PRODUCTION OF CELLULASE – A REVIEW

R. Sharada¹, G. Venkateswarlu², S. Venkateshwar³ and M. Anand Rao⁴

¹Government Junior College (G), Sangareddy, Dist.Medak, Andhra Pradesh, India.
²Bharat Heavy Electronics Limited, R.C.Puram, Hyderabad, Andhra Pradesh, India.
³University College of Technology, O.U, Hyderabad, Andhra Pradesh, India.
⁴University College of Science, O.U, Hyderabad, Andhra Pradesh, India.

ABSTRACT
Cellulases are the enzymes which hydrolyze 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 α – 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 (SSF) generally includes C₁ (EC 3.2.1.9), (exo-1, 4-β-D-glucanase, cotton lyase), C₃ (EC 3.2.1.4), (endo-1, 4 - β-D-glucanase, carboxymethyl cellulase, or CMC ase), C₅ (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 C₁ and Cₓ, the cellulose system also contains cellobiase, which eventually breaks down cellobiose, the building unit of cellulose, to glucose.

**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 (Arrifin. 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 *Chuchinsonii* were demonstrated on different substrates (Clifford Louime et al., 2006)

*Acetivibrio cellulolyticus* 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-glucaanse (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.,...
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 hydrolyze 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.
**Fungi**

These organisms grow under suitable conditions to produce cellulase; e.g. *Trichoderma viride* and *Trichoderma reesei* produce active cellulase when grown in solid submersed culture. *Aspergillus niger* produces highly active cellulase when grown in liquid media by both surface and submersed 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 was 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 triiensis* 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 *Stachybotrys, Pestalotia, Palmarum, Merulius, Lneryms, 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 paradoxa* 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 cellulose biomass to fermentable sugars needs economical process for the production of cellulases. Soil is inhabited 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), Goodard (1913), Dale (1914), Werkentin (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; McCleary 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, 2012, Shazia Shafique et al., 2009), Trichothecium 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

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pesalottia</td>
<td>Metha et al., 1975</td>
</tr>
<tr>
<td>Stachy</td>
<td>Mandels et al., 1976</td>
</tr>
<tr>
<td>Botryosphaera</td>
<td>Shoemaker et al., 1978</td>
</tr>
<tr>
<td>Polyspores</td>
<td>Hyashida et al., 1980</td>
</tr>
<tr>
<td>Palmarum</td>
<td>Kassim, 1983</td>
</tr>
<tr>
<td>Neuraspora</td>
<td>Adikanae et al., 1983</td>
</tr>
<tr>
<td>A. ustus</td>
<td>Macris et al., 1985</td>
</tr>
<tr>
<td>Merulius</td>
<td>Takao et al., 1985</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>Heptinstall et al., 1986</td>
</tr>
<tr>
<td>A. heteromorphus</td>
<td>Anitha Singh et al., 2009, Rajesh Singh et al., 2009.</td>
</tr>
<tr>
<td>A. terreus</td>
<td>Araujo et al., 1986</td>
</tr>
<tr>
<td>A. aculeatus</td>
<td>Murao et al., 1988</td>
</tr>
<tr>
<td>T. aureoviride</td>
<td>Mercedes Zaldivar et al., 2001</td>
</tr>
<tr>
<td>A. flavus</td>
<td>Ojumu et al., 2003</td>
</tr>
<tr>
<td>Scopulariopsis</td>
<td>Bharathi Kodali et al., 2006</td>
</tr>
<tr>
<td>P. chrysogenum</td>
<td>Nwodo-chinedu et al., 2007</td>
</tr>
<tr>
<td>T. viride</td>
<td>Benkun Qi et al., 2007, Shazia Shafique et al., 2009.</td>
</tr>
<tr>
<td>Trichothecium roseum</td>
<td>Prashanth shanmugam et al., 2008</td>
</tr>
<tr>
<td>A. wentii</td>
<td>Panda et al., 1987; Srivastava et al., 1984</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>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.</td>
</tr>
<tr>
<td>Alternaria alternate</td>
<td>Bailey et al., 1988; Macris, 1984</td>
</tr>
<tr>
<td>A. japonicus</td>
<td>Sharma et al., 1985; Sanyal et al., 1988</td>
</tr>
<tr>
<td>A. niger</td>
<td>Gokhale et al., 1984; McCleary et al., 1988; Okada, 1989; Poutanen et al., 1984; Jan-ichi Abe et al., 1999; Milala, M.A. et al., 2005; Narasimha, G. et al., 2006; Shazia Shafique et al., 2009.</td>
</tr>
<tr>
<td>A. candidus</td>
<td>Milala, M.A. et al., 2009</td>
</tr>
<tr>
<td>Acrophialophora maniana and</td>
<td>Barros R R. et al., 2010</td>
</tr>
<tr>
<td>Ceratoctys parasordaxa</td>
<td>Chinnarjan Ravindran et al., 2010</td>
</tr>
<tr>
<td>Chaetomium sp. NIOCC 36</td>
<td>Deswal D. et al., 2011</td>
</tr>
<tr>
<td>Fomitopsis sp. RCK 10</td>
<td>A. oryzae (Adebace Johnson Adeleke et al., 2012)</td>
</tr>
<tr>
<td>A. flavus NRRL5521</td>
<td>Hussein Azzaz Murad et al., 2013</td>
</tr>
</tbody>
</table>
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 Cel lulase. 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 Tharzi um 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 Trichod erma Species Asper gillus 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 atrventen um, 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).

