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Ligninolytic Enzymes for Application in Treatment of Effluent from Pulp and Paper Industries

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Abstract:
The growing concern over the pollution caused by the rapid industrialization has posed a serious problem forcing researchers around the world to seek alternative eco-friendly technologies. The situation has been met with suitable regulatory steps in the developed western countries where public awareness coupled with stringent government regulations have stimulated the paper and pulp industry to take on newer and greener technologies but the problem remains the same in the developing parts of the world. Despite the impact of the industry on the environment, the world of pulp and paper industry continues to expand at alarming rates and more and more paper mills are booming up in the newly industrialised countries. Available industrial waste treatment processes are expensive and pose a major threat to the environment. Therefore to meet the demand of paper at the same time reduce the hazardous impact of industrial effluent on environment, the search for a sustainable and inexpensive biological intervention is indispensable. The present review therefore aims to bring out a comprehensive analysis of available biological agents which can be employed for effluent treatment from various industries like pulp and paper industry and dye industries.

Keywords: Biological treatment; Effluent; Paper and pulp industry; Waste treatment.

1.0 Introduction:
There have been rapid strides in the past few decades which have witnessed spectacular advances due to industrialization and globalization that have led to the high standard of living and betterment of mankind. The new ideas coupled with the scientific progress and rapid transformation of laboratory design into fruitful findings that had practical implications have together contributed largely to the commercial-scale manufacturing processes. Field of chemical technology involves production of a variety of products on large scale which has significantly resulted in serious effluent and hazardous and non-biodegradable waste disposal problems and causing the pollution of natural resources viz. air, water, soil which cannot be ignored or let off without mention. One such area needing immediate attention is the pulp and paper industry which processes large quantities of raw materials involving the use of naturally hazardous chemicals which are then let out in the natural water resources (Srinivasan and Meenakshi, 1999).

The extent of pollution caused by the paper and pulp industry can be adjudged by the fact that overgrowing demand of the paper makes paper and pulp industry among the world's largest contributors of air and water pollutants, hazardous waste products and the toxic gases that cause the considerable change in the climate (Thompson *et al.*, 2001; Sumathi & Hung, 2006). The pressure of demand exceeding the supply of required wood is leading to the forest being logged for timber and cleared for growing plants that have reduced ecological value, and use of toxic chemical fertilizers and herbicides that leads to secondary consequences (Centre for a New American Dream, 2002). The situation becomes worse considering the fact that industrialised and developed and industrialised nations with just 20 percent of the world's total population, consume 87 percent of the world's paper (Toepfer, 2002). According to the reports of OECD Environmental outlook, 2002 the global scenario of paper and pulp production is expected to be increased by 77% from 1995 to 2020. Indian paper mills use a wide variety of cellulosic and non cellulosic based raw materials for paper production accounting for about 43% from forest wood, 28% from agro based product,

and 29% from recycling of waste paper (Balakrishanan, 1999). Industrial pollution is a global concern. In India, more than 55% of industries do not have any proper treatment and around 20% of them have partial treatment facilities (Srivastava *et al*., 1994).

One of the processes involved in paper manufacture is pulping which can be done by chemical (Kraft pulping) or mechanical methods, however former is generally preferred (Schumacher and Sathaye 1999; Ali and Sreekrishnan, 2001). The waste stemming from the pulping process is called black liquor and it contains various organic, inorganic chemicals, chlor-phenols compounds, and fibre residues which contribute significantly to the high BOD, COD, suspended solids and organic matter together giving a deep brown colour to the effluent. The main component constituting black liquor is chlor-lignin. The vexing aspect associated with treatment of effluent from these industries is that the treatment procedures available for the paper and pulp industry are very expensive and the small scale paper and pulp industries do not treat their waste water to bring the BOD and COD to the standards set by the government agencies (Pokhrel and Viraraghavan, 2004).

With the onus rightly felt by the future chemical technologists, it is indispensable to transform paper and pulp production as well as consumption worldwide towards processes that are environmentally and socially responsible and sustainable. Therefore the need for safer and 'ecosystem friendly' technologies has become imminent. The scientists around the world are attempting to learn from the natural processes giving rise to the entirely new aspect of microbiology and biotechnology. The age old concept and realization of the potential of micro organisms to perform the large number of biochemical reactions under suitable conditions without toxic and hazardous end products is now being put to application. Therefore biotechnology based approaches that started initially as an idea have now started to gain ground and therefore they can be considered as valuable and useful technologies of the future. A simmer of hope to save the natural environment from the harmful impact of the paper and pulp industry and yet trying to realize the objectives of chemical technology is gaining impetus. Therefore the present review is focussed on studying potential biological agents which can bring about efficient lignin degradation and with possible application in large scale treatment of effluent discharged from pulp and paper industries.

