CHAPTER ONE

INTRODUCTION

Enzymes find lot of applications in the production of various useful commodities to meet human needs. The research work on enzyme production, its applications, identification and separation of novel enzymes are constantly increasing. Enzymes have become a part of everyone’s life because of its wide variety of applications in various industries like food industry, detergent industry, pharmaceutical industry and leather industry. Microbial enzymes are more useful than enzymes derived from plants or animals because of their variety of catalytic activities. Several other advantages include maximum yield of enzymes, ease of genetic manipulation for higher activity, rapid growth of microorganisms on inexpensive media and easy to isolate and purify. Microbial enzyme production is more convenient, safer and is more stable than the plant or animal enzymes (Wiseman, 1995).

The history of enzyme technology began in 1874 when the Danish chemist Christian Hansen produced rennet by extracting dried calves stomach with saline solution was the first enzyme used for industrial purpose. The fermentation using microorganisms was discovered in 18th century by French Scientist Louis Pasteur. In 1878 German physiologist Wilhelm Kuhne (1837 - 1900) coined the term enzyme from Latin words- which means “in yeast”. The first commercial enzyme trypsin was prepared by Rohm in Germany in 1914. Enzymes were used in 1930 in fruit juice manufacturing for clarification of juices. The major usage started in 1960 in starch industry. The first commercial bacterial protease was marketed in 1959 by Novozymes in Denmark and major detergent manufacturers started to use it around in 1965. Discovered in the latter half of the 19th century as an effective biocatalyst especially in the field of protein engineering & genetics, enzymes found their way in several new industrial processes (Hoondal et al., 2002). Industrial biocatalysis is widely viewed as the ‘third wave’ of biotechnology following the pharmaceutical and agricultural waves (Marrs et al., 1999).
Microbial enzymes are fast growing field in biotechnology. The global market of industrial enzymes was close to a billion dollars in 1990 and crossed the $2.0 billion mark in 2005. The market has been estimated at $3.3 billion in 2010 and is expected to reach $4.4 billion by 2015 (www.bccresearch.com). The production cost and the yields of enzymes are considered the major problems in commercial exploitation.

Microbial enzymes have tremendous potential for different applications. Over the years due to their remarkable features, enzymes have occupied the central stage of all the biochemical and industrial processes. Enzymes can carry out their myriads of biochemical reactions under ambient conditions, which make their use eco-friendly and often the best alternative to polluting chemical technologies. Currently, the chemical additives are being replaced by the enzymes in numerous food applications. The use of enzymes has become a need of the time, because they promote effects similar to those of chemical additives with the advantage of being considered as safe natural additives (Penstone, 1996). Microorganisms, in particular, have been regarded as a good source of useful enzymes because they multiply at extremely high rates and synthesize biologically active products that can be controlled by humans. In recent years, there has been a phenomenal increase in the use of enzymes as industrial catalysts.

1.1 Insight on Industrial Enzymes, Applications and Global Market

Although enzyme preparations have been used by mankind over a long history, breakthroughs are needed to extend their uses in broader areas with more superior performance. Enzymes are applied in various fields, including technical use, food manufacturing, animal nutrition, cosmetics, medication, and as tools for research and development. At present, almost 4000 enzymes are known, and of these, approximately 200 microbial original types are used commercially. However, only about 20 enzymes are produced on truly industrial scale. With the improved understanding of the enzyme production biochemistry, fermentation processes, and recovery methods, an increasing
number of industrial enzymes can be foreseeable. The world enzyme demand is satisfied by about 12 major producers and 400 minor suppliers. Nearly 75% of the total enzymes are produced by three top enzyme companies, i.e. Denmark-based Novozymes, US-based DuPont and Switzerland-based Roche (Shahn Li et al, 2012).

According to a research report from Austrian Federal Environment Agency (Australian federal agency, 2002), about 158 enzymes were used in food industry, 64 enzymes in technical application and 57 enzymes in feedstuff, of which 24 enzymes are used in three industrial sectors. Almost 75% of all industrial enzymes are hydrolytic enzymes. Carbohydrases, proteases and lipases dominate the enzyme market, accounting for more than 70% of all enzyme sales.

The global market for feed enzymes is definitely one promising segment in the enzyme industry. It was estimated at around $344 million in 2007, and expected to reach $727 million in 2015 (Feed enzymes, 2007). The market for feed enzymes globally is growing on in regions like U.S, China and South – East Asia.

The food and beverage enzymes segment is expected to reach about $1.3 billion by 2015 with the highest sales occurred in the milk and dairy market (Global market, 2011). Food enzymes are mainly used in baking industry, fruit juice and cheese manufacturing, as well as wine making and brewing to improve their flavor, texture, digestibility, and nutritional value. Global enzymes market is estimated to rise 7 percent at a healthy pace to $8.0 billion in 2015 (Global market, 2011).

1.1.1 Enzymes - Global market

As illustrated in Fig. 1.1 (BCC Research), the global enzyme market is dominated by the food and beverage industry, which benefits from the expansion of the middle class in rapidly developing economies. This is just the start of the industrial enzyme era, which is preparing itself to put known enzymes to novel uses and novel enzymes, discovered or tailored, to catalyze unexploited reactions. As the demand is for cleaner and greener technology to preserve our mother earth for our descent, the use of enzymes that can replace harmful chemical reactions are extremely importance and most of the current R & D on
enzymes is directed towards this issue. Similarly use of enzymes in extreme harsh conditions such as high and low temperatures and pH are also more prevalent. The use of enzymes in chemical processes saves the vast amount of water like in the textile industry, enzymatic treatment of textiles can save 70,000 – 90,000 litres of water for every ton of knitwear produced. With approximately 9 million tons of knitwear being produced annually, the world could save 630 billion litres of water every year if all knitwear were produced by enzymes [Pandey et al., 2008].

Fig. 1.1 Global Industrial Enzymes market, 2008-2015

America remains the leading consumer of enzymes, while the Asia/Pacific region is forecasted to surpass Western Europe as the second largest consumer of enzymes. The major contribution of the Asia/Pacific regions demand is attributed to China and India as the size and strength of these countries economies grow at a torrid pace. Western Europe would still occupy the largest producer of enzymes in the future ten years. However, Asia/Pacific companies, especially for the Japanese and Chinese manufactures, are playing an increasing important role.

Major enzyme producers are located in Europe, USA and Japan. Denmark is dominating, with major players like Novozymes (45%), Danisco (17%), Genencor (USA), DSM (The Netherlands) and BASF (Germany) (Binod et al., 2008, BCC-Business Communications Company, Inc., 2009). The pace of development in emerging markets suggested that companies from India and China can join this restricted party in a very near future (Ogawa et al, 2002, Chandel et
Enzymes are a very well established product in biotechnology (Norus, 2006), where sales from US have been from $1.3 billion in 2002 to US $5.1 billion in 2009 and is anticipated to reach $7 billion by 2013 (Norus, 2006, Agrahari, 2011, Schafer, 2005, El Enshasy et al., 2008). A recent survey on world sales of enzymes ascribes 31% for food enzymes, 6% for feed enzymes and the remaining for technical enzymes [Agrahari, 2011, Schafer, 2005, El Enshasy et al., 2008, Berka, 2006].

