2. REVIEW OF LITERATURE

2.1. Overview of the textile industry

One of the largest and oldest sectors in terms of economy, output, investment and employment of the country is textile industry. This sector provides employment nearly 35 million people and after agriculture, is the second-highest employer in the country. Textile industry accounts for around 4% of Gross Domestic Product, 14% of industrial production, 9% of excise collections, 18% of employment in the industrial sector and 16% of the country’s total exports earnings. With direct linkages to the rural economy and the agriculture sector, it has been estimated that one of every six households in the country depends on this sector, either directly or indirectly, for its livelihood. Fundamentally the textile industries are classified on the basis of the types of textile fiber they use. These are cellulose fibers, protein fibers and synthetic fibers. Cellulose fibers are obtained from plant sources such as cotton, rayon, linen, ramie, hemp and lyocell (Bledzki and Gassan, 1999). Protein fibers are obtained from animals and include wool, angora, mohair, cashmere and silk. Unnaturally synthesized fibres include polyester, nylon, spandex, acetate, acrylic, ingeo and polypropylene.

2.2. Classification of dyes

In 1856 chemist William Perkins accidentally discovered the synthetic dye, mauve, during the synthesis of quinine from aniline. There are several ways for classification of dyes. Based on the chemical structure or chromophore, 20-30 different dye groups are identified. Azo (monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine and
triaryl methane dyes are the most important chromophores (Fig.1) (Gregory, 1990; Manu, 2003; Suteu et al., 2011).

**Fig.1. The most important chromophores.**

Based on color, structure or method of application in the Color Index (C.I.), most of the commercial dyes are classified. From 1924 at every three months these dyes are edited by the "Society of Dyers and Colourists" and the "American Association of Textile Chemists and Colorists". The last edition of the Color Index lists about 13000 different dyes (http://www.colour-index.com/about). Each dye is assigned to a C.I. generic name determined by its application and color. Some of the dyes and its characteristics are given in Table 1.

**Table 1. Dyes and its characteristics.**

<table>
<thead>
<tr>
<th>Application</th>
<th>Class</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid dyes</td>
<td></td>
<td>These dyes are highly water-soluble better light fastness than basic dyes due to the presence of sulphonic acid groups. These dyes forms ionic bonds between the protonated functionalities of the fibers (-NH3+) and the negative charge of the dyes. These dyes also form Van-der-Waals, dipolar and hydrogen bonds. The most common structures are azo, anthraquinone and triarylmethane.</td>
</tr>
</tbody>
</table>

Cont…
### Application Class | Characteristics
--- | ---
Reactive dyes | These dyes forms covalent bonds with -OH, -NH or -SH groups in cotton, wool, silk and nylon. The problem of colored effluents associated with these dyes is due to the hydrolysis of the reactive groups that occurs during the dyeing process. The most common structures are azo, metal complex azo, anthraquinone and phthalocyanine.
Direct dyes | Their flat shape and length enables them to bind along-side cellulose fibers and maximize the Van-der-Waals, dipole and hydrogen bonds. Only 30% of the 1600 structures are still in production due to their lack of fastness during washing. The most common structures are sulphonated azo dyes.
Basic dyes | Basic dyes work very well on acrylics due to the strong ionic interaction between dye functional groups such as -NR₃⁺ or =NR₂⁺ and the negative charges in the copolymer. The most common structures are azo, diarylmethane, triarylmethane and anthraquinone.
Mordant dyes | Mordants are usually metal salts such as sodium or potassium dichromate. They act as “fixing agent” to improve the color fastness. They are used with wool, leather, silk and modified cellulose fibers. The most common structures are azo, oxazine or triarylmethane.
Disperse dyes | These dyes are non-ionic in structure, with polar functional groups like -NO₂ and –CN that improve water solubility, Van-der-Waals forces, dipole forces and the color. They are usually used with polyester. The most common structures are azo, nitro, anthraquinones or metal complex azo.
Pigment dyes | These dyes are insoluble, non-ionic compounds or salts, representing 25% of all commercial dye names, retain their crystalline or particulate structure throughout their application. The most common structures are azo or metal complex phthalocyanines.