Stolova. LS 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 cellulase under optimum conditions using Aspergillus flavus NRRI 5221 utilizing agricultural waste as carbon source.

Omojasola.P.F et al., 2008 studied cellulase production from cellulotic 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 °C on day 7 of fermentation. Optimization of the media components for cellulase production using Trichoderma reesei
was carried out. Optimization of cellulase production using pineapple waste as substrate was performed with statistical methodology (P. Saravanan et al., 2013). Omajiasola, F.F et al., 2008 studied production of cellulase from the orange peel using *Trichoderma longibrachiatus*, *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.longibrachiatus* (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.

Muhammad I. Massadeh et al., 2001 used sugarcane bagasse as substrate with *Trichoderma reessei* QM9414 and *Aspergillus terreus SUK-I* 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, Cori 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 *Aniger* 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 cellulase production using green gram husk as substrate in solid state fermentation by *Aspergillus niger*. Ikram-ul-Haq et 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 *Aniger* 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 H₂SO₄ and HCl pretreated wheat straw substrate in comparison to HNO₂ and HClO₄ 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 cellobiose 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 cellulolytic 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 NaN03 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 comparison 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 cellulase 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 products 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 – I052 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 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. Henkeleikian et al., (1925) have shown that Trichoderma and Penicillium possess the ability to decompose cellulose completely, with CO₂ 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 diluents 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 cellulotic 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 cellulotic 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 MC G77 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 acremonium 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 citata 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>
<thead>
<tr>
<th>Table 3: Various strains and substrates used for cellulase production</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strain</strong></td>
</tr>
<tr>
<td>T. koningii</td>
</tr>
<tr>
<td>T. reesei</td>
</tr>
<tr>
<td>T. reesei QMY-1</td>
</tr>
<tr>
<td><em>Sporotrichum</em></td>
</tr>
<tr>
<td><em>purpureulentum</em></td>
</tr>
<tr>
<td><strong>Strains of Basidiomycetes</strong></td>
</tr>
<tr>
<td>Polyporus sp.</td>
</tr>
<tr>
<td>Neurospora crassa</td>
</tr>
<tr>
<td>A. heteromorphus</td>
</tr>
<tr>
<td>A. niger</td>
</tr>
<tr>
<td>T. viride, A. niger</td>
</tr>
<tr>
<td><strong>Cerrena unicolor</strong></td>
</tr>
<tr>
<td><strong>Penicillium citrinum</strong></td>
</tr>
<tr>
<td>Mesophilic fungal strain</td>
</tr>
<tr>
<td>A. heteromorphus</td>
</tr>
<tr>
<td>Cerrena unicolor, Cortolaus hircatus, Pleurotus ostreatus</td>
</tr>
<tr>
<td>A. niger</td>
</tr>
<tr>
<td>Lentinus edodes</td>
</tr>
<tr>
<td><em>Trichoderma</em> reesei+Aspergillus phoenicis*</td>
</tr>
<tr>
<td><strong>Phanerochaete chrysosporium</strong></td>
</tr>
<tr>
<td>T. reesei</td>
</tr>
<tr>
<td><em>Gliocladium</em> sp.</td>
</tr>
<tr>
<td>T. reesei</td>
</tr>
<tr>
<td>A. jumigatus</td>
</tr>
<tr>
<td>A. niger</td>
</tr>
<tr>
<td>F. sutor caju</td>
</tr>
<tr>
<td><strong>Cerrena unicolor</strong></td>
</tr>
<tr>
<td><strong>Bacillus subtilis</strong></td>
</tr>
</tbody>
</table>