2.0 Biological Treatment of Paper and Pulp Industry effluent- An Overview

Recent advancement in the biotechnological techniques has led to the biological treatment of the effluent from paper and pulp industry where large number of microorganisms including bacteria, fungi and actinomycetes have been implicated in the biodegradation of lignin involving an oxidation process(Kirby, 2005; Gao *et al*., 2011; Amr *et al.*, 2009). Various enzymes present in fungi like lignin peroxidases, manganese peroxidases, can effectively degrade lignin but their efficiency decreases considerably under extreme environmental conditions of high temperature, pH and presence of any toxic chemicals which are present in the treatment plants. In addition to these, fungal filaments and hyphae cause structural impediment, so can not be used for the biological treatment of effluent emanating from pulp and paper industry (Amr *et al.*, 2009).

2.1 Microbial Lignin Degradation

Lignin is a complex polymer and its degradation takes a number of years. It constitutes the second largest sink next to cellulose for fixed carbon therefore lignin biodegradation occupies a significant portion in the global carbon cycle (Eriksson *et al.*, 1990). Microbial degradation has been described in detail by many researchers around the world (Blanchette, 2000; Garg and Modi, 1999; Hatakka, 2001). Anaerobic degradation of lignin by micro flora was found to be very limited. The degradation that occurred was mainly attributed to biodegradation of the non-lignin components of the plant tissues or low molecular weight materials which were biotically free (Benner and Hodson, 1985).

2.2 Bacterial Degradation

Lignin degradation by bacteria has not been studied much because lignin and cellulose together cannot support the growth of the bacteria. However some recent reports have reported delignification by certain bacteria like *Pseudomonas*, *Arthrobacterium*, *Xanthomonas, Aeromonas*, *Flavobacterium*, and *Streptomyces* (Crawford and Crawford, 1980; Amer and Drew, 1980). Some *Pseudomonas* spp. have been reported to be the most efficient bio-degraders of lignin as reported by Zimmerman, 1990, and Vicuna, 1988. However non filamentous bacteria have very less capacity to demineralise lignin and are restricted to small portion of lignin which has

less molecular weight (Vicuna *et al.*, 1993; Ruttimann *et al.*, 1991).

Marchand in 1978 has demonstrated alkali lignin utilization from sulphate waste water by species of *Pseudomonas, Corallina*, *Nocardia*, *Torula* and however so far no studies are available for lignin degradation to be carried out by thermophilic or anaerobic bacteria. It has been reported by Deschamps *et al.*, 1980, that *Aeromonas spp*. can utilise industrial kraft lignin as a sole source of carbon and degrade it up to 98%. Cyanobacteria have also been known to degrade lignin from paper mill effluent as reported by Bharti *et al.,* 1992. Tuomela *et al.*, 2000 have reported lignin demineralization by mixed cultures of bacteria, actinomycetes and fungi. Perez *et al.*, 1997 has reported *Streptomyces viridosporous* strain T7A to degrade lignosulphonates upto 25%. Janshekar and Fiechter, 1982 reported that *Pseudomonas, Nocardia,* and *Corynebacterium* could easily grow on lignin related phenols but could not degrade lignin. Available literature on bacterial degradation shows that so far no degradation has been reported efficiently by aerobic bacteria while anaerobic bacterial degradation of lignin might be limited by the complex lignin structure.

2.3 Degradation of lignin by fungi

Fungi are the only known micro organisms found to degrade lignin and have been extensively studied (Hatakka, 2001; Evans and Hedger, 2001). Based on the nature of degradation, wood-decaying fungi are classified as soft rot fungi, brown rot and white- rot fungi.

Soft rot fungi include imperfect fungi (Deuteromycetes) and molds of Ascomycetes which are known for degradation of lignin (Blanchette, 1995; Daniel and Nilsson, 1998). Soft-rot fungi include species of *Monodictys, Allescheria*, *Monodictys*, *Graphium, Papulospora*, *Paecilomyces* and *Thielavia.* Soft rot can be of two types: Type I and Type II: Type I consist of cavities formed within secondary walls whereas Type II is Eroded form of degradation. Lignin degradation by fungi is better in hardwood than in softwood. According to reports by Rodriguez *et al.*, 1996, soil fungi *Fusarium oxysporum, Penicillium chrysogenum*, and *Fusarium solani* degraded 23.5%, 27.4%, and 22.6% of lignin, from wheat straw, respectively. Another soil fungus *Fusarium proliferatum* had been reported to secrete aryl alcohol and laccase in liquid cultures (Regaldo *et al.*, 1999).

