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Review Article

# PRODUCTION OF CELLULASE – A REVIEW

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**ABSTRACT**

Cellulases are the enzymes which hydrolysis cellulosic biomass and are being produced by the microorganisms grown over cellulosic matters. Cellulase is an important enzyme which can be obtained from cheap agrowastes,as well as cellulose as substrates by using submerged fermentation and solid state fermentation. Cellulose protein can be degraded by cellulase enzyme produced by cellulolytic bacteria and fungi. This enzyme has various unique industrial applications and it has been considered as major group of industrial enzyme. In this present review paper, discussion attempted on "cellulase production" by submerged and solid state fermentation using various types of bacteria and fungi with different types of agro wastes.

## INTRODUCTION

Enzymes are among the most important products obtained for human needs through microbial sources. A large number of industrial processes in the areas of industrial, environmental and food biotechnology utilize enzymes at some stage or other. Current developments in biotechnology are yielding new applications for enzymes (Ashok Pandey *et al*., 1999).

In the present techno- economic era, procurance of energy is one of the major problems which humanity is facing. All the waste cellulose is a source of food and is also a potential source of energy (Elder *et al*., 1986).

Cellulose present in renewable lignocellulosic material is considered to be the most abundant organic substrate on earth as chemical feed stock (Krishna, 1999).

Cellulose is a branched glucose polymer composed of an -1,4 glucose units linked by a – 1, 4- D- glycosidic bond (Gielkens *et al*., 1999; Han *et al*., 1995, Acharya. P.B *et al*., 2008). The breakdown of cellulose into sugar can be achieved by acid hydrolysis as well as by enzymatic hydrolysis. But enzymatic hydrolysis is mostly preferred because it produces fewer by-products and proceeds under milder condition reported by Mandels *et al*., (1974).

Cellulase, a group of enzymes which catalyze the hydrolysis of cellulose and related oligosaccharide derivatives, is considered a potential tool for industrial saccharification if cellulosic biomass (Berry *et al*., 1990), and an economic process for its production is thought to be critical for the successful utilization of cellulosic materials (Soloman *et al*., 1999, Wu and Lee, 1997, Nwodo-Chinedu, S. *et al*., 2007).

The cellulase complex used in simultaneous saccharification and fermentation systems (SSFS) generally includes $C_1$ [(EC 3.2.1.9), (exo- 1, 4-β-D- glucanase, cotton lyase), $C_x$ (EC 3.2.1.4), (endo- 1, 4 - β-D- glucanase, carboxymethyl cellulase, or CMC ase), $C_b$ (EC 3.2.1.21), (β- glucosidase)], and pectinase. (Debing Jing *et al*., 2007).

Cellulases have been used and studied for most of the 20th century and are the most commercially important of all the enzyme families. The enzyme activities were increased about 30-80% when produced by SSF in comparison with conventional SmF enzyme production. Cost of cellulase production may be brought down by multifaceted approaches which include the use of cheap lignocellulosic substrates and the use cost efficient fermentation strategies like solid state fermentation (Rajeev *et al*., 2008) The enzyme production is good by most of the fungi like *Aspergillus* and *Trichoderma* Sp.

Enzymolysis of native cellulose is carried out by three components of cellulase as:

a. Exo- β-1-4, glucanase: It acts on the non-reducing end of the cellulose chain and successively removes single glucose units.

b. Endo-β-1-4, glucanase: It randomly attacks the internal β-1-4, linkages.
3. β-glucosidases or Cellobiases: In addition to $C_1$ and $C_x$, the cellulose system also contains cellobiase, which eventually breaks down cellobiose, the building unit of cellulose, to glucose.

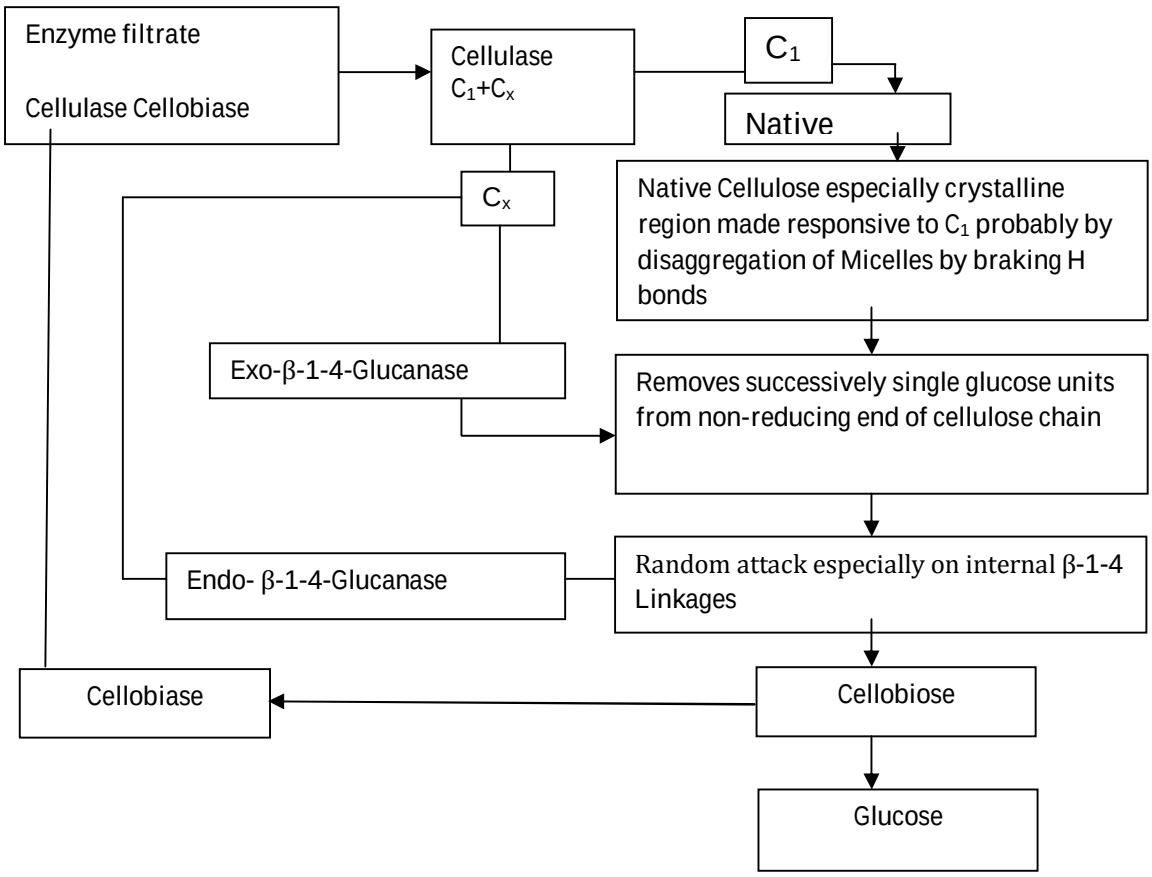


**Fig. Enzymolysis of native Cellulose**

**Microbial Sources of Cellulases**

Cellulase is one of the most useful enzymes in industry. Cellulase can be produced by fungi, bacteria or actinomycetes, but the most common producer is fungi (Arriffin. H., *et al*., 2006)

**Bacteria**

The bacterial systems have also been investigated for saccharification of the biomass and might have advantage because of the fast growth rate of bacteria. It has also been reported that the enzyme preparation from cellulolytic bacteria can effectively saccharify different cellulosic substrates (Choundry *et al*., 1980, 1981; Bynd *et al*., 1987; Rajoka *et al*., 1984; Waldron *et al*., 1986).