Regarding the global market for the industrial enzymes, a recent report published by BBC Research [BBC Research, 2011] states that the global market for industrial enzymes was estimated to reach a value of $3.3 billion in 2010. This market is expected to reach $4.4 billion by 2015, a compound annual growth rate (CAGR) of 6% over the 5-year forecast period. Technical enzymes are valued at just over $1 billion in 2010. This sector will increase at a 6.6% compound annual growth rate (CAGR) to reach $1.5 billion in 2015. The highest sales of technical enzymes occurred in the leather market, followed by the bioethanol market (Figure 4). On the other hand, the food and beverage enzymes segment is expected to reach about $1.3 billion by 2015, from a value of $975 million in 2010, rising at a compound annual growth rate (CAGR) of 5.1%. Within the food and beverage enzymes segment, the milk and dairy market had the highest sales, with $401.8 million in 2009.

The same report emphasized that, in terms of end-use, food and feed represents the largest segment for industrial enzymes. Developing regions are expected to emerge as the fastest growing consumers of industrial enzymes for food and feed applications. Market researchers highlights the fact that industrial demands for enzymes is being driven by new enzyme technologies and increase use of organic compounds in place of petrochemical-based ingredients.

1.1.2 Indian Enzyme Market

The biotech industry in India accounts for just 2% of global biotech markets. But it is gaining global visibility because of the investment opportunities. During 2010-2011 the Indian biotech sector grew at 21.5 % to reach Rs.17, 400
crores in revenues. The view of Indian Biotech market is shown in Fig 1.2 (Biospectrum 2011).

![Indian Biotech market](image)

**Fig 1.2 : Indian Biotech market**

Even though there is a domestic demand, the enzyme segment is largely export oriented. Major export market include the US (global share -40%), Europe (25 %), China (20 %), others include Rest of Asia (25 %) is shown in Fig 1.3. Fig 1.4 shows the sector wise demand for enzymes. Realising the potential of the opportunities outside India, many Indian companies are expanding their base outside the country even difficult markets such as China.

On the basis of application, industrial enzymes could be divided into four major categories, i.e. detergent enzymes, technical enzymes, food enzymes and feed enzymes. The technical enzymes segment could further be divided into textile enzymes, leather enzymes, pulp and paper enzymes, fine chemicals enzymes, fuel ethanol enzymes and others (Van Beilen, 2002).

India posses a skilled man power with network of research laboratories such as CSIR, IIT’s, IISER and universities make the country a potential knowledge hub. It is blessed with rich biodiversity Western ghats and Eastern Himalayas of rich microbial diversity which can be exploited for new enzymes with diverse properties. The cost of manufacturing on the Indian soil is approximately 40 % less than in USA. Also the overall cost of manpower and low installation charges further make India a preferred destination.
Fig 1.3: Industrial enzymes – export scenario

Fig 1.4: Sector wise demand for enzymes

1.1.3 Chinese Enzyme Market

Sales of enzymes in China have grown at a fast pace in the past decades, accounting for roughly 10% of the global enzyme market in 2010 (Research and market 2011). China was also the world’s second largest enzyme market in 2010. In China, the largest segment of enzyme market occurred in the feed and other technical applications accounting for about 50%. It is shown in Fig.1.5 (Research and Market 2011). During 2008-2013, consumption of feed enzymes in China is expected to grow at an average annual rate of about 7.5%, increasing to 46.03 thousand metric tons in the year 2013 (Frost and Sullivan, 2007). Fig: 1.6 shows the consumption and production of enzyme in China.

Fig 1.5 Chinese versus global enzyme market in 2010 (Roughly estimated)

Global demand for enzymes is expected to rise almost 7% annually from 2010 to $8 billion in 2015. The three major commercial enzymes – amylase,
carbohydrase and alkaline protease, occupy more than 90% of the Chinese enzyme market. After 2006, China transformed into a net exporter of enzymes by increasing the scale, quality and diversity of domestic production.

![Graph showing consumption and production of enzymes in China]

**Fig 1.6 Consumption and production of enzyme in China**

The exported volume of enzymes continues to grow year by year (Fig. 1.7). By comparing the growth rate of export in volume (Fig. 1.7(a)) and (Fig. 1.7 (b)), it can be speculated that major exported enzymes are most low value-added products.

![Graph showing import and export trends of enzyme]

**Fig 1.7. Import and export trends of enzyme**

(a) metric tons, (b) trade value in US dollars

**1.1.4 Enzymes - Future perspectives**

Industrial enzymes represent the heart of biotechnology processes. The field of industrial enzymes now is experiencing major R&D initiatives, resulting both in the development of a number of new products and in improvement in the process and performance of several existing products. Currently, new and
emerging applications are driving demand and the industry is responding with a continuous stream of innovative products. Significant future growth will require investments by all the participants in research and applications development. Enzymes contributed to more environmentally adapted clean and green technology due to their biodegradable nature and replace harsh chemicals. Future trend may be the development of more effective systems that use much smaller quantities of chemicals, less water and less energy to attain maximum product yield and performance. New enzymes through modern biotechnology will lead to enzyme products with improved effects at diverse physical conditions such as low/high pH, temperatures, which may allow various industrial processes to carry out at low temperature, less harm to the environment, greater efficiency, lower costs, lower energy consumption and the enhancement of product properties (Ee Taek Hwang, 2013).

1.2 Classification of Enzymes

The International Union of Biochemistry and Molecular Biology (IUBMB) classified enzymes into six major groups (classes), according to the type of reaction they catalyze (Enzyme nomenclature).

1. **Oxidoreductases**: All enzymes catalyzing oxido-reduction reactions belong to this class. The substrate oxidized is regarded as hydrogen donor.

2. **Transferases**: Transferases are enzymes which transfer a group, e.g. a methyl group or a glycosyl group, from one compound (generally regarded as donor) to another compound (generally regarded as acceptor).

3. **Hydrolases**: These enzymes catalyse the hydrolytic cleavage of C-O, C-N, C-C and some other bonds, including phosphoric an-hydrde bonds.

4. **Lyases**: Lyases are enzymes cleaving C-C, C-O, C-N, and other bonds by elimination, leaving double bonds or rings, or conversely adding groups to double bonds.
5. **Isomerases**: These enzymes catalyse geometric or structural changes within one molecule.

6. **Ligases**: Enzymes that catalyze the joining together of two molecules coupled with the hydrolysis of a diphosphate bond in ATP or a similar triphosphate.