ISSN: 2249-9504

1080
<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neura bicuscula</td>
<td>Sugarcane bagasse</td>
<td>Muhammad I. Mussadeh et al., 2001</td>
</tr>
<tr>
<td>A. fives, Nigrospora sp.</td>
<td>Orange waste</td>
<td>Shahera H. Attyia et al., 2002</td>
</tr>
<tr>
<td>Phytophthora cinnamomi</td>
<td>Mulch</td>
<td>Ben Faber et al., 2003</td>
</tr>
<tr>
<td>A. flavus Linn NSPR 101</td>
<td>Saw dust, bagasse and corn cob</td>
<td>Ojumu et al., 2003</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>Banana stalk</td>
<td>Siafuque, S. et al., 2004</td>
</tr>
<tr>
<td>A. niger K52</td>
<td>Rice straw, Wheat bran</td>
<td>Kang S.W., et al., 2004</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>Wheat straw, Wheat bran</td>
<td>Deschamps, F., et al., 2004</td>
</tr>
<tr>
<td>A. niger MSK-7, T. viride MSK-10</td>
<td>Wheat bran</td>
<td>Ikrar ul Haq et al., 2005</td>
</tr>
<tr>
<td>Thermoascus aureantiacus, A. niger</td>
<td>Oats straw, Wheat bran</td>
<td>Stoilova, I., et al., 2005</td>
</tr>
<tr>
<td>A. niger</td>
<td>Millet, guinea corn straw, rice husk and maize straw</td>
<td>Miala, M.A., et al., 2005</td>
</tr>
<tr>
<td>A. acidus</td>
<td>Rice husk, millet straw, guinea corn stalk and saw dust</td>
<td>Miala, M.A., et al., 2009</td>
</tr>
<tr>
<td>Thermoascus aureantiacus miche</td>
<td>Wheat bran, sugarcane bagasse, orange bagasse, corn cob, green grass, dried grass, saw dust and corn straw</td>
<td>Robutoda Silva et al., 2005</td>
</tr>
<tr>
<td>A. niger, T. viride</td>
<td>Rice bran, wheat bran, cotton</td>
<td>Ikrar ul Haq et al., 2005</td>
</tr>
<tr>
<td>T. reesei</td>
<td>Sugarcane bagasse, rice straw</td>
<td>Muthuvelayudham, R., et al., 2006</td>
</tr>
<tr>
<td>T. harzianum</td>
<td>Wheat bran, wheat rice bran, rice husk and soybean</td>
<td>Ikrar ul Haq et al., 2006</td>
</tr>
<tr>
<td>T. reesei LW1</td>
<td>Corn straw</td>
<td>Wang J., et al., 2006</td>
</tr>
<tr>
<td>Scopulariopsis</td>
<td>Rice bran</td>
<td>Bharathi Kodali et al., 2006</td>
</tr>
<tr>
<td>A. niger, A. terreus and Rhizopus stolonifer</td>
<td>Cassava waste</td>
<td>Pothiraj, C., et al., 2006</td>
</tr>
<tr>
<td>Penicillium eichiaum</td>
<td>Bagasse, wheat bran</td>
<td>Camassola, M., et al., 2007</td>
</tr>
<tr>
<td>T. koningiA3 4262</td>
<td>Wheat bran, vinegar waste</td>
<td>Jian Liu et al., 2007</td>
</tr>
<tr>
<td>T. viride ZY-01</td>
<td>Rice straw, wheat bran</td>
<td>Benkun Qi et al., 2007</td>
</tr>
<tr>
<td>T. reesei QM9414, T. reesei MCG77</td>
<td>Rice bran</td>
<td>Maryam Latifian et al., 2007</td>
</tr>
<tr>
<td>Humicola insolens TAS-13</td>