*Cytophaga hutchinsonii* revealed an unusual collection of genes for an organism that can attack crystalline cellulose. Location, formation and biosynthetic regulation of cellulases in *C.hutchinsonii* were demonstrated on different substrates (Clifford Louime *et al*., 2006)

*Acetivibrrio cellulolytieus* has been studied by many workers investigated that both endo-glucanase and exo-glucanase can be regulated by induction and catabolite repression. It differs from most of the cellulolytic organisms in its ability to utilize only cellulose, cellobiose or salicin for growth (Saddler *et al*., 1980). This organism has attracted special interest because of high specific activities for endo- and exo -glucanase (Mackenzie *et al*., 1985).The endocellulase activity of the culture broth was determined during growth of *Acinetobacter anitratus* and *Branhamella* Species by measuring release of reducing sugars from CMC (Ekperigin.M.M., *et al*., 2006). The enzyme preparations from *A.cellulolyticus* have the ability to saccharify cellulose (Mackenzie *et al*.,

1985); the condition for maintaining high specific activity have also been determined.

Cellulase system of *Pseudomonas* Sp. has been fairly well studied. Yamane *et al*., (1970); Yoshikawa *et al*., (1974) studied the biogenesis of multiple cellulose components of *P. fluorescens var*. cellulose with special emphasis on effects of culture conditions on the multiplicity of cellulose titres produced by the organism. Tewari *et al*., (1977) reported production, purification and properties of cellulase (extracellular) from *Pseudomonas* Sp. Localization of cellulase components in *Pseudomanas* Sp., isolated from activated sludge have been investigated (Ramasamy *et al*.,1980). Endo-glucanase are the major components of cellulase complexes with three being from *P.fluorescens var*. cellulose (Yashikawa *et al*., 1974) and four or more by *Pseudomomas* Sp. (Ramasamy *et al*., 1980). Gene for D-galactose dehydrogenase has been cloned in *E.coli* (Buckle *et al*., 1988). *Pseudomonas fluorescens, Bacillus subtilis, E.coli* and *Serratia marcescens,* cellulase producing bacreia was isolated from soil and optimization of the fermentation medium for maximum cellulase production was studied and found *Pseudomonas fluorescens* as best cellulase producer (Sonia Sethi et al., 2013).

The cellulolytic enzymes of *Bacillus* Species have been the focus of many due to their potential use in the conversion of agricultural wastes into useful products (Ozaki *et al*., 1990). It was reported that *Bacillus subtilis CBTK 106* can produce a considerable amount of cellulase activity (Chundakkadu Krishna, 1999). Femi-Ola. T.O *et al*., 2008 studied that aqueous extract of the woods inhibited the growth and production of cellulase in the strains of *B.subtilis* significantly. Muhammad Salem Akhtarif *et al*., 2001 studied the usage of *B.subtilis* in saccharification of wheat straw, rice straw and bagasse. Production of cellulase using carboxy methyl cellulose as substrate by *B.pumilus EB3* was studied by H.Ariffin *et al*., 2006.Saraswati Bai et al., 2012 studied production of cellulose using *Bacillus subtilis CEL PTK 1* from cow dung. Mohammed S.A.Shabab et al., 2010 studied cellulose productivity by *Bacillus subtilis KO* strain using CMC zone and dinitro salicyclic acid.

Effect of some nutritional and environmental factors on production of cellulases was studied using *Bacillus licheniformis MVS1* and *Bacillus Sp. MNS3* isolated from an Indian hot spring (Somen Acharya et al., 2012). Deepmoni Deka et al., 2011 attempted to optimize the medium components for enhanced production from *Bacillus subtilis AS3* and significant variables for enhancing alkaline cellulase production were screened and selected using the Plackett – Burman design.

*Clostridium thermocellum*, a thermophilic anaerobic bacterium, has attracted increased interest for conversion of LC biomass. Being thermophilic, it offers several advantages over mesophilic organisms because; the former produces enzymes with increased thermostability and significantly higher specific activity (Johnson *et al*., 1982; Ng and Zeikus, 1981, 1988). Moreover, the cellulases produced by *Clostridia* are not inhibited by moderate concentrations of glucose or cellobiose. The organism produces exo and endo- glucanase having multiforms (Bisaria *et al*., 1981) which act together during saccharification. The organism forms high molecular weight aggregated (Ait *et al*., 1979; Lamed *et.al*., 1983). Somen Acharya et al., 2012 isolated a thermophilic bacterium *Aneurini bacillus thermoaerophilus WBS2* which produces extracellular thermophilic cellulases from hot spring in India in order to enhance cellulase production various fermentation parameters was also optimized. Thermophilic bacteria strain *Geobacillus pallidus* was successfully isolated from empty fruit bunch (EFB) and palm oil mill effluent compost and characterized by Azhari Samsu Baharuddin et al., 2010.

Rumen cellulolytic bacteria have been studied extensively (Varel, 1984; Ohmiya *et al*., 1985). Cellulase produced by *Ruminococcus albus* can hydrolyse CMC and acid swollen cellulose causing a rapid fall in degree of polymerization while affecting only a small degree of hydrolysis (Wood *et al*., 1984). Groleu *et al*., 1981 have shown that Bacterium *Succinogens*, another anaerobic rumen cellulolytic bacterium producing high CMC-ase and cellobiase activity.

**Table 1: Cellulase producing Bacterial strains**

| Bacterial strain | Reference |
|---|---|
| *Ruminococcus albus* | Varel, 1984; Ohmiya *et al.*, 1985 |
| *Succinogens* | Groleu *et al.*,1981 |
| *Cytophaga hutchinsonii* | Clifford Louime *et al.*, 2006 |
| *Acetivibrrio cellulolytieus* | Saddler *et al.*, 1980 |
| *Acinetobacter anitratus* | Ekperigin, M.M., *et al.*, 2006 |
| *Branhamella* | Ekperigin, M.M., *et al.*, 2006 |
| *Pseudomonas* Sp. | Yamane *et al.*, 1970; Yoshikawa *et al.*, 1974 |
| *P.fluorescens* | Yashikawa *et al.*, 1974 |
| *Bacillus subtilis (CBTK 106)* | Chundakkadu Krishna, 1999 |
| *B.pumilus EB3* | Ariffin, H., *et al.*, 2006 |
| *Clostridia* | Bisaria *et al.*, 1981 |
| *Aneurini bacillus thermoaerophilus WBS 2* | Somen Acharya et al., 2012 |
| *Geobacillus pallidus* | Azhari Samsu Baharuddin et al., 2010. |
| *Bacillus subtilis CEL PTK 1* | Saraswati Bai et al., 2012 |
| *Bacillus subtilis KO* | Mohammed S.A.Shabab et al., 2010 |
| *Bacillus licheniformis MVS 1* and *Bacillus Sp. MNS3* | Somen Acharya et al., 2012 |
| *Bacillus subtilis AS3* | Deepmoni Deka et al., 2011 |
| *Pseudomonas fluorescens* | Sonia Sethi et al., 2013 |

