximum cellulases (Cₓ and C₂).

The influence that yeast extract had on cellulase production was noted by Reese et al. (1950) when they observed the maximum production of cellulase (Cₓ) by some microorganisms when grown on a mineral solution medium containing 0.01% yeast extract. Fergus (1969) however, reported that yeast extract did not induce cellulase synthesis by thermophilic fungi like *Chaetomium thermophile* var. *coprophile*, *J. thermophile* var. *dissitum*, *Humicola grisea* var. *thermoidea*, *Torula thermophile* and actinomycetes. Haenssler (1973) observed that the addition of yeast extract
to the culture medium had a great influence on C<sub>x</sub> activity by 20 isolates of *Cercosporella herpotrichoides*.

The effect of KH<sub>2</sub>PO<sub>4</sub> on the production of cellulase by *Myrothecium verrucaria* was studied by Whitaker (1953) using 0.02 % in the basal medium. The optimum concentration of KH<sub>2</sub>PO<sub>4</sub> for cellulase production by *Trichoderma lignorum* was 0.2 % (Wang and SuYuanchi, 1968). Fergus (1969) used 0.1 % concentration of KH<sub>2</sub>PO<sub>4</sub> in the medium and observed the cellulase production secreted by thermophilic fungi and some actinomycetes. Prasad and Bilgrami (1973) observed that secretion of cellulase (C<sub>x</sub>) by five *Aspergillus* spp. was best on a medium containing 0.34 % potassium dibasic phosphate.

As regards the effect of MgSO<sub>4</sub> on cellulase production, Reese and Levinson (1952) observed cellulase production in the case of several fungi studied by them to be at 0.02 % concentration. Other workers who supported the presence of MgSO<sub>4</sub> include Thomas (1956), Wang and SuYuanchi (1968), Gupta and Heale (1970), Mandels and Reese (1956), Whitney et al. (1969) and Prasad and Bilgrami (1973) in the case of several fungal species.

Little work has been reported with regard to the effect of the other salts. Thomas (1956) used KCl at 0.05 % and CaCl<sub>2</sub> at 0.002 % for *Stachybotrys atra*. Mandels and Reese
(1957) used 0.003 % CaCl₂ for *Trichoderma viride*, Gupta (1963) used 0.1 % NaCl for *Ozonium taxanum* var. *parasiticum*, likewise, Prasad and Bilgrami (1973) for several *Aspergillus* spp.

As regards vitamins, Sinden et al. (1948) for *Gliomastix convoluta* and Basu (1948) for *Stachybotrys atra* observed that neither thiamine, nicotinic acid, riboflavin, pantothenic acid, pyridoxine, inositol nor biotin were capable of stimulating cellulolytic activity and growth. However, Siu and Sinden (1951) reported that 0.2 % biotin stimulated cellulolytic activity of *Aspergillus flavipes*.

Whitaker (1953) has used the following trace elements in the medium: mg/litre FeSO₄·7H₂O, 0.054; H₂SO₄, 0.06; CuSO₄·5H₂O, 0.20; ZnSO₄·7H₂O, 0.20; MnCl₂·4H₂O, 0.40 and observed cellulase production by *Myrothecium verrucaria*. Mandels and Reese (1957) reported in the case of *T. viride* that trace elements must be added to the medium for cellulase production as they had a marked effect on cellulase yield. The best yields were obtained in the presence of Fe, Mn, Zn, and Co. Whitney et al. (1969) and Gupta and Heale (1970) found that *Verticillium albo-atrum* produced Cₓ enzyme when FeSO₄·7H₂O at 0.001 % was in the medium.

