Microbial enzymes are known to play a crucial role as metabolic catalysts, leading to their use in various industries and applications. The end use market for industrial enzymes is extremely wide-spread with numerous industrial commercial applications (Adrioand Demain, 2005). Over 500 industrial products are being made using enzymes (Johannesand Zhao, 2006; Kumar and Singh, 2013). The demand for industrial enzymes is on a continuous rise driven by a growing need for sustainable solutions. Microbes have served and continue to serve as one of the largest and useful sources of many enzymes (Demain and Adrio, 2008). The microbial enzymes have widespread uses in industries and medicine. The microbial enzymes are also more active and stable than plant and animal enzymes. In addition, the microorganisms represent an alternative source of enzymes because they can be cultured in large quantities in a short time by fermentation and owing to their biochemical diversity and susceptibility to gene manipulation.

Industries are looking for new microbial strains in order to produce different enzymes to fulfil the current enzyme requirements. However, the level of production of a particular enzyme varies in different microorganisms and more over the enzymes often differ in composition and properties. One usually finds that the closely related organisms have
enzymes with nearly similar properties, while unrelated organisms have enzymes system that differ widely. The most critical feature of the organisms for producing industrially significant enzymes is their GRAS (generally regarded as safe) status, which implies that they must be non-toxic, non-pathogenic and generally should not produce antibiotics. The GRAS listed microorganisms include fewer than 50 bacteria and fungi. Examples are the bacteria including *Bacillus subtilis*, *B. licheniformis* and various other bacilli, *lactobacilli*, *Streptomyces* species, the yeast *Saccharomyces cerevisiae* and the filamentous fungi belonging to the genera *Aspergillus*, *Mucor*, *Rhizopus*, etc. In case of *Bacillus*, mutants are selected that can no longer form spores.

Many industrial processes, including chemical synthesis for production of chemicals and pharmaceuticals have several disadvantages: low catalytic efficiency, lack of enantiomeric specificity for chiral synthesis, need for high temperature, low pH and high pressure. Also, the use of organic solvents leads to organic waste and pollutants. Enzymes are more useful for these applications as they work under mild reaction conditions (e.g., temperature, pH, atmospheric conditions), do not need protection of substrate functional groups, have a long half-life, a high stereo-selectivity yielding stereo- and regio-chemically-defined reaction products at an acceleration of 105 to 108-fold, and, in addition, they work on unnatural substrates (Johnson, 2013). Furthermore, enzymes can be selected genetically and chemically-modified to enhance their key properties: stability, substrate
specificity and specific activity. There are drawbacks however, to the use of enzymes, e.g., certain enzymes require co-factors. However, various approaches such as cofactor recycling and use of whole cells can solve this problem. About 150 industrial processes use enzymes or whole microbial cell catalysts.

1.1. Lignocelluloses

Lignocellulosic biomass from agricultural residues is produced in large quantities, approximately 73.9 Teragrams/year in the world (Kim and Dale, 2004). These wastes are mostly left in the field, causing a disposal problem for the local producing agro-industries. However, lignocellulosic biomass actually has a great potential as feedstock for production of more value-added products such as slow price chemicals, e.g., xylitol, xylose, glucose, furfural (Rahman et al., 2006; Sanchez, 2009), fuels (Kim and Dale, 2004), biofibres (Reddy and Yang, 2005), ruminant feed (Okano et al., 2009), biopulp (Chen et al., 2002; Scott et al., 2002; Yaghoubi et al., 2008), or even for enzyme production (Holker et al., 2004). Lignocelluloses are composed of cellulose, hemicelluloses, lignin, extractives, and in general, minor amounts of inorganic materials (Sjostrom, 1993). Cellulose and hemicelluloses are polysaccharides that can be hydrolysed to produce simple sugars. However, many factors such as lignin (content and composition), cellulose crystallinity,
degree of polymerization, pore volume, acetyl groups bound to hemicellulose, surface area and biomass particles size limit the digestibility of the hemicellulose and cellulose (Anderson and Akin 2008; Zhu et al., 2008; Alviraa et al., 2010).

The aromatic barriers in lignocelluloses, including lignins (consisting of phenylpropanoid units of various types) and low molecular weight phenolic acids, limit the degradation of fibres. Cell walls with syringyl lignin, e.g. leaves of sclerenchyma, are often less recalcitrant. However, coniferyl lignin appears to be the most effective limitation to biodegradation (Anderson and Akin, 2008). Therefore, pretreatment methods start to eliminate or break down the lignin will generally increase the digestibility of cellulose fractions of lignocellulosic biomass.