Chrysonilia sitophila caused up to 25% loss of lignin within 3 months in pine wood (Rodriguez *et al.*, 1997). Degradation of lignin indicated oxidative cleavage of Cα–Cβ and β-Oaryl bonds. Report by Machuca et. al., 1998 indicated lignin degradation of extracts of *Eucalyptus grandis* and bleached *Eucalyptus* kraft pulp by a strain *Thermoascus aurantiacus.*

2.4 Brown-rot Fungi

Brown-rot fungi include several species of Basidiomycetes. These fungi have the capacity to easily eliminate cellulose and hemi cellulose from the wood and leaving behind only brown lignin residue. The brown colour of lignin is actually the modified lignin. All brown- rot fungi produce hydroxyl radicals (Fenton type catalytic system) that degrade wood components. Brown-rot fungi can be classified into two important groups based on the difference in the mechanism: one belonging to *Gloeophyllum trabeum,* which accumulates oxalic acid required for the hydrolysis of polysaccharides and also as a chelator for a Fe(II)-H_2O_2 system generating hydroxyl radicals (Shimada *et al.*, 1997) and, the second includes *Poria* (*Postia*) *placenta* and *Coniophora puteana*. *Poria placenta* was found to de methoxylate lignin but no evidence of ring opening was found by Davis *et al.*, 1994.

2.5 White-rot Fungi

White-rot fungi are believed to be the only and most effective degraders of lignin which comprises of several hundred species of Basidiomycetes and a few species of Ascomycetes. They are most capable of completely degrading lignin component of wood to CO_2 and water. Some white rot fungi remove lignin and carbohydrates in the same proportion in contrast to some selective species which can perform delignification much faster than removing cellulose. White rot fungi are known to colonize cell-lumina and cause cell wall erosion and with the progression of decay process further, the eroded areas tend to coalesce and void areas are filled with mycelia. This process is called as non-selective or simultaneous rot and *Trametes versicolor* is an excellent example of this. Some white rot fungi degrade lignin without loss of cellulose and cause white-pocket rot and is commonly seen in *Phellinus nigrolimitatus*(Singh, 2006). Excellent example of both types of wood rot occurs in *Ganoderma applanatum* and *Heterobasidion annosum* (Eriksson *et al.*, 1990). A report by Arora *et al.*, 2002 showed seven species i.e. *Phlebia fascicularia*, *Daedalea flavida*, *Dichomitus squalens*, *T. versicolor*, *P. radiata*, *P.*

floridensis and *Phanerochaete chrysosporium* of whit rot fungi degrading up to 25% of lignin from wheat straw after 32 days. As per the report of Gilbertson, 1980, white rot fungi are known to occur less predominantly on wood species of Gymnosperms than compared to Angiosperms.

Lignin component Syringyl units are degraded more easily whereas guaiacyl units are more recalcitrant and resistant to degradation. TEM data from Burlat *et al.*, 1998 showed *Ceriporiopsis subvermispora* and *Pleurotus eryngii* partially removed middle lamella while *Phlebia radiata* removed lignin from the secondary cell walls. Marine fungi have also been reported to decompose spruce lignin (Sutherland *et al.*, 1982).Of late more diverse and taxonomically distinct fungi have been studied for their lignin degrading capacity and brought under the purview of useful application in effluent treatment. It has been documented that lignin degradation involved fungal specific physiological processes and differences might be due to ecology of fungi and its taxonomic position.

Kraft pulping method is employed for pulp production which is then bleached to remove residual lignin. The white–rot fungi were tested for their degradation potential of the toxic and chlorinated Kraft bleach mill effluents which contains high molecular weight chlor lignin and chlorinated organic compound with less molecular weight. It was found that *P. chrysosporium* decolourised bleach plant effluent which was obtained from the first alkali extraction stage and it also required ligninolytic system (Sundman, 1981). Different reactors have also been designed for mycelial colour removal (MyCoR) and other related processes and have been assessed using *P. chryosporium* as a biocatalyst (Messner *et al.*, 1990). Colour reduction (upto 80%) of effluent has been observed in 2 days of operation by Chang *et al.*, 1983. Comparative study of the decolourization ability of different microorganisms was studied by Bajpai and Bajpai (1994). Sasaki *et al.* (2001) designed a new pulp bio bleaching system involving immobilized MnP enzyme in a silica support with controlled pore sizes.

Dev and Thankamani, 2012 have recently reported a multi potent fungus MVI.2011, isolated from soil sample which has enhanced capability to degrade lignin(Fig. 1). The isolated fungus had the capacity to grow at wide range of pH (5-10) at ambient temperatures. MVI.2011 showed remarkable property of lignin decolourization within 12 hours of inoculation when compared to other conventional fungi used for industrial treatment of waste which takes a few weeks to bring about the required lignin degradation. Fungus MVI.2011 also showed increases lignin tolerance of up to 2%.

3.0 Lignin Degrading Enzymes from Fungi

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 combination 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 non phenolic compounds are oxidised via cation radicals. *Phanerochaete chrysosporium*, one of the important representatives of white-rot fungi and 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 several ligninolytic enzyme systems. Leonowicz *et al.*, 2001 has even proposed a hypothetical mechanism of enzymatic transformation of lingocellulose by white-rot fungi.

