CHAPTER II

LITERATURE REVIEW

Biotechnology is finding its claims in various industrial domains like health care, crop production and agriculture, non-food uses of crops and other products (e.g. biodegradable plastics, vegetable oil, bio fuels etc.) and environmental uses. In view of rapidly growing economic demand, it is imperative to exploit all indigenous resources at their maximum to attain the goal. The production of value added products from agro-industrial wastes can diminish to some extent the cost of production (Durrani et al., 2002).

Huge amount of agro-based wastes/by products are engendered every year over the globe and their improper controlling causes environmental pollution. Being a co-partner the food industry is contributing a significant share in producing agro-based biological wastes. The elimination of wide range of pollutants and wastes is an absolute requirement to encourage a sustainable and friendly environment. Industrialization contributed a considerable share to the environment as far as initiation of pollutants and environmental toxins are concerned. Biotechnology plays a major role in the elimination of contaminants and is taking advantage of the greater adaptability of microorganisms to degrade/convert such compounds (Masse et al., 2008).

2.1 Importance and need for cellulase enzyme

The growing use of enzymes to replace traditional chemical transformation processes is obsessed by an objective for better production economics, new product functionalities, improved safety, and an increasing desire to reduce the environmental pollution. Often, chemical transformations produce non-specific reactions that may result in poor product yields and by products that are difficult and costly to dispose. The processes involving organic solvents, acidity or alkalinity at high temperatures and pressures need expensive, specially designed equipment and control systems. Public, regulatory and private industrial forces are stimulating for their substitutes. All of these drawbacks can potentially be eliminated using enzymes. Industrial enzymes fit into this category, as enzymes after reaction, become inactive, and break down into simple and nontoxic component. They are increasingly being used in industrial process development and manufacturing, thereby avoiding the hazardous chemical and toxic loads often put into the environment by traditional
methods. To meet this increasing demand for enzymes, most new enzymes are produced from fungal or bacterial kingdom grown in large-scale fermenters using agro-industrial waste products (Cherry et al., 2001).

In recent years, more attention is given to the process of biodegradation of cellulose to soluble sugar (Chen et al., 2004). The advent of biotechnology, specifically fungal biotechnology, with its inexpensive mode of application, has been used as tool for the effective conversion of agro-based wastes into certain valuable products (Ahuja et al., 2004). In the last few decades, there is a mounting drift to produce different types of enzymes by using such cellulosic materials as substrates with the aid of microorganisms. Fabrication of these enzymes is valuable as such enzymes in turn can be used in the processing of various foods.

2.2 Cellulase

Cellulase production in fungi is found to be extra cellular and has three components such as endoglucanase (endo-1, 4-β-D-glucanase, EC 3.2.1.4), exoglucanase (exo-1, 4-β-Dglucanase, EC 3.2.1.91) and β-glucosidases (1, 4-β-Dglucosidase, EC 3.2.1.21). Cellulases are inducible enzymes which are synthesized by microorganisms during their growth on cellulosic materials (Lee et al., 2010).

Cellulase degrades cellulose to yield glucose and other soluble sugars which can be used either in juice liquefaction or as fuel. Higher saccharification on efficiency, mild operating conditions with respect to pH and temperature, absence of by products and avoidance of pollution makes enzymatic hydrolysis superior over chemical processes in industry. Therefore, using cellulosic waste as substrate rather than expensive pure cellulose is better economically viable strategy. Cellulases are capable of the extensive solubilisation of highly ordered form of cellulose and are reported to be produced from well-known microbial sources such as aerobic and anaerobic fungi (Onsori et al., 2005). The cost of enzyme production can be significantly reduced if low value biological substrates like fruit processing waste (Kojiam et al., 2000) are used.

There are reports of using various agriculture waste including rice bran (Lee et al., 2010), wheat straw (Singh et al., 2009), cassava (Pothiraj et al., 2006) banana waste and food process waste like oil palm (Alam et al., 2005) and apple waste (Hang et al., 1994) as
substrate for cellulase production. Banana waste which is cellulose is use by different research but activity is very low and it was found because pH is alkaline at normal condition (Baig et al., 2005).

They are studied extensively due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars. This serves as a raw material in the production of chemicals and fuel. Cellulases have a wide range of industrial applications. The main application of these enzymes in textile, paper and pulp, food and animal feed, fuel and chemical industry, demand highly stable enzymes, able to excel at extreme conditions of pH and temperatures. Additionally, they can be used in waste management, pharmaceutical industry, protoplast production, genetic engineering and pollution treatment (Bhat et al., 2000).

2.3 Submerged fermentation

Majority of industrial enzymes are produced by large-scale submerged fermentation. Agro-industrial wastes can eventually be used as substrate and act as good sources of carbon and nitrogen. This involves growing selected microorganism in closed vessels in which all the conditions critical for growth are carefully controlled. Selected microorganisms are either bacteria (*Bacillus* species) or fungal (*Aspergillus* or *Trichoderma* species) that have been carefully chosen and optimized. Generally, these organisms secrete the enzymes directly into the growth medium from where they can be purified by filtration and centrifugation (Ali et al., 1991).

2.4 Microorganisms producing cellulase

During the past few decades, many researchers worked on isolation, characterization, estimation and application of cellulases from fungal and bacterial sources. The history of cellulose degradation began with the understanding the role of *Trichoderma* in cellulase production. The fungus *Trichoderma reesi* is traditionally used by various enzyme manufacturers to produce fungal cellulases. It is one of the best characterized fungal cellulase systems. The fungus produces large amount of different cellulases that act synergistically for complete hydrolysis of crystalline cellulose to glucose. The fungus produces at least three different types of endoglucanases, two different cellubio hydrolases and beta glucosidases (Berhardseiboth et al., 2006). The filamentous fungus *Trichoderma reesei* is a paradigm for commercial scale production of different cellulases and hemicellulases and is well adapted to
fermenter cultivations. Beside well established applications of these enzymes in pulp, paper, food, feed or textile processing industries, these plant cell wall degrading enzymes are also employed for the saccharification of cellulosic plant biomass to simple sugars for biofuel production (Bouws et al., 2008; Kumar et al., 2008). The cellulyolytic potential of this pantropical fungus was already recognized during WWII through the deterioration of cotton fabrics of the US Army. Strain QM6a (originally named *T. viride*) was isolated from the cotton canvas of an army tent from Bougainville Island (Solomon Islands). After identification of the fungus as the cause for the massive destruction, it was put under quarantine in the eponymous quarter master collection of the US army at Natick. Strain QM6a was later recognized as an own species and named after its principal investigator in those years Elwyn T. Reese (Reese, 1976). Lignocellulose is a mixture of cellulose associated with hemicellulose, lignin, pectin and other substances in minor amounts. It is the most abundant renewable biomass, produced by the photosynthesis of plants directly from CO$_2$ and accumulates to approximately 200 billion metric tons worldwide. The complex structure of this solid and heterogeneous substrate hampers an efficient conversion to simple sugars and presents a number of technical and economic challenges in bringing cellulosic biofuels to the market. One of the major economic barriers for the production of biofuels is the intrinsic recalcitrance of lignocellulosic plant matter (Himmel et al., 2007).