Pretreatment of lignocellulosic biomass can be performed by physical, mechanical, chemical and biological methods (Mosier 2005; Taherzadeh and Karimi 2008; Hu et al., 2008; Hendriks and Zeeman, 2009; Alviraa et al., 2010). Physical/mechanical pretreatments are based on milling, irradiation and hydrothermal treatments. Examples of chemical pretreatments are ammonium fibre explosion (AFEX), alkali, acid and organosolvent treatments. Chemical pretreatments may produce toxic substances, interfering with the microbial fermentation, in addition to producing wastewater that need treatment prior to its release to the environment (Keller et al., 2003; Shietal., 2008). In view of these facts, biological
pretreatment has attracted interest because of its potential advantages over physical/chemical pretreatments such as: (a) greater substrate and reaction specificity, (b) lower energy requirements, (c) lower pollution generation, and (d) higher yield of desired products (Kirk and Chang, 1981).

Biological pretreatment employs microorganisms and their enzymatic machineries to break down lignin and alter lignocellulose structures. Some of the most promising microorganisms for biological pretreatment are white-rot fungi that can mineralise lignin to CO$_2$ and water in pure culture (Lundquist et al., 1977; Hatakka, 1983).

Certain white-rot fungi such as *Phanerochaete chrysosporium*, *Ceriporiopsissubvermispora*, *Phlebia subserialis* and *Pleurotus ostreatus* are capable of efficiently metabolising lignin in a variety of lignocellulosic materials (Kirk and Chang, 1981; Hatakka, 1983; Keller et al., 2003). The fungi have been studied in connection with several ligninolytic enzymes such as lignin peroxidases (LiP), manganese peroxidases (MnP), laccase (Lac) and versatile peroxidases (VP) (Higuchi, 2004; Wong, 2009). Having these enzymes, white-rot fungi can have many applications in biopulping, biobleaching, ruminant feeds, xylose, ethanol, biogas and enzymes productions (Kirk and Chang, 1981; Reid, 1989).
There are many whiterot fungi which remain unexplored for ligninolytic enzymes.

Biological pretreatments using whiterot fungi have mostly been carried out by solid state fermentation (SSF). In SSF, production of ligninolytic enzymes has been higher than in submerged fermentation (SF) (Xu et al., 2001). The enzyme activity and lignin degradation are influenced by a number of factors such as fungal strain, nutrient composition (nitrogen, Mn$^{2+}$ and Cu$^{2+}$), moisture content, aeriation, pH, and temperature (Dorado et al., 2001; Snajdr and Baldrian, 2007; Patel et al., 2009). Controlling these factors leads to an optimum condition in the pretreatment process, which results in good performance of whiterot fungi.

Most known white rot fungi are basidiomycetes and are capable of white rot decay. White rot decay derives from the appearance of attack of wood by these fungi, in which lignin removal results in a bleached appearance of the substrate. The ability to catabolize lignocellulose and hemicellulose is fairly common as a primary metabolic process among WRF. As a result it is not regarded as a rate limiting step in the carbon flux. Lignin is extremely recalcitrant and is mineralized in an obligatorily oxidative process, carried out appreciably only by WRF (Zabel and Morrell, 1992). The degradation of lignin yields no net energy gain, and so lignin is degraded during secondary metabolism in order to access wood polysaccharides locked in lignin–carbohydrate complex.
1.2. Lignin Degrading Enzymes

The natural process of lignin degradation involves predominantly white rot fungi and they contain specific enzymes necessary for lignin degradation. Important classes of enzymes involved in degradation of lignin are lignin peroxidase (LiP), manganese peroxidase (MnP), laccase, and hydrogen peroxide-generating enzymes. Along with these enzymes, ROSs (Reactive oxygen species) is also considered to be an important agent for wood decay by fungi. Different combinations of these enzymes are produced which suggest different mechanisms of lignin degradation (Singh, 2006). Lignin degrading enzymes bring about the oxidation of phenolic compounds to phenoxy radicals whereas nonphenolic compounds are oxidised via cation radicals. *Phanerochaetechrysosporium*, one of the important representatives of white-rot fungi, extensively studied model for lignin degradation research and production of LiP. Lot of literature is available discussing the oxidative mechanism, molecular genetics and application of ligninolytic enzyme systems of a few organisms (Kersten and Cullen, 2007; DorivKnop et al., 2015; Rameshaiah and Jagadish Reddy, 2015). Leonowicz et al., (2001) has even proposed a hypothetical mechanism of enzymatic transformation of lignocellulose by white-rot fungi.

1.3. Extracellular fungal ligninolytic enzymes

White-rot fungi produce several types of extracellular oxidative enzymes (oxido-reductases) that are involved in the degradation of lignin content in a plant cell wall. Two major families of enzymes are involved in
ligninolysis by white-rot fungi: peroxidases and laccases. Apparently, these enzymes act using low-molecular-weight mediators to carry out lignin degradation. Several classifications of fungi have been proposed based on their ligninolytic enzymes. Some of them produce all of the major enzymes, others only two of them, or even only one (Camarero et al., 2005).