3.1 Lignin Peroxidases (LiP; EC 1.11.1.14)

LiP enzymes are produced by the fungi during secondary metabolism in nutrient starved cultures and are glycosylated heme proteins. LiP was first discovered in *Phanerochaete chrysosporium* and since then has become one of the most studied peroxidases (Glenn *et al.*, 1983; Tien and Kirk, 1983).LiP has been reported to be produced by many white rot fungi including *Phanerochaete flavido-alba* (Hamman *et al.*, 1999), *Bjerkandera* sp. strain BOS55 (ten Have *et al.*, 1998), *Trametes trogii* (Vares and Hatakka, 1997), *Phlebia tremellosa* (Vares *et al.*, 1994) and *Phlebia ochraceofulva* (Vares *et al.*, 1993). Several isozymes have also been detected in cultures of *P. chrysosporium*, *Trametes versicolor*, *Bjerkandera adusta* and *Phlebia radiate.* There are several factors responsible for activity and number of LiP isozymes produced by *P. chrysosporium* such as strain, age of culture, medium composition and method of cultivation. The mechanism of action for oxidation of lignin model compounds by LiP involves H_2O_2. For reducing substrates, LiP is

relatively non-specific and reacts with several lignin model compounds. LiPs can catalyse the oxidative cleavage of Cα–Cβ linkages, β-O-4 linkages, and other bonds present in lignin and its model compounds. The enzyme also catalyzes side-chain cleavages, benzyl alcohol oxidations, de methoxylation, ring-opening reactions and oxidative de chlorination (Tien and Kirk, 1983).

3.2 Manganese Peroxidases (MnP; EC 1.11.1.13)

White rot fungi and soil litter-decomposing fungi are known to produce manganese peroxidase in multiple forms. Certain distinct groups of Basidiomycetes such as the families Meruliaceae, Polyporaceae, Coriolaceae, and the soil litter families Tricholomataceae and Strophariaceae are known to secrete MnP. Similar to LiP, production of MnP enzyme is greatly favoured by nitrogen deficient conditions. The catalysis by MnP is initiated by binding organic peroxide or H_2O_2 to the native ferric enzyme which in turn forms an iron-peroxide complex (Hofrichter, 2002).

3.3 Laccases (benzenediol:oxygen oxidoreductase; EC 1.10.3.2)

Laccases are another most largely found enzymes for lignin degradation present in many white rot Basidiomycetes and several fungi belonging to Ascomycetes and Deuteromycetes and higher plants. They cause lignin breakdown and are involved in several functions like fungal pathogenicity, pigmentation, sporulation, fructification and detoxification. The optimum temperature for the laccase produced by the fungus *Marasmius quercophilus*, that decomposes litter is very high, 75°C (Dedeyan *et al.*, 2000). There are three types: type I, type II and type III. Each of these types of laccase has an important role in lignin degradation. Laccase enzyme can catalyze oxidation of aromatic amines, phenolic compounds and other compounds via reduction of molecular oxygen to H_2O_2. Laccase is also known to cleave alkyl phenyl and Cα–Cβ linkage of phenolic group β-1 and β-O-4 lignin model dimers. It is also involved in de-methylation of several lignin model compounds.