**Fungi**

These organisms grow under suitable condition to produce cellulase; e.g. *Trichoderma viride* and *Trichoderma reesei* produce active cellulase when grown in solid submerged culture *Aspergillus niger* produces highly active cellulase when grown in liquid media by both surface and submerged culture methods and recently by solid state fermentation (Ikram-ul-Haq *et al*., 2005). The detailed study was made on production of cellulase using *Trichoderma reesei* (Muthuvelayudham *et al*., 2003). Production of cellulase also reported by using substrates like cellulose, xylose and lactose using *T.reesei* (Muthuvelayudham *et al*., 2005). Saravanan et al.,2013 studied production of cellulose using *Trichoderma reesei* in solid state fermentation.Some species of *Penicillium* i.e. *Penicillium iriensis* and *P. citriviride* produce significant quantities of cellulase, when grown under different conditions. *Penicillium funiculosum* is capable of dissolving cotton completely by cellulase production. Fungi like *Stachy, Botsysatra, Pesalotia, Palmarum, Merulius, Lnerymans, Polyspores, Neuraspora* have been reported to produce cellulase (Mandels *et al*., 1976; Shoemaker *et al*., 1978; Metha *et al*., 1975; Kassim, 1983; Takao *et al*., 1985; Brown *et al*., 1987; Hyashida *et al*., 1980; Adikanae *et al*., 1983).*Chaetomium sp.NIOCC 36* was found better for production of cellulase(Chinnarjan Ravindran et al., 2010). Barros R R. et al., 2010 study evaluated cellulases, xylanases and beta – glucosidases produced by two fungi, the thermotolerant *Acrophialophora nainiana* and *Ceratocystis parodoxa* using submerged fermentation. Production of cellulase was also reported by Deswal D. et al., 2011 in solid state fermentation using brown rot fungus, *Fomitopsis sp. RCK 2010.* Eggin *et al*., (1968); Malik *et al*., (1970) studied the cellulase production by certain fungi and found that microorganisms have the ability to degrade native cellulose. Since the first observation of the action of conversion of cellulosic biomass to fermentable sugar needs economical process for the production of cellulases. Soil is inhibitated by a large number of microorganisms like bacteria, actinomycetes, algae and fungi; which form a major component of soil. Although these microorganisms form a very small part of the soil, they are responsible for many of the chemical transformations and even for some of the physical changes that take place in soil. Fungi especially make a unique contribution in these changes which result in the proper maintenance of soil fertility.

The first attempt to isolate fungi from soil was made by Adametz (1866) after which various investigators studied the soil fungus flora by various methods.

Among these investigators the names of Lender (1908), Hagem (1910), Bachwith (1911), Jensen (1912), Goddard (1913), Dale (1914), Werkenthin(1916), Paine (1927), Gilman *et al*.,(1932), Wajid (1985) are more prominent. But the work of Waksman (1922, 1927) gave the soil dilution plate methods to isolate soil fungi, which is still being extensively used. Both qualitative and quantitative studies were made by these investigators. They found the data on kind of soil, soil reaction (pH), depth of soil, moisture, and season of year, tillage and manuring practices. They also found that the result of isolation of fungi is also influenced by

methods of investigation, nature of medium and material employed.

The work has also been carried out on the isolation and identification of *Trichoderma* Species from soil sample which determine the antagonistic properties of the mold cultures (Dennis, 1970; Dennis *et al*., 1971; Rifai, 1964, 1969)

Among *Trichoderma* Species, *T. harzianum* (Deschamp *et al*., 1985; Macris *et al*., 1985; Mes-Hartree *et al*., 1988; Saddler *et al*., 1982; Saddler *et al*., 1987; Md.Zahinger Alam *et al*., 2005; Ikram-ul-Haq *et al*.,2006, Shazia Shafique et al., 2009), *T.aureoviride* (Mercedes Zaldivar *et al*., 2001), *T.reesei* (Muthuvelayudham,R., *et al*., 2006,2007; Maryam Latifian *et al*., 2007; Wang.J.Sh *et al*., 2006, Shazia Shafique *et al*., 2009), *T.viride* (Benkun Qi *et al*., 2007, Shazia Shafique *et al*., 2009) and *T.konningii* (Wood, 1988; Wood *et al*.,1982, 1986) has been studied. Other well studied fungi are *A.terreus* (Araujo *et al*.,1986), *A.aculeatus* (Murao *et al*., 1988), *A.fumigatus* (Heptinstall *et al*., 1986), *Aspergillus heteromorphus* (Anitha Singh *et al*., 2009, Rajesh Singh *et al*., 2009), *Alternaria alternate* (Bailey *et al*., 1988; Macris, 1984), *A.japonicus* (Sharma *et al*., 1985; Sanyal *et al*., 1988), *A.niger* (Gokhale *et al*., 1984; MeCleary *et al*., 1988; Okada,1989; Poutanen *et al*., 1984; Jun-ichi-Abe *et al*.,1999; Milala. M.A *et al*., 2005; Narasimha,G., *et al*., 2006; Sharada,R et al.,2012), *A.ustus* (Macris *et al*., 1985), *A.wentii* (Panda *et al*., 1987; Srivastava *et al*., 1984), *A.flavus* (Ojumu *et al*., 2003), *Aspergillus candidus* (Milala,M.A., *et al*., 2009),*Tricothecium roseum* (Prashanth shanmugam *et al*., 2008),*Scopulariopsis* (Bharathi Kodali *et al*., 2006),*Penicillium chrysogenum* (Nwodo-chinedu *et al*., 2007),*A.oryzae* (Adebace Johnson Adeleke et al., 2012),*A.flavus NRRL5521* (Hussein Azzaz Murad et al., 2013).

**Table 2: Cellulase producing fungal strains**

| Fungal strain | Reference |
|---|---|
| *Pesalotia* | Metha *et al*., 1975 |
| *Stachy* | Mandels *et al*., 1976 |
| *Botsysatra* | Shoemaker *et al*., 1978 |
| *Polyspores* | Hyashida *et al*.,1980 |
| *Palmarum* | Kassim, 1983 |
| *Neuraspora* | Adikanae *et al*.,1983 |
| *A.ustus* | Macris *et al*., 1985 |
| *Merulius* | Takao *et al*., 1985 |
| *A.fumigatus* | Heptinstall *et al*., 1986 |
| *A. heteromorphus* | Anitha Singh *et al*., 2009, Rajesh Singh *et* al., 2009. |
| *A.terreus* | Araujo *et al*.,1986 |
| *Lnerymans* | Brown *et al*., 1987 |
| *A.aculeatus* | Murao *et al*., 1988 |
| *T.aureoviride* | Mercedes Zaldivar *et al*., 2001 |
| *A.flavus* | Ojumu *et al*., 2003 |
| *Scopulariopsis* | Bharathi Kodali *et al*., 2006 |
| *P. chrysogenum* | Nwodo-chinedu *et al*., 2007 |
| *T.viride* | Benkun Qi *et al*., 2007, Shazia Shafique *et al*., 2009. |
| *Tricothecium roseum* | Prashanth shanmugam *et al*., 2008 |
| *A.wentii* | Panda *et al*., 1987; Srivastava *et al*., 1984 |
| *T. harzianum* | Deschamp *et al*., 1985; Macris *et al*., 1985; Mes-Hartree *et al*., 1988; Saddler *et al*., 1982; Saddler *et al*., 1987; Md.Zahinger Alam *et al*., 2005; Ikram-ul-Haq *et al*., 2006, Shazia Shafique *et al*., 2009. |
| *Alternaria alternate* | Bailey *et al*., 1988; Macris, 1984 |
| *A.japonicus* | Sharma *et al*., 1985; Sanyal *et al*., 1988 |
| *A.niger* | Gokhale *et al*., 1984; MeClearyet *et al*., 1988; Okada,1989; Poutanenet *et al*., 1984; Jun-ichi-Abe *et al*.,1999; Milala,M.A., *et al*., 2005; Narasimha.G *et al*., 2006; Sharada,R et al., 2012. |
| *A. candidus* | Milala,M.A., *et al*., 2009 |
| *T.konningii* | Wood, 1988; Wood *et al*., 1982, 1986. |
| *T.reesei* | Muthuvelayudham,R., *et al*., 2006,2007; Maryam Latifian *et al*., 2007; Wang.J.Sh *et al*., 2006, Shazia Shafique *et al*., 2009, Saravanan et al.,2013 |
| *Acrophialophora nainiana* and *Ceratocystis parodoxa* | Barros R R. et al., 2010 |
| *Chaetomium sp.NIOCC 36* | Chinnarjan Ravindran et al., 2010 |
| *Fomitopsis sp. RCK 2010* | Deswal D. et al., 2011 |
| *A.oryzae* | (Adebace Johnson Adeleke et al., 2012) |
| *A.flavus NRRL5521* | Hussein Azzaz Murad et al., 2013 |

