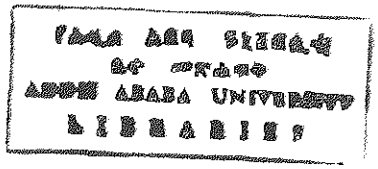
ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

LIGNOCELLULOSICS FOR CELLULASE AND FUNGAL BIOMASS PRODUCTION



By
Ameha Kebede
June 1994

LIGNOCELLULOSICS FOR CELLULASE AND FUNGAL BIOMASS PRODUCTION

A Thesis Submitted
to the School of Graduate Studies
Addis-Ababa University

In Partial Fulfillment of the Requirements for
the Degree of Master of Science in Biology.

June 1994

ACKNOWLEDGEMENT

I am highly indebted to my advisor Dr. Berhanu A. Gashe, without whose constant help and unreserved guidance the research work as well as the preparation of this thesis would have been impossible.

I am also grateful to Ato Mekuria Lakew, Dr. Dawit Abate, and Dr. Zerihun Woldu for allowing me to use their equipment and laboratory facilities.

Due thanks are also extended to Ato Amare Gessesse and Ato Abera Geyid for their valuable advice and cooperation.

I would like also to express my gratitude to Ato Kassa Semegn for the material support and technical assistance that he rendered.

My sincere appreciation and gratitude goes to my wife, W/o Nigist Getahun, who has been consistently helping and encouraging me throughout the work towards the realization of this thesis.

Finally, the financial support from the German Academic Exchange Service (DAAD) and the Swedish Agency for Research Cooperation with Developing Countries (SAREC) is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The effects of teff straw (*Eragrostis teff*) or corn stalk (*Zea mays*) on cellulase and biomass production by *Trichoderma* sp. BDCC-1, *Penicillium* sp. BDCC-2 and *Cladosporium* sp. BDCC-3 were studied and compared with those of cellulosic substrates.

In *Trichoderma* sp. both teff straw and corn stalk were found to be superior to filter paper for inducing ß-GDase as well as for improving fungal biomass production. CMCase and FPase productions were, however, lower on these substrates than they were on filter paper.

In *Cladosporium* sp. teff straw and corn stalk were found to be better than CMC for inducing both CMCase and FPase. However, no ß-GDase activity was detected when only these lignocellulosics were used in the media. It`s biomass production was also relatively lower on these substrates as compared to that it produced on 1% CMC.

Penicillium sp. produced relatively large amounts of biomass, CMCase, FPase and ß-GDase on 2% corn stalk as compared to those it produced on 2% CMC.

Lye- and 2% NaOH-pretreatments were generally effective in improving biomass production in the test fungi. But their effect on CMCase, FPase and ß-GDase production was not similar for the three fungi. There was, however, no significant difference between Lye- and 2% NaOH-pretreatment in their performance on biomass and cellulase production.

65-80% of the polysaccharides of alkali-treated teff straw and corn stalk were hydrolysed to reducing sugars in 72 hours using culture filtrate of *Trichoderma* sp. BDCC-1 which consisted of 1.7 IU/ml of FPase. The hydrolysates were found to support good growth of *Candida utilis* BDCC-25 (16.7-24.8mg dry wt./100ml) and *Saccharomyces cerevisiae* BDCC-24 (14.6-15.6mg dry wt./100ml). The yields of yeast biomass produced on these hydrolysates were higher than those produced on D-glucose (0.02%). It was , therefore, concluded that teff straw and corn stalk could provide cheap substrates for mold biomass production as well as for single-cell protein production if they are pretreated with Lye.

1. INTRODUCTION

Lignocellulosic materials comprise the largest portion of photosynthetic products on earth. They are the most abundant and continuously produced resources in nature. As a result, man has already used them for the production of countless artifacts. Some of them are being used directly for fuel, construction, cattle feed, paper and fibre production etc. . Much of them, however, remain buried or burned as agricultural, industrial and municipal wastes (Anonymous, 1974).

The efficient utilization of such cheap and renewable materials for the production of protein, chemicals and animal feed and at the same time combating pollution of the environment is one among the major objectives of biotechnological studies.

The conversion of these lignocellulosic materials to useful products can be done by employing microbial assimilation and fermentation processes. These processes, however, require four major steps: Pretreatment, cellulase production, enzymatic hydrolysis and assimilation or fermentation (Tanaka and Matsuno, 1985). All these steps involve the expenditure of a considerable amount of energy, materials and time. Hence, large scale production of fermentable sugars through these processes apparently does not seem to be economically feasible due to the high cost involved during production. Since, most of the production costs for fermentation products depend on the cost of the carbohydrate raw material (Dale, 1987), there is still a claim that lignocellulosic conversion can potentially provide less expensive fermentable sugars. Thus, a variety of lignocellulosic materials have been tested world wide as growth substrates for microorganisms in the production of chemicals (Flickinger, 1980; Ng *et al.* 1983; Christakopoulos *et al.* 1990), cellulase and single-cell protein (SCP) (Spencer-Martin and van-Uden, 1977; Kristensen, 1978; Tanaka and Matsuno, 1985; Ghanem *et al.*, 1991).

Some of the lignocellulosic materials that have undergone solid and liquid fermentation by fungi include beet pulp (Ghanem *et al.*, 1991), barley straw (Kristensen, 1978), wheat

straw (Detroy *et al.*, 1980; Margaritis and Merchant, 1986; Milstein *et al.*, 1981; Agosin *et al.*, 1987; Viesturs *et al.*, 1981; Szczodrak, 1989; Tangnu *et al.*, 1981), rice straw (Taniguchi *et al.*, 1982; Tangnu *et al.*, 1981), bagasse (Mishra *et al.*, 1984; Rodriquez *et al.*,1993; Dekker, 1983), sugarcane straw (Ortega *et al.*, 1993), corn stover (Tangnu *et al.*, 1981), water hyacinth (Ali *et al.*, 1991), wheat bran, rice bran and jute powder (Roy *et al* ., 1993), aspen wood (Mes_Hartree *et al.*, 1987a), bermuda and orchard grass (Akin and Rigsby, 1985).

Teff straw (*Eragrostis teff*) and corn stalk (*Zea mays*) are abundantly available and are commonly utilized as cattle fodder in many parts of Ethiopia. However, there has been little or no information on the bioconversion of these lignocellulosic materials. If these materials could be converted biologically to useful products like single_cell protein (SCP), it would then be possible to alleviate problems of protein-rich cattle feed in the country.

Moreover, the quality and the quantity of cellulases produced have been shown to depend substantially on the type of inducers used. For instance, the highest levels of C_1 and CMCase activities were achieved when *Thielvia terrestris* was grown on wheat straw (Margaritis and Merchant, 1986). Teff straw and corn stalk could probably have the same effect in the production of cellulases.

Effective utilization of these materials depends, among other things, on the use of very cheap method of pretreatment. The soap manufacturing factory (Gulelie Soap Manufacturing Factory) in Addis-Ababa continuously disposes of an alkaline solution (lye), which consists of NaOH as the major constituent of it`s industrial effluents. Using this solution, which would otherwise be pollutant, as a means of pretreatment would thus be advantageous both from the economic and pollution-abatement point of view.

Therefore, this study has been initiated by and large with the aforementioned perspective and in particular with the aim of achieving the following specific objectives :

1. Optimization of cellulase production using untreated

teff straw (*Eragrostis teff*) and cornstalk (*Zea mays*) as carbon and energy sources.

2. Assessment of the use of lye (spent liquor from soap manufacturing factory) in the pretreatment of teff straw and corn stalk.

3. Determination of mold biomass (biomass of *Trichoderma* sp., *Cladosporium* sp., and *Penicillium* sp.) produced using untreated and treated teff straw and corn stalk as carbon and energy sources in their growth media.

4. Evaluation of yeast biomass (biomass of *Candida utilis* and *Saccharomyces cerevisiae*) produced using the enzymatic hydrolysates of pretreated teff straw and corn stalk as carbon and energy sources.

2.2.1. Biological pretreatment

Biological pretreatment is a method used to break the crystalline structure of cellulose as well as the lignin seal by enzymes produced by micro-organisms. This method is said to have the possibility of upgrading the nutritive value of the substrates for ruminants. Although it is time consuming, it is generally accepted that it has important advantages to make it potentially useful, i.e. it is capable of removing the lignin and making it and other components of the lignocellulosics vulnerable to microbial attack (Mes-Hartree *et al.*, 1987a; Agosin *et al.*, 1987).

2.2.2.Chemical pretreatment

The chemical method of pretreatment involves delignification and transformation of the crystalline cellulose to amorphous cellulose by using acidic-, alkaline-, or organic-type solvents (Detroy *et al.*, 1980; Mandels *et al.*, 1974). However, the method seems to be costly unless a means is sought to recover the solvents used during the pretreatment process.

2.2.3. Physical pretreatment

Physical pretreatment includes the use of high temperature, high pressure, milling, radiation, or freezing to break the crystalline structure. The method, while altering crystallinity, also alters surface area and makes possible direct physical contact between the molecules and the substrate (Mandels *et al.*, 1974; Tanaka and Matsuno, 1985). Nevertheless, it has also a disadvantage for it demands for a very high supply of energy.

2.3. Cellulase and its mode of action

Cellulase is an enzyme complex consisting of three classes of enzymes, namely, exo-1,4-ß-glucanase (cellobiohydrolase or avicelase), endo-1,4-ß-glucanase (carboxymethylcellulase) and ß-glucosidase (cellobiase or salicinase) (Wood and McCrae, 1972; 1982; Mandels, 1982) which act co-operatively and synergistically to degrade cellulose.

2.3.1. Cellobiohydrolase

Cellobiohydrolase, also known as Avicelase or C_1 enzyme, degrades cellulose by splitting off cellobiose units from the non-reducing end of the chain (Halliwell and Griffin, 1973). The enzyme is capable of hydrolysing crystalline cellulose such as Avicel (microcrystalline cellulose) and cotton. It is often induced by cellulose or sophorose and strongly inhibited by cellobiose or glucose (Sasaki,1989). This enzyme is thought to play a key role in cellulose degradation since it alone can catalize the hydrolysis of microcrystalline cellulose up to 80% (w/w) and of cotton up to 40% (w/w) under a condition where there is a continuous removal of cellobiose (Petterson, 1975).

2.3.2. Endoglucanase

Endoglucanase, often called C_x-enzyme or carboxymethylcellulase (CMCase), attacks the internal bonds of soluble derivatives of cellulose and amorphous cellulose such as phosphoric acid-swollen cellulose randomly resulting in shorter chain lengths (cellodextrin, cellobiose) or glucose (Sasaki, 1989; Araujo and D'Souza, 1986). It is also capable of degrading carboxymethylcellulose (CMC). But it does not hydrolyse avicel, cotton, and the likes. Endoglucanase can act on such crystalline cellulosic substrates only when it exists together with cellobiohydrolase and it is also induced by cellulose or sophorose (Sasaki, 1989).

2.3.3. ß-Glucosidase

ß-Glucosidase, also known as cellobiase or salicinase, hydrolyses cellobiose to glucose (Mullings, 1985). It is also capable of hydrolysing a variety of ß-glucosides such as methyl-ß-glucoside, salicin, phenyl-ß-glucoside etc (Sternberg *et al.*, 1977) and cello-oligosaccharides (Sasaki, 1989).

2.3.4. Synergism

Synergism is an important phenomenon observed during cellulolysis. As indicated above, endoglucanase does not attack crystalline cellulose without the participation of exo-

glucanase (cellobiohydrolase). The later is also strongly inhibited by the accumulation of cellobiose. The presence of ß-glucosidase, however, can avoid the problem of cellobiose accumulation. Therefore, for complete solubilization of cellulosic materials it is vital to have a mixture of all the three components in the cellulase system.

The mechanism of the synergistic action of the cellulase components is not yet fully understood. As early as 1950, some people suggested a possible mechanism for the synergism between the two components of cellulase (endo-glucanase and exo-glucanase). According to this early hypothesis C_x enzymes (endo-glucanases) hydrolyse ß-1,4 bonds in cellulose molecules that are activated by the non-hydrolytic factor (so called C_1 component). But since other people have identified the C_1 component as an exo-ß-1,4-glucanase, a hydrolytic enzyme that is capable of removing successive units of cellobiose from the non-reducing end of the chain, the C_1-C_x hypothesis of the mechanism of cellulose hydrolysis together with its nomenclature seems no longer to be acceptable (Wood and McCrae, 1972). After years of extensive work, the recognition of the fact that exo-glucanase (cellobiohydrolase) is the only enzyme that is capable of degrading crystalline cellulose to a very high extent (80%) made some people to reconsider other suggestions. Accordingly, Petterson (1975) proposed a new and simple scheme for the mechanism of the synergistic action of the cellulase components as follows:

1. Native cellulose Endo-glucanase → Cellulose(modified)
2. Cellulose (modified) Exo-glucanase ----→ Cellobiose.
3. Cellobiose ß-glucosidase -----→ 2 Glucose.

From the above scheme it is to be understood that :

1. Regions of low crystallinity in the cellulose fibre are attacked by endo-glucanases and free chain ends are created;
2. Exo-glucanases start the degradation from the chain ends by hydrolytically removing cellobiose units;
3. Cellobiose is hydrolysed to glucose through the action of ß-glucosidase.

Although the above scheme has been proposed as a general mechanism for the co-operation of endo- and exo-glucanases in the degradation of pure cellulose, there is no substantial evidence to show that the endo-glucanases initiate the hydrolysis of native cellulose.

2.4. Cellulase production

The complex nature of lignocellulosics necessitates the use of a lot of cellulase for saccharification. In order to meet this demand, there has to be an effective method of improving cellulase production. According to Tanaka and Matsuno (1985) three methods have contributed to the improvement of cellulase production, viz., 1. selection and isolation of micro-organisms 2. optimization of culture conditions and 3. control of the cellulose fermentation process.

2.4.1. Selection and isolation of micro-organisms

Several mutants of *Trichoderma reesei*, which produce very high cellulase specific activities, have been isolated in United states and Japan (Sasaki, 1989). However, there still remains much to be done with the isolation and selection of mutants of fungal species other than *Trichoderma reesei* and applying the techniques of genetic engineering to promote cellulase production to a practical level.

2.4.2. Optimization of culture conditions

Optimization of culture conditions with respect to pH, nutrient, carbon source, temperature requirements, type, age and amount of inoculum, stirring, etc, greatly increases cellulase productivity (Desrochers *et al.*, 1981; Enari and Markannen, 1977). A particular condition which is optimal for one organism may not be optimal for another. Moreover, the addition of different chemicals into the media can have different effects on the cellulase productivity of different organisms (Tanaka and Matsuno, 1985).

2.4.3. Control of fermentation processes

Control of fermentation processes to increase cellulase productivity has been possible by employing any one of the following fermentation processes : fed-batch culture (Allen and Andreotti, 1982), continuous culture (Allen and Andreotti, 1982), cell-holding continuous culture (Taniguchi *et al.*, 1983), continuous culture with cell recycling (Ghose and Sahai, 1979).

In general, cellulase production would be expected to improve by employing and further refining the above-mentioned methods and, on top of that, through the elucidation of the genetic information on the control mechanism of cellulase synthesis.

2.5. Xylanases

Xylan polysaccharides are major hemicellulose components of lignocellulosics. Thus, it would be very important to have xylanases together with cellulases for maximum solubilization of lignocellulosics.

Xylanases, like cellulases, are complex enzymes consisting of components such as exo- and endo-1,4-ß-xylanases, which cleave the glycosidic linkages in the xylan back bone, ß-xylosidases, which hydrolyse the soluble xylo-oligosaccharides, and several of other enzymes which cleave the substituents from the xylan backbone (Biely, 1985). The enzymes are inducible and produced in good amount when the micro-organisms are grown on xylans (Dekker and Richards, 1976).