<td>Sugarcane bagasse</td>
<td>Muhammad Mohsin Javed et al., 2007</td>
</tr>
<tr>
<td>Penicillium crysophenum PCL 501</td>
<td>Saw dust</td>
<td>Nwado-Chinedu et al., 2007</td>
</tr>
<tr>
<td>T. koningi, A. niger, Lactobacillus</td>
<td>Pea shrub biomass</td>
<td>Debing Jia et al., 2007</td>
</tr>
<tr>
<td>T. longibrachiatum, A. niger, Saccharomyces cerevisiae</td>
<td>Palm oil mill effluent</td>
<td>Wong Kok Mun et al., 2008</td>
</tr>
<tr>
<td>T. longibrachiatum, A. niger, Saccharomyces cerevisiae</td>
<td>Orange waste</td>
<td>Omajasola, P.F., et al., 2008</td>
</tr>
<tr>
<td>A. niger</td>
<td>Pine apple waste</td>
<td>Omajasola, P.F., et al., 2008</td>
</tr>
<tr>
<td>T. longibrachiatum, A. niger, Saccharomyces cerevisiae</td>
<td>Pine apple waste</td>
<td>Omajasola, P.F., et al., 2008</td>
</tr>
<tr>
<td>A. niger</td>
<td>Saw dust</td>
<td>Acharya, P.B., et al., 2008</td>
</tr>
<tr>
<td>Penicillium roqueforti</td>
<td>Pumpkin oil cake</td>
<td>Draginja peric et al., 2008</td>
</tr>
<tr>
<td>A. niger</td>
<td>Green gram husk</td>
<td>Shafiq, S. et al., 2012</td>
</tr>
<tr>
<td>A. niger</td>
<td>Corn cob</td>
<td>Jahir Alam Khan et al., 2011</td>
</tr>
<tr>
<td>A. niger</td>
<td>Vigna mungo</td>
<td>Umbrin Ilyas et al., 2011</td>
</tr>
<tr>
<td>T. reesei NCIM 1186</td>
<td>Antigonum leopopus leaves</td>
<td>Tappa Mohammad Munawar et al., 2011</td>
</tr>
<tr>
<td>T. reesei NCIM 1052</td>
<td>Ground nut</td>
<td>Satyavarathan et al., 2013</td>
</tr>
<tr>
<td>T. reesei SEMCC</td>
<td>Water hyacinth</td>
<td>Shi hao Zhao et al., 2011</td>
</tr>
<tr>
<td>Rhizopus oryzae ME01</td>
<td>Palm kerne cake</td>
<td>Mohd.Firdaus Othman et al., 2013</td>
</tr>
</tbody>
</table>

**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 protease 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.

ACKNOWLEDGEMENTS
The authors are thankful to research scholars K.Swathi, J.Swarnalatha, Anamika, Neeraja for their friendly co-operation.

REFERENCES
10. Azhari Samsu Baharuddin, Razak, Mohamad Nafis Abd; Lim Slong Hock; Ahmad, Mohd Najib; Abd-Aziz, Suraini; Rahman, Nor’Aini Abdul, Md Shah, Umi Kalsom; Hassan, Mohd Ali; Sakai, Kenji; Shirai, Yoshitomo., 2010. Isolation and Characterization of Thermophilic Cellulase-Producing Bacteria from Empty Fruit Bunches-Palm Oil Mill Effluent Compost. American Journal of Applied sciences, 7(1): 56
16. Bharathi Kodali and Ravindra Pogaku., 2006. Pretreatment studies of rice bran for the effective production of
cellulase. EJEAF Che., 5(2): 1253-
1264.
61. Henkelekian, H., Dondero, N.C., 1925. Principles and applications in aquatic