Each enzyme described receives a classification number, known as “EC” (Enzyme Commission of the IUBMB), which is composed of four digits (Enzyme nomenclature):

1. Class
2. Subclass within the class
3. Specific chemical groups that participate in the reaction.
4. The enzyme itself

Examples of the classification numbers of each enzyme can be referred in Figure 1.8 (Sarrouh et al., J Bioproces Biotechniq 2012)

The major enzymes used in industrial enzymes market are amylase, lipase, protease, ligase, phytase, cellulase, xylanase, between others (Cowan 1996, Kirk et al., 2002, Howard et al., 2003, Eijsink et al., 2008). Food enzymes constitute the major market share of all industrial enzymes (Market Research news, 2011). Market trends revealed that xylanase and cellulase takes the major chunk of share amounting to 20% of the world enzyme market, together with pectinases (Polizeli et al, 2005). Xylanolytic enzymes from microorganism have attracted a great deal of attention in the last decade, particularly because of their biotechnological potential in various industrial processes.
An overview of the groups of fungal cellulolytic enzymes and their main features are given in Table-1.1 (Banerjee et al., 2010).

The enzymes that degrade cellulose, hemicellulose and lignin, location of action of the enzymes on the particular substrate, mode of action of the particular enzyme on the substrates structure and the enzyme classification (E.C) number are well explained in the table.

**Fig 1.8: General Classification of enzymes based on their catalytic functions**
Table 1.1: Enzymes involved in lignocellulose degradation and their mode of action

<table>
<thead>
<tr>
<th>Lignocellulosic Fraction</th>
<th>Enzymes</th>
<th>Location of action</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>Endo-1,4-β-gluconases (EG) (or) CMCase</td>
<td>Cellulose (amorphous regions)</td>
<td>Attack the amorphous regions of the cellulose and produce glucose.</td>
</tr>
<tr>
<td></td>
<td>Cellulobiolydrolases (CBH) (Exo-1,4-p-glucanase)</td>
<td>Cellulose (crystalline regions)</td>
<td>Hydrolyze β-1,4-glycosidic bonds from chain ends, producing cellobiose as the main product.</td>
</tr>
<tr>
<td></td>
<td>β-glucosidases (BGL)</td>
<td>Cellobiose, cellobextrins</td>
<td>Hydrolyze soluble cellobiose and cellobextrins to glucose.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>Endo-xylanase</td>
<td>Xylan main chain</td>
<td>Hydrolyzes primarily interior β-1,4-xylose linkages of the xylan backbone.</td>
</tr>
<tr>
<td></td>
<td>Exo-xylanase</td>
<td>Xylan main chain</td>
<td>Hydrolyzes the terminal β-1,4-xylose linkages releasing xylobiose.</td>
</tr>
<tr>
<td></td>
<td>β-Xylosidase</td>
<td>Xylooligosaccharides</td>
<td>Releases xylose from xylobiose and short chain xylooligo-saccharides.</td>
</tr>
<tr>
<td></td>
<td>α-Arabinofuranosidase</td>
<td>α-L-arabinofuranosyl compounds attached to the xylan main chain</td>
<td>Hydrolyzes terminal non-reducing α-arabinofuranose from arabinoxylans.</td>
</tr>
<tr>
<td></td>
<td>α-Glucuronidase</td>
<td>α-1,2-linked glucuronic or 4-O-methylglucuronic acid substituents attached to xylan main chain</td>
<td>Releases glucuronic acid from glucuronoxylans.</td>
</tr>
<tr>
<td></td>
<td>Acetylxylan esterase</td>
<td>O-Acetyl groups attached to the side ends of xylan main chain</td>
<td>Hydrolyzes acetylester bonds in acetyl xylans, liberating acetic acid.</td>
</tr>
<tr>
<td></td>
<td>Ferulic acid esterase</td>
<td>feruloyl group on the arabinofuranosyl side chain attached to the terminal non-reducing xylose</td>
<td>Hydrolyze the ester linkages between arabinose side chain residues and phenolic acids (ferulic acid).</td>
</tr>
<tr>
<td>Lignin</td>
<td>Laccase (phenol oxidase)</td>
<td>Phenolic compounds found in the lignin structure</td>
<td>Oxidizes phenolic subunits of lignin.</td>
</tr>
<tr>
<td></td>
<td>Lignin peroxidase</td>
<td>Aromatic compounds found in the lignin structure</td>
<td>Oxidation of benzilc alcohols, cleavage of C-C bonds, cleavage of C-O bonds. Oxidation of Mn^{2+} to Mn^{3+}, which then binds to an appropriate ligand, diffuses from the enzyme, and, in turn oxidizes phenolic substrates.</td>
</tr>
</tbody>
</table>
Plants are the major source of fixed carbon in nature and have three major polymers in their cell wall especially secondary cell wall are cellulose (insoluble fibres of β 1,4-glucan), hemicellulose (noncellulosic polysaccharides), and lignin (a complex polyphenolic structure). These structures are commonly referred to as lignocellulose.

Lignocellulose occurs within plant cell walls, which consist of cellulose microfibrils embedded in lignin, hemicelluloses and pectin. The plant cell wall is a composite material in which cellulose, hemicelluloses (mainly xylan) and lignin is closely joined together (Whistler RL et al., 1970).

1.3 Xylanase

Xylanases are genetically single chain glycoproteins, ranging from 6–80 kDa, active between pH=4.5–6.5. Xylanase (Endo-β-1,4-xylanase EC 3.2.1.8) is an industrially important enzyme which degrades xylan randomly and produces xylooligosaccharides, xylobiose and xylose. It is mainly present in microbes and plants but not in animals. Endo-1,4-β-xylanase has been extensively studied from fungal and bacterial sources and it has been commercially produced. Over 100 years ago the role of enzymes in the breakdown of xylan was observed by Hoppe-Seyler (Bastawade, 1992). Xylanases, the xylan hydrolyzing enzymes, are ubiquitous and diverse by nature [Collins et al., 2005]. The enzymes which break down hemicelluloses are referred as hemicellulases. Due to the heterogeneity and complex chemical nature of plant xylan, its complete breakdown requires the action of a complex of several hydrolytic enzymes with diverse specificity and modes of action. These have been defined and classified according to the substrates on which these act. These are collectively grouped as glycan hydrolases [Digvijay et al., 2012]. It consists of a mixture of hydrolytic enzymes including β-1,4 endoxylanase, β-xylosidase, α-glucuronosidase, α- L-arabinofuranosidase, acetyl xylan esterase and phenolic acid esterase.

Xylanases (endo-β-1, 4-xylanases, EC 3.2.1.8) cleave the xylan backbone into smaller oligosaccharides. They are the key enzymes for xylan degradation and differ in their specificities toward the xylan polymer. Many cleave only at unsubstituted regions whereas others have a requirement for side chains in the
vicinity of the cleaved bonds (Coughlan and Hazlewood, 1993). Xylanases are endo type enzymes that hydrolyse internal linkages in xylan and act by a random attack mechanism yielding a mixture of xylooligosaccharides from the polymer. However, some xylanases may also have an ability to hydrolyze xylooligomers to xylose, especially in the cross-linked enzyme crystal form (Finell et al., 2002). The depolymerization action of xylanase results in the conversion of polymeric substances into xylo-oligosaccharides and xylose (Subramaniyan and Prema, 1998; Bajpai, 1999; Omar et al., 2008).

