“ISOLATION OF CELLULASE ENZYME BY ASPERGILLUS NIGER FROM SUGARCANE BAGASSE ”

Dissertation submitted to Mahatma Gandhi University in partial fulfilment of the requirements for the award of degree of master of vocation studies

M.Voc Food Processing Technology

By

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Undertaken at

CASHEW EXPORT PROMOTION COUNCIL OF INDIA, KOLLAM

DEPARTMENT OF FOOD PROCESSING TECHNOLOGY

ST. TERESA’S COLLEGE ERNAKULAM

(AUTONOMOUS), COLLEGE WITH POTENTIAL FOR EXCELLENCE

Nationally Re-Accredited by NAAC A++ level (4th cycle)

Affiliated to Mahatma Gandhi University

Kottayam- 686560

July 2022

DECLARATION

I, ANJANA C. D(VM20FPT004) hereby declare that this dissertation entitled “ISOLATION OF CELLULASE ENZYME BY ASPERGILLUS NIGER FROM SUGARCANE BAGASSE ” is a bonafide record of research work done by me during the course of research and that the dissertation has not previously formed the basis for the award to me for any degree, diploma, fellowship or other title of any other university or society.

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ACKNOWLEDGEMENT

First of all, I humbly bow my head before God almighty who is my strength in all aspects.

I wish to express my sincere gratitude to Dr. Prabhakumari C, SENIOR SCIENTIST CEPC LABORATORY AND TECHNICAL DIVISION, KOLLAM. Who allowed me to my project finely under her able guidance and supervision.

I thank for the staff members of CEPC Laboratory and Technical division, Kollam for their help and co-operation during the period of my project work.

I also wish to express hearty thanks to miss. VISMAYA, CEPCLABORATORY AND TECHNICAL DIVISION, KOLLAM. My project coordinator for all the timely help and advice rendered.

I express my sincere gratitude to miss BHAVYA EP, HEAD OF FOOD PROCESSING TECHNOLOGY, ST TERESAS COLLEGE ERNAKULAM for permitting me to do this outdoor project successfully.

I extremely grateful to Miss. PRABHA,SENIOR SCIENTIST CEPC LABORATORY AND TECHNICAL DIVISION, KOLLAM for her valuable support throughout my project.

Finally, I thank my parents, and all my friends for their inspiration and encouragement in all efforts.

ANJANA CD

ABSTRACT

Enzymes arc proteins,which catalyze chemical reactions with great specificity and rate enhancements.Cellulase is an enzyme produced by the hydrolysis of cellulose,the most abundant biological compound on terrestrial earth.Microorganisms such as bacteria and fungi degrades cellulose and produces cellulase enzyme.One of the most important cellulolytic fungi is Aspergillus niger.

Here in this experimental work cellulase enzyme was produced from Aspergillus niger using solid state fermentation of sugarcane bagasse. The enzyme activity was assayed by DNS method.Maximum activity was obtained at 30 minutes of incubation,50°C temperature and pH 5.The ideal enzyme concentration.

Keywords : Cellulase enzyme, aspergillus Niger, sugarcane bagasse

INTRODUCTION

Enzymes are proteins that catalyse the chemical reactions, both biosynthetic and degradative, occurring in living cells. Almost all process in a biological cell needs enzymes in order to occur at significant rates. The enzyme activity is also affected by temperature, chemical environment and the concentration of substrate. Enzymes are among the most important products obtained for human needs through residual sources. A large number of industrial processes in the area of industrial, environmental and food bio technology utilize enzyme at some stages. Solid state fermentation holds tremendous potential for the production of enzyme. It can be of special interest in these processes where the crude fermented products may be used directly as enzyme source.

Cellulase is a complex multi enzyme system acts collectively to hydrolyze cellulose from agricultural waste to produce simple glucose units. This multi enzyme system comprises endo-1,4, β -D glucanase, exo-1, 4, β glucanase and β -D glucosidase enzymes. Endocellulases breaks internal bonds to disrupt the crystalline structure of cellulose and expose individual cellulose polysaccharide chains. Exo cellulase cleaves 2-4 units from the ends of the exposed chains produced by endo cellulases, resulting in the tetrasaccharides or disaccharides such as cellobiose. Cellobiose or beta glucosidase hydrolyses the endo cellulase products into individual monosaccharide. Within the above types there are also progressive and non progressive types. Progressive cellulase will continue to interact with a single polysaccharide strand. Non progressive cellulase will interact once then disengage and engage another polysaccharide strand.

Production of cellulase enzymes has gained considerable importance in view of possibility of hydrolysis of cellulose into monosaccharide which could later be fermented into ethanol for use as the alternative energy source. Enzymatic degradation of cellulose requires the synergistic activity of at least three enzyme endoglucanase, cellobiohydrolase and β - glucosidase.

Cellulases have many applications in several areas. They are in paper industry, biofuel industry, as biological detergent, in textile industry. It is used for commercial food processing in coffee, animal health care, pharmaceutical applications etc.Potential application of cellulase is in the large scale production of glucose by erizvni_ atic hydrolysis of cellulose to be fermented into alcohol, or used as carbon source for the production of various feeds and chemicals. Cellulase are also used in cereal Pmcessing- brewing. al h A co o. production, plant extraction, fruit processing, wine manufacture and waste treatment. In brewing cellulase speeds up mash filtration and can increase extract yield. In alcoholic fermentations, depending on the nature of the raw material, cellulase can increase the yield of alcohol produced, when used as a supplement to starch degrading enzymes. Cellulase, used to supplement pectinases. speed up important application as part of an enzyme complex in total liquefaction or maceration of vegetables and fruits. Cellulases are also used for extraction or refining of cereal proteins and for recovery of alginates from seaweed. Cellulase combined with pectinase and hemicellulase is used to hydrolyse mucilage during coffee extraction. Cellulase have a significant role in clarifying fruit or vegetable juices.Juice is a liquid naturally contained in fruit or vegetable tissue. It is prepared by mechanically squeezing or macerating fresh fruits or vegetables without the application of heat or solvents. Popular juices include apple, orange, n, cashew apple, tomato, carrot.strawberry e.t.c.Cellulose is the most abundant theticall is stored in the orm material on earth. About 50% of the Coe fixed photosyny f of cellulose and annually about 10" tons of plant dry material is produced as a result of total photosynthetic activity.Cellulosic material have a corresponding wide use as lignified cellulose (wood) for construction purposes, and as more or less pure cellulose in paper, fibres and textiles.The degradation of cellulosic materials has gained increasing attention due to its world wide availability and immense potential to sugars, fuels and chemical feed stocks.

Cellulose is a homopolysaccharide of D glucose units joined in a linear fashion through β — 1, 4 — glycosidic linkage. The chain length may go upto1.5x 10^4 glucose units. The cellulose

molecules are joined to each other through hydrogen bonds and van der wall forces. The cellulose is insoluble in water and does not give characteristic colour with iodine. Cellulose is the structural compound of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form bio films. It is the most common organic compound on earth. Native crystalline cellulose is insoluble and occurs as fibers Of densely packed hydrogen bonded unhydroglucose chains of 10-15000 glucose UM"' Its density and complexity make it very resistant to hydrolysis without preliminary chemical or mechanical degradation of swelling.