**Production of cellulase**

Agricultural and industrial wastes are among the main causes of environmental pollution. Their conversion into useful products may reduce the intensity of the problems caused by them. These wastes include green gram husk, black gram husk, rice bran, wheat bran etc. are underutilized in India especially in Andhra Pradesh. In most parts of A.P these materials are mainly used as animal feeds. A large quantity is left in farm lands to be decomposed by microorganisms such as bacteria and fungi (Okafor *et al*., 1987).Economically, the most important industrial material other than food stuffs affected by microorganisms are cellulose and wood products (Wainright, 1992; Debing Jing *et al*., 2007). Proper utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products (Milala *et al*., 2005).

Cellulose is commonly degraded by an enzyme called Cellulase. This enzyme is produced by several microorganisms, commonly by bacteria and fungi (Bahkali *et al*., 1996; Shin *et al*., 2000; Immanuel *et al*., 2006).Filamentous fungi are preferred for commercially important enzymes production, because the level of the enzymes produced by these cultures is higher than those obtained from bacteria (Bakri *et al*., 2003).

Fungal genera like *Aspergillus* and *Trichoderma* are taught to be cellulase producers and crude enzymes produced by these microorganisms are commercially available for agricultural use (Peig *et al*., 1998).The microorganism which appear to be most promising at present are *Trichoderma* mutant (Jecu *et al*., 2000), Shazia Shafique *et al*., 2009 studied evaluation of cellulase production potential by *Trichoderma* strains, in particular *T.viride, T.reesei* and *T.harzianum* available in First Fungal Culture Bank of Pakistan (FCBP). However, the cellulase systems of hypercellulase producing mutants of *Trichoderma reesei* are deficient in β-glucosidase (Duff *et al*., 1986).One of the problems related to the economic viability of the enzymatic hydrolysis of cellulose is due to low β -glucosidase levels (Umikalsom *et al*., 1997).Unlike *Trichoderma* Species *Aspergillus* Species exhibited high β -glucosidase activity (Wong Kok Mun *et al*., 2008).

There are several reports describing co-culturing of two cultures for enhanced cellulase production. Gupte,A., *et al*., 1997 cultivated two strains of *Aspergillus ellipticus* and *A.fumigatus* and reported improved hydrolytic and β-glucosidase activities compared to when they were used separately using SSF, improved enzyme titres were achieved by Kanotra,S., *et al*., 1995 when a mutant of *Trichoderma reesei* was co-cultured with a strain of *Pleurotus sajor caju* with wheat straw as the substrate.

Fungi was isolated and identified from soil and decomposing orange peels. Out of thirteen isolates, three highest producers of enzymes were isolated and identified as *Penicillium atrovenetum, Aspergillus flavus and Aspergillus oryzae*, these isolates were used to ferment orange peels in solid state fermentation. Maximum production was given by *A.oryzae* (Adebace Johnson Adeleke et al., 2012).

Stoilova. I.S *et al*., 2005 studied the enzyme production of two novel mixed cultures of mycelia fungi *Thermoascus aurantiacus* and *Aspergillus niger* in solid state fermentation. Enzymatic hydrolysis of palm oil effluent solid using mixture of locally isolated fungi *Aspergillus niger EB5* and *Trichoderma sp.EB6* was reported by Wong Kok Mun *et al*., 2008.They also observed effect of substrate pretreatment, different ratio of cellulase mixture and incubation pH on the enzymatic hydrolysis of POME solids. Ikram-ul-Haq *et al*., 2005 reported cotton saccharifying activity of cellulases obtained from mono and co-culture fermentation of *A.niger* and *Trichoderma viride.* Four carbon sources i.e. CMC, cellulose powder, wheat bran and rice bran were used as substrates, among them cellulose powder was having highest cotton degrading ability (963 U/h/L) after 72 hrs, compared to wheat bran (657 U/h/L).

Local isolated fungal cultures including *A.niger, Fusarium oxysporum, Fusarium avenaceum and Cephalosporium acremonium* were employed for cellulase production. Wheat straw was used as carbon source and *Aspergillus niger* was chosen on the basis of best mean cellulase activity (H.H.Azzaz et al., 2011), Hussein Azzaz Murad et al., 2013 studied production of celulase under optimum conditions using *Aspergillus flavus NRRl 5221* utilizing agricultural waste as carbon source.

Omojasola.P.F *et al*., 2008 studied cellulase production from cellulosic pineapple waste using *Trichoderma longibrachiatum*, *Aspergillus niger* and *Saccharomyces cerevisiae*. The amount of glucose produced was optimized by varying the fermentation parameters: time, pH, substrate concentration, inoculum size and temperature. Among three cultures, *Trichoderma longibrachiatum* found to highest producer of glucose (0.92 mg/0.5 ml) at pH 4.5 and temperature of 45 ºC on day 7 of fermentation.

Optimization of the media components for cellulase production using *Trichoderma reesei*

was carried out. Optimization of cellulase production using pine apple waste as substrate was performed with statistical methodology (P.Saravanan et al., 2013).

Omajasola. P.F *et al*., 2008 studied production of cellulase from the orange peel using *Trichoderma longibrachiatum*, *Aspergillus niger* and *Saccharomyces cerevisiae* after treatment with alkali and steam. The activity of the test organisms cellulase against CMC on the orange wastes was also reported. *T.longibrachiatum* (3.86 mg $ml^{-1}$), *A.niger* (2.94 mg $ml^{-1}$) and *S.cerevisiae* (2.30 mg $ml^{-1}$) glucose amounts from orange pulp.

Muhannad I. Massadeh *et al*., 2001 used sugarcane bagasse as substrate with *Trichoderma reesei* QM9414 and *Aspergillus terreus SUK-1* to produce cellulase and reducing sugars. The percentage of substrate degradation achieved employing mixed culture was 26% compared to 50% using separate cultures of two moulds.