*Aspergillus sp.* is another important soil fungus having a great potential of producing a range of primary and secondary metabolites like citric acid, gluconic acid, cellulases and other enzymes. *Aspergillus sp.* is used as an organism source by many researchers for the media optimization and fermentation of cellulases family. Researchers have taken different kinds of substrates for designing of media protocols for higher yield. *Aspergillus sp.* is basically group of nine genera according to fungal taxonomists, and among these genera some are having great potential of enzyme production. For the production of cellulases wheat bran, (Kang et al., 2004) wheat straw, rice straw, corn cob (Abostate et al., 2010), cotton flower shell, groundnut shell, wheat and sorghum straw (Mohite et al., 2010), water hyacinth blend (Usama F. Ali et al., 2008) saw dust (Acharya et al., 2008) and so many type of substrates are used by researchers. (Abostate et al., 2010) reported that after isolation of twenty nine fungal strains from agricultural waste of *Aspergillus* species five potent strains were tested for solid state fermentation with standard control strain of *Trichoderma viride*. Their findings shows that isolated strain MAM-F23 gave the highest carboxy-methyl
cellulase production on rice straw i.e. 309 U/ml. They further suggest that the highest cellulases were produced on wheat straw and lowest on corn cob.

Primarily carbohydrate degraders are cellulolytic microbes and are generally unable to use proteins or lipids as energy sources for growth (Lynd et al., 2002). Cellulolytic microbes, bacteria, and most fungi can utilize any carbohydrates including cellulose (Rajoka et al., 1997), while the anaerobic cellulolytic species have a restricted carbohydrate range, to cellulose and or its hydrolytic products. The ability to secrete large amounts of extracellular protein is characteristic of certain fungi and such strains are most suited for production of higher levels of extracellular cellulases.

Most commonly studied cellulolytic organisms include: Fungal species- Aspergillus (Milala et al., 2005; Oshoma et al., 2005; Juwaied et al., 2010), Trichoderma (Vintila et al., 2010; Juwaied et al., 2010), Fusarium (Ramanathan et al., 2010), Bacteria- Bacilli (Femi-Ola et al., 2008; Shabeb et al., 2010), Pseudomonads (Bakare et al., 2005), Acinetobacter (Ekperigin et al., 2007) and Actinomycetes- Streptomyces, Actinomucor, and Thermoactinomyces (Aboul-Enein et al., 2010). Although several fungi can metabolize cellulose as an energy source, only few strains are capable of secreting a complex of cellulase enzymes, which could have practical application in the enzymatic hydrolysis of cellulose. Besides T. reesei, other fungi like Humicola, Penicillium and Aspergillus have the ability to yield high levels of extracellular cellulases. Aerobic bacteria such as Cellulomonas, Cellovibrio and Cytophaga are capable of cellulose degradation in pure cultures (Lynd et al., 2002).

However, the microbes commercially exploited for cellulase preparations are mostly limited to T. reesei, H. insolens, A. niger, Thermomonosporafusca, Bacillus sp. and few other organisms.

2.5 Utilization of Agro-industrial wastes for cellulase production

Agro-horticulture and industrial by product which are rich in lignocelluloses has enormous potentials for enzyme production. Their transformation into useful products may better the environmental problems. Some agro-wastes like cereal straw, leaves corncobs etc (Shafique et al., 2004; Shabab et al., 2010) and horticulture food process waste (Rashad et al., 2009) and other cellulose rich waste like paper, coir and wood extract waste (Siham et al.,
In most parts of the country, these materials are mainly used as animal feeds or cause environment pollution. A large quantity is left on farmlands to be decomposed by microorganism such as bacteria (Chen et al., 2004; Femi-Ola et al., 2008) and fungi (Milala et al., 2005; Narasimha et al., 2006; Juwaied et al., 2010).

Economically, the most important industrial material other than food stuffs affected by microorganisms is cellulose and wood products including the wood itself (Siham et al., 2007; Ojumu et al., 2003; Femi-Ola et al., 2008). Production of wood products such as pulp, paper, textiles from natural fibers such as cotton flax and jutes are enhanced by microorganisms specifically fungi. Cellulose which forms about 40-50% of plants composition is the most abundant organic matter on earth. Proper utilization of these wastes in the environment will eliminate pollution and convert them into useful by-products is a matter of concern.

Pineapple has the second highest production volume of all tropical fruits in the world. Costa Rica is one of the main producers and exporters, with approximately 110,000 acres of this fruit. Of all the fresh pineapple harvested, approximately 25% is processed to make added value products like concentrated juice, canned pineapple and jelly. From these processing activities, over 65% of the whole pineapple is unused, and big amounts of waste products are produced and need to be treated, turning into an economical and environmental problem for the producers. The use of pineapple waste as an inexpensive underutilized agricultural by-product for the biotechnological production of cellulase is an interesting option. This alternative process can reduce the cost of production of this cellulase and, moreover, can add value to an agro-industrial waste as high carbohydrate fermentable source. During pineapple processing, the crown and stem are cut off before peeling. The core is then removed for further processing. These wastes (peel, core, stem, crown and leaves) generally account for 50% (w/w) of total pineapple weight. Therefore, with increasing pineapple production, pineapple wastes are also proportionally increasing. Waste disposal represents a growing problem since it is usually prone to microbial spoilage and it causes serious environmental problems. The utilization of waste would be an innovation to handle the great deal of waste from processing. Pineapple wastes are found to have potential uses as raw materials that can be converted into value-added products. In agricultural, waste is occasionally utilized as a fertilizer or animal feed. The peel is a rich source of cellulose, hemicelluloses and other carbohydrates.
The core waste could be used for the production of frozen pineapple juice concentrates or extracted juice for alcoholic beverages or for vinegar. In addition, the waste from pineapple has been used as a nutrient substance in culture broth (Nigam et al., 2000) and cellulase production (Omojasola et al., 2008).