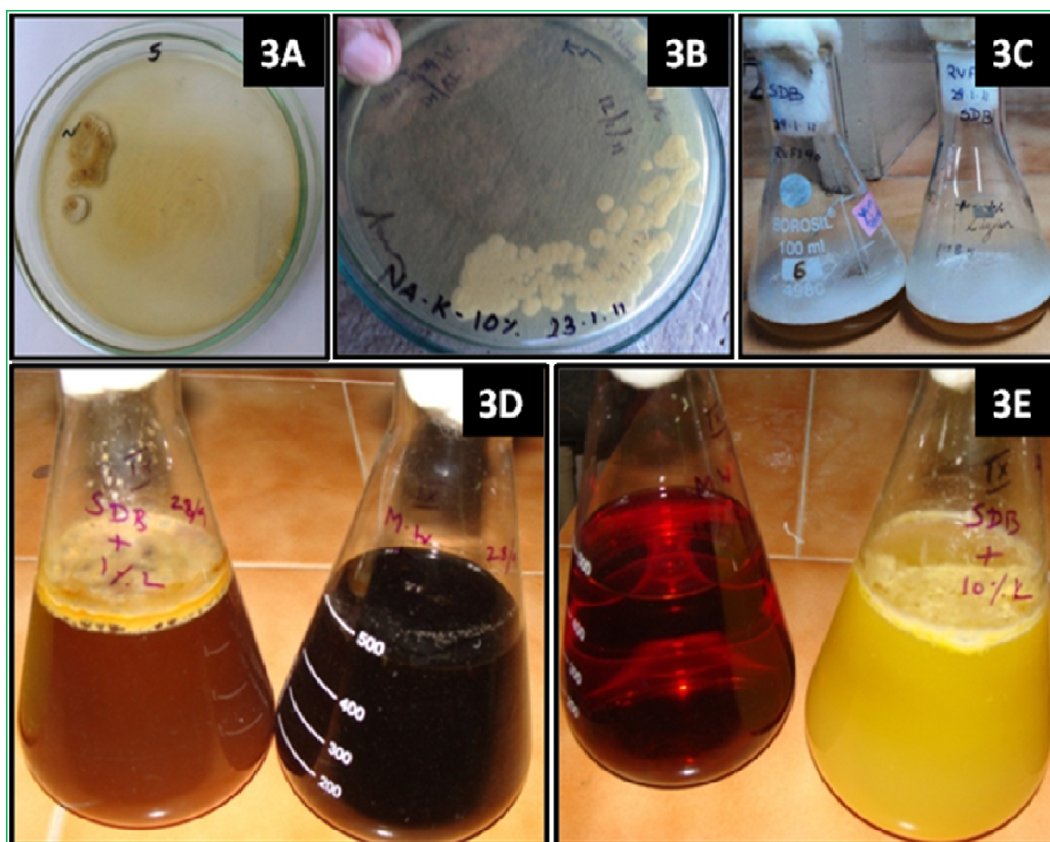

Fig. 1- Screeining of MVI.2011 for lignin breakdown; **3A-**Growth on SDA with 0.1% lignin; **3B**- Growth on SDA with 1% alkali wood extract; **3C-** Growth in SDB and SDB + 0.1% lignin; **3D –** SDB with 1% lignin (pH8.5) with heavy surface growth, deposited biomass and high decolourisation; uninoclulated medium control; **3E-** SDB with 10% alkaline wood extract (pH 8.5) control; 18 hours at ambient temperature, with high biomass, complete decolorisation and uniformly turbid and heavy biomass deposits(Dev and Thankamani, 2012).

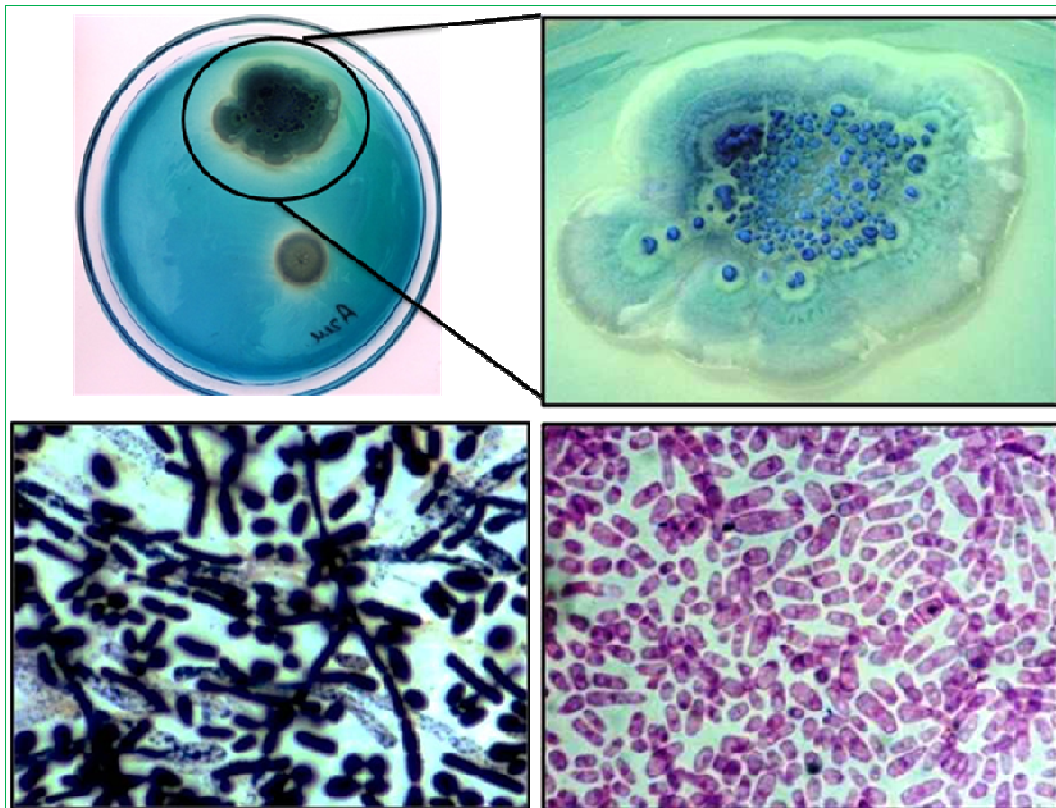

Fig. 2: Colonies grown on SDA containing 0.1% azur dye, uptake of dye attributed by the respective colour of the colonies and a zone of clearance Dev and Thankamani, in press)