2.6. Ligninases

Ligninase is a collective name for a family of extracellular isoenzymes produced by fungi that act on lignin. These enzymes are mostly secreted by fungi, in particular by some members of the basidiomycetes. Although bacteria of several genera including *Pseudomonas* and *Arthrobacter* and some actinomycetes are also known to degrade the single-ring aromatic compounds that build up the lignin macromolecule (Zimmermann, 1990; Antai and Crawford, 1981), no counterparts

of fungal ligninases have been found so far in these micro-organisms.

Ligninases bring about the degradation of lignin by involving a number of biochemical reactions. Some of these reactions are : cleavage of the intermonomeric linkages, demethylations, hydroxylations, side chain modifications, and aromatic ring fission followed by dissimilation of the aliphatic metabolites produced.

2.7. Pectinolytic enzymes

Pectinolytic enzymes also assist in the degradation of plant biomass. These enzymes are mostly known to cause tissue maceration by attacking the α-1,4 glycosidic bonds found in uronic polymers of pectic substances and by removal of methyl groups from pectin (Bilgrami and Dube, 1976). The components that participate in such activities are pectate lyase, pectin lyase, polygalacturonase, polymethylgalcturonase, and pectin methyl esterase (Bilgrami and Dube, 1976; Schlemmer *et al.*, 1987).

2.8. Cellulolytic micro-organisms

Several species of bacteria (Ramasamy *et al.* 1981; Akin and Rigsby, 1985; Shiang *et al.*, 1991; Rodriquez *et al.*, 1993) , actinomycetes (Crawford, 1978; Antai and Crawford, 1981; Mackenzie *et al.*, 1984; Sasaki, 1989), and fungi (Mandels *et al* ., 1974; Sternberg *et al.*, 1977; Viesturs *et al.*, 1981; Wood and McCrae, 1982; Macris, 1984; Sasaki, 1989; Szczodrak, 1989) are known to grow and elaborate cellulases on lignocellulosic substrates. However, only a limited number of fungal species are capable of excreting large amounts of cellulase (Wood, 1985).

Members of the genera, viz., *Trichoderma* (Mandels *et al.*, 1974; Herr, 1979; Tangnu *et al.*, 1981; Warzywada *et al.*, 1983), *Penicillium* (Mishra *et al.*, 1984; Funaguma *et al.*, 1986), *Aspergillus* (Sternberg *et al.*, 1977; Singh *et al.*, 1990), *Sclerotium* (Shewale and Sadana, 1978), *Cladosporium* (Abrha and Gashe, 1992) and a number of others have been reported to

produce high cellulase activities. Of all the investigated fungi, *Trichoderma reesei* and its mutants are the most powerful inducers of cellulases (Sasaki, 1989). Although this species is known to excrete high levels of cellulose-solubilizing activities, the extramycelial yields of ß-glucosidase in the culture filtrates are low (Sternberg, 1976). Therefore, much attention is now given to the search for a new and efficient endo-glucanase, cellobiohydrolase and ß-glucosidase hyperproducing organisms through isolation, mutation and genetic engineering. In addition to this the production of other enzymes like xylanases, ligninases and pectinases by such organisms would be of great importance to enhance and promote saccharification processes to the level of practical application.

Fortunately many cellulase-producing micro-organisms are also xylanase and pectinase producers (Harmova *et al.*, 1991; Stutzenberger, 1991; Bailey and Poutanen, 1989; Dekker, 1983; Tangnu *et al.*, 1981), which is a very useful property for maximum utilization of lignocellulosics using enzymatic hydrolysis.

2.9. Single-cell protein production from lignocellulosics

The sugar syrups produced by enzymatic hydrolysis of lignocellulosics can be used for single-cell protein (SCP) production (Tanaka and Matsuno, 1985; Spencer-Martin and van-Uden, 1977; Taniguchi *et al.*, 1982).

There are two ways of producing SCP: 1. by direct growth of micro-organisms on pretreated lignocellulosic materials using either a monoculture or a mixed culture; 2. by the growth of micro-organisms, preferentially yeasts, in the sugar solution obtained by enzymatic hydrolysis of pretreated lignocellulosic materials (Tanaka and Matsuno, 1985).

The organisms mostly used for SCP production are yeasts. The reason that they are preferred to other fungi, bacteria or algae is partly due to cultural bias (Humphrey, 1975) and partly a consquence of the good nutritional quality of yeast proteins (Young and Scrimshaw, 1975).

Some of the common yeast species that have been reported in SCP production are *Candida utilis* (Kristensen, 1978; Taniguchi *et al.*,1982), *Saccharomyces cerevisiae* (Taniguchi *et al.*,1982), *Trichosporon cutaneum* (Sandhu and Waraich, 1983; Taniguchi *et al.*, 1982), *Lipomyces starkeyi* and *Lipomyces kononenkoae* (Spencer-Martins and van-Uden, 1977), *Candida tropicalis, Torulopsis xylinus, Candida guilliermondii* (Taniguchi *et al.*, 1982).

material was then exposed to Lye or 2% NaOH (w/v)-pretreatment.

Filter paper (Whatman No.1), obtained from the department's chemical store, was also pulverized in a similar manner before use.

Commercially produced carboxymethylcellulose sodium salt (CMC, high viscosity, Sigma), avicel (microcrystalline cellulose, Merck), salicin (Sigma), xylan (oat spelt, Sigma), pectin (citrus pectin, Sigma), polygalacturonic acid (Sigma), D-glucose (Sigma) were all obtained in powder forms from the donations of the Biotechnology project of the Department of Biology.

3.3.1. Growth substrate for molds

The substrates used as carbon and energy source for *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp. were D-glucose, carboxymethylcellulose (CMC) or filter paper, and untreated or alkali-treated teff straw and corn stalk.

3.3.2. Growth substrates for yeasts

D-Glucose and enzymatic hydrolysates of alkali-treated teff straw and corn stalk were used as growth substrates for *Candida utilis* and *Saccharomyces cerevisiae*.

3.3.3.Assay substrates

The assay substrates included filter paper, avicel, carboxymethylcellulose, salicin, xylan, pectin, and polygalacturonic acid.

3.4. Pretreatment of lignocellulosics

Alkali-pretreatment of lignocellulosics was carried out using the method described by Mandels *et al.*, (1974) with slight modification.

Four grams of shredded teff straw or corn stalk were added to 100 ml of 2% NaOH (w/v) or Lye (spent liquor from soap manufacturing factory) in a 500 ml capacity Erlenmeyer flask. The flask was kept in a water bath (Gallenkamp,UK) for 90 minutes at 70°C. The suspension was then washed several times

with tap water to neutrality and dried in an oven overnight at 80°C.

3.5. Growth and cellulase induction media

The media used for growth and induction of cellulases were prepared as previously described by Erku (1990), Gashe (1992), and Abrha & Gashe (1992).

3.5.1. Media for *Trichoderma* sp.

The media contained the following ingredients (g/100 ml) : KH_2PO_4, 0.62; $MgSO_4.7H_2O$, 0.05; KCl, 0.05; $FeSO_4$, 0.005; KNO_3, 1.0; yeast extract, 0.2; Tween-80 (to increase porosity), 2% (v/v); 2 ml of trace mineral solution whose composition was (μg/100 ml): HBO_3, 0.5; $CaCO_3$, 10.0; $CuSO_4.5H_2O$, 1.0; $FeSO_4(NH_4)_2SO_4$, 50; KI, 1.0; $MnSO_4$, 2.0; MoO_3, 1.0 and $ZnSO_4.7H_2O$, 5.0.

3.5.2. Media for *Cladosporium* sp. and *Penicillium* sp.

The growth and cellulase induction media of *Cladosporium* sp. and *Penicillium* sp. consisted of all ingredients of the medium of *Trichoderma* sp. except for KCl and 0.2% Tween-80. These were replaced with 0.05g of $CaCl_2$ and 0.1% Tween-80 (v/v), respectively.

All media, including those of *Trichoderma* sp., were autoclaved at 121°C and 15 lbs./sq.in. for 15 minutes before use. These were then aseptically inoculated and incubated for 6-30 days, at room temperature in an orbital shaker (Gallenkamp, UK) operating at 150 RPM.

3.5.3. Media optimization

In addition to the above-mentioned mineral medium, carbon sources of varying quality and quantity, viz., 0.5% filter paper (for *Trichoderma* sp.), 1% or 2% carboxymethylcellulose (for *Cladosporium* sp. and *Penicillium* sp., respectively), 0.5-3% untreated or alkali-treated lignocellulosics, and cellulosics (filter paper or carboxymethylcellulose) supplemented with 0.5-3% untreated or alkali-treated lignocellulosics, were used to find the optimum concentration

4. RESULT

4.1. Production of extracellular enzymes by *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp.

Trichoderma sp., *Cladosporium* sp. and *Penicillium* sp., which were grown on different media containing cellulosics, lignocellulosics or cellulosics supplemented with lignocellulosics as carbon and energy sources, produced various extracellular degradative (hydrolytic) enzymes that were active against various components of plant parts (Table 1).

As can be seen from the table, all the test fungi were able to excrete cellulase(endoglucanase, exoglucanase and ß-GDase), xylanase, and polygalacturonase into the culture media. With the exception of *Cladosporium* sp., all were also capable of releasing pectinase into the media. In comparison *Trichoderma* sp. produced the highest cellulase, xylanase and polygalacturonase activities while *Penicillium* sp. produced the highest pectinase activity under the specified culture conditions.

In all cases, when three of the components of the cellulase were compared with each other, it was found that the activities of CMCase were much higher than those of ß-GDase and Avicelase. In *Trichoderma* sp. the activity of Avicelase was slightly higher than that of the ß-GDase. But in *Cladosporium* sp. and *Penicillium* sp. the activities of ß-GDase were much greater than those of the avicelase (Table 1).

The activities of FPase were generally lower than those of the above three components of the cellulase for all fungi studied.

Time course studies on the production of cellulase by the three fungi revealed that the components of cellulase (CMCase, avicelase and ß-GDase) show different optima at different periods of growth (Figures 1-3). The patterns of the enzyme production were, however, similar for all components of the cellulase system.

In *Trichoderma* sp. production of all three components of the cellulase started on the 6th day of incubation and reached

Table 1: Detection of extracellular enzymes from *Trichoderma* sp*., *Cladosporium* sp*. and *Penicillium* sp*. growth media.

ORGANISM	ENZYME	ACTIVITY (IU/ml)	GROWTH SUBSTRATE
Trichoderma sp.	Cellulase:		
	CMCase	75	FP[1]
	ß-GDase	3.15	FP
	Avicelase	3.6	FP
	FPase	2.9	FP
	Xylanase	1.51	LTrTS[2] (2%)
	Pectinase	0.20	LTrTS (2%)
	Polygalacturonase	0.37	LTrTS (2%)
Cladosporium sp.	Cellulase:		
	CMCase	20.4	CMC[3] (1%)
	ß-GDase	2.6	CMC (1%)
	Avicelase	0.9	CMC (1%)
	FPase	0.7	CMC (1%)
	Xylanase	0.47	LTrTS (2%)
	Pectinase	0	LTrTS (2%)
	Polygalacturonase	0.11	LTrTS (2%)
Penicillium sp.	Cellulase:		
	CMCase	16.7	CMC (2%)
	ß-GDase	2.0	CMC (2%)
	Avicelase	0.5	CMC (2%)
	FPase	0.4	CMC (2%)
	Xylanase	1.04	LTrTS (2%)
	Pectinase	0.29	LTrTS (2%)
	Polygalacturonase	0.28	LTrTS (2%)

[1]Filter paper

[2]Lye-treated teff straw

[3]Carboxymethylcellulose

*Growth was allowed to proceed for 6-30 days, at room temperature, before activity was determined.

Figure-1. The effect of filter paper (2%) on the production of cellulase by *Trichoderma* sp..

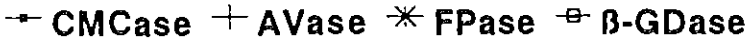


Figure-2. The effect of CMC (1%) on the production of cellulase by *Cladosporium* sp..

Figure-3. The effect of CMC (2%) on the production of cellulase by *Penicillium* sp.

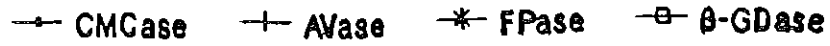
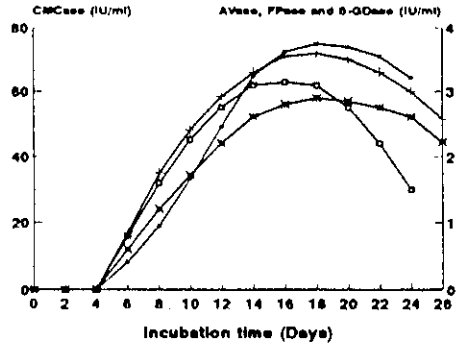


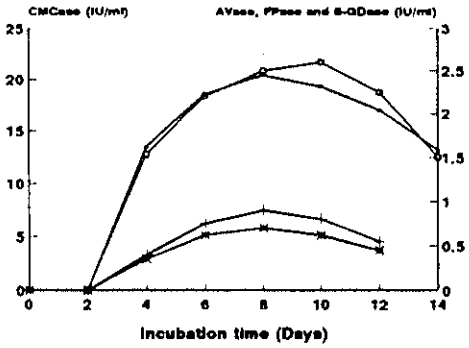
Figure-4. The effect of alkali-pretreatment of teff straw on the production of : A. CMCase B. FPase and C. ß-GDase by *Trichoderma* sp.

Note : See Tables 1-12 for abbreviations.

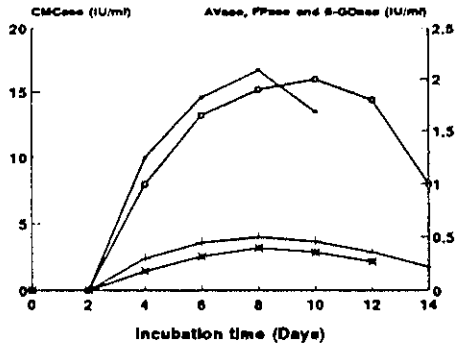
1


CMCase (IU/ml)
AVase, FPase and B-GDase (IU/ml)
Incubation time (Days)

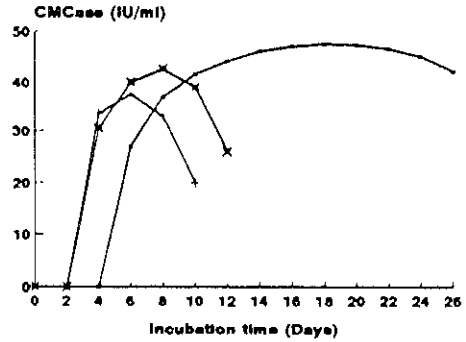
2


CMCase (IU/ml)
AVase, FPase and B-GDase (IU/ml)
Incubation time (Days)

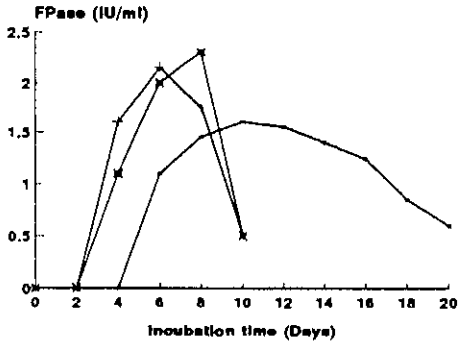
3


CMCase (IU/ml)
AVase, FPase and B-GDase (IU/ml)
Incubation time (Days)

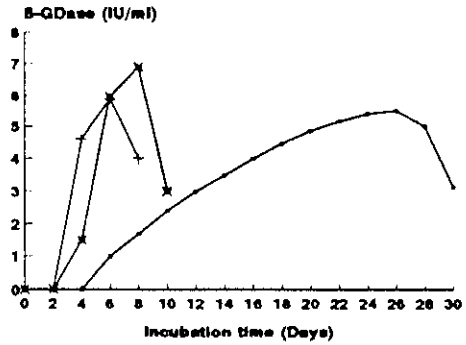
4-A


CMCase (IU/ml)
Incubation time (Days)

4-B


FPase (IU/ml)
Incubation time (Days)

4-C


B-GDase (IU/ml)
Incubation time (Days)

their peaks on the 16th to 18th day of incubation (Fig. 1).