Cont…
<table>
<thead>
<tr>
<th>Application Class</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vat dyes</td>
<td>Vat dyes are insoluble in water, but may become solubilized by alkali reduction (sodium dithionite in the presence of sodium hydroxide). The produced <em>leuco</em> form is absorbed by the cellulose (Van-der-Waals forces) and can be oxidized back, usually with hydrogen peroxide, to its insoluble form. The most common structures are anthraquinones or indigoids.</td>
</tr>
<tr>
<td>Ingrain dyes</td>
<td>The term <em>ingrain</em> is applicable to all dyes formed <em>in situ</em>, in or on the substrate by the development, or coupling, of one or more intermediate compounds and a diazotized aromatic amine. In the Color Index the sub-section designated Ingrain is limited to tetraazaporphin derivatives or precursors.</td>
</tr>
<tr>
<td>Sulphur dyes</td>
<td>Sulphur dyes are complex polymeric aromatics with heterocyclic -S-containing rings representing about 15% of the global dye production. Dyeing with sulphur dyes (mainly on cellulose fibers) involves reduction and oxidation processes, comparable to vat dyeing.</td>
</tr>
<tr>
<td>Solvent dyes</td>
<td>Non-ionic dyes that are used for dyeing substrates in which they can dissolve as plastics, varnish, ink and waxes. They are not often used for textile processing. The most common structures are diazo compounds that undergo some molecular rearrangement, triarylmethane, anthraquinone and phthalocyanine.</td>
</tr>
<tr>
<td>Other dye classes</td>
<td>Food dyes are not used as textile dyes. Natural dyes use in textile processing operations is very limited. Fluorescent brighteners mask the yellowish tint of natural fibers by absorbing ultraviolet light and weakly emitting blue light. Not listed in a separate class in the Color Index, many metal complex dyes can be found (generally chromium, copper, cobalt or nickel). The metal complex dyes are generally azo compounds.</td>
</tr>
</tbody>
</table>

Source: Adinew, 2012
Among various synthetic dyes, azo dyes are the most important synthetic dyes and dyes without appropriate treatment left are harmful for many living organisms (Hao et al., 2000; Pinheiro et al., 2004; Puvaneswari et al., 2006; Ghaly et al., 2014; Lavanya et al., 2014). Azo dye synthesis and its impact discussed below.

2.3. Azo dyes

Azo dyes are the largest group of synthetic dyes and pigments with industrial application due to their relatively simple synthesis and showing the largest spectrum of colors (Carliell et al., 1995; Fang et al., 2004; Khehra et al., 2006; Bae and Freeman 2007; Kusic et al., 2011). Worldwide over 10,000 different dyes and pigments are used in dyeing and printing industries and it is estimated to be 8,00,000 tons per year. Atleast 10% of the used dyestuff enters the environment as waste water (Cha et al., 2001; Levin et al., 2002; Palmeiri et al., 2005; Zhao and Hardin, 2007; Saratale et al., 2011). Azo dyes with only one N=N double bond is called monoazo dyes while diazo, triazo and polyazo dyes contain two, three or more N=N double bonds, respectively. The azo groups are normally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocyclic or aliphatic groups (Zollinger, 2003). The general structure of azo dye was given in Fig.2.

**Fig.2. General structure of azo dyes**

\[(R_1\text{--}N=N\text{--}R_2)\]

(where R can be an aryl, heteroaryl or - CH = C (OH) - alkyl derivative).
The azo dye synthesis (Fig. 3) involves diazotization of a primary aromatic amine to give a diazonium salt. Diazonium compound is coupled with one or more nucleophiles such as amino and hydroxyl groups generally at para position (Zollinger, 2003).

Fig.3. Azo dye synthesis

2.4. Azo dyes and its impact on environment

Due to their low cost, versatility and synthetic accessibility, azo dyes are often the colourants of choice. These are photolytically stable, highly visible even at the concentration < 1 mg/L (Nigam et al., 2000; Rieger et al., 2002). During the dyeing process it has been estimated that the losses of colorants to the environment can reach 10–50%. Some dyes are highly toxic and mutagenic, and also affects light penetration and photosynthesis (Vaidya and Datye 1982; Rajaguru et al., 1999; Forgacs et al., 2004; Przystaś et al., 2012).