The food and agricultural industries produce large volumes of wastes annually worldwide, causing a serious disposal problem. This is especially problematic in countries where the economy is largely based on agriculture and where the farming practice is very intensive. Currently, these agro-wastes are either allowed to decay naturally on the fields or are burnt. However, these wastes are rich in cellulose due to their organic nature, are easily assimilated by microorganisms and hence, make them potential substrates for exploitation as raw materials in the production of industrially relevant compounds through microbial conversion. In addition, the reutilization of biological wastes is of great interest since, due to legislation and environmental reasons; the industry is increasingly being forced to find an alternative use for its residual matter (Rodríguez-Couto et al., 2008). One of the agro-wastes currently causing pollution problems is the peels of the mango fruit. Mango is one of the most important fruits marketed in the world with a global production exceeding 26 million tons in 2004 (FAOSTAT, 2004). It is cultivated or grown naturally in over 90 countries worldwide (mainly tropical and subtropical regions) and is known to be the second largest produced tropical fruit crop in the world (Joseph et al., 1997). The edible tissue makes up 33–85% of the fresh fruit, while the peel and the kernel amount to 7–24% and 9–40%, respectively (Wu et al., 1993).

In fact, mango peel as a by-product of mango processing industry could be a rich source of bioactive compounds, and enzymes such as protease, peroxidase, polyphenol oxidase, carotenoids, vitamins C and E, dietary fibers, enzymes and carbohydrate content of 20.80 – 28.20% in dry weight samples of mango peel. While the utilization of mango kernels as a source of fat, natural antioxidants, starch, flour and feed has extensively been investigated, studies on peels are scarce. Their use in biogas production or making of dietary fiber with a high antioxidant activity (Larrauri et al., 1997) has been described in the past. However, mango peels are not currently being utilized commercially in any way, though a large quantity is generated as waste (20 – 25% of total fruit weight) during mango processing thus, contributing to pollution (Berardini et al., 2005). Most studies on the exploitation of
mango peels have been dealing with their use as a source of pectin, which is considered a high quality dietary fiber (Pedroza-Islas et al., 1994; Tandon et al., 1999). Recently, a screening study of 14 mango cultivars had demonstrated the content and degree of esterification of mango peel pectins to range from 12% to 21% and 56% to 66%, respectively (Berardini et al., 2005). Furthermore, mango peels have been shown to be a rich source of flavonol O- and xanthone C-glycosides (Berardini et al., 2005), gallotannins and benzophenone derivatives (Berardini et al., 2004). However, reports on the use of mango peels for the production of industrially relevant metabolites such as cellulase through fermentation processes are rare. Thus, cultivation of microorganisms on these wastes may be a value-added process capable of converting these materials, which are otherwise considered to be wastes, into valuable products through processes with techno-economic feasibility.

Pomegranate (*Punicagranatum* L.) is an important fruit crop of tropical and subtropical regions of the world. Pomegranate fruit is consist of three parts: the seeds (about 3% of the weight of the fruit); the juice (about 30% of the fruit weight); and the peels which include the husk and interior network membranes (Prakash et al., 2011). This fruit is either consumed fresh or used in the juice industries. Increasing agro-industrial units for producing pomegranate juice leads to the accumulation of a new by-product, namely, pomegranate peel (Shabtay et al., 2008) usually huge amounts of this by-product produced in pomegranate producing regions and countries. Annual production of this by-product exceeds 120,000 metric tons in India (Mirzaei-Aghsaghali et al., 2011). If it cannot used by farmers and industries as well as medical activities cause serious environmental problems.

In an attempt, (Beldman et al., 1985) produced cellulolytic enzymes by *Aspergillus niger* in submerged culture with millet, guinea corn straw, rice husks and maize straw as substrates. Effects of factors, such as pH and substrate concentrations were reported. Optimal cellulase secretion by *Aspergillus niger* was achieved at a time (growth period) of 72 hours in maize straw and rice husk media. 96 hours and 120 hours were the growth period in millet and guinea corn straws respectively. Substrate concentration of five percent (5%) w/v and pH 3 resulted in optimal enzyme secretion.

The production of cellulases by *Aspergillus niger* AS 101 was carried out using 2% alkali treated corn cobs under various culture and environmental conditions. The maximum
cellulase production was observed after 7 days of incubation at pH 5 under continuous
shaking conditions (Singh et al., 1992).

In another study, ammonia treated bagasse (5%) was subjected to mixed culture
fermentation using *Trichoderma reesei* and *Aspergillus phoenicis* at 30°C with ammonium
sulphate as nitrogen source for cellulases production (Duenas et al., 1995). Significantly
higher activities of all the enzymes of cellulase complex were achieved in 4 days of mixed
culture fermentation than that in single (*T. reesei*) fermentation.

*Trichoderma reesei* is one of the best cellulase producers but has low potential for the
production of β-glucosidase. It has been reported earlier that the mixed culture of *T. reesei*
and *Aspergillus* can produce increased amount of β-glucosidase (Duenas et al., 1995).
Therefore, *Trichoderma reesei* LM-UC4 and its mutant LM-UC4E1 were co-cultured with
*Aspergillus phoenicis* QM329 for cellulase production on bagasse. A mutual synergism was
observed between the parent *Trichoderma* strain and *Aspergillus*, resulting in enhanced yields
of CMCase (115.3 IU/L/h; specific activity 73.8) as compared to the mutant strain i.e. 31.3
IU/L/h and specific activity, 20. For β-glucosidase as well, the parent strain and *Aspergillus*
gave better results (28.3 IU/L/h with specific activity 18.1) than mutant and *Aspergillus*
together i.e. 13.3 IU/L/h with specific activity 8.5 (Gutierrez-Correa et al., 1997).

With the objective to increase the yields of cellulase and xylanase, (Kim et al., 1997)
used different reactors to grow *Aspergillus niger* on rice-straw and compared the activities,
yields and productivities of both enzymes. In general, better yield and productivity was
observed in bubble-column and external-loop air-lift reactors. The highest yield and
productivity of β-glucosidase were 84 FPA IU/g and 9.7 FPA IU/l/h, obtained by fed-batch
mode in the bubble-column and in the stirred-tank reactor, respectively. In case of β-
glucosidase, highest yield and productivity were 370 U/g and 26 U/l/h in an external-loop air-
lift reactor.