In nature cellulose is usually associated with other polysaccharides such as xylan or lignin. Cellulose is the most abundant organic source of food. fuel and chemicals. However, its usefulness is dependent upon its hydrolysis to glucose. Acid and high temperature degradation are unsatisfactory in that the resulting sugars are decomposed, also waste cellulose contains impurities that generate unwanted byproducts under these harsh conditions.

Microorganisms that degrade cellulose are both abundant and ubi quitous in nature. Both bacteria and fungi produce cellulase enzymes and can be classified into non-aggregating and aggregating enzymes. The anaerobic bacteria which are capable of breaking down cellulose to monosaccharides include *bacteroids, cellulosolvens, Bacillus spp, Clostridium, Fibrobacter succinogenes, cellvibrio gilvus, Ruminococcus flavefaciens* etc.Fungi are the main cellulase producing microorganisms. This includes *Aspergillus niger, Tree sei, T.viridae, Afiimigatu.s. Acremonium cellulolyticus, F.solani* etc. The crude enzyme produced by *Trichoderma* and *Aspergillus* are commercially available for agricultural use.

Four culture methods are in common use for the production of cellulases.

a. Submerged culture in a liquid medium which is aerated from spargers.
b. Shake cultures in a liquid medium
c. Stationery cultures in a liquid medium and

Koji - type processes in which the organism is grown on moist solid medium from which enzymes are subsequently extracted.

Solid State Fermentation

Fermentation processes which take place in the absence or near absence of free water in the substrate are termed as solid state fermentation. It is imperative, however, that the substrate contain enough moisture absorbed in the substrate particles within the solid bed (substrate).Enzymes are among the most important products obtained for human needs through microbial sources. Solid state fermentation holds tremendous potential for the production of enzymes. It can he of special interest in those processes where the crude fermented product may he used directly as the enzyme source. This system offers numerous advantages over submerged fermentation system including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipment etc.

Ideally almost all known microbial enzyme can he produced under solid state fermentation system. Application of solid state fermentation is the production of several metabolites relevant for food processing industry, centered on flavours, enzymes(amylase, lipase, pectinase, cellulase, protease etc)organic acids (lactic acid, citric acid etc) and xanthan guns. Selection of a suitable strain for the required purposes (SSF) depends upon the nature of the substrate and environmental conditions. Filamentous fungi seems to be the most suitable organisms for solid state fermentation.

Agro industrial residues are generally considered the best substrate for the solid state fermentation processes. A number of such substrates have been employed for the cultivation of micro organisms to produce rest of enzyme. Two types of the substrates are used in solid state fermentation, one in which the substrate itself is used by the micro organisms as the energy (carbon) source, and the other in which substrate acts as support only. While in former case, most of the naturally occurring agricultural by products, eg:- wheat bran, rice bran, corncobs, sugarcane bagasse, wheat

and paddy straw, fruit pulps and peels, sugar beet pulp and cassava etc. are most commonly used, in latter case, it may be an inert material of synthetic nature eg: polyurethane foam or even of natural origin like sugar cane bagasse. Other important substrates includes cashew shell cake, maize bran, grain bran, rice husk, trimmings dust, coconut coir pith, banana waste, tea waste, palm oil cake, steamed rice etc. In general, all the major polymeric materials viz. polysaccharides, proteins and lignin, can be used by the micro organisms as substrate (carbon source) in SSE. These substrates are insoluble in water and the water with nutrient remains absorbed on the particles of the substrate. There are large number of physical like accessibility of the substrate to the micro organisms, particle size of the substrate, film and mass effects etc and factors like the chemical nature of the substrate, Pit of the fermentation

medium etc which affect the utilization of substrate by the micro organisms. Substrates with finer particle size generally result in a better growth and activity of the micro organisms as they get large surface area

The major factors that affect microbial synthesis of enzymes in solid state fermentation system include selection of a suitable substrate and microorganisms, pretreatment of the substrate, water content and aw of the substrate, relative humidity, type and size of the inoculum, control of temperature of fermenting matter or removal of temperature of heat, period of cultivation, maintenance of uniformity in the environment of SSF system, and the gaseous atmosphere ie, oxygen consumption rate and Coe evolution rate. The fungi used in this experiment is Aspergillus niger

Aspergillus niger

A large number of microorganisms including bacteria, yeast and fungi produce different groups of enzymes. A strain of Aspergillus niger was used for the production of cellulase in solid cultures. The Aspergillus niger goup is wide spread with many strains capable of producing amylases.

Aspergillus is among the best known and most frequently recognized fungal genera on earth.Many useful enzymes are produced using industrial fermentation of Aspergillus niger.It is one of the most common species of the genus Aspergillus .Aspergillus niger is cultured for the industrial production of many substances. Various strains of A. niger are used in the industrial preparation of citric acid and gluconic acid. The minimum temperature for growth is 6-8°C, optimum is 25-30° C and maximum temperature is 45 — 47° C..Aspergillus is also used for the production of chemicals for biosynthetic transformations and enzyme production. The enzymes produced by Aspergillus niger are ligase, cellulases, p- glucosidase, Amyloglucosidase, hemi cellulases, pectinases, xylanases. It is also used in the production of high fructose corn syrup.

Sugarcane bagasse

Sugarcane bagasse is the major by-product of the sugar cane industry. It contains about 50% cellulose, 25% hemicellulose and 25% lignin. Due to its abundant availability, it can serve as an ideal substrate for microbial processes for the production of value-added products such as protein enriched animal feed, enzymes, amino acids, organic acids and compounds of pharmaceutical importance etc. Since untreated bagasse is degraded very slowly by micro-organisms, a pre-treatment step may be useful for improved substrate utilization.

AIM AND OBJECTIVES

AIM

To extract and partially purify cellulase enzyme from sugarcane bagasse

OBJECTIVES

- Development of a suitable process for the production of cellulase using sugarcane bagasse as substrate
- Partial purification of crude enzyme
- Assays for the presence of cellulase

REVIEW OF LITRATURE

Cellulase (a multi enzyme system) acts collectively to hydrolyze cellulase from agricultural waste to produce simple glucose units (Milala *et al.,* 2005).

Production cost of cellulases may be brought down multifaced approaches which include the use of cheap lingo cellulosic substrates for fermentation production of the enzyme and the use of cost efficient fermentation strategies like solid state fermentation .(Reeta *et* al.,2007).

Generally hydrolytic enzymes, eg: cellulases, xylanases, pectinases etc are produced by fungal cultures. Since such enzymes are by used in nature by fungi for their growth. *Aspergillus spp.* and *Trichoderma spp.* have most widely been used for these enzymes.(Ghanem *et al.,* 2000).

Microorganisms of the genera *Trichoderma* and *Aspergillus* are thought to be cellulase producers, and crude enzymes produced by these micro organisms are commercially available for agricultural use. (Khalid Mahmood *et al.,* 2006).