While extensive work on inhibition of cellulase activity by different chemical substances has been reported
by Siu (1951), Finholt et al. (1952) and Reese and Siu (1954), less has been done on cellulase production. Gupta and Bilgrami (1969) have evaluated the effect of some chemicals on the production of cellulolytic ($C_X$) enzyme by Alternaria tenuis and Pythium aphanidermatum and observed that 4 chemicals like naphthol, kaththa, pyrogallol and tannic acid when separately added to the basic medium in 0.01% concentration reduced the $C_X$ production. Very recently Mall (1973) studied the effect of phenols like phloroglucinol, pyrocatechol, phenol and m-creosol and found that in the presence of m-creosol, Fusarium oxysporum f. tuberosii and F. solani var. eumartii did not grow at 500 and 1000 ppm and very poor enzyme activity was observed at 100 ppm. Phenol has also reduced the activity by 40-50%, but reduction in case of phloroglucinol and pyrocatechol was not very significant. Lusis and Becker (1973) have worked on the $\beta$-glucosidase system of the thermophilic fungus Chaetomium thermophile var. coprophile where they have observed that the inhibitors of protein synthesis (cyclohexamide) and of metabolism (azide, dinitrophenol) prevent the induction of cellobiase.

In-vivo cellulase production:

There are many reports by various workers on plant parasites producing cellulases in-vivo (Hussain, 1958; Winstead and McCombs, 1961; Bateman, 1963; Hancock et al.,
1964; Horton and Keen, 1968). During pathogenesis by certain bacteria and fungi, cellulases are known to degrade celluloses in plant cell walls (Bateman, 1964; Kelman and Cowling, 1965). The importance of hydrolytic enzymes in the pathogenesis of many wilt inducing phytopathogens have been reported by Dimond (1955), Sadasivan (1961) and Wood (1960). Bhagwat (1973) has recorded the involvement of pectolytic and cellulolytic (C)<sub>x</sub> enzymes in producing potato tuber rots by Fusarium sp. Eidoa (1974) has studied the in-vivo cellulase from the brown rot fungus Coniophora cerebella. Hancock et al. (1964) found that cellulase production was very high in tissues of detached onion leaf sections that had been inoculated with any one Botrytis allii, B. cinerea or B. squamosa. Szecsi (1969) observed cellulase in Fusarium stalk rot of corn. Wood (1971) observed that when cotton muslin placed for about 3 weeks in contact with tissue infected by Ascochyta pisi and Mycosphaerella pinodes, it became fragile and broke easily. This suggested that spreading lesions contained enzymes that attacked native cellulose. Helminthosporium apattarnae caused maximum rot of potato tuber and produced cellulase (C)<sub>x</sub> and C<sub>2</sub> of highest activity on 7th day. Carbohydrates, amino acids and vitamins were found to be inhibitory for cellulase production (Ghewande, 1973). Nemec (1974) has observed the production of pectinases and cellulases by 6 Pythium spp. isolated from necrotic strawberry roots. Nilsson (1974) has
reported the cellulose degradation and production of cellulase by 12 out of 20 species of wood attacking microfungi grown on agar media or in liquid cultures.

Properties of in-vitro cellulase:

It was perhaps Sorensen (1909) who first observed that the hydrogen ion concentration of a medium has a profound effect on enzymic activity, and that, each enzyme is to react best at a definite pH, which is known as its optimum pH. The pH optima of cellulases from different fungal species are as follows:

*Myrothecium verrucaria*, 5 to 6 (Saunders et al., 1948);
*Helminthosporium oryzae*, 5.5 to 6.0 (Akai, 1951);
*Aspergillus oryzae* for Cₓ, 3.5 (Jermyn, 1952); *Penicillium pusillum* on CMC had two different pH optima (Reese et al., 1952), *Aspergillus niger*, 4.5 (Whistler and Smart, 1953);
*Trichoderma koningi* on CMC 4.5 and on cellulose 5.0 (Toyama, 1953, 1955, 1956 and 1957); *Polyporus annosus*, for intra-cellular and extra-cellular 5.5 (Nokrants, 1956);
*Stachybotrys atra* 6.0 to 7.5 (Thomas, 1956). The cellobiase from *Poria vaillantii* studied by Sison and Schubert (1958) has an optimum activity at pH 4.2 and remains active between 3.5 to 5.0. *Rhizoctonia solani*, 3 to 8 (Bateman, 1964);
*Aspergillus fumigatus*, 4.5 (Loginova and Tashpulatov, 1965);
*Piricularia oryzae*, 5.4 to 6.4 (Jothianandan and Shanmugasundaram, 1968); *Fusarium lateritium* f. *cajani*, 6.0
(Singh, 1963), *Corticum* sp., 4.0 (Ghosh et al., 1968); *Diplodia zeae*, 4.5 (Bekiller et al., 1968); *Trichoderma lignorum*, 4.0 to 4.4 (Wang and SuYuanchi, 1968); *Fusarium vasinfectum*, 5.4 to 6.0 and 8.0 to 8.4 (Sampathnarayanan and Shanmugasundaram, 1970); *Stereum gausapatum*, 4 to 5 (Jensen, 1971); *Helminthosporium spattarnae* for $C_\chi$ at 2.6 and 4.6 (Ghewande, 1973); *Phoma strasseri*, 4.5 to 5.5 for $\beta$-glucosidase (Melouk and Horner, 1973); *Aspergillus awamori* and *A. usami* for $C_1$, $C_2$, $C_\chi$ and $\beta$-glucosidase 5 to 6 (Surhova and Tsyperovych, 1973).

Temperature has a direct influence on enzyme activity. While the velocity of the enzyme reaction is increased by heat, it also causes the denaturation of the enzyme and has a temperature optimum for maximum activity as well. Almost all enzymes are irreversibly denatured at 80°C. For cellulase activity temperature optima between 29 to 40°C have been reported by Saunders et al. (1948), Greathouse (1950), Siu and Sinden (1951), Toyama (1952), Aschan and Morkrans (1953), Whitaker (1953), Thomas (1956), Verma (1962) and Bateman (1964) for various fungi. Reese et al. (1950) recorded an optimum of 50 to 60°C for $C_\chi$ activity by a number of cellulolytic and non-cellulolytic microorganisms. While Akai (1951) reported 35°C for cellulase activity by *Helminthosporium oryzae*, Kooiman et al. (1953, 1957) and Aitken et al. (1956) have recorded that cellobiase from *Myrothecium verrucaria* are inactivated on heating above 60°C.
The cellobiase from *Poria vaillantii* had an optimum of 50°C and inactivation was at 70°C (Sison and Schubert, 1958). Pal and Basu (1961) found the temperature optima of different fungal cellulases to be in the range of 47 to 52°C; *Trichoderma koningi*, 45 to 55°C (Iwasaki et al., 1964); *Fusarium lateritium* f. *cajani*, 20 to 25°C (Singh, 1968); *Corticium* sp., 58°C (Ghosh et al., 1968); *Trichoderma lignorum*, 50 to 60°C (Wang and SuYuanchi, 1968). Haenssler (1973) has reported that in the case of *Cercosporella herpotrichoides*, a temperature range higher than 45°C for 30 min. reduces the \( C_X \) activity. However, the activity is not completely lost even at 90°C. Prasad and Bilgrami (1973) recorded 30°C as optimum for cellulase activity for several *Aspergillus* spp. The temperature optimum for \( C_X \) activity in *Helminthosporium spattarnae* from CMC and cellulose medium was recorded at 25°C (Ghewande, 1973).

As regards the effect of dialysis very little has been reported. Fahraeus (1947) showed that dialysis of cell-free solutions for *Cytophaga* resulted in considerable decrease in cellulase activity after dialysing the culture filtrates obtained from *Fusarium lateritium* f. *cajani*. Though the enzyme prior to dialysis was activated considerably by chlorides of sodium and potassium, activity lost on dialysis could be restored only by KCl. Heath and Wood (1971) showed that dialysed culture filtrates of *Mycosphaerella pinodes* decreased the cellulase activity. The activity in the
culture filtrates of Helminthosporium apattarnae from CMC and cellulose medium decreased during dialysis (Ghewande, 1973). The activity was not restored either by the addition of any salt or of non-dialysed autoclaved culture filtrate.