In *Cladosporium* sp. and *Penicillium* sp., however, the release of these enzymes were detected on the 4th day of incubation. Apart from these, the time needed for optimum production of all components of cellulase in the later two fungi was shorter than that required for *Trichoderma* species.

4.2. The effects of untreated and alkali-treated lignocellulosics on the production of CMCase, FPase and ß-glucosidase by *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp.

The effects of using varying concentrations of untreated and alkali-treated lignocellulosics or cellulosics supplemented with lignocellulosics, as carbon and energy sources, on the synthesis and excretion of CMCase, FPase and ß-glucosidase by *Tichoderma* sp., *Cladosporium* sp. and *Penicillium* sp. are shown on Tables 2-12.

When filter paper and CMC (cellulosics) were used as carbon and energy sources, optimum production of CMCase, FPase and ß-GDase occurred at 0.5% filter paper for *Trichoderma* sp., 1% CMC for *Cladosporium* sp. and 2% CMC for *Penicillium* sp.. The values for the enzyme activities are shown in Tables 2 - 12.

4.2.1. The effect of untreated teff straw on CMCase, FPase and ß-GDase production by *Trichoderma* sp.

Table 2 shows the effect of varying concentrations of untreated teff straw (i.e. 0.5%, 1%, 2% and 3%) on CMCase, FPase and ß-GDase production by *Trichoderma* sp..

At 0.5% concentration of untreated teff straw the amounts of CMCase, FPase and ß-GDase were 23, 0.7 and 2.0 IU/ml respectively. Increasing the concentration of the straw to 1 or 2% brought about improvements in CMCase, FPase and ß-GDase activities. The activities of CMCase were increased by 67% and 107% and those of FPase were increased by 57% and 129%, respectively. Similarly, ß-GDase activities were improved by 65% and 175% when using 1% and 2% untreated teff straw, respectively. Concentrations higher than 2% did not bring about

Table 2: CMCase, FPase and ß-GDase production by *Trichoderma* sp.* grown on varying concentrations of untreated teff straw and on filter paper supplemented with different concentrations of untreated teff straw.

SUBSTRATE		ENZYME ACTIVITY (IU/ml)		
		CMCase	FPase	ß-GDase
0.5% FP[1]		75	2.9	3.15
UnTS[2] alone,	0.5%	23	0.7	2.0
	1%	38.5	1.1	3.3
	2%	47.5	1.60	5.5
	3%	49.3	1.60	5.3
0.5% FP Plus,	0.5% UnTS	36.3	0.9	5.0
	1% UnTS	54	1.4	7.25
	2% UnTS	44	0.9	5.9

[1]Filter paper

[2]Untreated teff straw

* -Growth was allowed to proceed for 8 to 26 days, at room temperature, before activity was determined.

by 34% and 7% respectively (Table 3). Similarly, NaOH-pretreatment increased the quantities of FPase and ß-GDase by about 44% and 26%, respectively. Neither lye- nor NaOH-pretreatment helped in improving CMCase production. The amount of CMCase was rather decreased by 21% and 11% by pretreating teff straw with lye and NaOH, respectively.

Maximum production of CMCase, FPase and ß-GDase was observed at a much earlier period in the growth of the test organism when alkali-treated teff straw was used as the sole carbon and energy source (Figures 4A-C) and as a supplement to filter paper (Figures 5A-C). Both lye and NaOH pretreatments reduced the time required for maximum production of CMCase by 12 and 10 days and that required for ß-GDase by 20 and 18 days , respectively (Fig. 4A & C). Similarly, the time of maximum production of FPase was also shortened by 4 and 2 days by lye- and NaOH-pretreatment, respectively (Fig. 4B).

4.2.3. The effect of untreated corn stalk on CMCase, FPase and ß-GDase production by *Trichoderma* sp.

Four levels of concentrations (0.5%, 1%, 2% and 3%) of untreated corn stalk were used as carbon and energy sources for the growth of *Trichoderma* sp..

At 0.5% level of concentration of untreated corn stalk there was no detectable quantity of CMCase, FPase and ß-GDase in the culture medium (Table 4). Increasing the concentration of untreated corn stalk to 1% or 2% resulted in the production of very small quantities of CMCase, FPase and ß-GDase. The activities of CMCase and ß-GDase generally increased with increase in the concentration of the growth substrate (i.e. CMCase and ß-GDase activities increased by about 309% and 175%, respectively, when the concentration of untreated corn stalk was increased from 1% to 2%). But FPase was detectable only when the concentration of untreated corn stalk was ≥ 2% in the medium. Concentrations higher than 2% did not significantly improve the activities of CMCase, FPase and ß-GDase. Therefore, 2% untreated corn stalk, being the optimum concentration, was used for pretreatment studies.

Table 4: CMCase, FPase and ß-GDase production by *Trichoderma* sp.* grown on varying concentrations of untreated corn stalk and on filter paper supplemented with different concentrations of untreated corn stalk.

SUBSTRATE		ENZYME ACTIVITY (IU/ml)		
		CMCase	FPase	ß-GDase
0.5% FP[1]		75	2.9	3.15
UnCS[2] alone,	0.5%	0	0	0
	1%	2.2	0	0.4
	2%	9.0	0.5	1.1
	3%	10.5	0.6	0.9
0.5% FP plus,	0.5% UnCS	0	0	0
	1% UnCS	0.7	0	0.2
	2% UnCS	1.9	0.2	1.1
	3% UnCS	1.7	0.2	1.0

[1]Filter paper

[2]Untreated corn stalk.

* -Growth was allowed to proceed for 8 to 14 days, at room temperature, before activity was determined.

However, the amounts of CMCase, FPase and ß-GDase produced in 2% untreated corn stalk were much less than those produced in 0.5% filter paper. The activities of CMCase, FPase and ß-GDase were decreased by 88%, 83% and 65% respectively.

When filter paper was supplemented with varying concentrations of untreated corn stalk the amounts of CMCase, FPase and ß-GDase still declined much more than they did in media containing only untreated corn stalk as carbon and energy source (Table 4).

No trace of CMCase, FPase and ß-GDase was detected at 0.5% concentration of untreated corn stalk added to filter paper. When, however, 1% or 2% untreated corn stalk was added to filter paper very small quantities of CMCase and ß-GDase were detectable in the culture medium. The highest activity was detected when 2% untreated corn stalk was added to filter paper. ß-GDase activity was detected only in filter paper supplemented with ≥ 2% untreated corn stalk. Therefore, 2% was considered as the optimum concentration for supplementing filter paper. Nevertheless, the yield of CMCase and FPase obtained from media containing filter paper supplemented with 2% untreated corn stalk was much lower than that obtained from media containing only 2% untreated corn stalk. Thus, the supplemented medium was not used for pretreatment studies.

As compared to media -containing only filter paper, filter paper supplemented with 2% untreated corn stalk was found to be a very poor substrate for the production of CMCase, FPase and ß-GDase. Supplementation, generally, reduced the activities of these enzymes by 98%, 93% and 65%, respectively (Table 4).

4.2.4. The effect of alkali-pretreatment of corn stalk on CMCase, FPase and ß-GDase production by *Trichoderma* sp.

Tremendous improvements were observed in CMCase, FPase and ß-GDase production when alkali-pretreated corn stalk was used as the sole carbon and energy source (Table 5). Lye-pretreatment improved the amounts of CMCase, FPase and ß-GDase by 278%, 160% and 355% respectively. Likewise, NaOH-

Figure-5. The effect of supplementation of filter paper with alkali-treated teff straw on the production of: A. CMCase B. FPase and C. ß-GDase by *Trichoderma* sp..

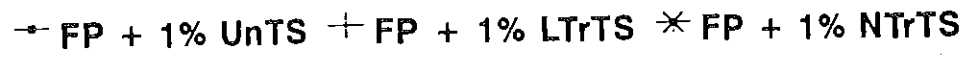
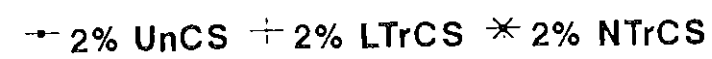
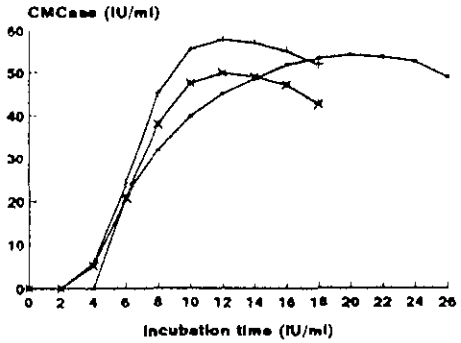


Figure-6. The effect of alkali-pretreatment of corn stalk on the production of: A. CMCase B. FPase and C. ß-GDase by *Trichoderma* sp..

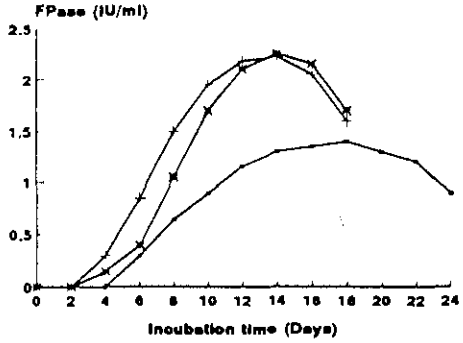


Note: See Tables 1-12 for abbreviations

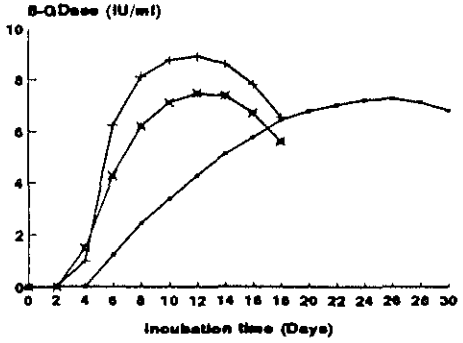
5-A



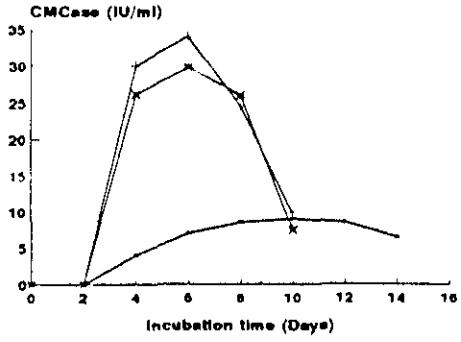
5-B



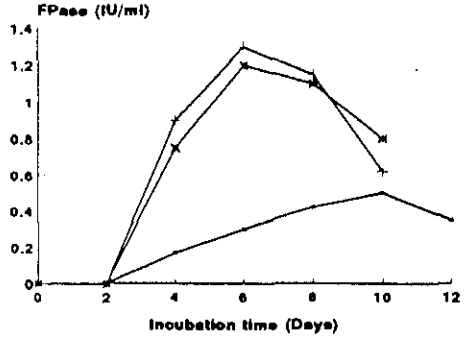
5-C



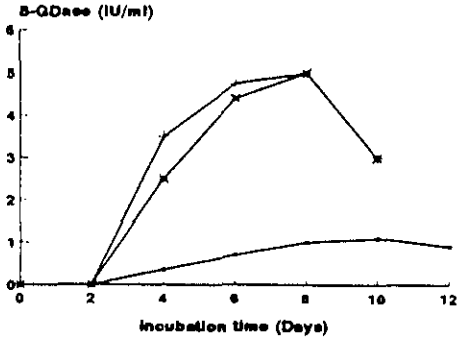
6-A



6-B



6-C



pretreatment increased the levels of these enzymes by 231%, 140% and 355%, respectively. Moreover there was a 4 days reduction in the time required for maximum production of both CMCase and FPase by pretreating corn stalk with either lye or NaOH (Fig. 6A & B). The time for maximum production of ß-GDase was also reduced by about 2 days in media containing only lye-treated or NaOH-treated corn stalk.

4.2.5. The effect of untreated teff straw on CMCase, FPase and ß-GDase production by *Cladosporium* sp.

Cladosporium sp. was grown in media containing different concentrations of teff straw and in media containing 1% CMC supplemented with varying concentrations of untreated teff straw.

When 0.5% untreated teff straw was used as a single carbon and energy source, the amounts of CMCase and FPase excreted into the culture media were 42.2 and 0.9 IU/ml, respectively (Table 6). Increasing the concentration of the untreated teff straw to 1% improved their activities by 30% and 33%, respectively. Further increase in concentration, however, did not improve the activities of CMCase and FPase. ß-GDase activity was not detected at all in any of the concentrations of the straw used. Thus, 1% untreated teff straw was found to be the optimum concentration with which further studies could be made on the effect of alkali-pretreatment on CMCase, FPase and ß-GDase production by *Cladosporium* sp..

The amounts of CMCase and FPase produced in media containing only untreated teff straw were generally higher than those produced in media containing CMC as a single carbon and energy source. In CMC-containing media *Cladosporium* sp. produced 20.4 and 0.7 IU/ml of CMCase and FPase, respectively (Table 6). When CMC was replaced with 1% untreated teff straw the activities of CMCase and FPase were increased by 233% and 71% respectively. However, ß-GDase was detectable only when CMC was used as a single carbon and energy source but not in media containing untreated teff straw.

Supplementing CMC with varying concentrations of untreated

Table 5: CMCase, FPase and ß-GDase production by *Trichoderma* sp.* grown on alkali-treated corn stalk.

SUBSTRATE	ENZYME ACTIVITY (IU/ml)		
	CMCase	FPase	ß-GDase
0.5% FP[1]	75	2.9	3.15
2% UnCS[2]	9.0	0.5	1.1
2% LTrCS[3]	34	1.3	5.0
2% NTrCS[4]	29.8	1.2	5.0

[1]Filter paper
[2]Untreated corn stalk
[3]Lye-treated corn stalk
[4]NaOH-treated corn stalk
* -Growth was allowed to proceed for 6-14 days, at room temperature, before activity was determined.

Table 6: CMCase, FPase and β-GDase production by *Cladosporium* sp.* grown on varying concentrations of untreated teff straw and on CMC supplemented with different concentrations of untreated teff straw.

SUBSTRATE		ENZYME ACTIVITY (IU/ml)		
		CMCase	FPase	β-GDase
1% CMC[1]		20.4	0.7	2.6
UnTS[2] alone,	0.5%	42.2	0.9	0
	1%	68	1.2	0
	2%	30.4	0.8	0
1% CMC plus,	0.5% UnTS	25.2	0.7	2.0
	1% UnTS	25.2	0.7	2.0
	2% UnTS	20.4	0.7	2.4

[1]Carboxymethylcellulose

[2]Untreated teff straw

* -Growth was allowed to proceed for 10 to 20 days, at room temperature, before activity was determined.

teff staw did not improve CMCase, FPase and ß-GDase production by *Cladosporium* species. When 0.5% untreated teff straw was added to 1% CMC the amounts of CMCase and FPase released into the media were more or less similar to those released when CMC was used as a single carbon and energy source (Table 6). Increasing the concentration of untreated teff straw added to CMC to 1% or 2% did not improve their activities. Furthermore, ß-GDase activity was not improved in any of the concentrations of untreated teff straw added to CMC. In media containing only CMC as carbon and energy source, the activity of ß-GDase was 2.6 IU/ml. When CMC was supplemented with 2% untreated teff straw its activity was decreased by about 8% (Table 6). Thus, no supplemented medium was used for further study on the effect of pretreatment of teff straw on CMCase, FPase and ß-GDase production by *Cladosporium* species.

4.2.6. The effect of alkali-pretreatment of teff straw on CMCase, FPase and ß-GDase production by *Cladosporium* sp.

When 1% teff straw was pretreated with lye and used as carbon and energy source for *Cladosporium* sp., the amounts of CMCase and FPase excreted into the culture medium were found to decrease by about 15% and 25% respectively (Table 7). Simlarly, pretreatment with NaOH also resulted in a decrease in the quantities of CMCase and FPase by 34% and 42%, respectively (Table 7). Furthermore, neither lye- nor NaOH-treated teff straw were able to induce the release of ß-GDase into the media.