Mahmood et al., (1998) investigated the possible use of orange and potato peels as
substrate for production of cellulase and also studied in terms of their chemical compositions
and their ability to support the growth and extracellular hydrolytic enzyme production of
*Bacillus subtilis*. The orange and potato peel substrates were prepared by blending and
removal of large particles by filtration. The chemical compositions of the filtrates were
similar to the crude peel ‘starting’ material and were shown to contain predominantly alcohol-insoluble solids (pectin, cellulose, and starch), soluble sugars and minerals (mainly Ca, K, P, and Si). The composition of the orange peel (substrate) was restraining to that of the potato peel (substrate) mainly in terms of low levels of starch and protein, and higher levels of sugars, pectin, and cellulose. Bacillus species 11089 was capable of growth in continuous culture on both orange and potato substrates when these were used as the carbon-energy source in a mineral salts basal medium. Potato filtrate supported the highest growth but lowest specific activities of cY-amylase, neutral and alkaline proteases, and polygalacturonate-lyase compared to orange filtrates substrate: however, when enzyme activity was expressed as units per volume of culture, potato filtrate supported the highest levels.

Van Wyk et al., (1999) studied the production of cellulase using used paper products. They produced cellulases from Penicillium funiculosum and Trichoderma reesei by saccharification of paper products such as foolscape paper, newspaper and microcrystalline cellulose. They found the cellulase concentration of 10.0 mg/mL for each cellulase system the strongest synergistic action was observed at a combination of 1:1 (m/m) during saccharification of all cellulose materials. The individual enzyme performance as well as their synergistic actions showed different rates of hydrolysis during degradation of the investigated cellulose substrates.

Xia Liming et al., (1999) produced cellulase using corn cob residue from xylose manufacture as substrate by Trichoderma reesei ZU-02. They found that on the same cellulose basis, the cellulase activity and yield produced on corn cob residue were comparable with that on purified cellulose. Under batch process, the optimum concentration of substrate was 40 g/l and the optimum C/N ratio was 8.0. In 500 ml flasks, cellulase activity reached 5.25 IU/ml (213.4 IU/g cellulose) after seven days’ cultivation. In a 30 m³ stirred fermenter for large scale production, cellulase and cellobiase activity were 5.48 IU/ml (222.8 IU/g cellulase) and 0.25 IU/ml (10.2 IU/ g cellulose), respectively, after four days’ submerged fermentation. The produced cellulase could effectively hydrolyze the corn cob residue, and the yield of enzymatic hydrolysis reached 90.4% on 10% corn cob residue (w/v) when the cellulase dosage was 20 IU/g substrate.

Dominguesa et al., (2000) used Trichoderma reesei Rut C-30, in submerged fermentation. They examined the influence of the size inoculum and the composition of the
fermentation medium on the morphology and cellulase production. Different inoculum sizes were studied but the significant change in fungus morphology was observed for spores concentration between $10^5$ and $10^7$ spores/ml (i.e. $10^2$ and $10^4$ spores/ml in pre-culture medium). In the medium without tween 80, at low inoculum size, the majority of the pellets were large and well individualized; in contrast, at higher inoculation densities small flocks were obtained, with higher production of soluble protein and higher filter paper activity. It was found that the average pellet size seems to be inversely proportional to the inoculum size. Medium composition, namely tween 80, also influences the morphology of *T. reesei* Rut Cut-30 and enzyme production. The presence of tween 80 in fermentation medium inhibited the pellet formation of this strain.

Shafique et al., (2004) used *Bacillus subtilis*, cultured in solid-state fermentation (SSF) of Banana stalk to produce exoglucanase. The fermented biomass was harvested after 72 h of SSF at pH 7 and temperature 35 °C. It was filtered and centrifuged at 10,000 rpm at −10 °C and supernatant was collected as crude enzyme extract. They found maximum activity of exoglucanase (3.48 IU/mL/min) was obtained from the medium fermented with 70% moisture content, 5 mL inoculum, 0.1% peptone, 0.4% yeast extract and 0.2% tween- 80 at pH 7 and temperature 35 °C.

Cellulase and hemicellulase enzymes were produced by *Trichoderma reesei* RUT C30 on steam pretreated spruce, willow, corn stover and delignified lignocellulose (SolkaFloc), as a reference. The enzymes produced were characterized by protein and various enzyme activity measurements. On steam pretreated corn stover higher cellulolytic enzyme activities were reached than on SolkaFloc, while the activities obtained on steam pretreated spruce and willow were considerably lower. The produced and two commercial cellulases were compared by determining specific activities. There were minor differences among the enzymes corresponding to their specific cellulase activities. In contrast, within hemicellulase and β-glucosidase activities, the differences were found to be more significant. It should be also noted that commercial cellulases had considerably higher specific acetyl xylan esterase activities than the produced enzymes. According to subsequent hydrolysis experiments, performed to characterize the produced enzyme complexes to evaluate their applicability for hydrolysis and enzyme production, it seems that the application of the enzyme that was produced on the same substrate as was used for hydrolysis can be advantageous in the case of some substrates. As a result, these experiments demonstrated that pretreated corn stover is a
good substrate both for enzyme production and hydrolysis, since high cellulolytic activities could be reached using it as carbon source. Moreover, high sugar yields could be obtained in the hydrolysis by the enzyme produced on steam pretreated corn stover (Juha’sz et al., 2004).

Xiao-Bin Yu et al., (2005) utilized a mutant strain Trichoderma reesei WX-112 and obtained high cellulase activity. The mutants ability to produce cellulase increased 1.95 times after the treatment with UV and N-methyl-N-nitrosoguanidine. Also, the medium composition was optimized using response surface methodology (RSM). A fractional factorial design was applied to elucidate the medium components that significantly affect cellulase production. They identified concentration of avicel and soybean cake flour in the medium was significant factors and the composition of fermentation medium optimized with response surface methodology.

Maryan Latifian et al., (2007) applied response surface methodology to evaluate the effects of fermentation parameters for cellulase production by Trichoderma reesei (QM9414 and MCG77) in solid state fermentation using rice bran as substrate. Initial pH, moisture content and temperature were optimized using filter paper activity as response. Statistical analysis of the results for T. reesei 9414 showed that only moisture content has significant effect on cellulase activity and had a linear effect on enzyme activity.