Jermyn (1952) observed that higher the enzyme concentration, maximum is the decomposition of Na-CMC in the case of Aspergillus oryzae. Levinson and Reese (1950) showed that in the case of filtrates of Myrothecium verrucaria and actinomycetes species the increase in production of reducing sugars is a direct function of the enzyme concentration. In the case of Stachybotrys atra, Youatt (1958) has reported the rate of hydrolysis was linear in relation to time and was proportional to the enzyme concentration. The filtrates from Verticillium albo-atrum cultures on CMC and on filter paper added at a concentration of 10% to a suspension of filter paper caused no increase in reducing groups after 72 hours incubation at 25°C (Blackhurst et al., 1963). These filtrates, therefore, had no detectable cellulase (C₁) activity. Tashputalov (1966) showed that the activity of the cellulolytic enzymes of both strains of Aspergillus fumigatus was highest when the concentration of the preparation was 0.8 to 0.9 mg/ml and the Na-CMC concentration was 0.8%. Ghewande (1973) reported the activity of Cₓ in the culture filtrates from both CMC and cellulose media showed gradual reduction with increasing dilution.
Quite a few workers have studied the purification of cellulase by fractional precipitation techniques. The effect of precipitation with ethanol on the activity of cellulase from *Myrothecium verrucaria* and other fungi was studied by Kooiman (1958) who showed that the activity of ethanol-precipitated enzyme was the same as that of the untreated enzyme. In the case of *Stachybotrys atra*, Thomas (1956) observed that a negligible precipitation was obtained with ammonium sulphate until 40% saturation was reached and at 80% saturation approximately half of the total activity was precipitated. Toyama (1960) obtained a highly active cellulase preparation from extracts of Koji culture of *Trichoderma viride* with different treatments of salts and organic solvents as precipitants. Gbewande (1973) has reported that both C₁ and C₂ activity of *Helminthosporium apollinaris* did not enhance with acetone precipitation, whereas precipitation with alcohol and lead acetate reduced it.

The available literature on chemical inhibition of cellulases is scattered and its study may be of help in the control of microbiological deterioration of cellulose, and therefore is of recent origin. The information relating to chemical inhibition of cellulase has been well covered in four reviews (Gascoigne and Gascoigne, 1960; Mandels and Reese, 1963, 1965 and Norkrans, 1963). The inhibition of
cellulases by heavy metal cations, specific organic reagents, dyes and various other compounds has been reported by many workers but little work has been carried out on inhibition of cellulase of plant pathogenic fungi. Basu and Whitaker (1953) have studied the inhibitory and stimulatory influence of heavy metals on the cellulase of *Myrothecium verrucaria* and observed that the hydrolysis of cellulose was inhibited by salts of heavy metals (HgCl₂, CuSO₄, AgNO₃, Pb(NO₃)₂, FeCl₃, CdCl₂ and ZnCl₂). Thomas (1956) showed that, of the compounds tested, mercuric acetate alone completely inhibited the activity of *Stachybotrys atra* cellulase, although partial inhibition was produced by copper sulphate and lead acetate at 0.02 % concentrations. Cellulases of *Gladosporium cucumerianum* are inhibited by mercury, silver, copper, chromium, lead and zinc salts at about 10⁻³ M (Hussain and Rich, 1958). Mercuric chloride is strongly inhibitory (Sison et al., 1958; Verma et al., 1963) for *Poria vaillantii* and *Curvularia lunata* cellulases respectively. The inhibition by heavy metals is probably due to a non-specific salt formation (Mandels and Reese, 1965). Singh (1968) observed that copper inhibited cellulase activity of *Fusarium lateritium* f. *cajani* at concentration of 10⁻² M. The inhibition of cellulases by mercuric ions was reported by Ghosh (1964) for *Aspergillus terreus* and *Penicillium verruabile* and by Eriksson and Petterson (1968) for *Penicillium notatum*. Basu
and Whitaker (1953) reported that cellulase of *Myrothecium verrucaria* was inhibited by oxidising and reducing agents like $\text{K}_3\text{Fe(CN)}_6$, KCN, glutathione, cysteine and Na$_2$S. While Thomas (1956) reported that partial inhibition of *Stachybotrys atra* cellulase was produced by potassium cyanide at 0.02% concentration, Sisson et al. (1958) reported its stimulation in *Foria vaillanti*.