The release of hazardous dyes in the environment is risky and through food chain it affects the man also (Van der Zee, 2002; Puvaneswari et al., 2006).

The acute toxicity of azo dyes is quite low and the mortality rate of algae and fish are not affected by dye concentrations below 1 mg/L, basic and acid dyes are toxic to them. In the mammal tests only a few azo dyes showed LD50 values below 250 mg/kg, whereas a majority showed LD50 values between 250 and 2000 mg/kg (Van der Zee, 2002). The
toxicity of azo dyes can be decreased by the sulphonation of dyes (Brown and DeVito 1993). Sensitization to azo dyes has been seen in textile industry since 1930, when 20% of the workers dyeing cotton with red azoic dyes, developed occupational eczema (Giusti et al., 2002). The majority of sensitizing dyes, present in cloth, practically all belong to the group of disperse dyes (Seidenari et al., 2002). Bioaccumulation of azo dyes has been extensively investigated in fish (Niimi et al., 1989; Erickson and McKim 1990; Brown and DeVito 1993; Puvaneswari et al., 2006). The majority of azo dyes, if highly purified are not mutagenic. However, many of the commercial available azo dyes, due to impurities, show metabolic activation and mutagenic activity in vitro (Jung et al., 1992; Arcos and Argus 1994; Brown and DeVito 1993). Many studies have been conducted showing the toxic potential of aromatic amines from azo dyes (Chung and Cerniglia 1992; Weisburger, 2002; Pinheiro et al., 2004).

2.5. Textile dye removal techniques

Textile dye effluents are complex, having a wide range of dyes, pH, BOD, COD and TDS and natural impurities such as dispersants, levelling agents, acids, alkalis, salts and sometimes heavy metals (Sreedhar Reddy et al., 2008; Ahmad et al., 2011; Varsha et al., 2013; Rajeswari et al., 2013; Sharma et al., 2013; Ghaly et al., 2014).

Many physico chemical processes such as membrane filtration, coagulation, flocculation, precipitation, flotation, adsorption, ion exchange, ultrasonic mineralization, electrolysis, chemical reduction and advanced chemical oxidation are being followed to treat textile dyeing effluent (Bossmann et al., 1998; Aplin and Wait 2000; Akbari et al., 2002; Bansode et al., 2003; Al-Ghouti et al., 2003; Gogate and Pandit 2004a; Aguilar
The advanced oxidation processes include chlorination, bleaching, ozonation, Fenton oxidation, photocatalytic oxidation and wet-air oxidation (Robinson et al., 2001a, Alaton and Ferry 2003, Kusvuran et al., 2004, Gogate and Pandit 2004a; Swaminathan et al., 2012, Ghaly et al., 2014). These processes generate large quantities of sludge having toxic by-products (Robinson et al., 2001; Kumar et al., 2006; Adinew 2012; Huber and Carre 2012; Huang et al., 2014). Biological techniques include biosorption and biodegradation in aerobic, anaerobic or combined anaerobic/aerobic treatment processes with bacteria, fungi, plants, yeasts, algae and enzymes (Nyanhongo et al., 2002; Ramalho et al., 2002; Mohan et al., 2002; Pearce et al., 2003; Blümel and Stolz 2003; Ramalho et al., 2004; Forgacs et al., 2004; Acuner and Dilek 2004; Aubert and Schwitzguebel 2004; Mbuligwe, 2005; Christian et al., 2005; Mohan et al., 2005; Shrivastava et al., 2005). For this reason, several factors decide the technical and economic feasibility of treatment process of textile dyeing effluent. The individual process may not be sufficient to achieve complete degradation of textile dyeing effluent because each treatment process has its own disadvantages.

An overview of the most important techniques is presented in the following chapters.