Benkun et al., (2007) investigated the production of cellulases from Trichoderma viride, by solid state fermentation (SSF) using different ratios of rice straw (RS) and wheat bran (WB) as substrate. The mixed support inoculated with spores was incubated under static conditions for 6 days and the enzyme extracts obtained at different time intervals were analysed. These results showed that the activities of Filter paper enzyme (FPase), Endoglucanase (CMCase) and β-glucosidase were significantly affected by substrate mixture. They found maximum activity of FPase and β-glucosidase were produced at 96 when the ratio of RS and WB is 3:2 and 1:4, respectively.

Marcel Gutiérrez-Correa et al., (2007) investigated the lignocellulolytic enzyme production by Aspergillus niger and compared both in submerged fermentation (SF) and biofilm fermentation (BF) at various water activities. They found maximal filter paper activity, endoglucanase and xylanase activities were much higher in BF (2.96, 4.7 and 4.61 IU ml⁻¹, respectively) than in SF cultures (1.71, 1.31 and 2.3 IU ml⁻¹, respectively) but
biomass yields were lower in BF than in SF (0.338 g g\(^{-1}\) and 0.431 g g\(^{-1}\), respectively). In the presence of 20% ethylene glycol (\(a_w = 0.942\)) the enzyme activities decreased in both systems but BF still had higher levels (1.0, 1.0 and 2.6 IU ml\(^{-1}\), respectively) than SF cultures (0.6, 0.7 and 1.5 IU ml\(^{-1}\), respectively). An increase in xylanase specific activity of more than 2 fold (from 4.2 to 10.2 IU mg\(^{-1}\) biomass) was observed in the presence of 20% ethylene glycol, suggesting differential regulatory mechanisms in biofilm fermentation related to cell adhesion.

Zahangir Alam et al., (2008) investigated the production of cellulase enzyme by \textit{Trichoderma harzianum} in liquid state bioconversion using domestic waste water sludge (DWS) as substrate. They studied the effects of six factors, i.e., substrate and co substrate concentration, temperature, pH inoculum size and agitation rate. They found the optimum process conditions and were: temperature - 32.5 °C, substrate concentration - 0.75% (w/w), co substrate concentration - 2%, initial pH - 5, inoculum size - 2% and agitation - 175 rpm. Biodegradation of DWS was also evaluated to verify the efficiency of the bioconversion process as a waste management method.

Wen-Chien Lee et al., (2008) investigated the production of cellulases using alkaline pre-treated rice straw and non-pretreated rice straw with cellulase-producing strain \textit{Trichoderma reesei} Rut C-30. Effect of factors such as medium with concentrated KH\(_2\)PO\(_4\) and low concentration of KH\(_2\)PO\(_4\) were studied. The maximum enzyme productions of the alkaline pre-treated rice straw and non-pretreated rice straw were found to be 1.07 and 0.71 FPU/ml respectively.

Omojasola Folakemi et al., (2008) assessed the cellulase production from cellulosic pineapple waste using \textit{Trichoderma longibrachiatum, Aspergillus niger} and \textit{Saccharomyces cerevisiae}. The Fermentations were carried out in flasks containing the MSM, the waste substrate and the inoculum at pH 5.0, 1% substrate concentration, 10% inoculum size and cultured on a rotary shaker at 29 ± 1°C initially for 5 days to verify cellulase production by the organisms from the waste substrates, then for 7 days or 9 days while varying different fermentation parameters. Cellulase activity and amount of glucose produced by the three test organisms from the waste substrates were determined and compared. The amount of glucose produced was optimized by varying the fermentation parameters: Time, pH, substrate concentration, inoculum size and temperature. The results obtained from the fermentations
showed that *Trichoderma longibrachiatum* produced the highest amount of glucose among the cultures tested (0.92 mg/0.5 ml). This was produced from pineapple pulp at pH 4.5 and temperature of 45 ºC on Day 7 of fermentation. The highest amount of glucose produced by *Aspergillus niger* was also from pineapple pulp (0.63 mg/0.5 ml) at pH 3.5 and temperature of 40 ºC on Day 5 of fermentation. The highest amount of glucose produced by *Saccharomyces cerevisiae* was from pineapple pulp (0.54 mg/0.5 ml) at pH 4.5 and temperature of 45 ºC on Day 5 of fermentation.

Aftab Ahamed et al., (2008) conducted the hydrolysis experiment for the production of cellulase by co-culturing *Trichoderma reesei* and *Aspergillus niger* in a bioreactor to convert cellulose substrate into soluble sugars complete hydrolysis of cellulose. The aim of these works was to investigate an approach to enhance the production of these enzymes through a synergetic action of enzyme complex simultaneously produced by these two fungi. The experiments were conducted as fed batch growth on a Cellulose–Yeast extract medium. A mixture of lactose and lactobionic acid was added into the bioreactor as cellulase inducers. The maximum production of *Trichoderma reesei* is reached volumetric enzyme activity (98.4 UL⁻¹ h⁻¹), filter paper activity (7.1 UmL⁻¹), carboxymethyl cellulase activity (4.7 UmL⁻¹), soluble proteins (2.1 mgmL⁻¹), dry biomass (21.4 gL⁻¹), and percentage of utilized cellulose (89.4%) as compared with *A. niger* monocultures.

Usama F.Ali et al., (2008) grown two strains of *Aspergillus niger* and *Aspergillus nidulans* on water hyacinth (*Eichhorniacrassipes*, Martin). They prepared various blends of water hayacinth, Fortified with Czapeck-Dox in different ratios. It was found that Water hyacinth blend with Czapeck-Dox medium with 4:1 ratio reach its maximum concentration of cellulase enzymes.

Acharya et al., (2008) studied the hydrolysis of saw dust at various culture conditions. They found that in alkaline pretreated conditions (2 N NaOH) saw dust at 9.6% concentration gave the optimum yield value 0.1813 IU/ml cellulase activity. They collected the saw dust from saw mill near Gandhinagar, Gujarat, India. It was sieved by mesh no. 60. To make uniform particle size. The saw dust was pretreated with NaOH solution of variable concentration of range 1-5 N. solution incubated for 12 hours. They got maximum cellulase activity at 2N, NaOH (0.1813 I.U./ml). Later on 2N NaOH pretreated saw dust at different dust concentration 9 ranges (2.4-12%) in wet weight conditions were used and among these
range the maximum activity was recorded at 9.6% was 0.1813 IU/ml. The finding of Acharya et al. were comparatively promising with the earlier work reported by Ojummu et al., (2003). They reported that *A. Flavus* grown on saw dust produced highest cellulase activity 0.0743 IU/ml at 12 hours treatment of 3% saw dust.