An increased attention was paid to the use of the various agro industrial wastes for value addition using solid state fermentation by filamentous fungi. Solid state fermentation is the most appropriate process in developing countries due to the advantages it offer. The hyphal mode of growth and good tolerance to low water activity and high osmotic pressure condition, make fungi most efficient for bio conversion of solid substrate (Alva *et al.,* 2007).

Mutations using sub lethal concentrations of mutagen for a prolonged period of growth has yielded mutants, which can produce more cellulase.(Chand *et* a/.,2005).

The cotton sacchanifying as a feasible alternate for the conversion of activity of cellulase obtained from mono and co-culture lignocellulosics into fermentable sugars and fuel fermentation of *Aspergillus niger.(*Ikram — ul-Haq *etal*.,2005).

The difference of conditions between solid state and submerged cultures can lead to altered expression of several genes, which in turn may affect various phenotypes, such as growth, development, mycotoxin and enzyme production (Soledad *et al.,* 2006).

Solid state fermentation system offers numerous advantages over submerged fermentation system; including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipment etc. (Hassetline **C.** W 1977, Pandey and Ashok, 1992; Chahal D .S and Mooyoung M. Dev 1987; Nigam P and Singh D, 1994).

Solid state fermentation is a kin to the natural habit of fungi. Studies have revealed that the productivity is sometimes better under such conditions than under submerged fermentation condition.(Wang *et al*.,1974).

The use of cellulases, hemicellulases and pectinases has increased considerably. especially in textile, food, brewery and wine as well as in pulp and paper industries. To day these enzymes account for approximately 20% of the world enzyme market mostly from *Trichoderma* and *Aspergillus(Uhlig.,1998).*

Degradation of plant cell wall polysaccharides is of major importance in the food and feed, beverage, textile, paper and pulp industries as well as in several other industrial production processes. The members of the fungal genus *Aspergillus* are commonly used for the production of polysaccharide degrading enzymes (Ronard de Vries and Viseer,2001).

A pan reactor requiring a small capital investment was developed for SSF of wheat straw. High yields of complete cellulase system were obtained in comparison to those in submerged fermentation (Zaldivar *et al.,* 2001).

Baggase, saw dust and corn cob were used as lignocellulosic substrates for the production of cellulase enzyme using *A. flavus* aver ballmilling and pretreatment with caustic soda. The maximum enzyme activities at about the l2th **Hrs is** the optinium time when the enzyme may be

harvested. (Ojumu *et* a1.,2003). Generally the carbon sources have been estimated as the major cost factor in **enzyme** production. A reduction in the production costs can be achieved by the usage of inexpensive waste materials, such as corncob and sugarcane bagasse that are often reliable and abundant.(Yang *et* a/.,2004).

Cellulase is an important enzyme required for breakdown of polysaccharides into monosaccharide, those can further converted into ethanol andother alcohols through fermentation process. (Chundakkadu.,1999). Cellulase is the major constituent of all plant materials.

Ghani B.A and Rickard P. A. D (1990) viewed that cellulose is degraded by a cellulose enzyme complex, consisting of cellobiohydrolases, endo-1, 4 beta - glucanases and beta-glucosidases. Industrial enzyme have been a spectaculars in their production in the last three decades. The growth of industrial enzymes which are currently in commercial production. With the discovery of a variety of new and more active enzyme, the enzyme extract market has been forecasted to go upto U.S and 1720 billion by 2006 (Godfrey T and Riechelt J.R., 1983).

Trichoderrna strains are in the center of attention in enzyme production, because they excrete high amounts of cellohiohydrolyses and endoglucanase However, their enzyme complex is deficient in the beta glucosidase to cellulase activity required for the complex degradation of cellulose is in the range of 0.8 - 1.5 IU of β - glucosidase per filter paper unit of cellulase activity (Duff *et al.,* 1985).

The selection of a substrate for enzyme production in SSF process depends upon several factors mainly related with cost and availability of the substrate and thus may involve screening of several agro industrial residues (Cuadra *et al.,* 2007) Fungal cellulases, however, one difficult to measure and have limited expressibility in heterologous hosts.(Chun - Yi Hu *et al.,* 2003). Growth and cellulolytic enzymes production were maximal when organic nitrogenous compounds were supplied. Enzymatic production was maximal at an initial PH of the growth medium of 6.5 (Paula Magnelli *et al.,* 1999).

Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level, water activity are the most critical. Generally smaller substrate particle provide larger surface area for microbial attack and are a desirable factor.

Larger particles provides a better respiration efficiently, but provide limited surface for microbial attack. This necessitates a compromised particle size for a particular process.(Pandey and Ashok, 1991; Nigam P and Singh D, 1994; Pandey and Ashok, 1992; Duenas *et al;* 1995).

Solid state fermentation was chosen for the present work because it has been reported that SSF with fungal strain results in much greater productivity than a submerged fermentation. (Malathi S and Chakraborty R,1991). In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells.(Camassola *et al.*, 2(07)

Multiple enzyme components, an insoluble substrate, and generally slow reaction rates have plagued cellulase researchers interested in creating cellulase mixtures with increased activities and enhanced biochemical properties. (Wen of al., 2005).

The lignocellulosic biomass (especially agricultural wastes) is known to he an excellent carbon source for microbial enzyme production. (I.uiza Jecu *et al.*, 2000). Reduction of particle size of lingo cellulosic leads to *an* increase in their susceptibility to microbial fermentation.(Weber *et al.*, 2005).

Celluloytic enzymes isolated from various sources differ in their molecular characteristics like Molecular weight, aminoacid composition and sequence, isoelectric point, carbohydrate content, absorbidity on cellulose, catalytic activity and substrate specificity. (Paul J., 1992).

Sharma A. Rao et al.,(1995)viewed that cellulases which have received attention from the point of cellulose degradation and formation of cellulolytic enzymes,are microbial origin.He recognized the importance of cellulolytic microbes for the production of glucose from cellulose.

Several studies were carried out to produce celluloytic enzymes from biowaste degradation process by many micro organisms including fungi such as *Trichoderma, penicillium, Aspergillus*

species etc. (Immanuel *et* a/.,2007). Cellulases are easily obtained from microbial and fungal sources, but vertebrates lack to produce endogenous cellulases and hence are reliant upon gastro intestinal micro organisms for cell wall degradation of ingested plants (Arun K. Ray *et* a/.,2007).

Cellulose is a polymer consisting of thousands of glucose units linked with β - glucoside bounds and the resulting biopolymers are associated by means of hydrogen bonding.Cellulose exhibits structural features such as crystalline sections and amorphous parts.(Caritas *et* a/.,2006).

Many fungi and bacteria secrete a multi component enzyme system called cellulose that exhibits the ability to saccharity cellulose (Yoshihiko Amano *et al.*,1998).

Cellulose is the most abundant organic source of food, fuel and chemicals. However, its usefulness is dependent up On its depolymerization to kintentable sugar using microbial system.(Villena *et* a1.2006). Complete enzymatic hydrolysis of enzymes requires synergistic action of 3 types of enzymes, namely cellobiohydrolase, endoglucanase or carboxymethyl cellulase and β - glucosidases. (Narasimha *et al.,* 2006).