2.5.1. Physical treatment methods for textile dyes and dyeing effluents

2.5.1.1. Membrane filtration for the treatment of textile dyes and dyeing effluents

The complex molecules such as dyes and salts can be separated and reused from textile dyeing effluents through nanofiltration and reverse osmosis treatment processes (Erslew et al., 1988; Crossley, 1995; Van't Hul et al., 1997; Sójka-Ledakowicz et al.,
1998; Koyuncu et al., 2004, Kim et al., 2005). The reverse osmosis process can separate impurities from effluents with greater than 100 daltons molecular weight using a semipermeable membrane. Whereas in nanofiltration, it is greater than 200 daltons. The limitations of these processes are maintenance of membranes and its high cost. (Cooper, 1993; Vandevivere et al., 1998; Rozzi et al., 1999; Hao et al., 2000; Marcucci et al., 2001; Koyuncu, 2003; Ciardelli et al., 2003).

2.5.1.2. Coagulation and flocculation for the treatment of textile dyes and dyeing effluents

Several research reports highlighted the usage of the inorganic coagulants such as lime, aluminum, magnesium and iron salts for coagulation in the treatment of textile-processing wastewater to partly remove TSS, BOD, COD and color (Sarasa et al., 1998, Semerjian and Ayoub 2003; Allegre et al., 2004; Peres et al., 2004; Aguilar et al., 2005; Golob et al., 2005; Verma et al., 2012). In this process the coagulant react with the pollutant and forms coagulates or flocks which are precipitated, removed by flotation, settling, filtration or other physical technique. This is further treated to reduce its water content and toxicity (Aguilar et al., 2002; Semerjian and Ayoub 2003; Papic et al., 2004; Golob and Ojstrsek 2005, Mishra and Bajpai 2005). An organic anionic, cationic or non-ionic coagulant forms less sludge than inorganic coagulants (Al-Mutairi et al., 2004; Sengil and Ozdemir, 2012).

2.5.1.3. Adsorption for the treatment of textile dyes and dyeing effluents

In this process ions or molecules present in one phase tend to accumulate and concentrate on the surface of another phase. Physical adsorption occurs when weak
interparticle bonds exist between the adsorbate and adsorbent. The removal of adsorbates from liquids depends on the equilibrium between the adsorbates and the adsorbents (Suzuki, 1997). Dye effluents are mixtures of components with different absorption degrees and concentrations. In these cases weaker bounds are formed with the adsorbent and some material can be released back into the stream. Several reports highlighted the usage of adsorbents such as activated carbons, high-surface-area inorganic materials, synthetic ion-exchange resins and cellulose-based adsorbents such as chitin (poly-N-acetylglucosamine), synthetic cellulose and other fiber-based bioadsorbents. Standard ion exchange systems have not been widely used for treatment of dye effluents due to the high cost of organic solvents to regenerate the ion-exchanger (Southern, 1995; Walker and Weatherly, 1997; Slokar and Le Marechal 1998; Robinson et al., 2001a; Wu et al., 2004). Several reports are highlighted that activated carbon is effective at removing many different dyes from aqueous streams (Slokar and Le Marechal 1998, Robinson et al., 2001a; Sun et al., 2012). Cost and formation of secondary sludge are the limitations of activated carbon adsorption for the removal of textile dyes (Pereira et al., 2003, Faria et al., 2004, Forgacs et al., 2004, Golob and Ojstrsek 2005).

Several reports have highlighted the low-cost adsorbents for the removal of textile dyes such as high-surface-area silica, cinder ash and clays, corn, wheat, rice husks, wood chips, sawdust, bark, bagasse pith, cotton waste, cellulose, bacterial biomass, fungal biomass, yeast biomass, etc. (Dönmez, 2002; Woolard et al., 2002; Waranusantigul et al., 2003; Shawabkeh and Tutunji 2003; Guo et al., 2003; Aksu and Dönmez 2003; Ho and McKay 2003; Sun and Yang 2003; Walker et al., 2003; Malik, 2003; Malik, 2004; Garg et al., 2004; Wibulswas, 2004; Gong et al., 2005; Delval et al., 2005; Janos et al., 2005;
Gupta et al., 2005; Alkan et al., 2005; Aksu and Dönmez 2005; Özacar et al., 2005; Zolgharnein et al., 2014).