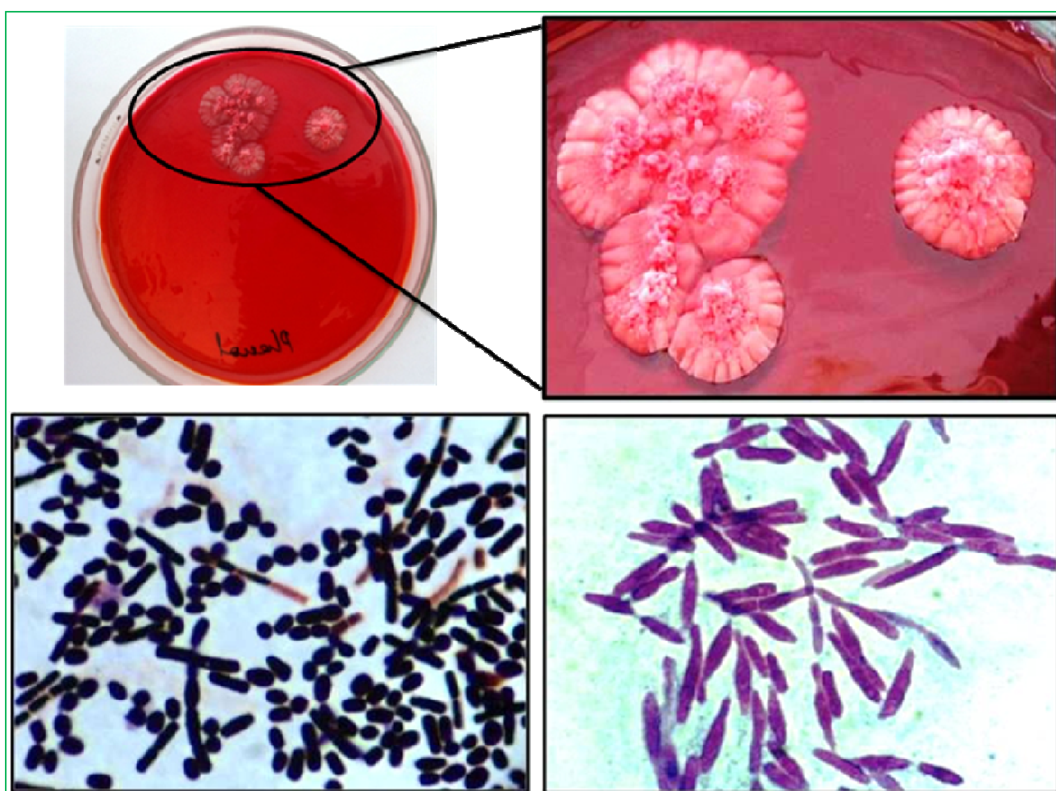

Fig. 3: Colonies grown on SDA containing 0.1% Phenol red, uptake of dye attributed by the respective colour of the colonies and a zone of clearance(Dev and Thankamani, in press)

The laccase finds its limited use in biodegradation of lignin due to its size limitation as it cannot have proper diffusion into pulp fibres. To overcome these limitations, often the mediators are used which enhance the oxidation potential of laccase. The role of mediators in lignin biodegradation has been well covered by Argyropoulos, 2001. *Pycnosporus cinnabarinus* does not secrete either LiP or MnP and does secrete only laccase required for lignin breakdown by producing a metabolite which works as a mediator for redox potential for the degradation of non phenolic lignin and synthetic lignin by laccase (Eggert *et al.*, 1996a; Eggert *et al.*, 1996b).

Dev and Thankamani, 2012 have studied the enzymatic activity of LiP, MnP and Laccase from MVI.2011. It has been reported that fungus like *Phlebia radiata* was an active wood degrader which produced large amounts of ligninolytic peroxidases and laccase (Krcmar *et al.*, 1999). So far *P.chrysosporium* is the best studied for lignin degradation by number of extracellular enzymes, the most important being LiP (Glenn *et al.*, 1983; Kirk *et al.*, 1986; Renganathan and Gold, 1986; Tien and Kirk, 1984). Despite much work, there is very little evidence that *P.chrysosporium* can cleave polymeric lignin. It has been concluded by a few that LiPs do not cleave lignin in vivo (Sarkanen *et al.*, 1991).

Lignin is composed of monolignols such as p-hydroxyphenyl, guaiacyl, and syringyl alcohols which are chemically distinct subunits and their proportions vary among different plant species. Different proportions of Laccase, lignin peroxidase and Manganese peroxidase are required to promote the efficiency of degradation (Masarin *et al.*, 2011; Weng *et al.*, 2008; Zhu *et al.*, 2002 ; Jordaan, 2005). Reports (Dube *et al.*, 2008) suggest that Laccase is the most preferred ligninase enzyme but can oxidise only phenolic lignin content (Bourbonnais and Paice, 1990; Breen and Singleton, 1999; Martínez *et al.*, 2009) which form very small fraction of total polymer content of natural lignin (Martínez *et al.*, 2009; Bourbonnais and Paice, 1990). In contrast, LiP is the most efficient ligninase enzyme catalysing the degradation of aromatic amines, ethers, phenolic and non-phenolic compounds, and polycyclic aromatic hydrocarbons (Breen and Singleton, 1999; Martínez *et al.*, 2009).