The crystallinity and lignifications limits the accessibility and susceptibility of cellulose to cellulolytic enzymes and other hydrolytic agents. However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported. (Lopez Contreras *et al.*,2004).

Changes in structure and properties of the cellulose caused by enzymatic treatment depend on the composition, the type of enzyme and the treatment condition. (Yucao *et al.,* 2002).

Major impediments to the commercial use of cellulases are low activity and high production cost of the available enzyme preparations. This has necessitated the search for cellulolytic organism with novel cellulases properties and strategies for low cost enzyme production (Ojumu *et al.,* 2003).

The cellulolytic organisms also grow rapid on media containing sugarcane pulp and saw dust as sole carbon sources with the result that based medium containing 2% of any of the two waste cellulosic materials was found adequate for its cultivation. (Boisset *et al.,* 2000).

Cellulase action is generally initiated by the random acting endoglucanase, at the amorphous regions within cellulose chain to produce cello oligosaccharides (Schaffner *et al.,* 2004).

Many useful enzymes are produced using industrial fermentation of *Aspergillus niger.* For example *Aspergillus niger* glucoamylases are used in the production of high fructose corn syrup and pectinases are used in cider and wine classification. (Klich M.A., 2002).

Analysis of cellular physiology under SSF conditions revealed accumulation of a mixture of polyols (glycerol, arabitol, erythritol and mannitol), while in SmF only mannitol was found(Rahardjo *et* a/.,2005).

Sun *et al*.,(1997)has developed novel fed-batch solid state fermentation process for cellulase production, which could overcome the problems associated with high initial nutrient concentration while retaining advantages from the high total effective salt concentration.

Siddique *et al.,* (1997) found that metal ions have an influence on cellulase production. Metal ions show both stimulatory as well as inhibitory effect on microorganisms. He found that in cellulomonas was found activated by Manganese ions. Reese E.T (1963) observed that cellulases are generally found to be inhibited by mercury, silver, copper, chromium, lead and zinc salts at about concentrations. Verma *et al.,* (1963) noted that the inhibition by heavy metals might be probably due to the non specific salt formation.

The importance of water activity in microbial physiological process is well recognized. It is known that aw is a critical factor affecting the SSF it is also considered as a fundamental parameter for mass transfer (Gretty K. Vallina *et al.,* 2007).

Cellulosic substrates hydrolysed by one type of cellulose are categorised as follows: acid swollen cellulose, carboxy methyl cellulose, cellulose azure and trinitro phenyl C-M cellulose are hydrolysed by endoglucanases (Kan *et* a/.,2006).

Generally, smaller substrate particles provide larger surface area for microbial attack but these small substrate particles may result in substrate accumulation, which may interface with microbial respiration/aeration, and therefore results in poor growth. (Solomon *et al.,1999).*

Most higher animal species lack the genes that encode enzymes capable of degrading plant cell wall polysaccharide. Herbivorous, Vertebrates, however, have evolved digestive systems which contain symbiotic microorganisms capable of degrading these compounds. (Hand *et* al.,2004).

Cellulase yields appear to depend on a complex relationship involving a variety of factors like inoculum size (carbon source and cellulose quantity), pH value, temperature, presence of inducers, medium additives, aeration and growth time, etc (Dominques *et al.,* 2000).

The particle size of cellulose can affect cellulose production by micro organisms. Release of enzyme is directly proportional to the particle size of the substrate used, ie, release of enzyme increases when the particle size of the substrate decreases. (Kotchoni *et* a/.,2002).

One of the most important organism used in bio technology are Fungai. Many important industrial products are produced from fungi using fermentation bio technology. Fungi can be used in new production process that are themselves less polluting than traditional chemical processes (Haltao *et al.*,2004)

Fungal genera like *Trichoderma* and *Aspergillus* are taught to be cellulase producers and crude enzyme produced by these microorganism are commercially available for agricultural use. (Kazuhisa

Miyamoto.,1997). In general bacterial cellulases are constitutively produced, where as fungal cellulase is produced only in presence of cellulose (Suto M and Tomito F., 2001).

Filamentous fungi particularly *Aspergillus* and *Trichoderma spp.* are well known efficient producers of cellulases. (Peil *et al*.,1998). Mutant strains from Aspergillus niger UAM-GSI were produced by UV radiation to increase their hemicellulolytic and cellulolytic activity production. (Szengyel *et al*.,2000).Strain improvement has been achieved through mutation, selection or genetic recombination. In many cases ,mutations are harmful, but occasionally may lead to a better adapted organism to its environmental with improved biocatalytic performance(Lynd *et al*.,2002).

Filamentous fungi are important in industrial enzyme production, since they are able to synthesize and secrete large amounts of extra cellular proteins. These organisms grow in liquid and solid state cultures by hyphal extension and branding. (Suto *et al*.,2001).

Cellulase with its immense importance is being imported for use in Nigeria at very high cost. The local production of such enzymes using locally available agricultural wastes which can serve as substrates may therefore reduce the cost of importation and encourage self reliance (Te Biese bake.,2005).

The conversion of cellulosic mass to fermentation sugars through biocatalyst cellulose derived from cellulolytic organisms has been suggested as a feasible process and offers potential to reduce use of fossil fuels and reduce environmental pollution. (Schin *et al*.,2000).

SSF using inert supports impregnated with chemically defined media has several potential applications in both scientific studies and in the industrial production of high value products such as metabolites and enzymes. As a result of its more defined system, SSF on inert support offers numerous advantages, such as improved process control and monitoring and enhance process consistency, compared with cultivation on natural solid substrates (Ooijkaas., 2000).

MATERIALS AND METHODS

CULTURE USED

The fungal culture used was Aspergillus niger. It was obtained from the C.E.P.C culture collection. This culture was used for the production of cellulase enzyme using solid state fermentation.

SUBSTRATE USED

Sugar cane bagasse was used as the substrate for solid state fermentation. It is a biowaste and is an abundantly available substrate.

PRETREATMENT OF SUBSTRATE

30gm of the substrate (Sugar cane bagasse) was weighed. The substrate was sun dried to reduce moisture content to make them more susceptible for crushing. It was sieved to get powdered form. It was then soaked in 1% NaOH solution in 1:1 ratio/substrate: solution for two hours at room temperature. Then they were washed with distilled water to free the chemicals.

FERMENTATION MEDIA

30gm of pretreated Sugar cane bagasse was autoclaved at 121°C for 1 hour. 20 ml of mineral salt medium was prepared and autoclaved at 121°C for 20 minutes. It was inoculated with the spore suspension of Aspergillus niger. This was then added to the conical flask containing the Sugar cane bagasse . The fermentation media was then incubated at room temperature for 5 days.

ENZYME EXTRACTION

After incubation, the fermented media was stirred and filtered through sterilized muslin cloth. The filtrate serves as the enzyme extract.

CELLULASE PURIFICATION

To the filtered enzyme extract (NH4)2 SO4 was added till saturation and kept at 4°C overnight

CENTRIFUGATION

The enzyme extract was then centrifuged at 5000 rpm for 15 minutes at The pellet was dissolved in phosphate buffer (pH 6) and was used as the enzyme source. It was used for the activity determination of cellulase. Assay for cellulose.