The time for maximum production of CMCase was reduced by 2 and 4 days when using lye-treated and NaOH-treated teff straw, respectively, in place of untreated teff straw (Fig. 7A). Similarly, there was a 4 and 6 days reduction in time for maximum production of FPase by replacing untreated teff straw with Lye-treated and NaOH-treated teff straw, respectively (Fig. 7B).

Table 7: CMCase, FPase and ß-GDase production by *Cladosporium* sp.* grown on alkali-treated teff straw and corn stalk.

SUBSTRATE	ENZYME ACTIVITY (IU/ml)		
	CMCase	FPase	ß-GDase
1% CMC	20.4	0.7	2.6
1% UnTS	68	1.2	0
1% LTrTS	58	0.90	0
1% NTrTS	45	0.7	0
1% UnCS	43	0.9	0
1% LTrCS	46.5	1.2	0
1% NTrCS	48.2	1.3	0

Note:

- Abbreviations for substrates are as in Tables 1-6.

* -Growth was allowed to proceed for 8-22 days, at room temperature, before activity was determined.

4.2.7. The effect of untreated corn stalk on CMCase, FPase and ß-GDase production by *Cladosporium* sp.

Varying concentrations of untreated corn stalk (0.5%, 1% and 2%) were used either as supplement to CMC or as single carbon and energy source for growth of *Cladosporium* species. At 0.5% level of concentration of corn stalk the activities of CMCase and FPase were 37% and 0.7% IU/ml respectively (Table 8). Increasing the concentrations to 1% improved the activities by 16% and 29%, respectively. Further increase in concentration of untreated corn stalk did not improve CMCase and FPase production. Although it was possible to harvest relatively large quantities of CMCase and FPase there was no trace of ß-GDase activity detected in any of the concentrations of untreated corn stalk used.

Since 1% was the optimum concentration for maximum production of CMCase and FPase this level of concentration of untreated corn stalk was used in the study of the effect of prtreatment on enzyme production.

The amount of CMCase and FPase produced in 1% untreated corn stalk were generally higher than the amounts produced in 1% CMC. However, CMC was superior to untreated corn stalk in inducing the release of ß-GDase.

When CMC was supplemented with different concentrations of untreated corn stalk, the production of CMCase and FPase was kept as low as that of the medium containing CMC as a single carbon and energy source. At the level of 0.5% concentration of untreated corn stalk added to CMC the activities of CMCase and FPase were 22.7 and 0.7 IU/ml, respectively (Table 8). Increasing the concentration of the substrate added to CMC to 1% increased their activities slightly by about 15% and 14%, respectively. Further increase to 2% did not improve their activities.

ß-GDase activity was also detected in all the concentrations of untreated corn stalk added to CMC. The level of its production was increased with increase in concentration of untreated corn stalk added to CMC. However, the activities were still much less than those produced in 1% CMC used as a

Table 8: CMCase, FPase and ß-GDase production by *Cladosporium* sp.* grown on varying concentrations of untreated corn stalk and on CMC supplemented with different concentrations of untreated corn stalk.

SUBSTRATE		ENZYME ACTIVITY (IU/ml)		
		CMCase	FPase	ß-GDase
1% CMC		20.4	0.7	2.6
UnCS alone,	0.5%	37	0.7	0
	1%	43	0.9	0
	2%	33	0.8	0
1% CMC plus,	0.5 UnCS	22.7	0.7	0.7
	1% UnCS	26.1	0.8	0.9
	2% UnCS	25.9	0.7	1.1

Note:

- Abbreviations for substrates are as in Tables 4 and 6.

* -Growth was allowed to proceed for 8 to 18 days, at room temperature, before activity was determined.

Figure-7. The effect of alkali-pretreatment of teff straw on the production of: A. CMCase and B. FPase by *Cladosporium* sp.

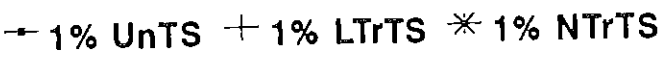
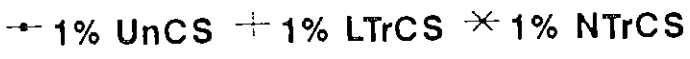
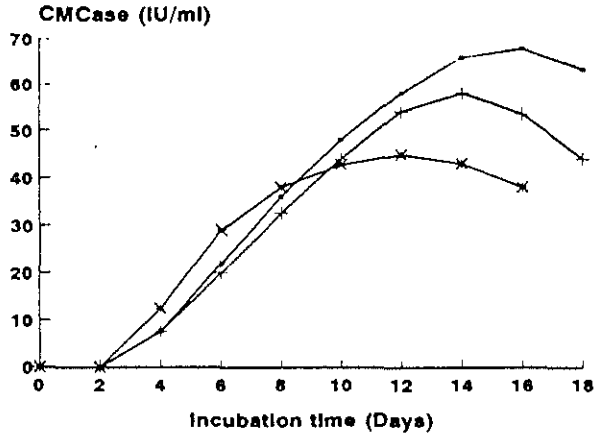


Figure-8. The effect of alkali-pretreatment of corn stalk on the production of: A. CMCase and B. FPase by *Cladosporium* sp.

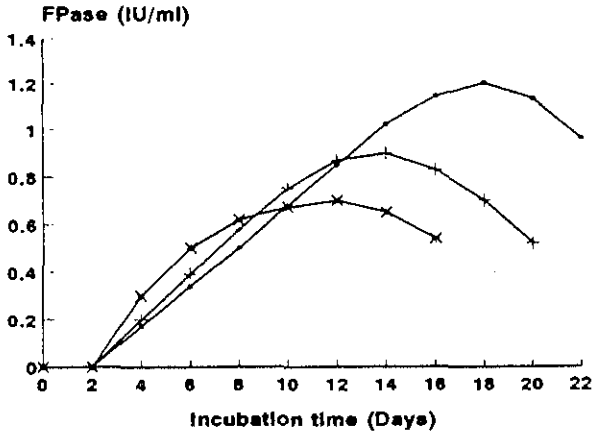


Note: See Tables 1-12 for abbreviations

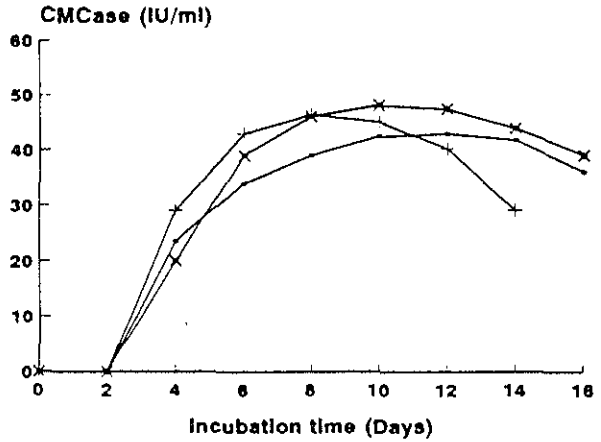
7-A


CMCase (IU/ml)
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18
Incubation time (Days)

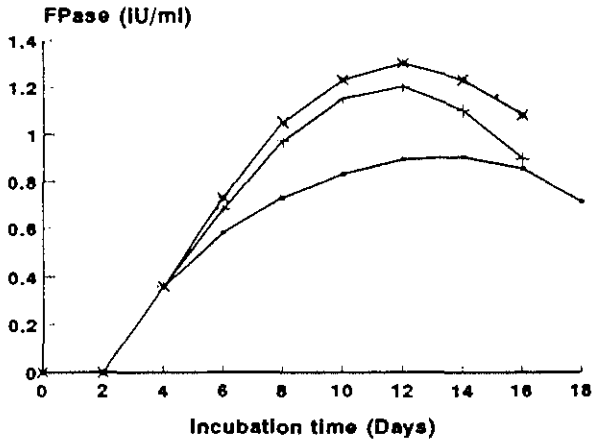
7-B


FPase (IU/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16 18 20 22
Incubation time (Days)

8-A


CMCase (IU/ml)
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16
Incubation time (Days)

8-B


FPase (IU/ml)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16 18
Incubation time (Days)

single carbon and energy source.

In general, supplementing CMC with untreated corn stalk was not better than either 1% CMC or 1% untreated corn stalk used as single carbon and energy sources in inducing CMCase and FPase. Moreover, the amount of ß-GDase was severely reduced by the addition of untreated corn stalk to CMC. Thus, none of the corn stalk-supplemented media was used for further study (i.e. study on the effect of pretreatment of corn stalk on CMCase, FPase and ß-GDase production).

4.2.8. The effect of alkali-pretreatment of corn stalk on CMCase, FPase and ß-GDase by production *Cladosporium* sp.

Pretreatment with lye and NaOH improved CMCase production by 8% and 12% and FPase production by 33% and 44%, respectively (Table 7). But it showed no improvement in ß-GDase production (i.e. there was no trace of ß-GDase activity in the culture medium containing alkali-treated corn stalk).

Slight changes were also observed in the time required for maximum production of CMCase and FPase (Fig. 8A & B). The time for maximum production of CMCase was shortened by 4 and 2 days when using lye-pretreated and NaOH-pretreated corn stalk, respectively. Likewise, maximum production of FPase occurred 2 days earlier in the alkali-treated corn stalk than in the untreated one.

4.2.9. The effect of untreated teff straw on CMCase, FPase and ß-GDase production by *Penicillium* sp.

Penicillium sp. was grown either in media containing only CMC or in media containing varying concentrations of untreated teff straw as the sole carbon and energy source. It was also grown in media containing CMC supplemented with different levels of concentrations of untreated teff straw.

When CMC was replaced with varying concentrations of untreated teff straw there was a sharp decline in the amounts of CMCase, FPase and ß-GDase (Table 9). At 0.5% concentration of untreated teff straw only low levels of CMCase (1.5 IU/ml)

Table 9: CMCase, FPase and ß-GDase production by *Penicillium* sp.* grown on varying concentrations of untreated teff straw and on CMC supplemented with different concentrations of untreated teff straw.

SUBSTRATE		ENZYME ACTIVITY (IU/ml)		
		CMCase	FPase	ß-GDase
2% CMC		16.7	0.4	2.0
UnTS alone,	0.5%	1.5	0	0
	1%	4.8	0	0.4
	2%	9.6	0	1.1
	3%	11.2	0	0.8
2% CMC plus,	0.5% UnTS	15.2	0.2	2.0
	1% UnTS	16.2	0.4	3.0
	2% UnTS	14.8	0.4	3.0

Note:

- Abbreviations are as in Table 6.

* -Growth was allowed to proceed for 6 to 14 days, at room temperature, before activity was determined.

were detected in the culture medium. There was no trace of either FPase or ß-GDase activity at this level of concentration. Increasing the concentration of the straw up to 2% improved the production of CMCase and ß-GDase. The activities were improved from 1.5 IU/ml to 9.6 IU/ml and from zero to 1.1 IU/ml, respectively. However, FPase activity was not detected in any of the concentrations of untreated teff straw used. Further increase in concentration of the untreated teff straw (≥ 2%) did not bring about any significant improvement in the production of CMCase, FPase and ß-GDase. Thus, 2% untreated teff straw was taken as the optimum concentration for maximum production of CMCase and ß-GDase, with which further studies were made on the effect of alkali-pretreatment on enzyme production by *Penicillium* species.

Supplementing 2% CMC with varying concentrations of untreated teff straw did not improve the production of CMCase, FPase and ß-GDase. However, the activities obtained in these media were far more better than those obtained when untreated teff straw was used as a single carbon and energy source. When 0.5% untreated teff straw was added to 2% CMC the amounts of CMCase, FPase and ß-GDase were 15.2, 0.20 and 2.0 IU/ml respectively (Table 9). Increasing the concentration of the substrate (untreated teff straw) added to CMC to 1% improved the activities of CMCase, FPase and ß-GDase by 7%, 100% and 50% respectively. Further increase in concentration of untreated teff straw, however, did not improve their activities. Thus, studies on the effect of pretreatment of teff straw on CMCase, FPase and ß-GDase production by *Penicillium* sp. were made using this level of concentration, i.e. 2% CMC supplemented with 1% untreated teff straw.

4.2.10. The effect of alkali-pretreatment of teff straw on CMCase, FPase and ß-GDase production by *Penicillium* sp.

Alkali-pretreated teff straw was either used as a single carbon and energy source or as a supplement to CMC for growth of *Penicillium* species.

Lye- and NaOH-pretreatment of teff straw generally were found to improve CMCase, FPase and ß-GDase production by *Penicillium* sp.(Table 10).

As can be seen in the table, in media containing only teff straw, the amounts of CMCase, FPase and ß-GDase were improved from 9.6, 0, and 1.1 IU/ml to 14.0, 0.2 and 1.9 IU/ml as a result of lye-pretreatment (a 46 - 73% improvement). Similarly, NaOH-pretreatment improved the quantities of these same enzymes from 9.6, 0, and 1.1 IU/ml to 15.5, 0.3 and 2.3 IU/ml, respectively (a 62 - 109% improvement).

There was also slight improvement in the time required for maximum production of CMCase and ß-GDase. Alkali-pretreatment generally reduced the time of maximum production of both enzymes by 2 days (Fig. 9A & C).

In media containing CMC supplemented with teff straw, pretreatment with lye improved the quantities of CMCase, FPase and ß-GDase by about 24%, 25% and 7%, respectively. Likewise, pretreatment with NaOH improved these enzymes by 61%, 75% and 47%, respectively (Table 10). Apart from these alkali-pretreatment shortened the time of maximum production of CMCase and FPase by about 2 days (Fig.10A & B). The time for maximum production of ß-GDase was also reduced by 4 and 2 days as a result of lye and NaOH-pretreatment, respectively (Fig.10C).

Enzyme production, particularly production of FPase and ß-GDase, was relatively higher in the supplemented media than in media containing only teff straw. Therefore, the supplemented media, i.e. CMC plus 1% alkali-treated teff straw, were selected for the study of fungal biomass production (i.e. for growth of *Penicillium* sp.).

4.2.11. The effect of untreated corn stalk on CMCase, FPase and ß-GDase production by *Penicillium* sp.

Penicillium sp. was found to grow and elaborate CMCase, FPase and ß-GDase when grown in media containing untreated corn stalk.

The amount of CMCase, FPase and ß-GDase produced from a medium containing optimum concentration of untreated corn stalk

Table 10: CMCase, FPase and ß-GDase production by *Penicillium* sp.* grown on alkali-treated teff straw and on CMC supplemented with alkali-treated teff straw.

SUBSTRATE	ENZYME ACTIVITY (IU/ml)		
	CMCase	FPase	ß-GDase
2% CMC	16.7	0.4	2.0
2% UnTS	9.6	0	1.1
2% LTrTS	14.0	0.2	1.9
2% NTrTS	15.5	0.3	2.3
2% CMC + 1% UnTS	16.2	0.4	3.0
2% CMC + 1% LTrTS	20	0.5	3.2
2% CMC + 1% NTrTS	26	0.7	4.4

Note:

- Abbreviations are as in Table 7.

* -Growth was allowed to proceed for 6-14 days, at room temperature, before activity was determined.

Figure-9. The effect of alkali-pretreatment of teff straw on the production of: A. CMCase B. FPase and C. ß-GDase by *Penicillium* sp.

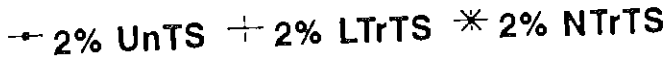
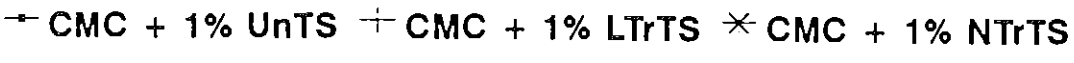
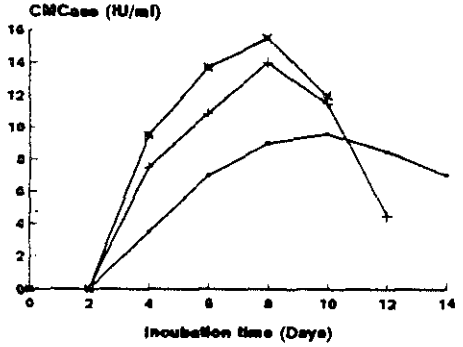


Figure-10. The effect of supplementation of CMC with alkali-treated teff straw on the production of: A. CMCase B. FPase and C. ß-GDase by *Penicillium* sp.