The fungus MVI.2011 used by Dev and Thankamani, 2012 had more powerful capability than a strain F-3 of *Aspergillus sp* (Yang *et al.*, 2011) to degrade lignin. The strain F-3 had shown only Laccase and MnP activity of 3.5 Ul-1 and 28.2 Ul-1 respectively, unlike MVI.2011 which showed LiP (9.39 Uml^{-1}), MnP (2.093 Uml^{-1}) and Laccase (3.5 Uml^{-1}) activities. As reported by Glenn *et al.*, 1983, LiP was demonstrated to act on a range of lignin compounds. Similarly a large number of documented literature has reported the presence of significant amounts of LiP and MnP activites in the ligninolytic cultures of *Bjerkandera*, *Phanerochaete*, and *Trametes* species whereas in contrast other white rot fungi including *Pleurotus*, *Phlebia*, and *Ceriporiopsis* species showed only MnP activity (Hattakka, 1994; Ruttimann *et al.*, 1992).

Although the enzyme activities of LiP have been studied to a considerable extent what still remains elusive is the location of binding sites (Du *et al.*, 1992). Though former two enzymes have been studied significantly the potential of laccase has not been well characterized (Cameron *et al.*, 2000). At the level of the above study the three enzymes did not present a holistic support for its biodegradation ability as there might be other enzymes which were not covered under the purview of their study (Emtiazi *et al.*, 2001). Thus the available data about the presence of three ligninolytic enzyme makes the fungus MVI.2011 as suitable alternative to the existing remediation system as it widens the scope for its potential to be used in degradation of persistent environmental pollutant from various industries and paper mills in particular.

4.0 Other enzymes of ligninolytic system

Beside the three main enzymes, other enzymes that are considered to be important for lignin breakdown are: Aryl alcohol dehydrogenase, Cellobiose, Aromatic acid reductase, Vanillate hydroxylase, Dioxygenase and Catalase**.** Aryl alcohol dehydrogenase is produced mainly *P. chrysosporium* and is an intracellular enzyme which is involved in aromatic ring cleavage and reduction of Cα aldehydes. This enzyme is believed to act in combination with lignin peroxidase for the degradation of non-phenolic β-o-4 lignin models. The possible requirement of this enzyme was due to non-accessibility of Cα-oxo compounds for LiP attribute to their high electro potential (Buswell & Eriksson, 1979; Muheim *et al.*, 1991). Cellobiose enzyme is involved in the reduction of quinones and cellobiose to cellobiono-l, 5-lactone. Westmark and Eriksson (1974) discovered this enzyme during cultivation of *Trametes versicolor.*

It was later purified from cellulose cultures of *P. chrysosporium* and identified to be a FAD enzyme. The aromatic reductase is involved in reduction of aromatic acids. Leisola and Fiechter (1985) had reported its production from *P. Chrysosporium,* while it was also detected in *Phlebia radiata* by Lundell *et al.* (1990).
Vanillate hydroxylase catalyses the oxidative decarboxylation of vanillic acid and has been reported from *P. chrysosporium* by Buswell and Eriksson (1988). De oxygenase enzyme is concerned with aromatic ring cleavage and found to be produced by *P. Chrysosporium* (Buswell & Eriksson, 1979). Catalase enzyme converts H_2O_2 to water and oxygen thereby preventing the inactivation of enzyme system by excess of H_2O_2 (Kwon & Anderson, 2001). It is produced under nutrient limited conditions by *P. chrysosporium* and not connected with the production of other ligninolytic enzymes.

5.0 Other Applications of ligninolytic system in dye degradation

Fungi are capable of acting upon wide range of environmental pollutants. Glenn and Gold, 1983 had reported for the first time the decolourization of several polymeric dyes by fungus *P. chrysosporium.* The experiments using dyes Poly B-411, Poly R-481 and Poly Y-606 suggested dye uptake by lignin degrading enzymes as substrates. Another report by Ulmer *et al.*, 1984, showed decolourization of Remazol Brilliant Blue R. Chet *et al.*, 1985 studied the correlation between the decolourization of dye Poly B-411 (poly-(vinylamine sulfonate)-anthraquinone) with lignin degradation by fungi.