As xylan is a large polymer that cannot penetrate into cells, xylanases have to be secreted to the extracellular environment to reach and hydrolyse it. Generally, xylanases are induced in most micro-organisms during their growth on substrates containing xylan. Small soluble oligosaccharides released from xylan by the action of low levels of constitutively produced enzymes are transported inside cells where they induce xylanase expression (Kulkarni et al., 1999).

Xylanase due to their multidimensional role in fermentation processes have gained immense importance. Sugars like xylose, xylobiose and xylooligomers can be prepared by the enzymatic hydrolysis of xylan.

1.3.1 Applications of Xylanase

Applications of microbial xylanase are described below:

**Feed industry**

The supplementation of corn-soybean meal based diet with xylanase significantly enhanced the egg production and quality in egg laying quail (Bayram et al., 2008).

**Lignocellulose bioconversions**

Saccharification of lignocellulosic materials using xylanase liberates sugars, which could be employed for producing products like xylooligosaccharides, ethanol, organic acids, SCP and hydrogen production (Barnard et al., 2010, Lo et al., 2010, Li et al., 2011).

**Pulp and paper industry**

The major current industrial application of xylanases is in the pulp and paper industry where xylanase pretreatment facilitates chemical bleaching of pulps, resulting in important economic and environmental advantages over the non-
enzymatic process (Viikari et al., 1994, Bajpai, 2004). Xylanase-boosted bleaching results in up to 20–25% savings on chlorine-based chemicals and a reduction of 15–20% in the generation of pollutant organic chlorine compounds from lignin degradation (adsorbable organic halogens, AOX).

**Bioethanol production**

The process of ethanol production from lignocellulosic biomass includes delignification of plant biomass and hydrolysis of cellulose and hemicellulose to monosaccharides (Beg OK et al., 2001). Endo-1, 4-β-xylanase has been reported to be a bifunctional enzyme having endo-1,4-β-xylanase as well as cellulase activity. Bi-functionality of endo-1,4-β-xylanase could result in more efficient and cheaper saccharification process of the agricultural residues, municipal and industrial wastes used for bio-ethanol production as it can degrade both cellulose and xylan residues. An increased possibility of fermentation of both hexoses and pentoses sugars present in lignocelluloses into methanol has also been reported (Senn T et al., 2001).

**Production of xylooligosaccharides in paper industries**

- Xylo-oligosaccarides have been shown to be useful as prebiotics and preventing dental caries.
- newly developed functional oligosaccharides feature many beneficial biomedical and health effects, such as the stimulation of human intestinal *Bifidobacteria* growth (Yang Ch et al., 2007).
- The end products of xylan degradation are xylose, arabinose, and methyl-glucuronic acid containing oligosaccharides. Xylooligosaccharides are sugar oligomers showing potential for practical applications in a variety of fields, including pharmaceuticals, feed formulations, agricultural purposes and food applications (Vazquez et al., 2000).
- As additives for functional foods, XOs have pre-biotic action showing positive biological effects such as improvement in the intestinal function by increasing the number of healthy *Bifidobacteria* (Fooks et al., 2002, Rycroft et al., 2001, Izumi yet al., 2003).
These xylooligosaccharides are used as dietary supplements to gastrointestinal health and reduce the risk of colon cancer (Whitehead et al., 2003).

As food ingredients, XOs have an acceptable odor, and are non-carcinogenic (Kazumitsu et al., 1987, Kazumitsu et al., 1997) have low-calorie value, allowing their utilization in anti-obesity diets (Taeko et al., 1998, Toshio et al., 1990).

in food processing, XOs show advantages over insulin in terms of resistance to both acids and heat, allowing their utilization in low pH juices and carbonated drinks (Modler et al., 1994).

Advantages of xyloooligosaccharides are the reduction of cholesterol, maintenance of gastrointestinal health, and improvement of the biological availability of calcium. They also inhibit starch retrogradation, improving the nutritional and sensory properties of food (Ghatora et al., 2006).

**Fruit juice and beer clarification**

- xylanase was useful for clarification of non-citrus fruit juice.
- Endo-1,4-β-xylanase helps in increasing juice yield from fruits or vegetables and also in the maceration process.
- Endo-1,4-β-xylanase reduces the viscosity of the fruit juice improving its filterability (Biely et al., 1985).
- Endo-1, 4-β-xylanase also improves extraction of more fermentable sugar from barley and therefore useful for making beer, also useful for processing the spent barley for animal feed.
- Endo-1, 4-β-xylanase reduces the viscosity of the brewing liquid, improving its filterability (Garg et al., 2010).
- Xylanase, together with pectinase, carboxymethylcellulase and amylase, can be used for the clarification of juices because the turbidity observed is due to both pectic materials and other materials suspended in a stable colloidal system.
Bioenergy

- efficient holocellulases (mixture of cellulases and xylanases) cocktail plays a significant role in commercialization of biorefinery, textile, detergent formulation and paper manufacturing industries (Chiranjeevi et al., 2012).
- Xylanase is capable of efficiently converting hemicelluloses and cellulose components of biomass into energy rich sugars and these sugars can be used to make fuels and high value chemicals.

Improving silage

- It is known that endo-1, 4-β-xylanase and cellulase treatment of forages produces better quality silage that improves the subsequent rate of plant cell wall digestion by ruminants.
- by endo-1,4-β-xylanase treatment, there is increased nutritive sugar and that is useful for digestion in cow and other ruminants.
- It is also known that endo-1,4-β-xylanase also produces compounds which are the nutritive source for many ruminal microflora (Garg et al., 2010).

9) Baking Industry

- The efficiency of xylanases in improving the quality of bread has been seen with an increase in specific bread volume. This is further enhanced when amylase is used in combination with xylanase (Maat et al., 1992).
- Xylanase decreases dough firmness, increasing volume and creates finer and more uniform crumbs. It significantly improves manufacturing conditions by making dough more 'machine-friendly' as it does not stick to the machinery parts (Rouau et al., 1993).
- Xylanases (hemicellulases) are used to enhance bread quality, extend shelf life by reducing the staling rate, and they appear to be particularly effective in straight dough process (Wang M et al., 2004, Sorensen et al., 2001, Monfort, et al., 1997).
- These enzymes also contribute in eliminating the use of chemical additive such as bromate (Kulkarni et al., 1999, Maat et al, 1992).
- Xylanases have an antistaling action during bread storage and most of the added xylanases, found to have a significant effect on softening of doughs, arabinoxylans due to water release. In spite of the softening, dough
tolerance, oven spring, as well as bread volume, shape and texture are all improved (Courtin CW et al., McCleary BV, 1986).