DETERMINATION OF REDUCING SUGAR BY DNS METHOD (MILLER, 1959).

PREPARATION OF STANDARD

0.1 gm of glucose was taken and dissolved in 100 ml distilled water. From this, 25 ml stock solution was taken in a standard flask and made up to 100 ml with distilled water. This serves as a working standard.

PROCEDURE FOR COLORIMETRY

0.45 ml of 1% CMC solution and 1 ml of enzyme extract was taken in a test tube, and was incubated at 55°C for 15 minutes. Then 0.2 ml of this mixture was transferred to a fresh tube.Made up the volume to 2 ml by adding distilled water.

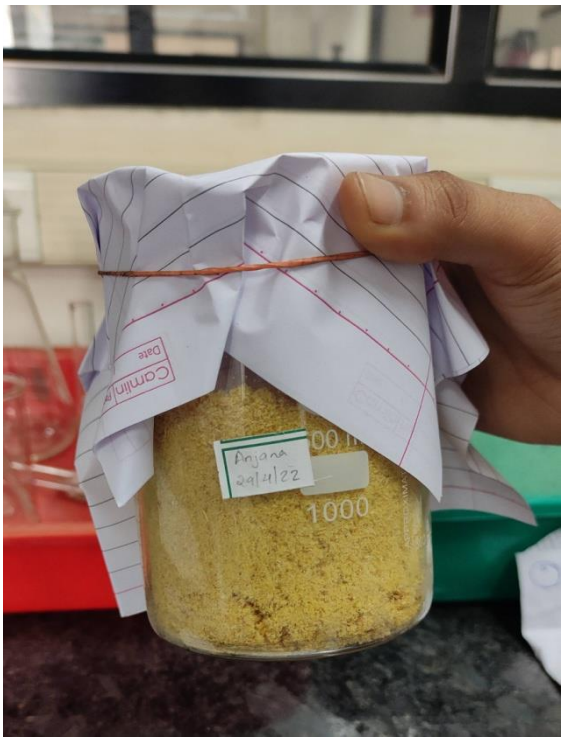
Then 0.5 ml DNS reagent was added to it.Incubated in a boiling water bath for 5 minutes. While the tubes were warmed, 1 ml sodium potassium tartarate solution was added. It was cooled to room temperature. The volume was made up to 5 ml with distilled water. The absorbance was measured at 540 nm. Run the standard to draw a standard graph.Cellulase activity was determined based on the standard graph using glucose concentration.

RESULT AND DISCUSSION

Cellulase is an enzyme of microbial origin, being able to convert crystalline amorphous and chemically derived celluloses quantitatively to glucose. It is a very important enzyme because the human body cannot produce it on its own. It has wide applications in the industrial field. The commercial use of celluloses is dependent on high titer, good enzymatic activity, low production cost and feasible mass production.

Solid state fermentation holds tremendous potential for the production of enzymes. Agro industrial residues are generally considered the best substrates for solid state fermentation process. In solid state fermentation ,the selection of suitable substrate for the fermentation process is a critical factor and thus involves the screening of agro industrial materials for microbial growth and product formation.

The solid state fermentation for the production of cellulase was carried out using the substrate sugarcane bagasse and the fungal species used was *Aspergillus niger .Many* useful enzymes are produced using industrial fermentation of *A.niger.*



1.powdered sugarcane bagasse

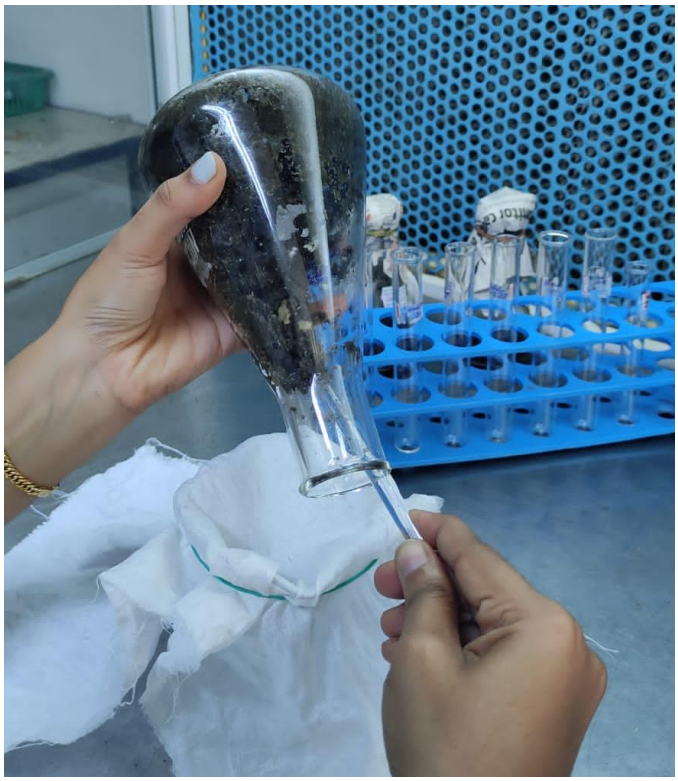
2.addition of 1% NaoH sol

3.soaking

4.wash out NaoH sol

5. Addition of aspergillus Niger

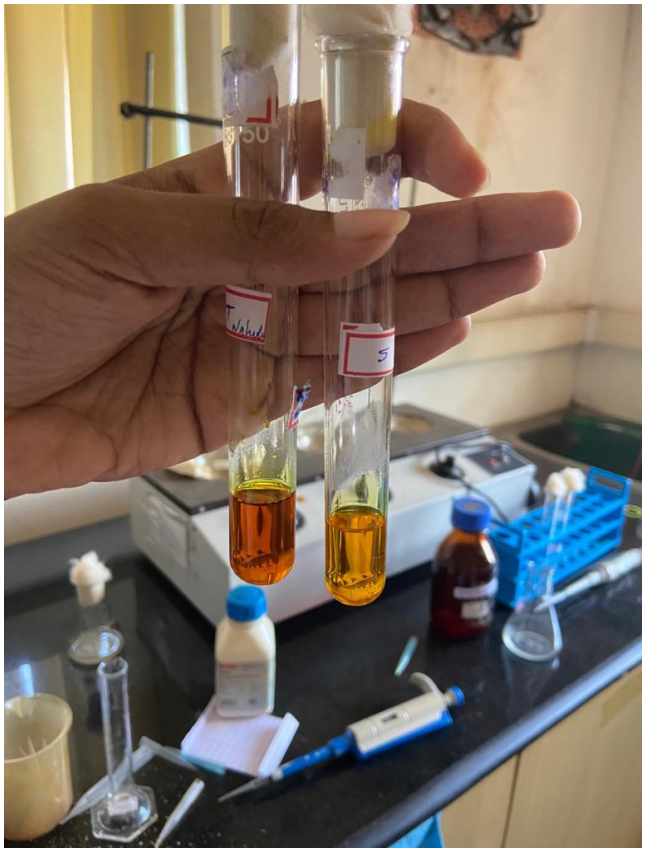
6. Incubated at room temperature (5 days)