Leonardo Faria Martins et al., (2008) compared *Penicillium echinulatum* and *Trichoderma reesei* cellulases in relation to the activity against various cellulosic substates. *Penicillium echinulatum* has been identified as a potential cellulase producer for bioconversion processes but its cellulase system has never been investigated in detail. In their work, the volumetric activities of *P. echinulatum* cellulases were determined against filter paper, carboxymethylcellulase, hydroxyethylcellulose, birch wood xylan, oat spelt xylan sigmacell type, cellobiose, and p-nitrophenylglucopiranoside. These values were then expressed in relation to the amount of protein and compared those of *Trichoderma reesei* cellulases.

Mohammad Sohail et al., (2009) collected 128 fungal stains from native environment of Karachi, Pakistan, from different sources like soil, plant material and spoiled juice. They screened these isolates for hydrolytic enzymes production and found that among these 128 strains of different genera of fungi majority of strains had shown the hydrolytic activity. *Aspergillus niger* group shown maximum output for hydrolytic activity. The production of endoglucanase and β – glucosidase was reported more than 1.5 IU/ml from those strains.

Sohail et al., (2009) have reported that all cellulase overproducing *Aspergillus* strains were from soil origin that indicates the property of degradation of biomass of *Aspergillus* group. Under the soil, Banana peel powder and coir powder, both bio wastes used as substrates by Usha Kiranmayi et al., (2011). They used solid substrate method for cellulase production and the higher values reported by them were 0.072 IU/ml for banana peel powder and 0.046 I.U. /ml for coir powder.

Patrick Vermette et al., (2009) reported that *Trichoderma reesei* was a good producer cellulase. They use fed-batch in a 7 L stirred tank bioreactor with four media with lactose and lactobionic acid. They found highest enzyme production with a volumetric enzyme activity of 69.8 U/ L, and a maximum fungal biomass of 14.7 g/L.
Rashid et al., (2009) studied to design a media composition to produce optimum cellulolytic enzyme where palm oil mill effluent (POME) as a basal medium and filamentous fungus, *Trichoderma reesei* RUT-C30 were used in the liquid state bioconversion (LSB). 2% (w/v) total suspended solid, TSS, of the POME supplemented with 1% (w/v) cellulose, 0.5 % (w/v) peptone and 0.02% (v/v) Tween 80 was estimated to produce the optimum CMCase activity of 18.53 U/ml through the statistical analysis followed by the faced centered central composite design (FCCCD). The probability values of cellulose (<0.0011) and peptone (0.0021) indicated the significant effect on the production of cellulase with the determination coefficient ($R^2$) of 0.995.

Anita Singh et al., (2009) investigated the production of cellulases from *Aspergillus heteromorphus*, by submerged fermentation using wheat straw as substrate. Influence and optimization of saccharification conditions like pH, temperature and time were studied. They found highest reducing sugar was released on 5th day at 5 pH, 30 °C temperature. When *A. heteromorphous* was grown on wheat straw in submerged fermentation after 5 days incubation at 30 °C, 3.2 IU/ml and 83 IU/ml, filter paper activity and CMCase activity respectively.

Vintila et al., (2010) used *Trichoderma* spores suspension obtained by washing the surface of cultures obtained with Mandels liquid medium by wheat bran as substrates. The liquid cultures were obtained by inoculation 50 ml Mandels media containing 1% cellulose in 300 ml flasks with 10% spores suspension of *Trichoderma*. The inoculated media were incubated in a water bath with shaker at 28°C, 180 r.p.m., for 21 days. Probes were harvested in regular basis to verify the purity of the cultures, development of fungi and cellulolytic activity.

Mohite et al., (2010) reported the potential of sorghum straw as a substrate for cellulase production. In submerged culture conditions, *Aspergillus niger* strain isolated from soil collected from Ankaleshwar-Gujrat, yields a maximum production of cellulase (0.77 units/ml). The lowest production was recovered on wheat straw medium i.e, 0.28 units/ml. These contrast finding suggest that the yield of cellulosases is depend on the potential of fungal strains and pretreatment to the media components.
Anita Singh et al., (2010) applied statistical design of experiments to plan experiments and optimize the microwave alkali pretreatment of rice straw and hulls. The influences of Process parameters important in pretreatment of biomass were identified by a Plackett–Burman design and the parameters with significant effects were optimized using a box–behnken design (BBD). Experimental results show that alkali concentration (AC), irradiation time (IT) and substrate concentration (SC) were main factors governing the saccharification of rice straw and hulls. Maximum enzyme productions at Optimum conditions of pretreatment were AC 2.75%, IT 22.50 min and SC 30 g/L, as optimized by BBD. The growth and production of lignocellulolytic enzymes from *Aspergillus heteromorphus*, solid state fermentation (SSF) was performed using rice straw and hulls pretreated under optimum conditions. Cellulases and xylanase reached the highest enzyme activity at 6th day of fermentation while maximum manganese peroxidase (MnP) and laccase activity occurred at 12th day.

Patrick Vermette et al., (2010) studied the production of cellulase using cellulose with *Trichoderma reesei* RUT-C30. Effect of factors such as mechanical agitation, a 35 L draft-tube airlift bioreactor equipped with a mechanical impeller was studied. They found Cultures carried out without mechanical agitation resulted in higher volumetric enzyme productivity (200 UL⁻¹ h⁻¹), filter paper activity (17 UmL⁻¹), cellulase activity (11.8 UmL⁻¹) and soluble proteins (3.2 mgmL⁻¹) when compared to those with agitation.

In another study cellulase was produced from *termittomycesclypeatus* with mustard stalk and straw (MSS). MSS with high cellulose and hemicellulose content was utilized as sole source of carbon by the fungus for productions of enzymes such as (CMcase, β-glucosidase, xylanase) in submerged fermentation. Production of enzymes were further increased by 2–10 folds on supplementation with common agro-residues such as wheat bran and rice straw (MWR) in 1:1:1 ratio and by using alkali treated MSS (TMSS). The results indicated that MSS can be used as a potential and cheap renewable raw material from India for production of bio-ethanol Swagata Pal et al., (2010).