- Xylanase along with protease, lipase and α-amylase are significantly effective for obtaining bread with higher specific volume in microwave oven, as compared to the bread with no enzyme added.
- Xylanases, cellulase, and glucanase improve the properties of wheat bread and reduce staling during storage.
- Rheological behaviour of dough made with different commercial enzyme preparations consisting of amylases, xylanases, lipases, and glucose oxidase, singly and in mixed combinations results in softening and weakening of the dough immediately after mixing and further during resting. Xylanases caused the main changes, when compared to the dough without the supplement during resting (M. Martínez-Anaya et al., 1997).
- Xylanases in bread making results in increased volume, reduced stickiness and staling, and increased shelf life. The enzyme can substitute the addition of different emulsifiers and other chemical additives used in bread production.

**Textile industry**

- Xylanases have been used for the pretreatment of low quality jute fibers, flex, hemp and ramie. Such fibers are rich in hemicellulosic content, and therefore, cause difficulties in proper spinning in the mill. The pretreatment of fibers with thermostable xylanases removes xylan without compromising the strength, and thereby, soften them up to a significant level (Patra and Madhu, 2010).
- Xylanases and cellulases are used for the softening of jute (Kundu et al., 1991).
- Cellulases, pectinases, hemicellulases, lipases and catalases are used in different cotton pre-treatment and finishing processes (Meyer-Stork, 2002)
Food industry

There are several applications of xylanases in the food industry:

- The processing of cereals and millets with xylanase resulted in the reduction in processing time during fermentation of these food materials.
- Treatment of wheat flour with xylanases reduces the dough water absorption as well as significant changes in the texture of loaf.
- Xylanases are also used in improving nutritional properties of silage and to achieve the desirable texture and loaf volume of bread in baking industry (Subramaniyan and Prema, 2002). The food industry does not require thermostable and alkalistable xylanases.

Bioconversion

- Xylitol, a five carbon sugar alcohol is used as a natural food sweetener (Nigam and Singh 1995), has applications such as teeth hardening, remineralisation, antimicrobial agent, used in toothpaste formulations (Roberto et al., 2003, parajo et al., 1998], bulk of xylitol produced is consumed in various food products such as chewing gum, candy, soft drinks and ice cream.
- 2, 3 ±butanediol, a product of hemicellulosic hydrolyzate is a valuable chemical feedstock, used as a solvent, liquid fuel, precursor of many synthetic polymers and resins.
- dehydration of 2,3 - butanediol yields industrial solvent methyl ethyl ketone which is used as a fuel due to its lower boiling point.
- dehydration of 2,3 –butanediol yields 1,3-butanediene, a starting material for synthetic rubber, used as a monomer in the polymer industry (Howard et al., 2003).

Detergent industry

Xylanase improves the cleaning ability of detergents, especially in cleaning fruits, vegetables, soils and grass strains (Kuhad et al., 1997).
Presently, efforts are being made to increase the production of hydrolytic enzymes by fermenting the agricultural waste materials through biotechnological approaches. Fermentation process gives promising yield of enzymes and is an economical method due to low cost and accessibility of waste materials as substrates. Currently, much interest has been generated in using non-starch polysaccharide hydrolyzing enzymes especially xylanases in baking industry. Xylanases of *Aspergillus niger* improve the overall bread quality characteristics (Maat *et al.*, 1992; Jiang *et al.*, 2005; Collins *et al.*, 2005). The production of microbial xylanases is preferred over plant and animal sources because of their availability, structural stability and easy genetic manipulation (Bilgrami and Pandey, 1992).

Commercial xylanases preparations are obtained from different microorganisms and diverse culture systems, which are applied according to the desired enzymatic profile. New fungal xylanases have interesting properties such as thermal, electrostatic, pH and catalytic stabilities (Pellerin *et al.*, 1991, Rani *et al.*, 1996, Gerber *et al.*, 1997, Loera *et al.*, 1999).

The Xylanase enzyme can be produced by a number of microorganisms including bacteria, yeast and filamentous fungi like Trichoderma, Aspergillus, Penicillium, Fusarium, Chaetomium, Humincola, Taloromyces, and many others (Haltrich *et al.*, 1996). In practice, the great majority of microbial enzymes come from a very limited number of genera, of which *Aspergillus* species, *Trichoderma* species, *Bacillus* species and *Kluyveromyces* species predominate (Chaplin *et al.*, 1990). Table 1.2 shows Commercial Xylanases and their industrial Suppliers (QK Beg, 2001).

Agricultural waste materials can be used as substrates which provide carbon and mineral nutrients to the organisms under the controlled conditions i.e., pH of the culture medium and temperature of incubation. Generally the organisms produce the extra cellular enzyme which is collected and purified (Ali *et al.*, 2002, Skowronek and Fiedurek, 2006). The various biotechnological techniques like submerged and solid state fermentation are generally employed for xylanase biosynthesis (Cai *et al.*, 1998, Gawande and Kamat, 1999, Kansoh and Gammel, 2001).
### Table: 1.2 Commercial Xylanases and their industrial Suppliers

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Product Trade name</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alko Rajamaki, Finland</td>
<td>Ecopulp cartazyme</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Sandoz, Charlotte, N.C. and Basle, Switzerland</td>
<td>Cartazyme HS 10, cartazyme HT, cartazyme SR 10, cartazyme PS 10, cartazyme 9407 E, cartazyme NS 10, cartazyme MP</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Clarient, UK</td>
<td>Cartazyme HS 10, cartazyme HT, cartazyme SR 10, cartazyme PS 10, cartazyme 9407 E, cartazyme NS 10, cartazyme MP</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Genercor, Finland, Ciba</td>
<td>Irgazyme 40-4X/Albazyme 40-4X, Irgazyme-10A, albazyme -10A, Multifect xylanase</td>
<td>Baking, food</td>
</tr>
<tr>
<td>Giegy, Switzerland</td>
<td>VAI xylanase</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Novo Nordisk, Denmark</td>
<td>Pulpzyme HA, Pulpzyme HB, Pulpzyme HC, Biofeed beta, Biofeed plus ceremix</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Voest Alpine, Austria</td>
<td>VAI xylanase</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Biocon India, Bangalore</td>
<td>Beachzyme F</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Rohm Enzyme</td>
<td>Ecopulp X-100, Ecopulp X-200, Ecopulp X-200/4, Ecopulp TX-100, Ecopulp TX-200, Ecopulp XM</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Primalco Finland, Finland</td>
<td>Xylanase</td>
<td>Research</td>
</tr>
<tr>
<td>Meito Sankyo, Nogayos, Japan</td>
<td>Xylanase</td>
<td>Research</td>
</tr>
<tr>
<td>Rohm, Germany</td>
<td>Rholase 7118</td>
<td>Food</td>
</tr>
<tr>
<td>Solvay Interox, USA</td>
<td>Optipulp L. -8000</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Thomas Swan, UK</td>
<td>Ecozyme</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Iogen, Canada</td>
<td>GS-35, HS-70</td>
<td>Pulp bleaching</td>
</tr>
<tr>
<td>Sankyo, Japan</td>
<td>Sanzyme PX, Alpelase F</td>
<td>Feed</td>
</tr>
<tr>
<td>Enzyme Development, USA</td>
<td>Enzoko, Xylanase</td>
<td>Baking, Food</td>
</tr>
</tbody>
</table>