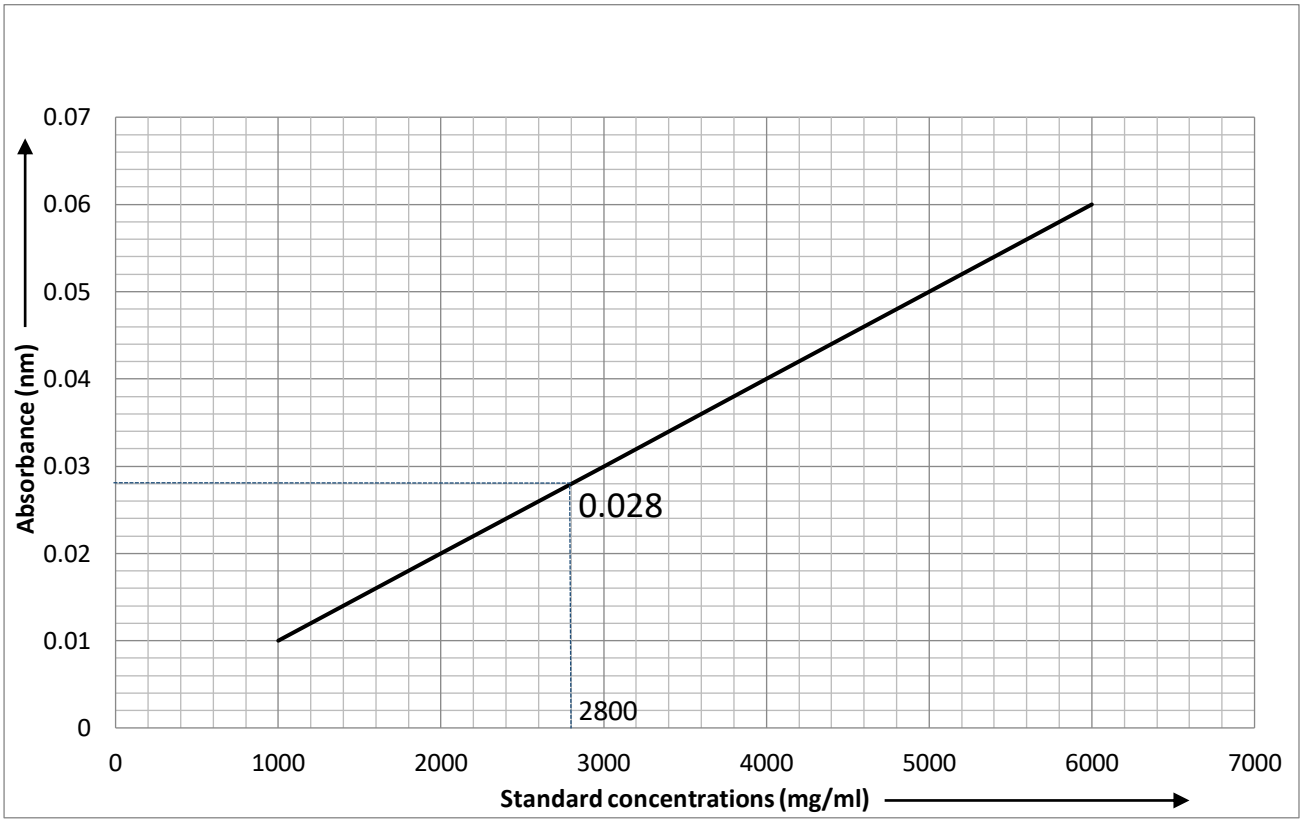
7. fermented media filtrate through a muslin cloth

8. Crude enzyme

9.Crude enzyme for centrifugation



10.Determination of reducing sugar by DNS method



The cellulase enzyme activity obtained is 2800 µ/ml. Hence, the 30g of sugarcane bagasse sample as substrate for solid state fermentation by Aspergillus niger gives 2800 µ/ml cellulase activity

SUMMARY AND CONCLUSION

Enzymes are biological catalysts that speed up the rate of reaction between substances without themselves being consumed in the reaction Enzymes are obtained from animal tissues.plants.baciena and fungincluding yeast

ellulase refers to a class of enzymes produced chiefly by fungi.bactena and protozoans that catalyze the cellulolysis or hydrolysis of cellulose The study of cellulase production is important in recent years due to their application in various industnes (ellulase have found various applications in food.paper.textile.biofucl industncs and widely used in laundry detergents

Cellulasc production from Aspergillus niger using solid state fermentation of sugarcane bagasse was studied.

REFERENCE

- Alva, S.,Anupama, J., Salva, J.,Chicc, Y.Y.,Vyshali, **P.,** Shruti,M.,Yogeetha, B.S., Bhavya, D., Puravi,J., Ruchi, K.,Kumudini,B.S., and Varalakshmi, K.N. 2007 . Production and Characterization of fungal amylase enzyme isolated from *Aspergillus* spiG112 in solid state culture .*African Journal of Biotechnology.* Vol .6(5): 576-581.

- Arun,K.Ray., Abhinanda Bairaji .,Keka Sarkar Ghosh and Sukanta.2007.Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY 5 and *Bacillus circulants* TP3 isolated from fish gut .*Actajchthgologicaet piscatoria;* 37 (1): 47-53.

- Boisset, C.,Franchini, C.,Schulein, M.,Henrissat, B. and Chanzy, H.2000.Imaging the Enzymatic Digestion of Bacterial Cellulose reveals the endocharacter of the cellobiohydrolase cel 6A from cellobiohydrolase Cell *7 A.Appl.Envir.Microbiol.* 66(4):1444-1452

- Camassola, M.,Dillon, A.J.P.2007. Production of cellulase and hemicellulase be *Penicillium echinulatum* grown on pretreated sugarcane bagasse and wheat bran in solid state fermentation. *J Appl.Microbio1.103* (6):2196-2204.

- Caritas, U., Okafoayu and Humphrey, C. Nzelibe.2006. Effect of acid hydrolysis of Gracinia a kola(bitter kola) pulp waste on the production of CM-Cellulase and B-glucosidase using *Aspergillus niger.African Journal of Biotechnology.* Vol.5, No: 10; PP. 819-822.

- Chand, P., Arena, A Maqsood,A .M..11(x),L.V.2005.Novel Mutation method fot increased cellulose production. *.1. Appl. Microbiol.* 98(2): 31S-:123.

- Chundakkadu.K 1990. Production of bacterial cellulase by solid state hopiwessing of banana wastes. *Rioresou.Techno1.69:231-239.*

- Chun-Yi Hu.1 . and Liang-Ping Lin. 1. 2003 .Characterization and Purification of hydrolytic enzymes in *Sinorhizobium fredii* CCRC 15-60. *Journal World Journal of Microbiology and Biotechnology.* klQ(5):515-522.