Haque *et al*., (1989, 1990, 1991) have reported the production of cellulase by *Aspergillus* and *Penicillium* by solid substrate fermentation using different cellulosic biomass. Of all the substrates however, enzymes production by all the cultures were maximum with wheat bran. The parameters such as depth of wheat bran, partial replacement of wheat bran by different agricultural by products selection of diluents and extraction of the enzymes from the fermented substrate, were optimized.

Rajesh Singh *et al*., 2009 reported cellulase production using rice straw as substrate by fungal strain *Aspergillus heteromorphus*. The RSM methodology was used for optimization of cellulase production.

Immanuel, G *et al*., 2007 studied the cellulase enzyme production ability of fungal strains *Aspergillus niger* and *Aspergillus fumigatus* against the bio wastes, coir waste and saw dust as carbohydrate source at varying environmental parameters of pH and temperature. Partial purification of the cellulase enzyme was reported using Poly Acrylamide Gel Electrophoresis (PAGE) showed that *A.niger* grown in coir waste supplemented media had two protein bands with the molecular weight of 36 and 23 kDa respectively and *A.fumigatus* grown in the same medium had two protein bands with molecular weight of 32 and 21 kDa respectively.

Cellulase enzyme production was studied by Charitha Devi.M et al., 2012 using fungal strain *Aspergillus niger* against the lignocellulosic bio wastes like sawdust, paper cellulose at varying environmental parameters. Sharada.R et al., 2012 studied production of cellulase using *Trichoderma reesei* and *Aspergillus niger* using black gram husk and green gram husk and reported maximum cellulose production using green gram husk as substrate in solid state fermentation by *Aspergillus niger*.

Ikram-ul-Haq *el al*., 2006 described the production of cellulolytic enzymes (CMC-ase, FP-ase, β-glucosidase) and hemicellulolytic enzyme (xylanase) along with total extracellular protein by *Aspergillus niger* and *Trichoderma viride* using submerged fermentation.

Debing Jing *et al*., 2007 studied optimization of cellulase inoculent mixture, to improve woody lignocellulosic hydrolysis using pea shrub woody biomass with cellulase inoculent mixture consisting of *Trichoderma koningii, Aspergillus niger*, *Lactobacillus* and observed that *Lactobacillus* inoculent resulted in lactic acid inhibitory effect on further improvement of cellulase hydrolysis and crude protein content in SSF fermentation.

Bokhary, H.A *et al.,* 1994 reported that, *Aspergillus* was a predominant genus, among 61 fungal species isolated as cellulose degraders, from rhizosphere soil mycoflora of Alfalfa, Date palm and Grape wine. The production of cellulase was examined by cleared zone technique.

Ajayi. A.A *et al*., 2007 described the effects of some chemicals and cations on the activity of partially purified cellulase from tomato fruits deteriorated by *Aspergillus flavus Linn*. The enzyme was partially purified by a combination of ammonium sulphate precipitation, molecular exclusion chromatography and ion exchange chromatography. They observed that uninfected tomato fruits did not possess detectable cellulase whereas tomato fruits infected with *Aspergillus flavus* have appreciable quantity of cellulase activity. The production of cellulase by *A.niger* on three different carbon sources was compared by Gautam. S.P. et al., 2010

Umar Dahot. M *et al*., 1996 made tests to utilize wheat straw as a carbon and energy source for the growth of *Aspergillus fumigatus* and production of cellulases. They observed that the maximum production of β- glucosidase and CM-cellulase was achieved by *Aspergillus fumigatus* grown on $H_2SO_4$ and HCl pretreated wheat straw substrate in comparison to $HNO_3$ and $HClO_4$ pretreated wheat straw.

*Aspergillus flavus* can be grown on different substrates such as bagasse, corncob and saw dust, but saw dust pretreated with caustic soda gave the best result with an enzyme activity value of 0.0743 IU/ml as reported by Ojumu *et al*., 2003. Acharya, P.B., *et al*., 2008 studied factors relevant for the improvement of enzymatic hydrolysis of saw dust pretreated

with NaOH using *Aspergillus niger*. Cellulase activity obtained was around 0.0925 IU/ml at 120 rpm after 96 hrs incubation period.

Fade, M., 2000 studied the production physiology of endoglucanase, exoglucanase and β-glucosidase on radicle waste medium by *Aspergillus niger* F-119 under condition of SSF. Cellulase production from radicle waste was markedly affected by different parameters such as moisture level, incubation period, initial pH, incubation temperature and inoculum size.

Rita Pye *et al*., 2003 studied conditions of the biosynthesis of pectinolytes, cellulolytes and hemicellulases by the filamentous fungi *Aspergillus niger* IBT-90 by a mathematical activity of factorial planning and gradient optimization. They found that the process optimization led to a three fold increase in the activity of pectinolytic enzymes and the double the activity of cellulolytic enzymes and xylanase.

King.K.W *et al*., 1963 studied distinctive properties like ultraviolet spectra, pH-activity responses, substrate specificities, thermal stabilities, and kinetic changes in the viscosity of substrate, adsorption characteristics on cellulose and exclusion characteristics on dextran gels of β-glucosidases and related enzymes from *Aspergillus niger*. Effect of pH on hydrolysis of carboxymethyl cellulose and cellohexaose was compared and suggested that a negative charge center on the substrate has pronounced inhibitory effect on the enzymes.

Gokhan Coral *et al*., 2002 studied some properties such as molecular weight, optimum pH, temperature and heat stability of carboxymethyl cellulase of new mutant strain of *Aspergillus niger* Z10 a cellulase producer. The crude enzyme preparation was subjected SDS-PAGE to determine the homogeneity and molecular weight of the enzyme. During the electrophoresis two bands were observed showing cellulolytic activity, the molecular weights of these proteins was calculated to be 83,000 and 50,000.These enzymes were not having ability to hydrolyse insoluble microcrystalline cellulose (Avicel) but active towards carboxy methyl cellulose.

Jun-ichi Abe *et al*., 1999 reported purification and characterization of endo-β-1,4-glucanase and β-glucosidase from cellulolytic fungus *Aspergillus* sp. K-27 by ion exchange chromatography and affinity chromatography. The molecular weight of endo-β-1,4-glucanase was calculated to be 21,000; the molecular weight of β-glucosidase was calculated to be 1,30,000 and 1,05,000 suggesting that the β-glucosidase was a hetero dimer enzyme.

1`Milala, M.A., *et al*., 2005 evaluated cellulosic agricultural wastes such as millet, guinea corn straw, rice husks and maize straw as suitable substrates for the production of cellulase by *Aspergillus niger*. Maize straw moistened with diluents containing mineral salts plus basal salt solution gave the highest cellulase activity of 102 (IU/ml) at about 72 hrs of fermentation.

Narasimha, G., *et al*., 2005 reported effects of nutrient on cellulase production by *Aspergillus niger* in submerged fermentation. Cellulase production by *Aspergillus niger* on three media (minimal, basal and Czapek-Dox) in liquid shake were compared. Czapek-Dox medium was found to be superior for the growth and cellulase production by *A.niger*. They also studied effects of different carbon and nitrogen sources to Czapek-Dox medium on cellulase production. On defined media carboxymethyl cellulose and $NaNO_3$ was found to best carbon and nitrogen sources respectively.