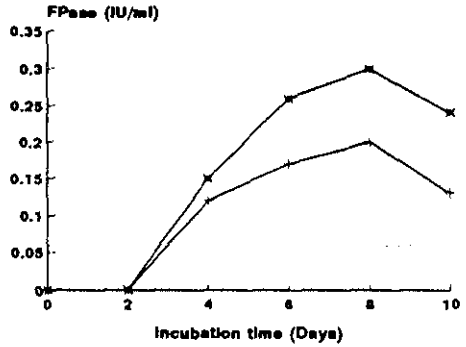


Note: See Tables 1-12 for abbreviations.

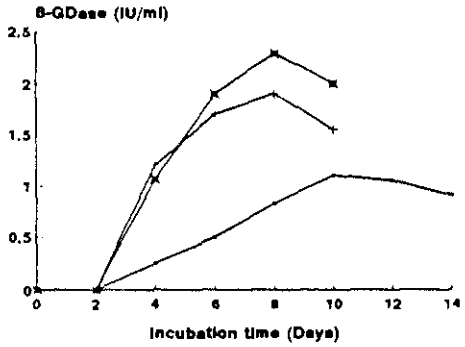
9-A



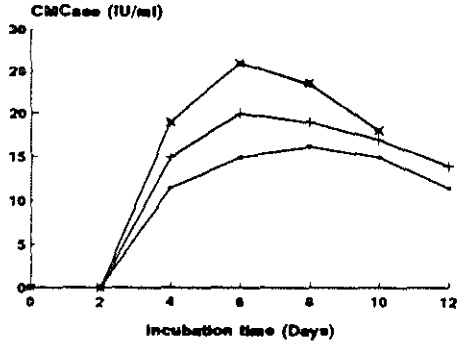
9-B



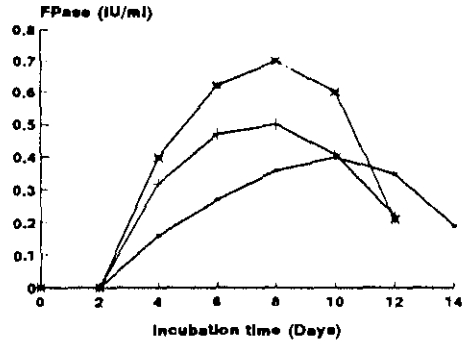
9-C



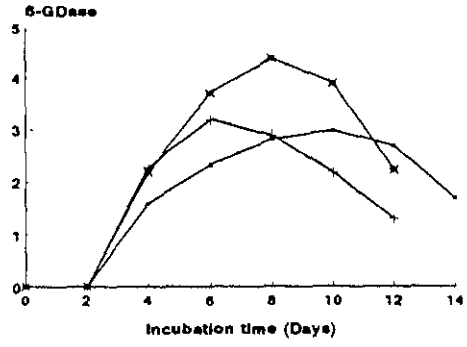
10-A



10-B



10-C



As can be seen from Table 11, the amounts of CMCase and FPase produced in 2% CMC supplemented with 1% untreated corn stalk were about the same as those produced in 2% CMC. Only the release of ß-GDase seemed to be slightly improved (a 50% improvement) by supplementing CMC with untreated corn stalk.

4.2.12. The effect of alkali-pretreatment of corn stalk on CMCase, FPase and ß-GDase production by *Penicillium* sp.

The amounts of CMCase, FPase and ß-GDase produced from media containing alkali-treated corn stalk were compared with those produced from media containing untreated corn stalk (Table 12).

In media containing only corn stalk, lye- and NaOH-pretreatment improved the quantities of CMCase by about 84% and 120%, respectively (Table 12). FPase activity, which was not detected in media containing untreated corn stalk, was produced relatively in large quantities. Nevertheless, alkali-pretreatment had no effect on the activity of ß-GDase (Table 12) and on the time required for maximum production of CMCase, FPase and ß-GDase (Fig. 11A-C).

In the supplemented media, alkali-pretreatment of corn stalk did not show much improvement in enzyme production. CMCase was only slightly improved by about 6% and 15% with lye- and NaOH-pretreatment of corn stalk (Table 12). But there was very little or no effect in the production of FPase as well as ß-GDase by pretreating corn stalk with alkali. In addition to these, neither lye-pretreated corn stalk nor NaOH-pretreated corn stalk was shown to reduce the time required for maximum production of CMCase, FPase and ß-GDase by *Penicillium* sp. (Fig. 12A-C).

However, media containing only alkali-treated corn stalk were better than the supplemented media for inducing CMCase and FPase. Therefore, these media were used for fungal biomass production studies.

Table 12: CMCase, FPase and ß-GDase production by *Penicillium* sp.* grown on alkali-treated corn stalk and on CMC supplemented with alkali-treated corn stalk.

SUBSTRATE	ENZYME ACTIVITY(IU/ml)		
	CMCase	FPase	ß-GDase
2% CMC	16.7	0.4	2.0
2% UnCS	30.7	0	3.5
2% LTrCS	36.7	0.9	2.85
2% NTrCS	33.2	0.7	2.5
2% CMC + 1% UnCS	17.2	0.5	3.0
2% CMC + 1% LTrCS	18.3	0.5	3.0
2% CMC + 1% NTrCS	19.7	0.4	3.0

Note:

- Abbreviations are as in Table 7.

* -Growth was allowed to proceed for 10-18 days, at room temperature, before activity was determined

Figure-11. The effect of alkali-pretreatment of corn stalk on the production of: A. CMCase B. FPase and C. ß-GDase by *Penicillium* sp.

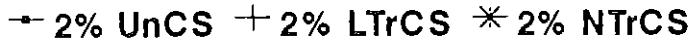
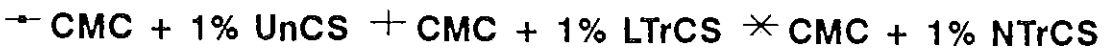
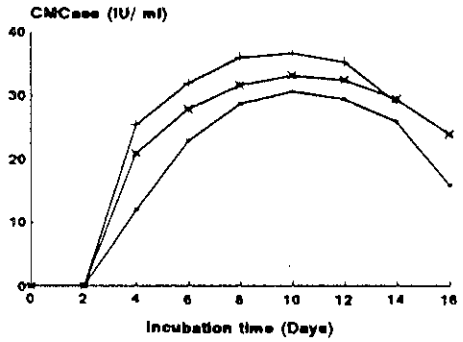


Figure-12. The effect of supplementation of CMC with alkali-treated corn stalk on the production of: A. CMCase B. FPase and C. ß-GDase by *Penicillium* sp.

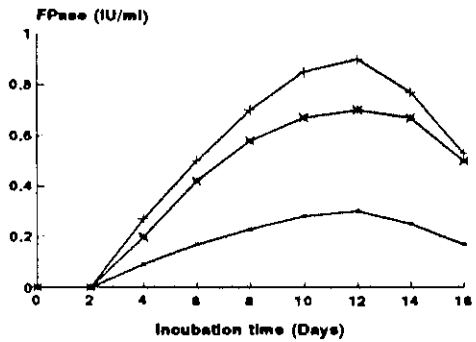


Note: See Tables 1-12 for abbreviations.

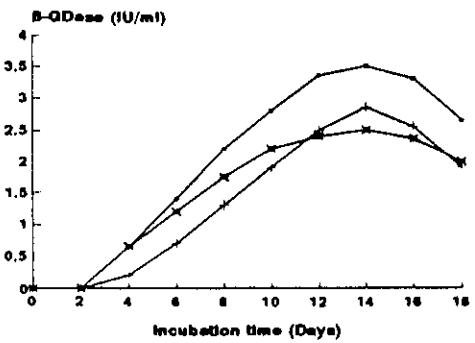
11-A



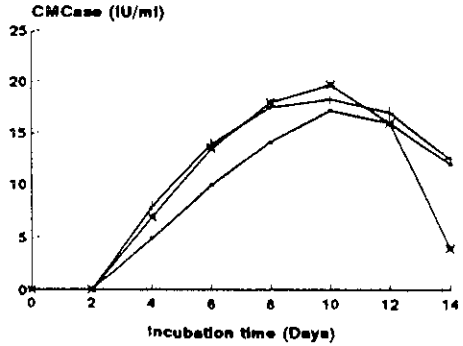
11-B



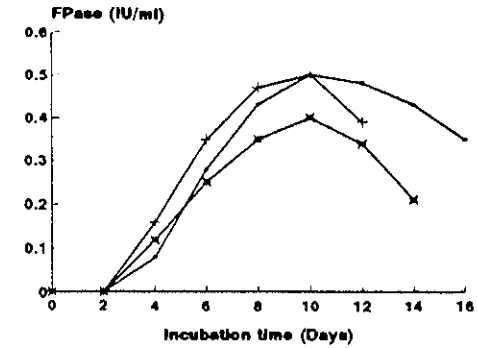
11-C



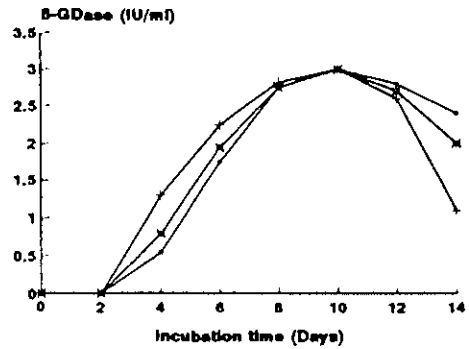
12-A



12-B



12-C



4.3. Mold biomass production

The amounts of cell mass and the crude protein produced as a result of the growth of *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp. on different growth substrates are shown on Tables 13 - 15. In addition to these, the tables also include the calculated values of the percentage protein and the percentage conversion of substrate to protein.

4.3.1. Biomass production by *Trichoderma* sp.

Table 13 shows the biomass produced in the form of *Trichodermal* cell mass when lignocellulosics, filer paper or D-glucose were used as growth substrates.

The highest biomass was produced in 1% D-glucose-containing medium (610 mg/ml medium at 6 days of growth).

When filter paper (0.5%) was used as the sole carbon and energy source, only 217 mg of mold biomass/100 ml growth medium was obtained. Supplementing filter paper with 1% untreated teff straw resulted in a slight improvement in biomass production. Addition of lye- or NaOH-pretreated teff straw (1%) to filter paper-containing medium, however, improved the yield of mold biomass greatly (305 mg and 341 mg/100 ml growth medium for Lye-treated and NaOH-treated teff straw, respectively).

Untreated corn stalk was generally a poor substrate for growth of *Trichoderma* sp. (31 mg of cell mass/100 ml growth medium was produced on untreated corn stalk).

Pretreatment of corn stalk with lye or NaOH, however, considerably improved the amount of *Trichodermal* cell mass production.

The highest crude protein content was also produced from D-glucose-containing medium (218.2 mg/100 ml medium) and the lowest was from filter paper-containing medium (79.7 mg/100 ml medium).

The percent protein of *Trichoderma* sp. remained more or less the same for the fungus when grown on different growth substrates. It ranged from 35.2% to 39.6%.

The percent conversion of substrate to protein was highest for D-glucose (21.8%) and lowest for lignocellulosics or for

Table 13: Biomass production by *Trichoderma* sp. on different growth substrates.

GROWTH SUBSTRATE	PARAMETERS				
	Protein (mg/100ml)	Protein (%)	Conversion (%)	Biomass (mg/100ml)	Relative Biomass (%)
1% D-Glucose[1]	218.2	36	21.8	609.9	100
0.5% FP[2]	79.7	36.7	15.9	217	35.8
0.5% FP + 1% UnTS[2]	ND	ND	ND	237	39.1
0.5% FP + 1% LTrTS[2]	107.3	35.2	9.3	305	50.3
0.5% FP + 1% NTrTS[2]	130.5	38.3	11.6	341	56.2
2% UnCS[2]	ND	ND	ND	31	5.1
2% LTrCS[2]	137	39.6	8.4	346	57
2% NTrCS[2]	138.5	37.3	8.5	371	61.1

[1]Growth was allowed to proceed for 6 days before biomass was determined.

[2]Growth on these substrates was allowed to proceed for 6-20 days.

Note:

- ND = Not determined
- Other abbreviations are as in Tables 1-12.

filter paper supplemented with lignocellulosics (8.4%-11.6%).

4.3.2 Biomass production by *Cladosporium* sp.

The highest biomass (822.5 mg/100 ml medium) was produced in 1% D-glucose-containing medium (Table 14).

The next preferred substrate was carboxymethylcellulose (CMC). In this medium alone it was possible to harvest more than 82% of the biomass produced from 1% D-glucose-containing medium.

The lignocellulosics, particularly the pretreated ones, were nearly as good as CMC for the growth of *Cladosporium* sp..

Pretreatment with lye and NaOH improved the biomass production for teff straw by about 18-22%. Similar pretreatments for corn stalk also showed improvements (40-45% improvement for lye and NaOH-treated corn stalk) in biomass production.

The highest crude protein was produced from D-glucose-containing and the lowest from alkali-treated corn stalk (246.2 mg and 178.7 mg or 203.6 mg/100 ml medium on D-glucose and on lye-treated or NaOH-treated corn stalk, respectively). CMC and alkali-treated teff straw gave more or less comparable values of crude protein.

It's percent protein was also more or less the same for the mycelia produced on different substrates. The percentage protein generally ranged from 32% to 36.7%.

The percentage conversion of substrate to protein was also highest for lye- and NaOH-treated teff straw followed by D-glucose.

CMC and lye-treated corn stalk showed the lowest percentage conversion values (23% and 21.7%, respectively).

4.3.3. Biomass production by *Penicillium* sp.

Penicillium sp. produced the highest biomass in 1% D-glucose-containing medium (Table 15).

It showed very poor growth on CMC-containing media. It's growth was not significantly improved by supplementing CMC with untreated teff straw.

Table 14: Biomass production by *Cladosporium* sp. on different growth substrates.

GROWTH SUBSTRATE	PARAMETERS				
	Protein (mg/100ml)	Protein (%)	Conversion (%)	Biomass (mg/100ml)	Relative biomass (%)
1% D-Glucose[1]	264.2	32	26.4	822.5	100
1% CMC[2]	230	33.8	23	680	82.7
1% UnTS[2]	ND	ND	ND	515	62.6
1% LTrTS[2]	224	36.7	34.5	610	74.2
1% NTrTS[2]	231.2	36.7	37.1	630.1	76.6
1% UnCS[2]	ND	ND	ND	417	50.7
1% LTrCS[2]	178.7	30.6	21.7	584	71
1% NTrCS[2]	203.6	33.7	25	605	73.6

[1]Growth was allowed to proceed for 6 days before biomass was determined.

[2]Growth on these substrates was allowed to proceed for 8-18 days.

Note:

- ND = Not determined
- Other abbreviations are as in Tables 1-12.

Table 15: Biomass production by *Penicillium* sp. on different growth substrates.

GROWTH SUBSTRATE	PARAMETERS				
	Protein (mg/100 ml)	Protein (%)	Conversion (%)	Biomass (mg/100ml)	Relative biomass (%)
1% D-Glucose[1]	234.6	27.5	23.5	851.8	100
2% CMC[2]	42.8	26.5	2.1	161.7	19
2% CMC + 1% UnTS[2]	ND	ND	ND	174.1	20.4
2% CMC+ 1% LTrTS[2]	74.2	26	2.8	285	33.6
2% CMC + 1% NTrTS[2]	89.8	29.1	3.4	309	36.3
2% UnCS[2]	ND	ND	ND	272.3	32
2% LTrCS[2]	123.3	30.6	7.5	403	47.3
2% NTrCS[2]	136.2	30.6	8.4	445	52.2

[1]Growth was allowed to proceed for 6 days before biomass was determined.