- Cuadra, T.F.J.. Fernandez, A., Tomasini, J.,Barnos- Gonzalez.2007.1nfluence of pH regulation and nutrient content on Cephalos porin C production in solid state fermentation by *.4erernonium Chrysogerum .Lett.Appl.Microbiol.* 143(1):63-79.
- Dominques, E.C.,Queiroz, J.A.,Cabral,J.M.S AND Fonseca,L.P.2000.The influence of culture conditions on mycelial structure and cellulase production by *T.reesei* Rut *E30.Enzyme Microbiol.* Technol.26:394-401.
- Duenas,R.,Tengerdy, R.P.and Gutierrez Correa,M.1995. *World Biotechno1.11:333-337.*
- Duff, S.J.B.,Coorper,D.G. and Fuller,D.M.1985.Appl.Environ. *Microb.934-938.*
- GhanemN.B.,Yusef, H.H.,Mahrouse, H.K.2000. Production of *Aspergillus niger* xylanase in solid state cultures: application of the placket-Burman experimental design to evaluate nutritional requirements.Bioresour. *Technol.* 73:113-121.
- Ghani,B.A and Rickard,P.A.1).1 990. Enzymatic hydrolysis of lignoccIlulose: contribution of li-glucosidase.A.seam *Food* J.5(2):51-70.
- Godfrcy,T.,Rcichclt,J.R.1983.1ndustrialEnzymology.Nature Press.New York.3:1-7.
- Gretty,K.Vallina and Marcel Guticrrenz Correa 2007.Production of lignocellulolytic enzymes by Aspergillus niger at variable water *activities.Electronic Journal of Biotechnologv.10(1):786-792.*
- Haltao,M.O.,Xlaoyong Zhang and Zuohu,L.J.2004.Control of gas phase for enhanced cellulase production by *Penicillium decumbens* in solid state culture.Process Biochemistry. 39(10):1293-1297.
- Hanif, A., Yasmeen, A., Rajoka, M. I. 2004. Induction, Production,Repression and De-repression of exoglucanase synthesis by *Aspergillus niger.Bioresour. Technol.* 94:311-319.
- Hassetline,C.W.1977. *Process Biochem.12:24-27.*
- Ilcram-U1-Haq.,Muhammad Mohsis Javed., Tehmina Saleem Khan and ZafarSiddiq.2005. Cotton Saccharifying Activity of cellulases produced by Co-culture of *Aspergillus niger* and *Trichoderma viride.Research Journal of Agriculture and Biological sciences.1(3):241-* 245.

- Paul,J.1992.Different aspect of microbial degradation of cellulosc./ndian *J.Microbiol.32:127-147.*
- Paula Magnelli.,Araceli,M.Ramos.,Favia Forchiassin.1999.Factors influencing cellulose production *Saccoholus saccoboloides. Bioresour. Technol;* 141(2-3):219-255.
- Pcil,N.,Geelkem,M.M.C.,Verles,R.P.Visser,K., Graff,L.H.1998.The transcriptional activator Xis R regulates both xylanolytic endoglucanase gene expression in *A.nigerAppl.Environ.Microbio1.64(10):3615-3617.*
- Rahardjo, Y.,Sie,S.,Weber,F.J.,Tramper,J.,Rinzema,A.2005.Effect of low oxygen on growth and alpha-amylase production of *Aspergillus oryzae* in model solid state fermentation systems.Biomolecular engineering;21 :163-172.
- Reese, E.T.1963.Advances in enzymatic hydrolysis of cellulose and related materials.Pergamon Press.London.
- Reeta Ram Singhania., Rajeev,K., Sukumaran.,Ashok Pandey.2007. Improved cellulose production by *Trichoderma reesei* RUT C30 under SSF through process *optimization,Appl.Biochem.Biotechno1;142(1):60-* 70.
- Ronard de vries and visser,J. 2001 .*Aspergillus* enzymes involved in degradation of plantcel polysaccharides.Microbiol *Bio.Rev.65(4):494-522.*
- Schaffner, D.W., Toledo,R.T.2004.Cellulase production by *Trichoderma reesei* when cultured on xylose-based media supplemented with sorbose.Biotechnology abd Bioengineering;37(1):12.16.
- Lynd,L.R.,Weiner,P.J.,Vanzyl W.H.,Pretorius,I.S.2002.Microbial cellulose utilization ,fundamentals anf Biotechnology,Microbiol .*Mot BiaRev.66:506-577.*
- Malathi, S. and Chakraborty, R,1991.Production of allealine protease by *Aspergillus flavus* isolate under solid substrate fermentation condition for use as a depilation agent.Appl. *and env.Microbio1.52(3):712-716.*
- Milala,M.A.,Shygaba,A.,Gidado,A.,Ene,A.C., and Wafer,J.A.2005.Studies on the use of agricultural waste for cellulose enzyme production by *Aspergillus niger.Research Journal of Agriculture and Biological sciences;* I (4):325-328.

- Narasimha, G.,Sridevi, A., Buddotla Viswanath.,Subhosh Chanda, M.,Rajasekar Reddy, B.2006.Nutrients effects on production of cellulolytic enzymes by *Aspergillus niger. African Journal of Biotechnology.5* (5):472-476.
- Nigam,P. and Singh,D.1994.J.Basic *Microbio1.34:201-237.*
- Ooijkaas,L.P., Webwe,F.J.,Butelaa,R.M.,Trampes). and Rinzema, J.2000. Defined media and inert supports their potential as solid state fermentation production systems,18:356-359.
- Pandey and Ashok.1991.Biores.Techno1.37;169-172
- Pandey and Ashok.1992.Process *Biochem.27:109-117*
- Paul,J.1992.Different aspect of microbial degradation of celluloseindian.
- Paul,J.1992.Different aspect of microbial degradation of cellulosc./ndian *J.Microbiol.32:127-147.*
- influencing cellulose production *Saccoholus saccoboloides. Bioresour. Technol;* 141(2-3):219-255.
- Pcil,N., Geelkem,M.M.C.,Verles,R.P.Visser,K., Graff,L.H.1998.The transcriptional activator Xis R regulates both xylanolytic endoglucanase gene expression in *A.nigerAppl.Environ.Microbio1.64(10):3615-3617.*
- Rahardjo, Y.,Sie,S.,Weber,F.J.,Tramper,J.,Rinzema,A.2005.Effect of low oxygen on growth and alpha-amylase production of *Aspergillus oryzae* in model solid state fermentation systems.Biomolecular engineering;21 :163-172.
- Reese, E.T.1963.Advances in enzymatic hydrolysis of cellulose and related materials.Pergamon Press.London.
- Reeta Ram Singhania., Rajeev,K., Sukumaran.,Ashok Pandey.2007. Improved cellulose production by *Trichoderma reesei* RUT C30 under SSF through process *optimization,Appl.Biochem.Biotechno1;142(1):60-* 70.
- Ronard de vries and visser,J. 2001 .*Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides.Microbiol *Bio.Rev.65(4):494-522.*
- Schaffner, D.W., Toledo,R.T.2004.Cellulase production by *Trichoderma reesei* when cultured on xylose-based media supplemented with sorbose.Biotechnology abd Bioengineering;37(1):12.16
- Schin·C.S.·Lec·J.P.·Lee,P.S.,Park,S.C.2000.1:.nzyme production of *Trichoderma reesei* Rut ('-300 on a various lignoccIlulomic substrates .*Appl.Biochem and Biotechnol;* 84-80:237-245.