### 1.4 Types of fermentation

#### 1.4.1 Submerged Fermentation (SmF)

SmF utilizes free flowing liquid substrates, such as molasses and broths. The bioactive compounds are secreted into the fermentation broth. The substrates are utilized quite rapidly hence needs to be constantly replaced/supplemented with nutrients. This fermentation technique is best suited for microorganisms such as bacteria that require high moisture content. An additional advantage of this technique is that purification of products is easier (Subramaniam and vimala, 2012). Most of the enzymes are being synthesized by utilizing the submerged
fermentation technique. Approximately 90% of all industrial enzymes are produced in SmF, frequently using specifically optimized, genetically manipulated microorganisms. In this respect SmF processing offers an insurmountable advantage over SSF. The submerged fermentation is most beneficial as compared to other techniques due to more nutrients availability, sufficient oxygen supply and less time required for the fermentation (Hoq et al., 1994, Gomes et al., 1994, Veluz et al., 1999, Bim and Franco, 2000, Gouda, 2000).

Biotechnological innovations bring many significant and successful efforts to convert lignocellulosic residues into valuable products including enzymes for biotechnological and industrial applications (Leite et al., 2008, Tengerdy & Szakacs, 2003). However, the cost to produce these enzymes is still an obstacle that needs to be overcome. Therefore, the use of agroindustrial residues as carbon source for fungal growth and ligno/hemi/cellulolytic enzyme production through solid state fermentation (SSF) has been reported in many studies as a way of significantly reducing process cost. Many agricultural and agroindustrial by-products are used in SSF processes for the production of cellulases, xylanases and ligninases, among other enzymes (Gao et al., 2008, Kang et al., 2004).

1.4.2 Solid State Fermentation (SSF)

Solid state fermentation processes (SSF) are characterized by the growth of microorganisms within a porous support without free water. This condition favors the development of filamentous fungi, given their unique capacity to colonize the interparticular spaces of solid matrices. In addition, the risk of bacterial contamination is reduced due to the low water (Sebastianos Roussos, 1997). Solid state fermentation has gained interest for researchers in recent years because of economic and engineering advantages (Pandey et al., 1999), since this method employs agricultural residues in their natural form, thus helping to prevent the environmental impact caused by the accumulation of these residues. Enzyme productivity in solid state fermentation (SSF) is usually much higher than that of submerged fermentation (Haltrich, 1996). Cultivation in solid substrate has been traditionally used by humankind for several centuries and the history and development of solid-state fermentation shown in Table-1.3 (Pandey, 1992).
Table 1.3. History and development of solid-state fermentation

<table>
<thead>
<tr>
<th>Period</th>
<th>Development Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>2600 B.C.</td>
<td>Bread making by Egyptians</td>
</tr>
<tr>
<td>In Asia (1000 B.C.)</td>
<td>Cheese making by <em>Penicillium roqueforti</em></td>
</tr>
<tr>
<td>2500 B.C.</td>
<td>Fish fermentation/preservation with sugar, starch, salts, etc., koji process</td>
</tr>
<tr>
<td>7th century</td>
<td>Koji process from China to Japan by Buddhist priests. Vinegar from pomace</td>
</tr>
<tr>
<td>18th century</td>
<td>Gallic acid used in tanning, printing, etc.</td>
</tr>
<tr>
<td>1860–1900</td>
<td>Sewage treatment, waste-water treatment</td>
</tr>
<tr>
<td>1900–1920</td>
<td>Fungal enzymes (mainly amylases), kojic acid</td>
</tr>
<tr>
<td>1920–1940</td>
<td>Fungal enzymes, gluconic acid, rotary drum fermenter, citric acid</td>
</tr>
<tr>
<td>1940–1950</td>
<td>Fantastic development in fermentation industry. Penicillin production by SSF and submerged Fermentation</td>
</tr>
<tr>
<td>1950–1960</td>
<td>Steroid transformation by fungal cultures</td>
</tr>
<tr>
<td>1960–1980</td>
<td>Production of mycotoxins, protein enriched feed</td>
</tr>
<tr>
<td>1980–present</td>
<td>Various other products like enzymes, alcohol, gibberellic Acid</td>
</tr>
</tbody>
</table>

Production of enzymes by solid-state fermentation (SSF) has potential advantages over submerged fermentation regarding to operation, simplicity, high productivity fermentation, less favorable for growth of contaminants and concentrated product formation (Gupta et al., 2001).

In recent years, SSF has received more and more interest from researchers, since several studies for enzymes (Pandey et al., 1999), flavours (Ferron et al. 1996), colourants (Johns & Stuart, 1991) and other substances of interest to the food industry have shown that SSF can give higher yields (Tsuchiya et al., 1994) or better product characteristics (Gutierrez-Rojas et al., 1995) than submerged fermentation (SmF). Among the several factors, which are important for microbial growth and activity in a particular substrate, particle size and moisture level/water activity are the most critical (Auria et al., 1992, Pandey et al., 1994, Zadrazil & Punia, 1995).

SSF has mainly been centered around agro-industrial residues due to their potential advantages for filamentous fungi, which are capable of penetrating into the hardest of these solid substrates, aided by the presence of turgor pressure at the tip of the mycelium (Ramachandran et al., 2004).
The selection of an appropriate substrate is a key factor for the success of SSF. Besides having the adequate composition to induce the desired product, the particles size should also be considered, since this is a factor that greatly influences SSF. Small particles offer more contact surface, allowing more access of the microorganism to the nutrients, however depending on the type of substrate and on the moisture level, it can get compacted, impairing aeration and oxygen availability, as well as heat dissipation, limiting microbial growth. Big particles, on the other hand, facilitate aeration, however may hinder microbial access, limiting substrate contact surface and making heat transfer difficult. Other parameters should also be evaluated and optimized for higher process efficiency, such as initial moisture and pH, incubation temperature, aeration, inoculum size, nutrient supplementation, extraction and purification of the final product (Pandey, 2003).