[2]Growth was allowed to proceed for 8-12 days.

Note:

- ND = Not determined
- Other abbreviations are as in Tables 1-12.

Supplementation of CMC with lye- or NaOH-treated teff straw showed, however, major improvements (a 76% and 91% improvement, respectively) in biomass production over CMC.

Penicillium sp. seemed also to have more preference for corn stalk than for teff straw. This can be seen from the fact that it produced about half of the biomass that was produced in 1% D-glucose-containing medium (Table 15). Even the untreated corn stalk was far more better than 2% CMC for it's growth. It`s biomass was further increased by pretreating corn stalk with Lye or NaOH (a 48% and 63% increase, respectively).

The highest and the lowest crude protein were produced on D-glucose and on CMC-containing media, respectively (234.6 mg and 42.8 mg/100 ml medium, respectively). Supplementing CMC with alkali-pretreated teff straw improved it's crude protein content on average by about 203%. However, the values were still lower than those obtained from alkali-treated corn stalk.

It's percentage protein ranged from 26% to 36.6%. The highest was obtained from alkali-pretreated corn stalk.

The percentage conversion of substrate to protein by this organism was highest for D-glucose and lowest for CMC (23.5% and 2.1%, respectively). Alkali-pretreated corn stalk was next to D-glucose in being the best converted substrate to protein (7.5% and 8.4% conversion for lye- and NaOH-pretreated corn stalk, respectively).

4.4. Hydrolysis of teff straw and corn stalk using crude enzyme obtained from *Trichoderma* sp.

Figure 13 shows the hydrolysis of alkali-pretreated teff straw and corn stalk over a range of time. The susceptibility of these lignocellulosics to enzymatic attack was also compared with that of avicel (microcrystalline cellulose).

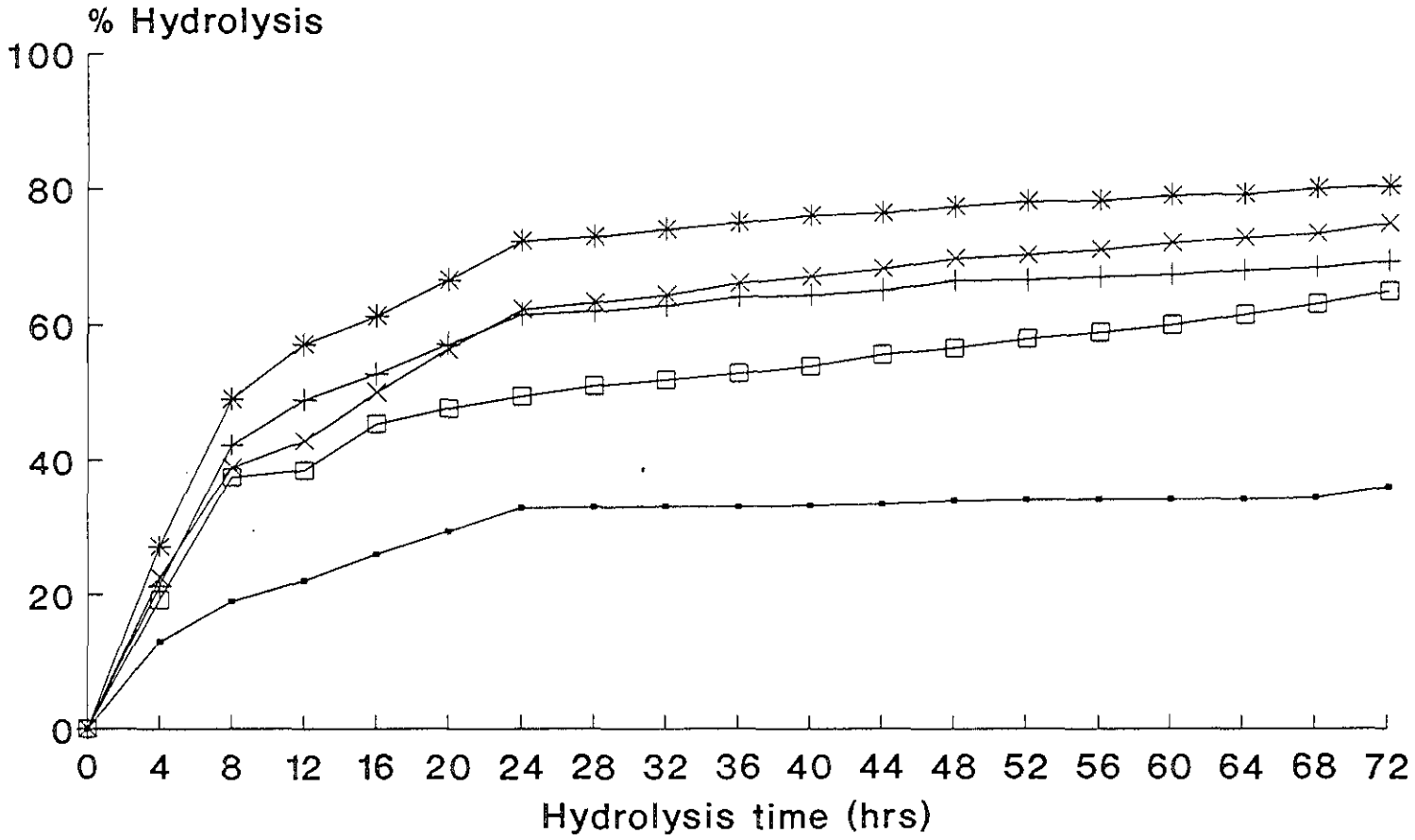
Of the three tested substrates, teff straw was the most degradable substrate by the crude enzyme from *Trichoderma* species.

Under the specified assay conditions 36% of avicel, 64.8% of lye-treated corn stalk, 74.8% of NaOH-treated corn stalk, 69.3% of lye-treated teff straw, 80.3% of NaOH-treated teff

Figure-13. Time course of the hydrolysis of Avicel, alkali-treated teff straw and corn stalk using crude enzyme (which contained 1.7 IU/ml of FPase) of *Trichoderma* sp.

Note: See Tables 1-16 for abbreviations.

13


% Hydrolysis
100
80
60
40
20
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72
Hydrolysis time (hrs)

straw , as polysaccharides, were hydrolysed to give reducing sugars in 72 hours.

As shown from the figure, over 65% of the polysaccharides contained in both the alkali-treated teff straw and corn stalk were hydrolysed while only 30% of the cellulose in avicel was hydrolysed in 72 hours.

Considering the amount of reducing sugar produced in 72 hours from NaOH-treated lignocellulosics as 100%, over 87.7% of these sugars can be obtained from corn stalk and teff straw pretreated with lye alone.

The hydrolysis data also showed that more than 76.6% of the reducing sugars were obtained in 24 hours time (i.e. 91,7%, 76.6%, 83.6%, 88.9% and 90% for avicel, lye-treated corn stalk, NaOH-treated corn stalk, lye-treated teff straw, and NaOH-treated teff straw, respectively).

4.5. Yeast biomass production using enzyme hydrolysates of teff straw and corn stalk as carbon and energy sources

Table 16 shows the biomass of *Saccharomyces cerevisiae* and *Candida utilis* produced from the enzyme hydrolysates of teff straw and corn stalk. For comparison, the yeasts were also grown in media containing D-glucose whose concentrations were equal to those of the reducing sugars in the hydrolysates.

As can be seen from the table, *Saccharomyces cerevisiae* and *Candida utilis* were able to grow in both D-glucose- and hydrolysate-containing media.

Both of the yeasts were more efficient in converting the hydrolysates than the D-glucose to cell biomass. When compared with each other, however, *Candida utilis* was found to be more efficient than *Saccharomyces cerevisiae* in converting these substrates to cell biomass. It's efficiency was exceedingly increased when it was grown in the hydrolysate of alkali-treated lignocellulosics, particularly in the hydrolysate of lye- and NaOH-treated teff straw.

Table 16: Comparison of the yeast* biomass production from enzymatic hydrolysates of alkali-treated lignocellulosics and from D-glucose.

YEAST	D-Glucose		ENZYME HYDROLYZATES							
			LTrTS+		NTrTS+		LTrCS+		NTrCS+	
	Biom.[1]	Yiield[2]	Biom.	Yield	Biom.	Yield	Biom.	Yield	Biom.	Yield
Saccharomyces cerevisiae	11.80	0.58	15.20	0.76	15.60	0.78	14.6	0.73	15.3	0.77
Candida utilis	15.2	0.78	24.8	1.24	27.7	1.39	16.7	0.83	18.9	0.95

[1]Biomass (mg/100 ml)

[2]Yield (g/g)

+Abbreviations are as in Table 8.

*Growth was allowed to proceed for 48 hours, at room temperature, before biomass was determined.

5. DISCUSSION

The three fungi (*Tichoderma* sp., *Cladosporium* sp. and *Penicillium* sp.) were found to elaborate extracellular enzymes (Table 1) that are capable of degrading a variety of substrates. The ability of these fungi to excrete xylanase, pectinase and polygalacturonase enables them to efficiently utilize heterogenous substrates (lignocellulosics). This suggests that the culture filtrates of such micro-organisms can be directly used in fermentation processes to get maximum saccharification of lignocellulosics.

Incubation time studies on the production of these enzymes (CMCase, FPase and ß-GDase) showed similar patterns for the test fungi (Tables 1-3). Although the values obtained vary, the patterns observed in this work were similar to those demonstrated earlier by these same organisms (Erku, 1990; Gashe, 1992; Abrha & Gashe, 1992).

In all cases, CMCase levels exceeded those of FPase, Avicelase and ß-GDase. CMCase is believed to be the first component of the cellulase enzyme system to act during the hydrolysis of cellulose by cleaving the ß-glycosidic bonds randomly in the non-crystalline regions of cellulose fibrils (Petterson, 1975). CMCase may, therefore, be synthesized in relatively large quantities to facilitate initiation of cellulose hydrolysis.

In *Trichoderma* sp. the production of relatively low quantities of Avicelase as compared to the quantity of it's ß-GDase suggests that there has been an accumulation of cellobiose in the culture medium (in the medium containing only filter paper as carbon and energy source). High concentration of cellobiose in the culture medium of fungi is known to repress cellulase induction (Mandels & Reese, 1960). It is therefore possible that the organism's full potential of cellulase production in filter paper-medium probably has not been attained due to repression resulting from cellobiose accumulation.

CMCase, FPase and ß-GDase production was generally higher in *Trichoderma* sp. than in *Cladosporium* sp. and *Penicillium* sp.. The values obtained were even higher than those reported for *Trichoderma reesei* 9414 (grown on avicel for 7 days, at

28°C) and *Thermoascus aurantiacus* A-131 (grown on walseth's cellulose or avicel for 4 days, at 45°C) (Kawamori *et al.*, 1987).

The type of growth substrate is known to affect both the quality and the quantity of cellulases produced by fungi (Norkrans, 1967; Olutiola, 1976). In this work, significant differences (at 5% level of significance) were found between cellulosics (filter paper or CMC) and lignocellulosics in their inducing abilities. Pure cellulose was the most effective substrate for inducing CMCase and FPase while untreated or alkali-treated lignocellulosics (teff straw and corn stalk) were the most effective ones for inducing ß-GDase in *Trichoderma* species (Tables 2-5). Similar results were also reported for *Trichoderma koningi* (Halliwell and Lovelady, 1981) and *Chaetomium globosum* (Lakshmikant *et al.*, 1990). Since organisms differ in the nature of the enzymes they produce, they will also differ in their response to various kinds of substrates (Reese *et al.*, 1969). The results in this work showed that pure cellulose cannot necessarily be the best inducer of cellulase production for all fungi. In *Cladosporium* sp. and *Penicillium* sp. it was the lignocellulosics rather than the cellulosics (CMC) that were found to induce the production of relatively large quantities of CMCase and FPase. Similarly, the lignocellulosics were able to induce the release of high levels of ß-GDase in *Trichoderma* sp. and *Penicillium* sp. while they failed to do so in *Cladosporium* species. Thus, the data indicate that the ability of a substrate to induce a particular enzyme is dependent on species difference.

The activities of CMCase and FPase were shown to decrease when filter paper was supplemented with either teff straw or corn stalk. This may be related, perhaps, to the presence of hemicellulose in the lignocellulosics. The hemicellulosic fraction of lignocellulosics has been shown to affect cellulase production by *Trichoderma harzianum* (Mes-Hartree *et al.*, 1987a). These workers observed that the cellulase production was higher in the steamed aspen wood, in which most of the pentosan has been removed during a water extraction step, than in the pentosan rich biologically delignified aspen

wood. The hemicellulose content (e.g. xylan) which may be sufficiently easily hydrolyzed to cause catabolite repression of cellulase production was probably responsible for the observed low activity of cellulase produced in media containing filter paper supplemented with lignocellulosics.

Comparisons between untreated and alkali-treated lignocellulosics showed that alkali-pretreatment generally improves, significantly (at 5% level of significance), mold biomass production (Table 13), ß-GDase, CMCase and FPase production (Table 3 & 5) and the duration of the incubation period for maximum enzyme production (Fig. 4-7) by *Trichoderma* species. However, there were some exceptions. Alkali-pretreatment of teff straw had no significant effect on the production CMCase and FPase by *Trichoderma* sp. eventhough it showed a significant improvement in fungal biomass production. Obviously it would have been very unlikely to get very high fungal biomass production without a concommitant production of increased levels of cellulase. This suggests that the real (actual) values of CMCase and FPase activities had not been determined in media containing alkali-pretreated teff straw probably due to adsorption of the enzymes on the surfaces of the lignocellulosics. This was supported by findings from previous works. Viesturs *et al.*, (1981) have demonstrated that enzyme adsorption can be increased by pretreatment. Several other workers have also reported the adsorption of fungal cellulases on lignocellulosics (Mes-Hartree *et al.*, 1987b; Hogan and Mes-Hartree, 1990), and cellulosic materials (Hagerdal *et al.*,1983; Soundar & Chandra, 1988; Ooshima *et al.*, 1990).

The increase in biomass production by the molds on pretreated teff straw and corn stalk, as compared to that on the untreated ones, was undoubtedly the result of the increase in the degradability of the lignocellulosics due to the removal of the lignin barrier and the increased available surface area for enzymatic attack. Such an increase in the biodegradability of lignocellulosics has been previously reported by Patros *et al.*, (1983).

There was no statistically significant difference (at 5% level of significance) between the performance of lye-

pretreatment and NaOH-pretreatment with respect to cellulase production and biomass production by the three fungi. Lye can, therefore, be utilized in place of 2% NaOH for pretreating lignocellulosics and using them as growth substrates for cellulolytic molds. However, the performance of lye was slightly lower than that of 2% NaOH in the hydrolysis of teff straw and corn stalk. Although no statistical analysis was done for the hydrolysis data the differences between pretreatments can be seen clearly from figure 13. The low performance of lye in the hydrolysis of lignocellulosics may be due to the fact that lye is highly rich in fatty acids and other ingredients which may interfere with the accessibility of the cellulose to cellulase attack. But since no supporting literature has been available so far, further investigation needs to be done in this respect to draw valuable conclusions.

Trichoderma's biomass was higher on teff straw or corn stalk than on filter paper-containing media. The synthesis (release) of cellulase was, however, more on filter paper than was on the lignocellulosics. One would expect to find more biomass in media producing large quantities of cellulase. But the result was quite the opposite. The degree of crystallinity is a major factor in the hydrolysis of cellulosic and lignocellulosic materials (Mandels *et al.*, 1974). Alkali-treated lignocellulosics are less crystalline than filter paper (Sasaki, 1989). The less crystalline materials would thus be expected to be degraded extensively resulting in a relatively large cell mass production. Apart from these the presence of other enzymes such as xylanase, pectinase and polygalacturonase (Table 1) and may be others, for which attempt has not been made to detect their presence in the culture filtrate, suggests that other readily utilizable sugars (such as the pentoses), in addition to glucose, may have been released into the media and contributed to the relatively high growth of the fungus in lignocellulosic-containing media.