Paul, L., *et al*., 1976 studied mechanism of action of cellulase and purification, physico-chemical and enzymatic properties of 1,4-β-glucan glucanohydrolase (3.2.1.4) from *A.niger*. They observed that the enzyme was rich in acidic and aromatic amino acids and kinetic studies gave pK values between 4.2 and 5.3 for groups involved in the enzyme-substrate complex. The molecular weight of the enzyme calculated was 26,000, and cellulase was found to be stable to heat treatment at pH 8.0. The mode of action and substrate specificity of a cellulase purified from *A.niger* was reported by Paul. L. Hurst *et al*., 1977. The specificity region of cellulase was five glucose units in length, indicating cellulase is an endoglucanase.

Gretty K. Villena *et al*., 2006 studied the production of lignocellulolytic enzymes (cellulase, endoglucanase and xylanase) of *A.niger* in submerged fermentation (SmF) and biofilm fermentation (BF) cultures. Maximal filter paper activity, endoglucanase and xylanase activities was higher in BF (2.96, 4.7, and 4.61 IU/ml respectively) but biomass yields was higher in SmF (0.431 g $g^{-1}$). Sharada.R et al.,2009 studied optimization of cultural conditions for cellulase production by *Aspergillus niger* (MTCC 2196) using submerged fermentation.

**Solid state fermentation**

The term solid state fermentation (SSF) denotes cultivation of microorganisms on solid, moist substrates in the absence of free aqueous phase. The possible advantages of the biotechnological uses of SSF relative to those of classical submerged fermentation (SmF), different aspects of the two, include: a) historical, b) biological, c) ecological, d) engineering and e) economical differences.

### A. Historical aspects

When compared with the SSF processes that have long been established in Asian countries, SSF in Western countries appears to still be in its infancy. SSF is the state of the art technology that is used in many applications in the food industry in Asia, i.e. SSF is used in the production of enzyme rich Koji (which is made of rice and uses *Aspergillus* sp. as an enzymatic starter for different hydrolytic processes), in the saccharification of rice used for the production of alcoholic beverages such as sake, in the production of cellulase, which uses *Trichoderma* and *Aspergillus* strains. In Asia, enzymes and metabolites are commonly produced on a large scale by SSF processes that may be thousands of years old. Today, SSF has been nearly completely abandoned because of the pressure of increasing industrial rationalization and standardization.

### B. Biological aspects

SSF processes simulate the living conditions of many higher filamentous fungi: Ascomycetes, basidiomycetes and deuteromycetes developed in terrestrial habitats on wet substrates. Studies of the production of fungal enzymes in SSF have shown that SSF, in comparision with SmF, provides higher volumetric productivities, is less prone to problems with substrate inhibition and yields enzymes with a higher temperature or pH stability. Also, the fermentation time may be shorter and the degradation of the enzymes by undesirable proteases is minimized (Holker.U *et al*., 2004).

### C. Ecological aspects

Ecological advantages of SSF reflect the fact that the processes are conducted in the absence of a free aqueous phase. Because the SSF processes are performed at water activities below 1, the growth of contaminating bacteria and yeasts is minimized. Which means that, in certain cases, energy and instrumentation demanding sterilization processes can be eliminated. Another additional environmentally friendly feature of SSF is, in many cases it can use agricultural wastes as carbon and energy sources. The production of enzymes or organic acids makes frequent use of plant remnants as carbon sources and inducers or mediators.

### D. Engineering aspects

The most serious reasons why SSF has not yet found a broad use in western countries are engineering problems, the low amenability of the processes to standardization and the limited reproducibility of the results.

### E. Economic aspects

Calculation of the costs of production of cellulase, the economical efficiency is higher by a factor of 100 than in the case of SmF (Tengerdy,R.P *et al*., 2003). This striking difference has several reasons: much cheaper growth substrates, minimized requirements for sterility and low requirements for instrumentation and equipment.

SSF hence offers advantages over fermentation in liquid broth (SmF) like higher product yield, better product quality, cheaper product recovery and cheaper technology (Oguntimein *et al*., 1992). Microbial degradation of the biological wastes is a natural process that has occurred since the on set on earth. In fermentation processes microorganisms utilize the wastes as potential energy source for synthesis of very useful products such as enzymes (Kishwar Hayat *et al*., 2001).

Solid state fermentation processes are distinct from submerged fermentation (SmF) culturing, since microbial growth and product formation occurs at or near the surface of the solid substrate particle having low moisture contents. Solid state fermentation (SSF) holds tremendous potential for the production of enzymes. It can be of special interest in those processes where the crude fermented products may be used directly as enzyme sources.

Tengerdy, R. P., *et al*., 1996 compared cellulase production in SmF and SSF techniques. While the production cost in crude fermentation by SmF was about $ 20/kg, by SSF it was only $ 0.2/kg. Nigam, P., *et al*., 1994 have reviewed processing of agricultural wastes in SSF systems for cellulolytic enzyme production. They also enumerated advantages of cellulase production together with the factors affecting the cellulase production in SSF systems.

Elisashvili, V.I., *et al*., 1998 studied on the lignolytic system of *Cerrena unicolor* 062 a higher basidiomycete upon supplementation of the medium with carbon sources and phenolic compounds in SSF system; it was observed that the growth of *C.unicolor* 062 could be regulated by the exogenous addition of these compounds. The efficiencies of the degradation of cellulose and lignin were dependent on the nature and concentration of the compounds added.

Solid state fermentation of palm kernel cake has been carried out by Mohd Firdaus Othman et al., 2013 using locally isolated *Rhizopus oryzae ME 01*.

Sun, T., *et al*., 1997 developed a novel fed batch SSF process for cellulase production which could overcome the problems associated with high initial nutrients concentration while

retaining advantages from the high total effective salt concentration.

In significant finding, Smits, J.P., *et al*., 1996 reported that glucosamine level of the fungi in liquid culture could not be used to estimate the biomass contents in SSF. They studied the SSF of wheat bran by *T.reesei* and reported that using glucosamine, correlation between the fungal growth and respiration kinetics could only partly be described with the linear growth model of Pirt.

Shi – hao Zhao et al., 2011 reported production of cellulose in solid state fermentation using Trichoderma reesei SEMCC – 3.217 strain with water hyacinth. Fractional factorial design showed that, the addition amount of wheat bran, ammonium sulphate, calcium chloride and Tween 80 had significant effect on cellulase production.

Ikram ul Haq *et al*., 2006 studied the exploitation of agricultural by products like wheat bran, wheat straw, rice bran, rice straw and soybean for the production of industrially important enzyme Cellulases by SSF using locally isolated *Trichoderma harzianum*. Of all the substrates wheat bran was found to be best substrate for production of cellulase. Kishwar Hayat *et al*., 2001 reported kinetics of cellulase and Xylanase production by culturing *Chaetomium thermophile* on wheat straw by SSF. They observed that the enzyme activity was affected by fermentation period, and found maximum at 72 hrs.

Sathyavarathan .P. et al., 2013 studied cellulase production using *Trichoderma reesei NCIM – 1052* and *Aspergillus niger NRRL – 322*. Maximum cellulase activity reported was 217.17 U ml $^{-1}$ using *T.reesei*.

Jian Liu *et al*., 2007 studied cellulase production in SSF using the waste from the vinegar industry as the substrate for *Trichoderma koningii* AS3 4262. The effects of water content, initial pH value in solid substrate and culture temperature on cellulase synthesis was observed for optimal production in flask fermentors.