Gautam et al.,(2010) studied the production cost of cellulase by optimizing the production medium and using an alternative carbon source such as municipal solid waste residue with the two cellulase producing fungi (*Aspergillus niger* and *Trichoderma sp.*) from
municipal solid waste. Municipal solid waste residue (4-5% (w/v)) and peptone and yeast extract (1.0% (w/v)) were found to be the best combination of carbon and nitrogen sources for the production of cellulase by *A. niger* and *Trichoderma* sp. They found optimum temperature and pH of the medium for the cellulase production by *A. niger* were 40°C and 6-7, whereas those for the production of cellulase by *Trichoderma* sp. were 45 °C and 6.5. Cellulase production from *A. niger* and *Trichoderma* sp. can be an advantage as the enzyme production rate is normally higher as compared to other fungi.

Majdinasab et al., (2010) used sugar beet pulp which has cellulosic components that can induce the production of cellulase when used as carbon sources for fungi growth. *T. reesei* and with the mutants *T. reesei* RUT-C30 and *T. reesei* QM 9414 having been identified as possessing improved filter paper activity. In this work *T. reesei* QM 9414 was grown in medium containing SBP as substrate. Both SBP and pure crystalline cellulose were used. It was found that these fungi could produce cellulase in medium containing SBP or pure crystalline cellulose. The pure crystalline cellulose resulted in a lower FPA than the SBP that indicates the increase in cellulose concentration causes a decrease in cellulase activity. The pattern of cellulase production indicated cellulase activity increased during the first four to five days, reached the maximum at day 5 and then decreased at the end of cultivation.

Hao Fang et al., (2010) studied enzymatic hydrolysis of steam exploded corn Stover (SECS). Two approaches, response surface methodology (RSM) and utilization of the cellulase from mixed cultures of *Trichoderma reesei* RUT Cut 30 and *Aspergillus niger* NL02. The RSM, the first approach, was consisted of Plackett–Burman Design (PBD) and Central Composite Design (CCD). After the optimization of RSM, a model was proposed to predict the optimum value 79.6% confirmed by the experimental result 80.1%. Mixed culture of *T. reesei* and *A. niger* was found to be an effective method to enhance cellulolytic enzymes production. Using the cellulase from mixed culture to optimize enzymatic hydrolysis was the second approach. The yield of 85.6% was obtained by the second approach using 25 IU/g glucanecellulase. The two approaches were compared and it was found that the second approach was a better one with higher hydrolysis yield and less enzyme dosage.

Jamal I. Daoud et al., (2010) identified the potential of low cost substrate such as Palm Oil Mill Effluent (POME) for the production of cellulase enzyme by liquid state bioconversion with the filamentous fungus *Trichoderma harzianum*. They studied the effect of parameters on production of cellulase in level fractional factorial design with six central
points. The maximum enzyme production (14 FPU mL\(^{-1}\)) was found to be at optimum conditions were: Temperature of 30 °C, substrate concentration of 2%, wheat flour concentration of 3%, pH of 4, inoculum of 3% and agitation of 200 rpm.

Jayant et al., (2011) tried to get cellulase production by inoculating strains of \textit{A.niger} and \textit{Pencillium chrysogenum} by co-culturing approaches of inoculation. In this new method for cellulase production they got cellulase production with maximum cellulase activity at solid state fermentation of 3.5 IU/ml on newspaper waste. This co-culturing approach gave higher production from the previous reports of solid state fermentation.

Aishwarya et al., (2011) studied the production of cellulase by submerged fermentation using cellulose as the substrate by \textit{Trichoderma reesei} in the form of slant culture. The influence of fermentation parameters like incubation period, temperature, pH and carbon source on cellulase production was examined. Cellulose in the form of filter paper was used as the substrate for the production of cellulase. They found optimized parameters for cellulase production is temperature 32 °C, incubation period of 8 days, pH of 7 and carbon source as 25% starch and 75% glucose cellulase produced using optimum parameters was purified using alcohol precipitation and ion exchange chromatography. From these studies, we are able to estimate the optimum fermentation parameters required for cellulase production and carry out its purification.

Satinder KaurBrar et al., (2011) used mixed cultures of \textit{Aspergillus niger} and \textit{Trichoderma reseei} for cellulase production on agricultural residues by solid substrate fermentation. The milled bagasse was treated with 1.2% NaOH. A mutual synergism was observed between the parent \textit{Trichoderma} strain and the \textit{Aspergillus}. It was observed that \textit{A. Phoenicis} boosted the cellulose production of \textit{Trichoderma} and which in turn enhanced biomass and β- glucosidase production of \textit{Aspergillus} resulting in increased activities of cellulase (38.3 IU/g DW of biomass), CMCase (210.9 IU/g DW of biomass) and β-glucosidase (93.0 IU/g DW of biomass). Such sort of synergism was absent in case of mutant \textit{Trichoderma} strain that may be attributed to loss of ability for cooperative interaction with other microbes as a consequence of hypermutation.

To assess the ability to produce cellulase by \textit{Aspergillus niger} on three different carbon sources were compared. Glucose containing media gave the highest mycelia weight of
1.294 mg/flask. Maximum cellulase enzyme activity (Filter paper activity, endoglucanase and β-glucanase) were obtained from the culture containing cellulose. They found waste cellulosic material can be used as low-cost carbon source for commercial cellulose production. (Sarsaiya et al., 2011). The production of cellulases by NCIM 992 was carried out using rice straw under various culture and environmental conditions. The maximum cellulase production 30.7 FPU/gds was observed after 7 days of incubation at pH 5 under solid state fermentation (Vimala Rodhe et al., 2011).

Tamires Carvalho dos SantosI et al., (2011) investigated the possible use of mango waste as substrate for production of cellulase. The effect of water activity and fermentation time with the fungus species *Aspergillus niger*. The maximum production of cellulase was found to be 7.26 U g⁻¹.

Somen Acharya et al., (2011) studied the effect of some nutritional and environmental factors on the production of cellulases, in particular endoglucanase (CMCase) and exoglucanases (FPase) from *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3 isolated from an Indian hot spring. The characterization study indicated that the optimum pH and temperature value was 6.5 to 7.0 and 50-55 °C, respectively. Maximum cellulases production by both the isolates was detected after 60 h incubation period using wheat and rice straw. The combination of inorganic and organic nitrogen source was suitable for cellulases production. Overall, FPase production was much higher than CMCase production by both of the strains. Between the two thermophiles, the cellulo-lytic activity was more in *B. licheniformis* MVS1 than *Bacillus* sp MVS3 in varying environmental and nutritional conditions.