**Lignin peroxidases** are glycosylated, heme containing enzymes which functionally require \( \text{H}_2\text{O}_2 \) for the oxidation of lignin related aromatic structures (Asgher et al., 2006; Papinutti and Forchiassin, 2007). LiPs are strong oxidizers capable of catalysing the oxidation of phenols, aromatic amines, aromatic ethers and polycyclic aromatic hydrocarbons (Tien and Kirk, 1998).

**Manganese peroxidases** have been found in most WRF studied till today. Some WRF even secrete MnP as a sole ligninolytic enzyme for lignin degradation. MnP is a heme containing glycoprotein which requires hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) as well as \( \text{Mn}^{+2} \) ions for its activities (Asgher et al., 2008a; Bermek et al., 2004). MnP oxidizes a wide range of substrates, including several phenolic compounds, high molecular weight chlorolignins and nylon, rendering the enzyme an interesting biocatalyst for potential applications in various industries such as pulp and paper industry and textile industry where it is utilized to degrade the pollutants (Bermek et al., 2004).

**Laccase** is a dimeric or tetrameric glycoprotein containing four copper atoms which are distributed in redox sites and has the advantage of not
needing H$_2$O$_2$ for substrate oxidation, which makes the enzyme to have a broader application spectrum than peroxidases (Mishra and Kumar, 2007). LiPs, MnPs and laccases have been applied to numerous processes such as pulp delignification, oxidation of organic pollutants, stabilization of fruit juices, biosensor development, biofuels cells, textile biofinishing, environmental protection processes, beverage processing, animal feed stuffs, biobleaching systems, cosmetics, enzyme immunoassays, wastewater detoxification, denim stone washing, detergent manufacturing and transformation of antibiotics and steroids (Tien and Kirk, 1998; Ryan et al., 2003; Boer et al., 2006; Papinutti and Forchiassin, 2007; Revankare et al., 2007; Asgher et al., 2008).

Ligninolytic enzymes are needed for large quantities for their application in a variety of industrial processes. Production of enzymes at cheaper rates by fermentation methods is gaining more importance. Certain lignocellulosic materials were tried as substrates for cultivation of certain white-rot fungi in fermentation methods for production of industrially important ligninolytic and cellulolytic enzymes (Reddy et al., 2003). Among different fermentation methods used for enzyme production, solid state fermentation (SSF) using agro-wastes is an attractive and cost effective option because it presents higher productivity involving a simpler operation, when compared with submerged cultures (SmF) (Pointing, 2001).

Many microorganisms and their enzymes have been discovered by means of extensive screening and are now commonly used in industrial
applications. The discovery of new microbial enzymes through extensive and persistent screening has brought about many new and simple routes for synthetic processes and provided one possible way to solve environmental problems. Screening for such enzymes, will offer further exciting possibilities for the discovery of new fungal strains producing such enzymes and their industrial use. Although the majority of previous studies on lignin degrading enzymes of organisms have been focused on only few white rot fungi – *Phanerochaetechrysosporium* (Kersten and Cullen 2006), *Pleurotus* (Cohen et al., 2002, Doriv-Knopet et al., 2015), *Trametesversicolor* (Rameshaiah and Jagadish Reddy, 2015), there has been a growing interest in studying ligninolytic enzymes of a wide array of white rot fungi from the standpoint of comparative biology but also with expectation of finding better lignin degrading system. According to a recent study (Viswanath et al., 2008), an unexplored white rot fungus *Stereumostrea* isolated from wood logs produced higher titres of laccase enzyme than the well-studied reference culture *P. chrysosporium* in liquid culture. Besides laccase enzyme it also produced MnP and LiP (Praveen et al., 2011). In the light of the above information, it is proposed to examine the performance of *S. ostrea* for production of ligninolytic enzymes in solid state fermentation on locally available lignocellulose materials with the following objectives

- To screen locally available and cheap agro-residues for suitability as solid substrates for growth of *S. ostrea* in solid state fermentation for production of ligninolytic enzymes.
➢ To find out the effect of different carbon and nitrogen sources on ligninolytic enzyme production by *S. ostrea*.

➢ To optimize the culture conditions like pH, temperature, moisture content and inoculum size for maximum yield of ligninolytic enzymes.

➢ To assess the influence of different surfactants on enzyme production.

➢ To study the effect of different inducers like guaiacol, veratryl alcohol, tannic acid, gallic acid etc., on the production of ligninolytic enzymes.

➢ To study the effect of different aromatic compounds on the production of ligninolytic enzymes.

➢ To study the influence of different concentrations of copper sulphate on laccase production.

➢ To explore a variety of leachate methods for maximum recovery of enzymes from the fermented bran.

➢ To further enhance the production of ligninolytic enzymes through Response Surface Methodology.