Benkun Qi *et al*., 2007 reported production of cellulases from *T.viride* using SSF by different ratios of rice straw and wheat bran as substrate. It was observed that activities of Filter paper enzyme, Endoglucanase and β-glucosidase were significantly affected by the substrate mixture.

Henkelekian *et al*., (1925) have shown that *Trichoderma* and *Penicillium* possess the ability to decompose cellulose completely, with $CO_2$ as the only waste product. They also observed a direct correlation between the amount of nitrogen transformed into ammonia by species of *Penicillium* and *Trichoderma* and the amount of cellulose decomposed.

Mandels and Reese (1957) produced cellulose by growing *Trichoderma viride* and observed that it was influenced by carbon source and metals. Cellulase was an adaptive enzyme in cultures of *T.viride*. It was produced on cellulose, lactose, glucose and cellobiose, but not on a wide variety of other substrates. Trace elements such as iron, manganese and zinc or cobalt were best for cellulose production.

*Trichoderma viride* can be grown on different substrates such as avicel, rice straw, filter paper, wheat straw, but wheat bran moistened with dilutents containing mineral salts plus CMC gave the best yield as reported by Haq *et al*., (1989, 1991a, 1991b).

Dissolving cellulose pulp, newspaper cellulose, avicel and cellophane are more reactive towards hydrolysis with cellulose of *T.viride*. Cellophane, coated on one side with nitrocellulose, would be hydrolysed to glucose in high yields in a reasonable time. Toymao *et al*., (1978) reported that in case of solid cultures, stronger enzyme activities were produced than were produced in the submerged culture. Chand *et al*., (1990) evaluate the agro-residues and grass as carbon source for cellulose production. Agro cultural wastes were used as carbon source for the production of cellulose. These wastes were subjected to NaOH treatment and fermentation parameters with *T.reesei* were detected. Properly treated cellulosic waste promises to be a reasonable substrate for cellulase production by *T.reesei*.

Tappa Mohammad Munawar et al., 2011 investigated cellulase production in solid state production using *T.reesei NCIM 1186* by Antigonum leptopus leaves as substrate gave maximum cellulosic activity.

Cellulase was produced from corn straw by solid state fermentation with the help of *T.reesei*. The corn straw was supplemented with wheat bran for the supply of nitrogen and carbon, by Wang, J., *et al*., 2006. They also studied the optimal conditions like temperature, pH, and water contents of substrate. Cellulase production studies was carried out using fungal strain *Trichoderma reesei NCIM 992* by using three different lignocellulosic materials by solid state fermentation and reported maximum production using wheat bran yielding 2.63 U ml $^{-1}$ during incubation time of 6 days(Devendra p. Maraya et al., 2012).

Muthuvelayudham, R., *et al*., 2007 used RSM to evaluate the effects of the medium parameters on cellulase production by using *T.reesei* Rut C30 and to evaluate the kinetic model for attaining a higher cellulase yield in SSF. Maryam Latifian *et al*., 2007 used RSM to evaluate the effects of fermentation parameters for cellulase

production by *T.reesei* QM 9414 and *T.reesei* MCG77 in SSF using rice bran as substrate.

H.H. Azzaz et al., 2012 studied cellulase production by locally isolated fungal cultures including *A.niger*, *Fusarium oxysporum*, *Avenaceum and Cephalosporium acrenonium* using wheat straw as a sole of carbon source and reported that the highest cellulase production obtained from *A.niger*.

Ben Faber *et al*., 2003 studied differences in the production of cellulase from different mulching materials in a field setting using SSF. Organic mulches are found to control root rot of avocado, caused by *Phytophthora cinnamomi.* The possible mechanism for this control was enzyme production specifically cellulase and glucanase.

Solid state fermentation of lignocellulosic material oil palm biomass generated from palm oil industries as waste was used for cellulase production through *T.harzianum* in lab scale by Md.Zahangir Alam *et al*., 2005. The parameters glucosamine and reducing sugar was observed to evaluate the growth and substrate utilization.

Shahera, H., *et al*., 2002 used SSF to evaluate the possibility of re-use of orange peel and pulp wastes as a source of enzymes production; cellulase, amylase, pectinases, lipases, esterases and peroxidases. Microorganisms were isolated from fermented waste and tested for their enzymes production. Maximal production of cellulase and lipase was reported by the use of orange peel waste with yeast strain.

The effects of glucose, crystalline cellulose and saw dust of *Mitragyna cilata* on the growth and cellulase production, inferred from cellulase activity of *Penicillium chrysogenum* PCL501 was studied (Nwodo-Chinedu *et al*., 2007). Saw dust is reported as a good inducer of cellulase activity in the organism.

There are several reports indicating production of cellulases in SSF using various substrates with *Aspergillus* sp. (Debing Jing *et al*., 2007; Omajasola, P.F., *et al*., 2008; Acharya, P.B., *et al*., 2008; Milala, M.A., *et al*., 2005; Fadel, M., 2000; Umar Dahot, M., *et al*., 1996, Jahir Alam Khan et al., 2011, Umbrin Ilyas et al., 2011,sharada.R et al., 2012).

**Table 3: Various strains and substrates used for cellulase production**

| Strain | Substrate | Reference |
|---|---|---|
| *T.koningii* | Saw dust, wheat bran | Arima, K., *et al*., 1964 |
| *T.reesei* | Wheat bran | Mudgett, R.E., *et al*., 1982 |
| *T.reesei QMY-1* | Wheat straw | Chahal, D.S., *et al*., 1985 |
| *Sporotrichum pulverulentum* , *T.reesei* | Wheat bran | Kim, J.H., *et al*., 1985 |
| *T.harzianum* | Wheat straw, Wheat bran | Deschamps, F., *et al*., 1985 |
| *Strains of Basidiomycetes* | Bagasse | Nigam, P., *et al*., 1986 |
| *Trichoderma sp. Botritis sp.* | Wheat bran, rice straw | Shamla, T.R., *et al*., 1987 |
| *Polyporus sp.* | Bagasse | Nigam, P., *et al*., 1987 |
| *Neurospora crassa* | Wheat straw | Macris, B.J., *et al*.,1987 |
| *A. heteromorphus* | Wheat straw | Anitha Singh *et al*., 2009 |
| *A.niger* | Wheat bran | Talukdar, S., *et al*., 1992 |
| *T.viride, A.niger* | Wheat bran, bagasse, rice and wheat straw, | Ikram ul Haq *et al*., 1992 |
| *Cerrena unicolor* | Grape wine trimming dust | Zakariasvili, N.G., *et al*., 1993 |
| *Penicillium citrinum* | Rice husk | Kuhad, R.C., *et al*., 1993 |
| *Mesophilic fungal strain* | Rice husk | Begum, A.A., *et al*., 1993 |
| *A.heteromorphus* | Rice Straw | Rajesh Singh et al., 2009 |
| *Cerrena,unicolor, Coriolus hircutus, Pleurotus ostreatus* | Tea production waste | Kokhreidze, N.G., *et al*., 1993 |
| *A.niger* | Coconut coir pith | Muniswaran, P., *et al*., 1994 |
| *Aspergillus sp.* | Bagasse, Wheat bran, rice bran | Gupte, A., *et al*., 1994 |
| *Lentinus edodus* | Wheat straw | Giovannozzisermanni, G., *et al*., 1994 |
| *T.reesei, A.niger* | Sweet sorghum silage | Castillo, M.R., *et al*., 1994 |
| *Trichoderma reesei+Aspergillus phoenicis* | Bagasse | Duenas, R., *et al*., 1995 |
| *Phanerochaete chrysosporium* | Soyhull | Jha, K., *et al*., 1995 |
| *T.reesei* | Paddy straw | Kanotra, S., *et al*., 1995 |
| *Gliocladium sp. Trichoderma sp. Penicillium sp.* | Sweet sorghum silage | Szakaca, G., *et al*., 1996 |
| *T.harzianum* | Cassava waste | Onilude, A.A.,*et al*., 1996 |
| *T.reesei* | Steam pretreated willow | Reczey, K., *et al*., 1996 |
| *A.fumigatus* | Wheat straw | Umar Dahot, M., *et al*., 1996 |
| *A.niger* | Palm oil mill waste | Prasertsan, P.,*et al*., 1997 |
| *P.sajor caju* | Sago hampas | Kumaran, S., *et al*., 1997 |
| *Cerrena unicolor* | Grape wine cutting waste | Elisashvili, V.I., *et al*., 1998 |
| *Bacillus subtilis* | Banana wastes | Chundakkadu Krishna 1999 |