2.5.2. Chemical methods for treatment of textile dyeing effluent

2.5.2.1. Electrolysis for the treatment of textile dyes and dyeing effluents

The effective technique for the removal of colour from dyes and pigments, reduction of BOD COD and TDS from waste water is electrochemical method (Vlyssides et al., 2000; Gürses et al., 2002; Daneshvar et al., 2004; Bayramoglu et al., 2004; Cerón-Rivera et al., 2004; Fernandes et al., 2004; Shen et al., 2005; Alinsafi et al., 2005; Carneiro et al., 2005; Phalakornkule et al., 2010). In this process an electric current passed through the wastewater by using sacrificial iron electrodes to produce ferrous hydroxide in solution. The formed Fe (OH)$_2$ removes soluble and insoluble acid dyes from the effluent. Moreover Fe (II) can reduce azo dyes to arylamines. Water can also be oxidized resulting in the formation of O$_2$ and O$_3$. The efficiency of the electrochemical system in pollutant removal can often reach 90%. However, the process is expensive due to large energy requirements, limited lifetime of the electrodes and uncontrolled radical reactions (Hao et al., 2000, Van der Zee, 2002; Cerón-Rivera et al., 2004).

2.5.2.2. Ozone oxidation for the treatment of textile dyes and dyeing effluents

Ozone is a powerful and rapid oxidizing agent that can react with most species that contains multiple bonds (such as C=C, C=N, N=N, etc.) and with simple oxidizable ions such as S$^2-$, to form oxyanions such as SO$_3$$_2^-$ and SO$_4$$_2^-$ (Gogate and Pandit 2004a). Ozone rapidly tends to decolorizes water-soluble dyes than non-soluble dyes (vat dyes and disperse dyes) and it can oxidize all components in textile-processing wastewater (Özelbege
Decomposition of ozone requires high pH values (pH >10). In alkaline solutions ozone reacts almost indiscriminately with all compounds present in the reacting medium (Aplin and Wait 2000; Chu and Ma Chi 2000) converting organic compounds into smaller and biodegradable molecules (Peralta-Zamora et al., 1999a). Ozone treatment provides a logic that the biological methods can mineralize dyes completely (Krull et al., 1998, Krull and Hempel 2001). A major limitation of the ozonation process is the high cost of ozone generation process coupled with its very short half-life (Gogate and Pandit 2004a).

2.5.2.3. Fenton reagents for the treatement of textile dyes and dyeing effluent

The oxidation system based on the Fenton's reagent (hydrogen peroxide in the presence of a ferrous salt) has been used for treating both organic and inorganic substances (Walling, 1998; MacFaul et al., 1998; Hao et al., 2000; Moura et al., 2005; Wang et al., 2005). The hydroxyl and ferryl complex are involved in the fenton’s mechanism during the oxidation of non-biodegradable toxic waste effluents (Bigda, 1996; Chen and Pignatello 1997; Bossmann et al., 1998; Apline and Wait 2000; Nesheiwat and Swanson 2000; Kang et al., 2002b; Hsueh et al., 2005). Fenton oxidation process can decolorize a wide range of dyes and in comparison to ozonation, the process is relatively cheap and have resulted in maximum COD reduction (Ince and Tezcanli 1999; Park et al., 1999). Fenton oxidation is limited to the fact the textile process wastewaters usually have high pH, while the Fenton process requires low pH. At higher pH, large volumes of waste sludge are generated by the precipitation of ferric iron salts and the process loses its effectiveness (Van der Zee, 2002).
2.5.2.4. Photocatalytic method for the treatment of textile dyes and dyeing effluents