Devendra P. Maurya et al., (2011) produced cellulase using the fungal strain *Trichoderma reesei* NCIM 992 by using three different lignocellulosic materials by solid state fermentation (SSF). The effect of basic fermentation parameters (pH, temperature, and moisture content, particle size of substrate and moistening agent) on enzyme production was studied. Maximum cellulase production was 2.63U ml⁻¹ using wheat bran as substrate. The optimal conditions for cellulase production for wheat bran were found to be: initial moisture content-70%, initial medium pH-5.0, temperature-30 °C, moistening agents (MSS) and particle size of substrate (500 micron meter). The optimal incubation time for production was six days. Results indicate the scope for further optimization of the production conditions to obtain higher cellulase titres using the strain under SSF.
Soma Mrudula et al., (2011) produced cellulose by *Aspergillus niger* in submerged (SmF) and solid state fermentation (SSF). They found maximum production of cellulase was obtained after 72 h of incubation in SSF and 96 h in SmF. The CMCase and FPase activities recorded in SSF were 8.89 and 3.56 U per g of dry mycelial bran (DBM), respectively. Whereas in SmF the CMase and FPase activities were found to be 3.29 and 2.3 U per ml culture broth, respectively. The productivity of extracellular cellulase in SSF was 14.6 fold higher than in SmF. Influence of physical and nutritional parameters of fermentation like pH, temperature, substrate, carbon and nitrogen sources were studied. They found optimal conditions for maximum biosynthesis of cellulase by *A. niger* to be at pH 6, temperature 30 ºC. The additives like lactose, peptone and coir waste as substrate increased the productivity both in SmF and SSF. The moisture ratio of 1:2 (w/v) was observed for optimum production of cellulase in SSF.

Anuradha Jabasingh et al., (2011) studied enhancement of the cellulase activity of *Aspergillus nidulans* by combinational optimization technique. The strain isolated from decayed, dry leaf of Ficuscaricus was compared for the first time for its ability to produce cellulolytic enzyme in submerged fermentation (SmF). The medium ingredients enhancing the cellulase production were optimized by combinational statistical approach by one factor at a time methodology (OFAT), Plackett Burmann methodology (PB) and response surface methodology (RSM). A four-factor-five-level central composite design (CCD) was employed to determine the maximum activity of cellulase at optimum levels of carboxy methylcellulose (CMC), ammonium nitrate and potassium dihydrogen phosphate at varying pH values. The optimum fermentation parameters were found to be 1.2 g/l CMC, 0.9 mg/l ammonium nitrate and 0.75 mg/l potassium dihydrogen phosphate at pH 6. The optimization of medium by combinational statistical approach led to the fine tuning of the cellulase production thereby enhancing the cellulase activity from 4.91 U/ml to 39.56 U/ml. The predicted results were in agreement with the actual experimental values. The cellulase activity obtained with this strain may be one of the best obtained in *Aspergillus nidulans*.

Anuradha Jabasingh et al., (2011) charity response surface methodology (RSM) to optimize the conditions for the production of endoglucanase, a component of cellulase by *Aspergillus nidulans* SU04 and *Aspergillus nidulans* MTCC344 under solid state fermentation, using pretreated bagasse as chief substrate. A four-factor-five-level central
composite design was engaged for the experimental design. The endoglucanase produced during the bioconversion of cellulose to glucose by these strains were powerfully dependent on the NaOH pretreatment given to bagasse before hydrolysis. Maximum cellulase activity was 32.59 U g⁻¹ and 28.96 U g⁻¹ (CMCase) for *A. nidulans* SU04 and *A. nidulans* MTCC344 respectively. The optimum conditions for cellulase production are 15 mm bagasse bed height, 60 % moisture content, pH 5 and temperature 40 °C in the solid state fermenter. *A. nidulans* MTCC344 and *A. nidulans* SU04 were able to hydrolyze pretreated sugarcane bagasse completely after 15 days and 6 days of incubation with significant endo-1,4glucanase activities. The results of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM) of bagasse showed structural changes through pretreatment, in favour of enzymatic hydrolysis. *A. nidulans* SU04 was found to be highly efficient compared to *A. nidulans* MTCC 344 in terms of endoglucanase, exoglucanase and glucosidase activities.

Cunha et al., (2012) utilized the sugarcane bagasse as substrate for cellulase production by *Aspergillus niger A12* was assessed by measuring endoglucanase activity using both sequential solid-state and submerged cultivation. An unconventional pre-culture with an initial fungal growth phase under solid-state cultivation was followed by a transition to submerged fermentation by adding the liquid culture medium to the mycelium grown on solid substrate. The results exposed the potential of sugarcane bagasse, as a low-cost substrate for the production of cellulase. Therefore, the methodology proposed here of a sequential fermentation process offers a promising alternative for cellulase production.

Tamires Carvalho dos Santos et al., (2012) analyses the effects of water content, temperature and time on the kinetic activity of cellulolytic enzymes produced during the solid state fermentation of potato peel, using *Aspergillus niger*. Three main analytical steps – analysis of variance, regression analysis and plotting of response surface – were performed to obtain an optimum condition for enzymatic activity. They found that statistical results indicated that the best activity time for enzyme CMCase (carboxymethylcellulase) is 82.88 h, with water content of 51.48% and temperature of 29.46 °C; for FPase (filter paperase), the best activity time is 80.62 h, water content of 50.19% and temperature at 30.00 °C; for xylanase, time is 81.92 h, water content is 50.72% and temperature is 28.85 °C. Pareto charts have shown that all variables were significant in enzymatic activity for CMCase and
xylanase. On the other hand, FPase shows that time and temperature has significant effect for this response variable.

Jagdish Singh et al., (2012) used bacterial strains for the production of cellulase enzyme. A total 30 bacterial isolates showed positive results for the cellulase production but highest enzyme activity was shown by isolate JS 14. From the morphological and biochemical reactions, the isolate was identified as Bacillus sp. Cellulase production was studied by this strain using response surface methodology (RSM).

Devendra Kumar et al., (2012) investigated the production of cellulase from Mango peel. The solid mango processing waste comprises 15-20% of total fruit weight. This, being a rich source of lignocelluloses, was used as substrate for carboxymethylcellulase (CMCase) production using Paenibacillus polymyxa, Maximum CMCase production (7.814 U mg⁻¹) was observed.