With regard to the influence of phenolic compounds, Reese and Mandels (1957) using filtrates from the fungi like *Trichoderma viride*, *Myrothecium verrucaria* and *Pestalotiopsis westerdijkii* reported that phenol itself was not inhibitory but substituted phenols were very efficient inhibitors of the enzymes decomposing CMC. This was supported by Lyr (1961) for *Fomes marginatus* and by Reddy and Mahadevan (1967) for *Fusarium oxysporum f. vasinfectum*. Gupta and Bilgrami (1969) studied the effect of some chemicals on activity of cellulolytic ($C_X$) enzyme of *Alternaria tenuis* and *Pythium aphanidermatum* and found 0.1% solutions of chemicals such as naphthol, kaththa, pyrogallol and tannic acid when mixed with enzyme in 1:9 proportion inhibited enzyme activity but the maximum inhibition was caused by tannic acid when it was allowed to react with the enzyme for 2 hours. Mall (1973) reported the inhibitory effect of phenolic compounds on the cellulolytic activity in the case of potato wilt *Fusaria*. Basu and Whitaker (1953) investigated the effects
of various dyes over a wide pH range. They found that basic dyes were inhibitory at higher pH ranges and acid dyes at lower pH values for *Myrothecium verrucaria* cellulases at $10^{-4}$ M concentration. Ghewande (1973) has reported that all the chemicals tested inhibited cellulase ($C_x$) activity of *Helminthosporium apattarnae*.

**Properties of in-vivo cellulase:**

The literature on the properties of in-vivo cellulase is quite meagre; however, a few workers like Kelman and Cowling (1965), Heath and Wood (1971) have made some study on the properties of these enzymes. Bateman (1964) has pointed out that the $C_x$ activity of both, culture filtrates and the diseased tissue extract caused by *Rhizoctonia solani* had a broad pH optima between 3.0 and 8.0. Ghewande (1973) has recorded the optimum pH for in-vivo cellulase ($C_x$) of *H. apattarnae* to be 4.6 and for $C_2$ at 5.6. Melouk and Horner (1973) have recorded the optimum pH for activity of $\beta$-glucosidase extracted from spores and mycelium of *Phoma strasseri* between 4.5 to 5.5.

As regards the influence of temperature on cellulase activity, Strider and Winstead (1961) showed that cellulase ($C_x$) of *Cladosporium cucumerianum* was thermolabile. Bateman (1963) found that when heat treatment at 45°, 55°, 60°, 65° and 70°C was given to the enzyme solution from *Rhizoctonia solani*, cellulases was not inactivated. The activity of
cellulase obtained from diseased tomato and tobacco caused by *Pseudomonas solanacearum* on CMC was highest between 60° and 70°C (Kelman and Cowling, 1965). Ghewande (1973) has recorded an optimum temperature of 25°C for cellulases (C_x and C_2) from *Helminthosporium apattarnae*.

Bateman (1963) observed that dialysed extracts of *Rhizoctonia solani*-infected bean tissue possessed "macerating enzyme" activity which contained cellulase. In the case of *H. apattarnae* dialysis was found (Ghewande, 1973) to reduce the activity of both C_x and C_2 cellulases.

As regards the effect of dilution, Ghewande (1973) has reported that as dilution increased, the activity decreased in the case of *H. apattarnae*. No work has yet been carried out with regard to the effect of precipitation. As far as inhibitors are concerned, Goel and Mehrotra (1974) have studied the effect of some fungicides including antibiotics on pectolytic and cellulolytic enzyme activity of *Rhizoctonia bataticola*. A number of fungicides and other chemicals have been reported to reduce or check the production and activity of cell wall degrading enzymes of pathogenic fungi (Grossman, 1962; Grover, 1963). Mehta (1974) obtained isolates of *Alternaria solani* and *A. tenuis* from diseased tomato fruits and studied the inhibitory effects of fungicides at 0.1 % concentration.