An important factor to be considered for the economic exploitation of a species is also the time required to reach the optimum cellulase production. In the present work it varied from 6 to 26 days (Fig. 1-12). Although the maximum

CMCase and FPase production in *Trichoderma* sp. was obtained in filter paper-containing medium, the time required for maximum production of these enzymes was relatively longer than in any of the alkali-treated lignocellulosic-containing media (Fig. 4-7). For example, maximum CMCase activity was attained on the 24th day of incubation in filter paper-containing media whereas in the pretreated corn stalk- or teff straw-containing media the time was shortened by more than 50%. Similar results were observed in *Trichoderma reesei* grown on untreated and treated wheat straw (Mahesewari *et al.*, 1993). The data indicate, therefore, that the pretreated teff straw and corn stalk can be useful and promising for biomass and cellulase production because of the added advantage of a shorter incubation period to the observed high ß-GDase-inducing ability.

Cladosporium sp., which had been previously reported to have elaborated large quantities of CMCase in media containing only CMC (Abrha & Gashe, 1992), was found to excrete CMCase and FPase in teff straw- or corn stalk-containing media better than it did in the former (Tables 1-4). This fungus was found to show many important features when grown in 1% teff straw. The biomass production, total crude protein production and the percentage conversion of substrate to protein by this organism were much more than those produced by *Trichoderma* sp. and *Penicillium* species. Particularly, it's CMCase production in 1% untreated teff straw was greater than the CMCase produced by *Trichoderma* sp. grown on alkali-treated teff straw or corn stalk. However, *Cladosporium* sp. was not able to release it's ß-GDase in both the untreated and alkali-treated lignocellulosics. It was not easy to explain why ß-GDase activity was completely absent in media containing only lignocellulosics when it was found that the organism is capable of excreting it in media containing only CMC or CMC supplemented with lignocellulosics.

In *Cladosporium* sp., although alkali-pretreatment improved the biomass production and reduced the time required for maximum production of CMCase and FPase, it didn't help to improve the activities of these enzymes as expected. It rather decreased the activities of CMCase and FPase (Table 7). Here again, adsorption is believed to have been responsible for the

reduced activities of the enzymes in media containing alkali-treated lignocellulosics, for it's cellulase has been previously known to adsorb on cellulosic substrates (Abrha & Gashe, 1992).

The other important aspect of *Cladosporium* sp. is that it did grow and elaborate cellulases (CMCase and FPase) on untreated lignocellulosics such as corn stalk, on which growth and cellulase production by the highly cellulolytic fungus, i.e. *Trichoderma* sp., were found to be extremely low (Table 4). Such an ability is a desirable characteristic in the selection of a cellulolytic micro-organism for it helps to reduce the cost of enzyme production.

In media containing only CMC, the biomass of *Cladosporium* sp. was relatively high and its cellulase production was low as compared to that produced in a medium containing only teff straw. The non-crystalline cellulosic substrate, i.e. CMC, which is obviously an easily degradable substrate (Mandels *et al.*, 1974) must have enabled a good growth for *Cladosporium* sp. and thus accounting for the high biomass produced in this medium.

In comparison, the lignocellulosics (teff straw and corn stalk) are superior to and nearly as good as CMC for CMCase and fungal biomass production by *Cladosporium* sp., respectively. Therefore, it can be safely concluded that these substrates as well as the organism (*Cladosporium* sp.) are potentially useful for the preparation of protein-enriched material and endoglucanase.

In *Penicillium* sp., alkali-pretreatment of teff straw was effective in improving the release of CMCase, FPase and ß-GDase. However, the quantiies of these enzymes were as low as those produced on 2% CMC. Supplementing CMC with alkali-pretreated teff straw resulted in a better yield of CMCase and ß-GDase. Therefore, the results suggest that alkali-treated teff straw can be used either as a supplement to or as a replacement for 2% CMC in the induction of CMCase and ß-GDase by *Penicillium* species.

Although pretreatment of corn stalk with alkali did not show any significant change in CMCase and ß-GDase activities, it brought about a significant improvement in the amounts of

FPase. The pretreatment was also effective in improving the fungal biomass production (biomass of *Penicillium* sp.). Thus, it can be said that either the CMCase and ß-GDase produced on the alkali-pretreated corn stalk had been adsorbed or the improved levels of FPase had been sufficient enough to account for the relatively high fungal biomass produced on this substrate.

Compared to teff straw, the alkali-treated corn stalk was, in general, a good substrate for growth and induction of CMCase, FPase and ß-GDase by *Penicillium* species. This substrate was found to be even much better than 2% CMC which had been reported earlier to be the best substrate for the production of cellulases by *Penicillium* sp. (Erku, 1990).

Considering the less costly means of pretreatment and the relatively high CMCase, FPase and ß-GDase production, crude protein production and the better percentage conversion of the substrate to protein than those recorded in media containing only CMC as well as in CMC supplemented with alkali-treated teff straw, the medium containing only lye-treated corn stalk in particular seems to be a good medium for growth and induction of cellulase by *Penicillium* species. However, it's over-all performance was found to be much lower than those of *Trichoderma* sp. and *Cladosporium* species.

The percentage crude protein of *Trichoderma* sp. and *Cladosporium* sp. grown on alkali-treated teff straw or corn stalk (30.6-39.6%) were higher than those reported for *Trichoderma reesei* (25.2%), *Trichoderma longibranchiatum* (22%), *Scytalidium lignicola* (21.6%), *Sporotrichum pulverulentum* (20.8%) and *Talaromyces vermiculatus* (20.2%) which were all grown on sludge of paper mills, at 28°C (Royer and Nakas, 1987); *Penicillium funiculosum* (26.6%), *Myrothecium verrucaria* (21.86%), *Aspergillus niger* (27.33%) grown for 7 days on apple pulp, at 28-30°C and pH 5 (Kuzmanova *et al.*, 1991) and favourably compare with that repoted for *Trichoderma reesei* (34%) grown in a medium containing beet pulp, at 28°C (Ghanem *et al.*, 1991); *A. niger* grown (35%) for 4 days in an optimized medium containing grape waste, apple pulp and beet pulp, at 28-30°C and pH 5 (Kuzmanova *et al.*, 1991). Particularly the percentage conversion of the lignocellulosics to protein

by *Cladosporium* sp.(Table 14) was much more than that reported for *Trichoderma reesei* (15% and 18% as reported by Royer and Nakas, 1978 Ghanem *et al.*, 1991). This finding together with their relatively high ability of cellulase and biomass production can qualify these two molds as well as the two lignocellulosics as potential candidates to produce and use them in a large scale as feed supplement. Especially, the fungal biomass together with the residual teff straw or corn stalk can be directly used as a feed if thorough studies for the presence of toxic compounds are made prior to use. Mycelia of *Trichoderma viride* (Peiterson, 1975), *Sporotrichum pulverulentum* (Thomke *et al.*, 1980) and *Trichoderma branchiatum* (Sidhu and Sandhu, 1980), for instance, have been shown to be suitable feed sources in animal feeding trials. It is also possible that *Trichoderma* sp. BDCC-1 and *Cladosporium* sp. BDCC-3 could be useful in the preparation of animal feed. Such a feed, if devoid of toxic compounds, will have an advantage in two ways: 1. it becomes highly enriched with quality protein (microbial protein); 2. the degradability of the lignocellulosic will be increased for it has been subjected to combined pretreatments (pretreatment with alkali as well as with fungal cellulase, xylanase, pectinase, polygalacturonase, etc.).

The alkali-treated teff straw and corn stalk were shown to be effectively hydrolysed by the crude enzyme of *Trichoderma* sp. to the extent of 50-72% in 24 hours (Fig. 13). This was, however, lower than that reported by Szczodrak (1989) for the hydrolysis of chemically modified wheat straw in 24 hours (77.8 - 87.9%) using *Trichoderma reesei* F-522 and *Trichoderma reesei* F-522-V-7 cellulase preparations. The differences might have been attributed to the differences in the composition of the lignocellulosics. But the most important factors that were responsible for the lower degree of hydrolysis in teff straw and corn stalk as compared to that in the wheat straw of Szczodrak (1989) were probably the assay conditions and the pretreatment methods employed. In this work relatively mild pretreatment, weak cellulase and low temperature were employed in the hydrolysis experiment.

The time course study of the hydrolysis of avicel,

alkali-treated teff straw and corn stalk, using the same enzyme source, showed a levelling-off in activity beyond 24 hours of incubation. Similar results were observed in the rate of saccharification of filter paper by *Trichoderma reesei* (Soundar and Chandra, 1988). Such results can happen due to the inhibitory effect of the end products. Sternberg *et al.*, (1977) have demonstrated, experimentally, the inhibition of ß-GDase by glucose accumulation. The inhibition of ß-GDase leads again to an accumulation of cellobiose which can result in product inhibition of the exoglucanase and a retardation in the rate of cellulase saccharification.

The data also show that teff straw and corn stalk were more easily degradable than avicel (microcrystalline cellulose) (Fig. 13). The use of such lignocellulosics, which are commercially inexpensive, will reduce the cost of enzyme production which accounts, according to Ryu and Mandels (1980), for 50% of the cost of glucose production from cellulose, in addition to alleviating the problem of single-cell protein production, fuel alcohol etc..

Many pretreatments involve harsh reaction conditions and toxic chemicals which are likely to reduce the fermentability of cellulose hydrolysates (Chahal *et al.*, 1982). In this work efforts have been made to test the growth of yeasts on the hydrolysates of pretreated teff straw and corn stalk. The results show that the hydrolysates support good growth for both *Saccharomyces cerevisiae* and *Candida utilis*. Interestingly, these two yeasts were found to grow better than they did in media containing comparable quantities of D-glucose (Table 16). This indicates that either the hydrolysates were consisting of additional non-reducing carbohydrates in solution or the yeasts may have preferred some compounds in the hydrolysates to D-glucose.

The results, in this work, favourably compare with those reported for *Saccharomyces cerevisiae* grown on wheat bran and rice bran by Roy *et al.*, (1993).

Comparison of the biomass yield of the two yeasts showed that *Candida utilis* produced more cell mass than *Saccharomyces cerevisiae* (Table 16). The ability of *Candida species* to utilize a variety of compounds has been documented in

literature (Peppler, 1978). *Candida utilis* is , for example, capable of utilizing pentose sugars (e.g. xylose) while *Saccharomyces cerevisiae* lacks this ability. It is, therefore, this ability that has allowed *Candida utilis* to vigorously grow in the hydrolysates.

RECOMMENDATIONS

1. Teff straw and corn stalk performed better than filter paper in inducing ß-GDase in *Trichoderma* sp. BDCC-1. It is possible that these lignocellulosics could even be made to induce high levels of CMCase and FPase if the presumed catabolite repression can be stopped. One way to do this is by continually removing the end product from the culture medium using selected strains of yeasts. It is, therefore, recommended that the simultaneous saccharification and fermentation of teff straw or corn stalk be studied using *Trichoderma* sp. BDCC-1 and *Candida utilis* BDCC-25.

2. Lye has been shown to be as effective as 2% NaOH for pretreating teff straw and corn stalk (with respect to cellulase and biomass production). Generally, it was found to improve fungal biomass production. However, in most cases, it showed little or no effect in improving CMCase and FPase production, particularly when using pretreated teff straw. Adsorption is assumed to be the main factor responsible for not getting increased levels of CMCase and FPase as expected. Therefore, it is suggested that further investigation be done in this area before lye can be used in the cellulase production processes. In addition to this, the effect of fatty acids and other compositions of lye on the production of cellulase should also be studied prior to use.

3. Pretreated teff straw and corn stalk have been shown to be valuable for mold biomass production. It is possible to increase the feed values of these substrates by

growing the molds on them using solid state fermentation processes. Most fungi, however, are known to produce toxic metabolites. It is not known whether these molds produce such toxic compounds or not. Therefore, recommendations are also made to assess the toxicity of their mycelia to use them as a feed supplement.

4. It is also recommended that the economic assessment of the feasibility of using teff straw and corn stalk hydrolysates as growth substrates for single-cell protein production be made.

5. It is also interesting to know what actually prevents the release of ß-GDase in *Cladosporium* sp. when it was grown on teff straw or corn stalk. Therefore, studies should also be conducted in this direction to make these lignocellulosics industrially valuable.

REFERENCES

Abrha,B. and B.A.Gashe. 1992. Cellulase production and activity in a species of *Cladosporium*. *World J. Microbiol. Biotechnol. 8*:164-166.

Agosin, E.,X.Rouau, and J.M.Brillouet. 1987. Fermentation of wheat straw xylan by the white-rot fungus *Dichomitus squalens*. *Can. J. Microbiol. 33*:1050-1054.

Akin, D.E., and L.L.Rigsby. 1985. Degradation of bermuda and orchard grass by species of ruminal bacteria. *Appl. Environ. Microbiol. 50*:825-830.

Ali, S., A.Sayed, R.I.Sarker and R.Alam. 1991. Factors affecting cellulase production by *Aspergillus terreus* using water hyacinth. *World J. Microbiol. Biotechnol. 7*:62-66.

Allen, A.L. and R.E.Andreotti. 1982. Cellulase production in continuous and fed batch culture by *Trichoderma reesei* MCG 80. *Biotechnol. Bioeng. Symp. 12*:451-459.

Anonymous. 1974. Making sugar and protein from trash. *Environ. Sci. Tech. 8(9)*:784-785.

Anonymous. 1993. *Manual. Tecator application note, AN 300*. Hoganas,Sweden (Unpublished). pp 1-12

Antai, S.P. and D.L.Crawford. 1981. Degradation of softwood, hardwood and grass lignocellulosics by two *Streptomyces* strains. *Appl. Environ. Micobiol. 42*:378-380.

Araujo, A. and J.D'Souza. 1986. Characterization of cellulolytic enzyme components from *Aspergillus terreus* and it's mutant. *J. Ferment. Technol. 64*:463-467.

Bailey, M.J. and K.Poutanen. 1989. Production of xylanases by strains of *Aspergillus*. *30* :5-10.

Biely, P. 1985. Microbial xylanolytic systems. *Trends Biotechnol. 3*:286-290.

Bilgrami, K.S. and H.C.Dube. 1976. *A Text Book of Modern Plant Pathology*. Vikas Publishing House PVT LTD. New Delhi. pp 344.

Chahal, D.S., S.McGuire, H.Pikor and G.Noble. 1982. Production of cellulase complex by *Trichoderma reesei* Rut-C30 on lignocellulose and it's hydrolytic potential. Biomass. 2: 127-138.

Christakopoulos, P., B.J.Macris and D.Kekos. 1990. On the

mechanism of direct conversion of cellulose to ethanol by *Fusarium oxysporum* : effect of cellulase and ß-glucosidase. *Appl. Microbiol. Biotechnol. 33*:18-20.

Crawford, D.L. 1978. Lignocellulose decomposition by selected *Streptomyces* strains. *Appl. Environ. Microbiol. 35*:1041-1045.

Dale, B.E. 1987. Lignocellulose conversion and the future of fermentation biotechnology. *TIBTECH. 5*:287-291.

Dekker, R.F.H. 1983. Bioconversion of hemicellulose: Aspects of hemicellulase production by *Trichoderma reesei* QM 9414 and enzymic saccharification of Hemicellulose. *Biotechnol. Bioeng. 25*:1127-1146.

Dekker, R.F.H. and G.N.Richards. 1976. Hemicellulases: their occurrence, purification, properties and mode of action. *Adv. Carbohyd. Chem. Biochem. 32*:277-352.

Desrochers, M., L.Jurasek, M.G.Paice. 1981. Production of cellulase, ß-glucosidase and xylanase by *Schizophyllum commune* grown on a cellulose-peptone medium. *Dev. Ind. Microbiol. 22*:675-684.

Detroy, R.W., L.A.Lindenfelser, G.ST.Julian, JR., and W.L.Orton. 1980. Saccharification of wheat straw cellulose by enzymatic hydrolysis following fermentative and chemical pretreatment. *Biotechnol. Bioeng. Symp. 10*:135-148.