One hundred and seventy strains of brown-rot, white-rot, soft-rot and xylophilous fungi were screened for their decolourization ability of dye Poly R-478 and to establish a correlation between decolourization and their phenol oxidase and peroxidase activity. However authors could not conclude any correlation between the production of MnP or LiP and the decolourization of dyes (Freitag & Morrell, 1992). However significant reports by other authors have shown correlation between decolourization of dye and production of peroxidase. The study by DeJong *et al.*, 1992 showed that decolourization of Poly R by three of the 67 new fungal strain isolates were significantly higher that that of *P. chrysosporium. P.chrysosporium* and have been reported to decolourize tri phenyl methane dyes, including para rosaniline, crystal violet, bromophenol blue, cresol red, malachite green, ethyl violet and brilliant green (Bumpus & Brock, 1988). Literature has also reported a group of azo dyes to be attacked by *P.chrysosporium* (Cripps *et al.*, 1990).

Sulpho and azo group do not occur in natural environment and thus sulphonated azo dyes cannot be acted upon by the microbe for degradation. Therefore the susceptibility of azo dyes to degradation was increased by attaching guaiacyl substituents analogous to the structures present in lignin to favour degradation (Paszczynski *et al.*, 1991). It was reported by Paszczynski and Crawford (1991) that veratryl alcohol is required for the oxidation of some azo dyes by LiP. Four different groups of dyes (polymeric, heterocyclic, azo, and tri phenyl methane) were reported to be decolourized by three major lignin degrading peroxidase isozymes (H2, H7, H8) (Ollikka *et al.* (1993). Spadaro *et al.*, 1992 have reported that *P. chrysosporium* was able to mineralise a variety of azo dyes even without sulpho groups. A number of other azo dyes have been studied for their use as potential substrates for assaying LiP and MnP of white rot fungi (Pasti-Grigsby *et al.,* 1994). Several other studies on the aerobic breakdown of the exotic dyes further demonstrated the ability of these enzymes to decolourize dyes (Gogna *et al.*, 1991; Pointing & Vrijmoed, 2000).

Dev and Thankamani, have found MVI.2011 (Article in press) to degrade Azure B and Phenol red dye and utilize these dyes as substrates. Interestingly, colonies of MVI.2011 even after prolonged growth, incubation for 3-4 weeks at ambient temperature, showed no asexual, sexual spores or any other reproductive structures. This property rendered the organism distinct from other major classes of fungi breaking down lignin. Literature showed that dye decolourization was associated with fungal growth and hyphal uptake and was strongly dependent on mycelial morphology(Fig. 2 & Fig. 3). At pH 5.0 and temperature 28°C the dye degradation obtained was up to 89% (Erdal and Taskin, 2010). Different classes of fungi have been reported to decolourize range of dyes including azo dyes. Most of the fungi reported so far have shown maximum decolourization within 1-2 weeks at optimum pH 6-7(Singh *et al.*, 2013; Saranraj *et al.*, 2010; Asgher *et al.*, 2008; Balaji *et al.*, 2012, Kumar *et al.*, 2011). Reports exist on the utilization of ligninolytic system of two bacterial isolates *Pseudomonas aeruginosa* and *Serratia marcescens* involved in the decolourization of textile dye based effluent by up to 58% though decolourization of paper-pulp effluent was more, up to 75 % (Bholay *et al.*,

2012). Similarly, fungi known for dye degradation have also been studied for their lignin degradation capability as reported by Abarnadevi *et al.*, 2013.

Most of the reports available have shown dye decolourization by fungus in 7-14 days, however fungus MVI.2011 could decolorize the dye within 24 hours which makes it all the more unique with respect to the available biological means which are slow growing. The microscopic studies of dye degradation by MVI.2011 showed that fungal hyphae absorbed the dye and produced very characteristic colony colour as well as marked influence on microscopic cell morphology depending on the dye used unlike most of the available reports which failed to show if growth on dye containing media was associated with dye degradation and dye utilization as a carbon source, which was very much evident in the fungal isolate MVI.2011 which utilized the dye (Azur B and Phenol Red) as a carbon source (in press).

Bio treatment process utilising the fungus strain MVI.2011 can offer an easy, inexpensive and effective alternative for colour removal of textile dyes at high pH conditions prevalent in the field as against the traditional waste water treatment which is expensive and requires large number of chemicals contributing to environmental pollution.

6.0 Conclusion:

The present review on ligninolytic system from microbes provides detailed information on the application of fungi and bacteria in the effluent treatment emanating from paper and pulp mills and dye industries. However, despite of large technological advancements and stringent government regulations, little has been done to promote safe environmental friendly treatment process especially in developing countries like India. Literature shows several significant studies done on the lignin degradation using approaches in biotechnology but the fungi used in various methods suffers drawbacks owing to the environmental conditions they are exposed to. Most of the fungi studied so far are acidophilic and thus they are inefficient to grow on medium containing effluents from pulp and paper industries that are highly alkaline. In view of the requirement for efficient biological system to degrade lignin biomass from paper pulp effluent, the need for some lignin degrading novel biological system gains added attention. Therefore characterization of novel fungus with broad spectrum action on effluent waste is the need of the hour and MVI.2011 hold such promise for future chemical engineers who are looking for better, safer and environment friendly “green Technology”.

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