The use of SSF for fungi cultivation presents many advantages, such as: simulation of their natural environment, which results in better adaptation to the medium and higher enzymatic productions, reduction of bacterial contamination, due to absence of free water, obtainment of more concentrated enzymes, once the enzymatic extracts may be extracted with small amounts of water (Bianchi et al, 2001). Other advantages of fungal enzyme production by SSF can also be mentioned when comparing it to submerged fermentation, such as use of lesser amount of water and consequently a decrease in effluent generation, lower requirement of space and energy, more stability of the obtained products, more biomass and enzyme production, less catabolic repression of enzymes and little protein degradation (Pandey et al., 2000; Viniegra-González et al., 2003). Fig : 1.9 shows the scheme of some micro scale processes that occur during solid state fermentation (SSF) (Holker & Lenz, 2005).
Fig: 1.9 A scheme of some microscale processes that occur during solid state fermentation (SSF)

Table 1.4 Main Applications of SSF Processes in Various economical sectors

<table>
<thead>
<tr>
<th>Economical Sector</th>
<th>Applications</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agro-Food Industry</td>
<td>Traditional Food Fermentation</td>
<td>Koji, Teznpch, Atticke, Fermented cheeses</td>
</tr>
<tr>
<td></td>
<td>Mushroom production &amp; spawn</td>
<td>Agaricus, Pleurotus, Shn-take</td>
</tr>
<tr>
<td></td>
<td>Bioconversion By-products</td>
<td>Sugarpulp Bagass Coffee pulp, Silage composting, Detoxification</td>
</tr>
<tr>
<td></td>
<td>Food Additives</td>
<td>Flavours, dyestuffs, Essential Fat and organic acids</td>
</tr>
<tr>
<td>Agriculture</td>
<td>Biocontrol, Bioinsecticide</td>
<td>Beauveria Metarhizum, Trichoderma</td>
</tr>
<tr>
<td></td>
<td>Plant Growth, Hormones</td>
<td>Gioberellins, Rhizobium, inchoderma</td>
</tr>
<tr>
<td></td>
<td>Mycorrhization, Wild Mushroom</td>
<td>Plant inocytation</td>
</tr>
<tr>
<td>Industrial Fermentation</td>
<td>Enzymes production</td>
<td>Xylanases, Amylases, Cellulases, Proteases, Pectinases</td>
</tr>
<tr>
<td></td>
<td>Antibiotic Production</td>
<td>Citric acid, Fumaric acid, Gallic acid, Lactic acid</td>
</tr>
<tr>
<td></td>
<td>Ethanol Production</td>
<td>Schwannomyces sp.sbrch Malting and Brewing</td>
</tr>
<tr>
<td></td>
<td>Fungal Metabolites</td>
<td>Hormones Alcaloides</td>
</tr>
</tbody>
</table>
1.5 Production of xylanases under SSF and SmF

Xylanases are produced by either solid-state or submerged fermentation (Agnihotri et al., 2010). Although most xylanase manufacturers produce these enzymes using submerged fermentation (SmF) techniques (nearly for 90% of the total xylanase sales worldwide) (Polizeli et al., 2005) the enzyme productivity via solid-state fermentation (SSF) is normally much higher than that of submerged fermentation (Agnihotri et al., 2010). The growing interest in using solid-state fermentation (SSF) techniques to produce a wide variety of enzymes, including xylanases from fungal origins, is primarily due to the economic and engineering advantages of this process (Pandey et al., 1999).

The advantages of SSF processes over SmF include a low cultivation cost for the fermentation, lower risk of contamination (Beg et al., 2001), improved enzyme stability, mimicking the natural habit of the fungus, production of enzymes with higher specific activities, generation of a protein-enriched byproduct, and easier downstream processing of the enzymes produced (Considine et al., 1989). SSF conditions are especially suitable for the growth of fungi, as these organisms are able to grow at relatively low water activities, contrary to most bacteria and yeast, which will not proliferate under these culture conditions (Corral et al., 2006).

Pineapple, mixed fruit, maosmi waste, wheat rawa with raspberry seed powder, broiler matter, corn stover, almond meal, apple pomace, corncob, barley husk, banana waste, soybean cake, cacao jelly, sweet lime rind, cassava, soybean, amaranth grain, eucalyptus kraft pulp, coffee residues, hardened chickpeas, lignite, rubber or orange bagasse are some of the substrates used for enzyme production using fungi (Hölker et al., 2004).

On the contrary, submerged fermentation allows better control of the conditions during fermentation (Pandey et al., 1999). The submerged fermentation of aerobic microorganisms is a well-known and widely used method for the production of cellulase and xylanase (Garcia-Kirchner et al., 2002). In general, SmF is the preferred method of production when the preparations require more purified enzymes, whereas synergistic effects from a battery of xylan-degrading enzymes can easily found in preparations obtained by SSF using
complex substrates, though the latter is commonly sought in improving animal feed (Corral et al., 2006).

1.6 CMCase (CMCellulase/Carboxy methyl cellulase) or Endoglucanase

Cellulose is the main polysaccharide found in the plant cell wall and is degraded by the cellulase enzyme complex. Cellulose is a linear homopolymer of D-glucose linked by β-1, 4 bonds with a crystalline structure stabilized by intramolecular and intermolecular hydrogen bonds (Hikaru et al., 2008). Cellulose is degraded by the cellulase enzyme complex which consists of 1) Carboxymethyl cellulase CMCase or Endoglucanase (E.C. 3.2.1.4) cleaves the β-1,4- linkage in amorphous region of cellulose to yield long chain oligosaccharides, 2) Cellobiohydrolase (E.C. 3.2.1.19) cuts in exo-manner on oligosaccharides to produce cellobiose, a dimer of glucose, and finally 3) β-glucosidase (E.C. 3.2.1.21) hydrolyses cellobiose to yield glucose. CMCase or Endoglucanase (E.C.3.2.1.4), exoglucanase or avicelase (E.C. 3.2.1.91) and β-glucosidase or cellobiase (E.C. 3.2.1.21), of which endoglucanases cut randomly at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends (Narasimha et al., 2006). Cellulose represents the most abundant renewable natural product in the biosphere with an estimated annual production of 4.0x10^7 tons (Bakare et al., 2005). It is a major polysaccharide constituent of plant cell walls and one of the most abundant organic compounds in the biosphere (Bhat and Bhat, 1997). It has also enormous potential as a renewable source of energy. Much of the cellulose in nature exists as waste material from agriculture industry in form of stalks, stems and husk (Immanuel et al., 2007). Large quantities of cellulosic wastes are generated everyday through forestry and agricultural processes (Camassola et al., 2004) which remain unutilized and accumulate as wastes in the environment thereby causing pollution problem (Milala et al., 2005). The bioconversion of cellulosic materials has been receiving attention in recent years. Complete enzymatic hydrolysis of enzymes requires synergistic action of three types of enzymes, namely, cellobiohydrolase, endoglucanase, and β-glucosidase (Bhat, 2000).
Cellulases have attracted much interest because of the diversity of their application and today, these enzymes account for approximately 20% of the world’s enzyme market. Since the cost of production is the major constraint, much attention is given to the utilization of low cost carbon source and an effective fermentation system (Das et al., 2008). Cellulases have potential application in the animal feed industry to improve the nutritional value of feed; and/or to supplement animals own digestive enzymes (Chesson, 1987). Cellulose being an abundant and renewable resource is a potential raw material for the microbial production of food, fuel, and chemicals (Coughlan, 1985). It becomes a potentially challenging area where cellulases would have a central role in the bioconversion of renewable cellulosic biomass to commodity chemicals (Ibrahim & El-Diwany, 2007).