- Sharma,A,Rao C C 5 N.,I3all and 1lasiza,S.K.1995.CcIlulose degrading bacteria from factory eftluents../.1nd.Bot.Sco.75:122.
- Siddique,K.S., Azkar,M.J.,Rashid,M.11. and Rajoka,M.1.1997.Stability and identification of active site residues of CMC from *A.niger* and *cellulomonas biazotea, Folio Microbiom.42:312-318.*
- Soledad De Necolas-Santiago., Carlos Regalado-Gonzalez.,Blanca GarciaAlmen-darez.,Francisco,J, Fernandez., Alejandro Tellez-jurado.,Sergio Huerta-Orchoa.2006.Physiological ,morphological and mannose production studies on *Aspergillus niger* Uam-gs 1 *Mutants,Electronic Journal of Biotechnology;9* CD:105-109.
- Solomon, B.O., Amigan, B.,Betiku, Ojumu,T.V.,Layokun,S.K.1999. Optimization of cellulase production by *Aspergillus flavus* Linn isolates NSPR101 Grow on Bagasse. *JNSchE.16:61-68.*
- Sun, T, Lire,B.H and Li ,Z.H.1997.J. *Chem.Technol.Biotechnology.69:492*- 432.
- Suto,M.,Tomito,F.2001. Induction and Catabolite repression mechanism of cellulases in fungi.J.Biosci.Bioeng:92:305-311.
- Szengyel,Z.,Zaochi,G.,Varga,A.,Reezey,K.2000.Cellulase production at *Trichoderma reesei* Rut C-30 steam pretreated spruce.Hydrolytic potential on different *substrates.Appl.Biochem.Biotechnol.* 84(86):679-691
- Te Biesebekc, R.2005.Moleeular analysis and improvement of protein production by *Aspergillus oryzae* grown on solid substrates../ourna/ *of biotechnology;121* :1-176
- t111lig, H.1998.1ndustrial enzymes and their applications.John Wiley and sons,New York ;435.
- Verma,G.M.,V erma,R.K.,Sahgal,D. D. and V ijayaraghavan,P.K.1963. Decomposition of cellulose by fungus *Curvularia lunata* 3 properties of cellulolytic *enzyme.Defense sci.J.13:215-224.*
- Villena, G.K.,Gutierrez-Correa, M.2006. Production of cellulase by *Aspergillus niger* biofilms developed on polyester cloth.lett.AppLMicrobiol;43(3):262-268.

- Wang, H.L., Yespa, J.B. and Hessetine,C.1974. Acid protease production by fungi used in soyabean food fermentation.Appi.Microbio/.27:906-911.
- Weber, J and Agblevo,F.A. 2005.Micro bubble fermentation of *Trichoderma reesei* for cellulase production.Process *Biochemist;40:669-* 676.
- Wen., Zh,Y.,Liao,W. and Chen,S.L.2005.Production of cellulose by *Trichoderma reesei* from dairy *manure.Bioresou.Techno1;96;91-99.*
- Yang,Y.H., Wang,B.C.,Wang,Q.H.,Xiag,L.J. and Duan, C.R.2004. Research on solid state fermentation on rice chaff with a microbial consortium.Colloids and surface B:Biointerfaces;34:1.6.
- Yoshihiko Amano.,Masahiro Shiroishi.,Kazatosi Nisizawa.,Eiichi Hoshino and Takahisa Kanda.1998.Fire substrate specificities of four exotype cellulose produced by *Aspergillus niger, Trichoderma reesei* Irper lacteus on (1-3),(1-4),B-D Glucans and *Xyloglucan.Journal of Biochemistry;120(6):1123-1129.*
- Yucao and Hulmin Tan.2002.Effects of cellulose on the modification of cellulose.Carbohydratc Research;33 F(14):1291-1296.

Zaldivar,M.,Velasquez, J.C.,Contreras, 1 and Perez, *L.M.2001.T.aureovide* T-121,a mutant with enhanced production of lytic enzymes its potential use in waste cellulos degradation and /orbiocontrol. *Electron.J.Biotechno1.3:* 160-168

APPENDICES

1. Mineral salt medium

Nlacronutrients:

Ammonium nitrate	-	1.65 g
Potassium nitrate	-	1.90 g
Magnesium sulphate	-	0.37 g
Potassium dihydrogen phosphate	-	0.17 g
Ammonium ferrous sulphate	-	0.05 g
Calcium chloride	-	4.50 mg
Distilled water	-	100 ml

Micronutrients:

Bromic acid	-	6.25 g
Magnesium sulphate	-	22.3 g
Zinc sulphate	-	8.6 g
Sodium molybdate	-	0.25 mg
Copper sulphate	-	0.25 mg
Calcium chloride	-	0.25 mg
Distilled water	-	100 ml

Take 1 ml micronutrients and add to 99 ml of macronutrients to produce mineral salt medium.

2. Phosphate buffer (pH 6)

Solution A:

Sodium hydrogen orthophosphate	-	2.7 g
Distilled water	-	100 ml

Solution B:

Disodium hydrogen phosphate	-	5.365 g
Distilled water	-	100 ml

Mix 8.77 ml of solution A and 1.23 **ml of** solution

3. 1% Carboxy methyl cellulose

carboxy methyl cellulose - 0.1 g

distilled water - 10 ml

4. DNS Reagent

DNS - 1 g

crystaitine phenol - 200 mg

Sodium sulphite - 500 mg

1N NaOH - 100 ml

Store in *cool* place (4°C).

5. 1 N NaOH

NaOH - 4 g

Distilled water - 100 ml

6. 40 % Sodium potassium tartarate

Sodium potassium tartarate - 40 g

Distilled water - 100 ml

7. SDS - PAGE — Reagents

Separating gel buffer (pH 8.8)

Tris buffer - 6.06 g

Distilled Water - 100 ml

Stock acrylamide solution

Acrylamide - 29.2 g

Bisacrylamide - 0.8 g

Distilled water - 100 ml

Stacking Gel buffer (P^H 6.8)

Tris - 6.06 g

Distilled Water - 100 ml

Electrode buffer

Tris	- 1.8g
Glycine	- 9.0 g
SDS	- 0.63 g

Sample loading buffer

1M Tris (p^H 6.8)	- 17 ml
10 % SDS	- 4.5 ml
100 % SDS	- 20 ml
P-mercaptoethanol	- 1.0 ml
0.1 % bromophenol blue	- 0.2 ml
Distilled water	- 0.6 ml

10 % Ammonium per sulphate

Ammonium per sulphate	- 1,0 g
Distilled water	- 10 ml

Staining solution

Coomassie brilliant blue R.250	- 0.1 g
Methanol	- 40 ml
Acetic acid	- 10 ml
Distilled water	- 50 ml

Destaining solution

Methanol	- 40 ml
Acetic acid	- 10 ml
Distilled water	- 50 ml