| | | |
|---|---|---|
| *A.niger* | Radicle waste (malt manufacture residue) | Fadel, M.,2000 |
| *T.reesei QM9414, A.terreus SUK-1* | Sugarcane bagasse | Muhannad I. Mussadeh *et al*., 2001 |
| *A.flaves, Nigrospora sp.* | Orange waste | Shahera H. Attyia *et al*., 2002 |
| *Phytophthora cinnamomi* | mulch | Ben Faber *et al*., 2003 |
| *A.flavus Linn NSPR 101* | Saw dust, bagasse and corn cob | Ojumu *et al*., 2003 |
| *Bacillus subtilis* | Banana stalk | Shafique, S.,*et al*., 2004 |
| *A.niger KK2* | Rice straw, Wheat bran | Kang, S.W., *et al*., 2004 |
| *T.harzianum* | Wheat straw, Wheat bran | Deschamps, F., *et al*., 2004 |
| *T.harzianum* | Oil palm biomass | Md.Zahangir Alam *et al*., 2005 |
| *A.niger MSK-7,T.viride MSK-10* | Wheat bran | Ikram ul Haq *et al*., 2005 |
| *Thermoascus aurantiacus, A.niger* | Oats straw, Wheat bran | Stoilova, I.S., *et al*., 2005 |
| *A.niger* | Millet, guinea corn straw, rice husk and maize straw | Miala, M.A., *et al*.,2005 |
| *A.candidus* | Rice husk, millet straw, guinea corn stalk and saw dust | Milala, M.A., *et al*., 2009 |
| *Thermoascus aureantiacus miche* | Wheat bran, sugarcane bagasse, orange bagasse, corn cob, green grass, dried grass, saw dust and corn straw | Robutoda Silva *et al*., 2005 |
| *A.niger, T.viride* | Rice bran, wheat bran, cotton | Ikram ul Haq *et al*., 2005 |
| *T.reesei* | Sugarcane bagasse, rice straw | Muthuvelayudham,R., *et al*., 2006 |
| *T.harzianum* | Wheat bran,wheat straw, rice bran, rice husk and soybean | Ikram ul Haq *et al*., 2006 |
| *T.reesei LW1* | Corn straw | Wang.J.Sh *et al*., 2006 |
| *Scopulariopsis* | Rice bran | Bharathi Kodali *et al*., 2006 |
| *A.niger,A.terreus and Rhizopus stolonifer* | Cassava waste | Pothiraj, C.,*et al*., 2006 |
| *Penicillium echinalatum* | Bagasse, wheat bran | Camassola, M., *et al*., 2007 |
| *T.koningiiAS3 4262* | Wheat bran, vinegar waste | Jian Liu *et al*., 2007 |
| *T.viride ZY-01* | Rice straw, wheat bran | Benkun Qi *et al*., 2007 |
| *T.reesei QM9414, T.reesei MCG77* | Rice bran | Maryam Latifian *et al*., 2007 |
| *Humicola insolens TAS-13* | Sugarcane bagasse | Muhammad Mohsin Javed *et al*., 2007 |
| *Penicillium chrysogenum PCL 501* | Saw dust | Nwado-Chinedu *et al*., 2007 |
| *T.koningii. A.niger, Lactobacillus* | Pea shrub biomass | Debing Jing *et al*., 2007 |
| *A.niger Eb5, T.sp.EB6* | Palm oil mill effluent | Wong Kok Mun *et al*., 2008 |
| *T.longibrachiatum, A.niger, Saccharomyces cerevisae* | Orange waste | Omajasola, P.F., *et al*., 2008 |
| *T.longibrachiatum, A.niger, Saccharomyces cerevisae* | Pine apple waste | Omajasola, P.F., *et al*., 2008 |
| *A.niger* | Saw dust | Acharya, P.B., *et al*., 2008 |
| *Penicillium roqueforti* | Pumpkin oil cake | Draginja pericin *et al*., 2008 |
| *A.niger* | Green gram husk | Sharada.R et al., 2012 |
| *A.niger* | Corn cob | Jahir Alam Khan et al., 2011 |
| *A.niger* | Vigna mungo | Umbrin Ilyas et al., 2011, |
| *T.reesei NCIM 1186* | Antigonum leptopus leaves | Tappa Mohammad Munawar et al., 2011 |
| *T.reesei NCIM 1052* | Ground nut | Satyavarathanetal.,2013 |
| *T.reesei SEMCC* | Water hyacinth | Shi hao Zhao et al., 2011 |
| *Rhizopus oryzae ME01* | Palm kerne cake | Mohd.Firdaus Othman et al., 2013 |

**CONCLUSION**

cellulases were produced by SmF and SSF using various bacterial and fungal strains. Development of an economical process for cellulase production is hindered because of the high costs of substrate (pure cellulose) and of some chemicals, such as proteose peptone, and also because of low yields of cellulases per unit of cellulose. To overcome these bottlenecks, cheap source of cellulose; lignocelluloses, agricultural wastes are used in SSF.

The microorganisms which appear to be most promising at present are *Aspergillus sp.* and *Trichoderma sp.* However, it is of interest to examine *Aspergillus sp.* to improve cellulase production which is a known good producer of cellulases (Jecu *et al*., 2000, Sharada.R. et al., 2012).

Many researches have been conducted on enzymatic hydrolysis of various lignocellulolytic substrates like Pumpkin oil cake, Saw dust, Pine apple waste, Orange waste, Palm oil mill effluent, pea shrub biomass, Sugarcane bagasse, Rice bran, Rice straw, wheat bran, vinegar waste, Cassava waste, Corn straw, wheat straw, rice husk, soybean, cotton, corn cob, green grass, dried grass, Millet, Oats straw, Oil palm biomass, Banana stalk, mulch, Radicle waste (malt

manufacture residue), Sago hampas, Grape wine cutting waste, Steam pretreated willow, Sweet sorghum silage, soyhull, Paddy straw, Coconut coir pith, Tea production waste, Grape wine trimming dust, green gram husk utilized cellulases from bacterial and fungal strains.

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