A number of biomass conversion methods have been proposed viz. acid hydrolysis, pyrolysis and enzymatic hydrolysis, but the latter being environmentally safe and can be performed at normal temperature and pH is the most preferred method (Bakare et al., 2005). By means of chemical or bioconversion methods, it is possible to transform this insoluble polymer into glucose, an excellent substrate for industrial fermentation. However, in nature, cellulolytic bacteria and filamentous fungi produce families of enzymes that synergistically hydrolyze crystalline cellulose to smaller oligosaccharides and finally to glucose. This supports their growth on smaller oligosaccharides and that of other microorganisms. The cellulases have attracted considerable attention in recent years due to their great biotechnological and industrial potentials.

Cellulases have a wide range of applications, and the main potential applications are in food, brewery, wine, pulp and paper, textile, detergent, feed and agriculture (Bhat, 2000). Cellulases are used in cotton preparations, wool and dyeing treatment and in effluent treatment. Majority of studies on cellulase production have been focused on fungi with relatively lesser emphasis on bacterial sources for cellulase production (Bhat, 2000, Bischoff et al., 2006, Camassola et al., 2004, Haakana et al., 2004).

Cellulases are widely used in textile applications for many years, and again received additional consideration in the enzyme market owing to their
powerful ability in the degradation of lignocellulosic feedstocks. The cost of cellulases is a significant technical barrier to the conversion of lignocellulosic biomass to fuels associated with commercializing processes. Cellulase preparations cost reduction attributed to two main strategies: i) economical improvement in production of cellulase by process and strain enhancement, i.e. cheaper medium and alternative inducer system and ii) improvement in the specific cellulase performance or activity to reduce grams of enzyme for achieving equivalent hydrolysis by cocktails and component improvement. Many companies have devoted themselves to developing new cellulase preparations by using genetic techniques and have streamlined production of those enzymes. After two generations of Cellic® release in 2009 and 2010, Novozymes launched a new enzyme for production of bioethanol from agricultural wastes and residues, called Cellic® CTec3 in Feb 2012. The new enzyme product has been claimed to be 1.5 times better than the previous Novozymes Cellic® CTec2 and five times less of enzyme dose compared to competing enzymes to make the same amount of ethanol. Cellic® CTec3 could make the cost of cellulosic biofuels to around $2.0/gal of ethanol, which is competitive with production of corn ethanol and gasoline. Cellulase enzyme is used in denim washing. Cellulase works by loosening the indigo dye on the denim in a process known as “Bio - Stonewashing”. A small dose of enzyme can replace several kilograms of pumice stones.

1.7 Need for the present study

Fermentation process gives promising yield of enzymes and is an economical method due to low cost and accessibility of waste materials as substrates. Presently, efforts are being made to increase the production of xylanolytic enzymes by fermenting the agricultural waste materials through biotechnological approaches. India, being an agriculture-based economy, generates huge quantum of agricultural/agro industrial residues which are difficult to dispose off, and their use as substrates for xylanase production not only reduce the production cost of enzyme but also combat environmental pollution.
The management of these wastes must be given a prime priority in the transformation of these wastes into useful raw materials for the production of value added products of industrial and commercial potential. Therefore it is of interest to study not only the production of xylanases, but also the ability to produce on low cost agricultural wastes.

1.8 Objectives of the Present study

The objectives of the present research work are given below:

1) To study the xylanase enzyme production by *Aspergillus fumigatus* (MTCC No - 343), *Aspergillus niger* (MTCC No - 16404), *Aspergillus terreus* (MTCC No - 1782) under SSF.

2) Studies on the effect of various substrates such as wheat bran, sugarcane bagasse, combination of wheat bran & sugarcane bagasse using the above three microorganisms.

3) Screening of the most significant medium components that affect xylanase production using two level Plackett Burman Design.

4) Optimisation of the most significant variables by central composite design (CCD) using response surface methodology (RSM).

5) Optimisation of the process parameters such as substrate concentration, temperature, Initial pH, Initial moisture content, Incubation Time, using RSM.

6) Experimental validation of the second order polynomial model of RSM.

7) Evaluating kinetic parameters (\(K_m\) and \(V_{max}\)) for the fungal strain which gives maximum xylanase activity under solid state fermentation.

8) To predict the rate of xylanase formation using Michaeli’s Menten model.

9) Application of Artificial Neural Network (ANN) model for the prediction of xylanase activity using the data obtained from RSM.

10) Comparison of experimental Values with RSM predicted and ANN predicted values for production of xylanase.
1.9 Organisation of the thesis

The thesis entitled “Studies on xylanase enzyme production – Kinetics and Modeling” consists of nine chapters as detailed below:

Chapter 1 – “Introduction” deals with the introduction and importance of xylanase production using low cost medium constituents, introduction and importance of CMCase production.

Chapter 2 – “Literature Review” describes a complete and exhaustive review of literature about the sources of xylanase, fermentation conditions, applications and its purification methods. This covers all the conventional methods of xylanase production. The work carried out so far in xylanase production using solid state production and submerged fermentation are discussed.

Chapter 3 – “Materials and Methods” describes the techniques adopted for microbial subculture cultivation, medium formulation and maintenance of operating conditions by batch solid state fermentation. The modeling and optimization using RSM and ANN are discussed. The quantification of xylanase and CMCellulase are discussed.

Chapter 4 - “Results and Discussion” describes research work carried out for xylanase production and the concomitant CMCase production along with xylanase. This includes the following:

i. Xylanase production using Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus.

ii. Screening of the medium components using Plackett Burman Design.

iii. Optimisation of the most significant medium components by Central composite design (CCD) a type of Response surface Methodology. Experimental validation of the second order polynomial model of RSM.

iv. Optimisation of various process parameters by Central composite design. Experimental Validation of the second order polynomial model of RSM.
Chapter 5 – “Kinetics and Modeling” describes the kinetics of xylanase enzyme production by SSF and the determination of kinetic parameters ($K_m$ and $V_{max}$) for the strain that gives maximum xylanase production. Michaeli’s Menten model is employed to predict the product formation.

Chapter 6 – “Artificial Neural Network Modeling” describes the application of Artificial Neural Network for modeling of xylanase production using RSM experimental data and Comparison of ANN predicted values with experimental values and RSM predicted values for xylanase enzyme & CMCase production.

Chapter 7 – “Conclusions” of this research work describes the conclusions from this work.

References - gives the important articles and research work published so far in reputed International Journals

Suggestions for future work