Dubois, M., K.A.Gilles, J.K.Hamilton, P.A.Rebers, and F.Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem. 28*:350-356.

Enari, T.M. and P.Markkanen. 1977. Production of cellulolytic enzymes by fungi. *Adv. Biochem. Eng. 5*:1-24.

Erku, Y. 1990. *Some physico-chemico factors affecting cellulase and ß-glucosidase production and activity by Penicillium BDCC-2*. Masters Thesis.pp 91.

Fan, L.T., Y.H.Lee and D.R.Beardmore.1981. The influence of major structural features of cellulase on the rate of enzymic hydrolysis. *Biotechnol. Bioeng. 23*:419-424.

Flickinger,M.C. 1980. Current biological research in conversion of cellulosic carbohydrates into liquid fuels: How far have we come? *Biotechnol. Bioeng. 22*:22-48.

Funaguma, T., H.Tsuji and A.Hara. 1986. Purification and some

properties of cellulase 1,4-ß-cellobiosidase from a strain of *Penicillium* sp..*J. Ferment. Technol. 64*:77-80.

Gashe, B.A. 1992. Cellulase production and activity by *Trichoderma* sp.A-001. *J. Appl. Bacteriology. 73*:79-82.

Ghanem, K.M., A.H.El-Refai and M.A.El-Gzaerly. 1991. Protein-enriched feedstuff from beet pulp. *World J. Microbiol. Biotechnol.* 7:365-371.

Gharpuray, M.M., Y.-H.Lee and L.T.Fan. 1983. Structural modification of lignocellulosics by pretreatments to enhance enzymatic hydrolysis. *Biotechnol. Bioeng. 25*:157-172.

Ghose, T.K. 1987. Recommendations on the measurement of cellulase activities. *Pure Appl. Chem. 59*:257-268.

Ghose, T.K. and V.Sahai. 1979. Production of cellulases by *Trichoderma reesei* QM 9414 in fed batch and continuous flow culture with cell recycle. *Biotechnol. Bioeng. 21*:283-296.

Gupta, C.B. and V.Gupta. 1973. *An introduction to statistical methods*. Vikas publishing house PVT, LTD. New Delhi, India. pp 758.

Hagerdal, B.G.R., J.D.Ferchak, and E.K.Pye. 1978. Cellulolytic enzyme system of *Thermoactinomyces* sp. grown on microcrystalline cellulose. *Appl. Environ. Microbiol. 36*:606-612.

Halliwell, G. and J.Lovelady. 1981. Utilization of carboxymethylcellulose and enzyme synthesis by *Trichoderma koningi*. *J. Gen. Microbiol. 126*:211-217.

Halliwell, G. and M.Griffin (1973). The Nature and mode of the cellulolytic component C_1 of *Trichoderma koningi* on native cellulose. *Biochem. J. 135*:587-594.

Harmova, M., E.Petrakova and P.Biely. 1991. Induction of cellulose- and xylan-degrading enzyme systems in *Aspergillus terreus* by homo- and heterodisaccharides composed of glucose and xylose. *J. Gen. Microbiol. 137*:541-547.

Herr, D. 1979. Secretion of cellulase and ß-glucosidase by *Trichoderma viride* ITTC-1433 in submerged culture on different substrates. *Biotechnol. Bioeng. 21*:1361-1371.

Higuchi,T. 1971. Formation and biological degradation of

lignin.*Adv. Enzymol. 34*:207-283.

Hogan, C.M. and M.Mes-Hartree. 1990. Recycle of cellulases and the use of lignocellulosic residue for enzyme production after hydrolysis of steam-pretreated aspenwood. *J. Indust. Microbiol. 6*:253-262.

Humphrey, A.E. 1975. *Product outlook and technical feasibility of SCP*. In: *Single-cell protein II. Tannenbaum, S.R., Wang, D.I.C.,(eds.)*. Cambridge, Ma, London; MIT press. pp.1-23.

Kawamori, M., K.Takayama and S.Takasawa. 1987. Production of cellulases by a thermophllic fungus *Thermoascus aurantiacus* A-131. *Agric. Biol. Chem. 51*:647-654.

Kristensen, T.P. 1978. Continuous single-cell protein production from *Cellulomonas* sp. and *Candida utilis* grown in mixture on barley straw. *J. Appl. Microbiol. Biotechnol. 5*:155-163.

Kuzmanova, S., E.Vandeska and A.Dimitrovsky. 1991. Production of mycelial protein and cellulolytic enzymes from food wastes. *J. Indst. Microbiol. 7*:257-262.

Lakshmikant, Kamal and S.N.Mathur. 1990. Cellulolytic activities of *Chaetomium globosum* on different cellulosic substrates. *World J. Microbiol. Bitechnol. 6*:23-26.

MacKenzie, C.R., D.Bilous, and K.G.Johnson. 1984. Purification and characterization of an exoglucanase from *Streptomyces flavogriseus*. *Can. j. Microbiol. 30*:1171-1178.

Macris, B.J. 1984. Production and characterization of cellulase and ß-glucosidase from a mutant of *Alternaria alternata*. *Appl. Environ. Microbiol. 47*:560-565.

Mahesewari, D.K., H.Jahan, J.Paul and A.Varma. 1993. Wheat straw, a potential substrate for cellulase production using *Trichoderma reesei*. *World J. Microbiol. Biotechnol. 9*:120-121.

Mandels, M. 1982. Cellulases. *Annu. Rep. Ferment. Proc. 5*:35-78.

Mandels, M. and E.T.Reese. 1960. Induction of cellulase in fungi by cellobiose. *J. Bacteriol. 79*:816-826.

Mandels, M., L.Hontz, and J.Nystrom. 1974. Enzymatic

hydrolysis of waste cellulose. *Biotechnol. Bioeng.* *16*:1471-1493.

Margaritis, A. and R.F.Merchant. 1986. Optimization of fermentation conditions for thermostable cellulase production by *Thielvia terrestris*. *J. Indust. Microbiol.* *1*:149-156.

Mes-Hartree, M., C.M.Hogan and J.N.Saddler. 1987b. Recycle of enzymes and substrate following enzymatic hydrolysis of steam-pretreated aspenwood. *Biotechnol. Bioeng.* *30*:558-564.

Mes-Hartree, M., E.K.C.Yu, I.D.Reid and J.N.Saddler. 1987a. Suitability of aspenwood biologically delignified with *Phlebia tremellosus* for fermentation to ethanol or butanediol. *Appl. Microbiol. Biotechnol.* *26*:120-125.

Miller, G.L. 1959. Use of dinitrosalicylic reagent for the determination of reducing sugars. *Anal. Chem.* *31*:426-428.

Milstein, O., Y.Vered, J.Gressel, and H.M.Flowers. 1981. Biodegradation of wheat straw lignocarbohydrate complex (LCC) II. Fungal growth on aqueous hydrolysate liquors and particulate residues of wheat straw. *Eur. j. Appl. Microbiol. Biotechnol.* *13*:117-127.

Mishra, C., M.Rao, R.Seeta, M.C.Srinivasan, and V.Desphande. 1984.Communications to the editor. Hydrolysis of lignocelluloses by *Penicillium funiculosum* cellulase. *Biotechnol. Bioeng.* *26*:370-373.

Mullings, R. 1985. Measurement of saccharification by cellulases. *Enzyme Microb. Biotechnol.* *7*:586-591.

Ng, T.K., R.M.Busche, C.C.McDonald and R.W.F.Hardy. 1983. Production of feedstock chemicals. *Science.* *219*:733-740.

Norkrans, B. 1967. Cellulose and cellulolysis. *Adv. Appl. Microbiol.* *9*:91-130.

Olitiola, P.O. 1976. A cellulase complex in culture filtrates of *Penicillium citrinum*. *Physiol. Plant.* *37*:313-316.

Ooshima, H., D.S.Burns, A.O.Conserve. 1990. Adsorption of cellulase from *Trichoderma reesei* on cellulose and lignacious residue in wood pretreated by dilute sulfuric acid with explosive decomposition. *Biotechnol. Bioeng.* *36*:446-452.

Ortega, G.M., E.O.Martinez, P.C.Gonzalez, D.Betancourt and M.A.Otero. 1993. Enzyme activities and substrate degradation during white-rot fungi growth on sugar-cane straw in a solid-state fermentation.*World J. Microbiol. Biotechnol. 9*:210-212.

Patros, J., M-L.Fardeau, and J-P.Belaich. 1983. Influence of pretreatments on the fermentation of barley straw by undefined mixed bacterial populations. *Eur. J. Appl. Microbiol. Biotechnol. 18*:308-314.

Peitersen, N. 1975. Production of cellulase and protein from barley straw by *Trichoderma viride. Biotechnol. Bioeng. 17*:361-374.

Peppler, H.J. 1978. Yeasts. In: *Ann. Reports Ferment. Process 2. Perlman, D. and G.T.Tsao. (eds)*. Academic Press, New York, San Francisco, London. pp 191-202.

Petterson, L.G. 1975. The mechanism of enzymatic hydrlysis of cellulose by *Trichoderma viride*. In: *Symposium on enzymatic hydrolysis of cellulose,* ed. Bailey, M., T.Enari and M.Linko. pp 255-261.

Ramasamy, K., M.Meyers, J.Bevers and H.Verachtert. 1981. Isolation and characterization of cellulolytic bacteria from activated sludge. *J. Appl. Bacteriol. 51*:475-481.

Reese, E.T., J.E.Lola, and F.W.Parrish. 1969. Modified substrates and modified products on inducers of carbohydrases. *J. Bacteriol. 100*:1151-1154.

Rodriquez, H., R.Alvarez and A.Enriquez. 1993. Evaluation of different alkali-pretreatments of bagasse pith for cultivation of *Cellulomonas* sp.. *World J. Microbiol. Biotechnol. 9*:213-215.

Royer, J.C. and J.P.Nakas. 1987. Production of mycelial protein and hydrolytic enzymes from paper mill sludges by cellulolytic fungi. *J. Indust. Microbiol. 2*:9-13.

Roy, P.K., U.Roy, and V.C.Vora. 1993. Hydrolysis of wheat bran, rice bran and jute powder by immobilized enzymes from *Macrophomina phaseolina. World J. Microbiol. Biotechnol. 9*:164-167.

Ryu, D.D.Y., and M.Mandels. 1980.Cellulases: Biosynthesis and applications. *Enzyme Microb. Technol. 2*:91-102.

Sandhu, D.K. and M.K.Waraich. 1983. Conversion of cheese whey

to single-cell protein. *Biotechnol. Bioeng. 25*:797-808.

Sasaki, T. 1989. Cellulase. In: Biomass handbook. Kitani, O. and C.W.Hall,(eds). Gordon and Breach Science Publishers. New York. pp345-354.

Schlemmer, A.F., C.F.Ware, and N.T.Keen. 1987. Purification and characterization of a pectin lyase produced by *Pseudomonas fluorescens* WP1. *J. Bacteriol. 169*:4493-4498.

Shewale, J.G. and J.C.Sadana. 1978. Cellulase and ß-glucosidase production by a basidiomycete species. *Can.J Microbiol. 24*:1204-1216.

Shiang, M., J.C.Linden, A.Mohagheghi, M.P.Tucker, K.Grohmann and M.E.Himmel. 1991. Cellulase production by *Acidothermus cellulolyticus*: Growth on solka floc cellulose and simple sugar mixtures. *Biotechnol. Appl. Biochem. 14*:30-40.

Sidhu, M.S. and D.K.Sandhu. 1980. Single-cell protein production by *Trichoderma longibranchiatum* on treated sugar cane bagasse. *Biotechnol. Bioeng. 22*:689-692.

Singh, A., A.B.Abidi, N.S.Darmwal and A.K.Agrawal. 1990. Saccharification of cellulosic substrates by *Aspergillus niger* cellulase. *World J. Microbiol. Biotechnol. 6*:333-336.

Sloneker, J.H. 1976. Agricultural residues including feed lot wastes. *Biotechnol. Bioeng. Symp. 6*:235-250.

Soundar,S. and T.S.Chandra. 1988. Production of cellulase and detection of Avicel-adsorbing carboxymethylcellulase from a mesophilic fungus *Humicola grisea* Fb. *Enzyme Microb. Technol. 10*:368-374.

Spencer-Martins and N.van-Uden. 1977. Yields of yeast growth on starch. *Eur.J.Appl.Microbiol.4*:29-35.

Sternberg, D. 1976. ß-Glucosidase:it's biosynthesis and role in saccharification of cellulose. *Appl.Environ.Microbiol.31*:648-654.

Sternberg, D., P.Vijayakumar and E.T.Reese. 1977. ß-Glucosidase: microbial production and effect on enzymatic hydrolysis of cellulose. *Can. J. Microbiol. 23*:139-147.

Stutzenberger, F. 1991. Polygalacturonate lyase production by

Thermomonospora curvata on protein-extracted lucerne fiber. *J. Indust. Microbiol.* 7:243-250.

Szczodrak, J. 1989. The use of cellulases from a ß-glucosidase-hyperproducing mutant of *Trichoderma reesei* in simultaneous saccharification and fermentation of wheat straw. *Biotechnol. Bioeng.* *33*:1112-1116.

Tanaka, M. and R.Matsuno. 1985. Conversion of lignocellulosic materials to single-cell protein (SCP): recent developments and problems. *Enzyme Microb. Technol.* 7:197-206.

Tangnu, S.K., W.B.Harvey and R.Charles. 1981. Enhanced production of cellulase, hemicellulase and ß-glucosidase by *Trichoderma reesei* (Rut C-30). *Biotechnol. Bioeng.* *23*:1837-1849.

Taniguchi, M., Y.Kometani, M.Tanaka, R.Matsuno, T.Kamikubo. 1982. Production of single-cell protein from enzymatic hydrolysate of rice straw. *Eur. J. Appl. Microbiol. Biotechnol.* *14*:74-80.

Taniguchi, M., T.Kato, R.Matsuno and T.Kamikubo. 1983. Continuous cellulase production by cell-holding culture. *Eur. J. Appl. Microbiol. Biotechnol.* *18*:218-224.

Thomke, S., M.Rundgren and S.Eriksson. 1980. Nutritional evaluation of the white-rot fungus *Sporotrichum pulverulentum* as a feed stuff to rats, pigs and sheep. *Biotechnol. Bioeng.* *22*:2285-2303.

Updegraff, D.M. 1969. Semimicro determination of cellulose in biological materials. *Anal. Biochem.* *32*:420-424.

Viesturs, U.E., A.F.Apsite, J.J.Laukevics, V.P.Ose, and M.J.Bekers. 1981. Solid-state fermentation of wheat straw with *Chaetomium cellulolyticum* and *Trichoderma lignorum*. *Biotechnol. Bioeng. Symp.* *11*:359-369.

Warzywoda, M., V.Ferre, and J.Pourquie. 1983. Development of a culture medium for large scale production of cellulolytic enzymes by *Trichoderma reesei*. *Biotechnol. Bioeng.* *25*:3005-3010.

Wenzel, H.F.J. 1970. *The chemical technology of wood*. Academic press, New York, NY. pp 692.

Wood, T.M. 1985. Properties of cellulolytic enzyme systems. *Biochem. Soc. Trans.* *13*:407-410.

Wood, T.M. and S.I.McCrae. 1972. The purification and properties of the component of *Trichoderma koningii* cellulase. *Biochem. J. 128*:1183-1192.

Wood, T.M. and S.I.McCrae. 1982. Purification and some properties the extracellular ß-glucosidase of the cellulolytic fungus *Trichoderma koningii*. *J. Gen. Microbiol. 128*:2973-2982.

Young, V.R. and N.S.Scrimshaw. 1975. Clinical studies on the nutritional value of single-cell protein. In: *Single-cell protein II*. Tannenbaum, S.R. and D.I.C.Wang (eds). Cambridge, Ma, London: MIT Press. pp 564-586.

Zimmermann, W. 1990. Degradation of lignin by bacteria. Minireview. *J. Biotechnol. 13*:119-130.