The photocatalytic or photochemical degradation processes are gaining importance in the area of wastewater treatment, since these process results in complete mineralization with operation at mild conditions of temperature and pressure. The photo-activated chemical reactions are characterized by a free radical mechanism initiated by the interaction of photons of a proper energy level with the molecules of chemical species present in the solution, with or without the presence of the catalyst (Gogate and Pandit 2004a). Using UV radiation the radicals can be easily produced. UV light has been tested in combination with H₂O₂, TiO₂, Fenton reagents, O₃ and other solid catalysts for the decolorization of dye solutions (Hao et al., 2000, Gogate and Pandit 2004b). While the UV/H₂O₂ process appeared too slow, costly and little effective for potential full-scale application, the combination UV/TiO₂ seems more promising. With UV/TiO₂ treatment, a wide range of dyes can be oxidized and generally not only decolorized but also highly mineralized (Gonçalves et al., 1999; Peralta-Zamora et al., 1999a; Gomes de Moreas et al., 2000, Bauer et al., 2001, Konstantinou and Albanis 2004, Forgacs et al., 2004, Hasnat et al., 2005, Toor et al., 2006). But UV penetration in dye solutions is limited due to the highly colored nature of the effluents, the best use of UV technology is a posttreatment after ozonation (Vandervivere et al., 1998).

2.5.3. Biological treatment methods for textile dyes and dyeing effluent

2.5.3.1. Role of bacteria in textile dye degradation

Biological treatment has been the most economical alternative when compared to other physical and chemical processes. Several reports highlighted that different bacterial

Azo dyes are not readily metabolized under aerobic conditions. It is been reported that, the bacteria are able to degrade certain azo dyes with specialized reducing enzymes, aerobically (Stolz, 2001). In contrast, under anaerobic conditions many bacteria reduce azo dyes by the activity of unspecific, soluble, cytoplasmatic reductase, known as azo reductases. The anaerobic reduction degrades the azo dyes that are converted into aromatic amines (Blümel *et al.*, 2002), which may be toxic, mutagenic, and possibly carcinogenic to mammalians (Pinheiro *et al.*, 2004). Therefore, to achieve complete degradation of azo dyes involves aerobic biodegradation of the produced aromatic amines is necessary (Haug *et al.*, 1991, Sishadri *et al.*, 1994, O’Neill *et al.*, 2000, Kalyuzhnyi and Sklyar 2000, Lourenço *et al.*, 2001, Shaw *et al.* 2002, Isik and Sponza 2003, Isik and Sponza 2004, Libra *et al.*, 2004, Supaka *et al.*, 2004, Sponza and Isik 2005). The bacterial degradation of different dyes reported by different authors were listed in Table 2.
Table 2. Bacterial biodegradation of mixture of dyes

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Decolorization (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture of dyes</td>
<td>87 (ADMI)</td>
<td>Saratale et al., (2013)</td>
</tr>
<tr>
<td>Amaranth, Acid Orange 52, Direct Blue 71</td>
<td>Efficient</td>
<td>Liu et al., (2013)</td>
</tr>
<tr>
<td>Sulphonated Azo dyes, Acid Orange 7 (AO7) and Acid Red 88 (AR88)</td>
<td>~100</td>
<td>Vasconcelos et al., (2000)</td>
</tr>
<tr>
<td>Mixture of dyes</td>
<td>High</td>
<td>Naik et al., (2012)</td>
</tr>
<tr>
<td>Remazol Red, Rubine GFL, Brown 3REL, Scarlet RR, Golden Yellow HER, Methyl Red, Brilliant Blue GL</td>
<td>87</td>
<td>Kurade et al., (2011)</td>
</tr>
<tr>
<td>8 textile dyes</td>
<td></td>
<td>Joshi et al., (2010)</td>
</tr>
<tr>
<td>Congo red, Bordeaux, Ranocid Fast Blue and Blue BCC</td>
<td></td>
<td>Tony et al., (2009)</td>
</tr>
<tr>
<td>Dyes</td>
<td>Percentage</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------</td>
<td>------------------</td>
</tr>
<tr>
<td>16 Azo dyes</td>
<td>90</td>
<td>Joshi et al., (2008)</td>
</tr>
<tr>
<td>Azo dyes</td>
<td>-</td>
<td>Asad et al., (2007)</td>
</tr>
<tr>
<td>Naphthalene-containing sulfonated azo dyes</td>
<td>100</td>
<td>Vijaykumar et al., (2007)</td>
</tr>
<tr>
<td>Amaranth, Fast Red E and Ponceau S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Blue 74, Acid Orange 7, Acid Red 106,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Yellow 4 and Direct Yellow 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Acid Black 172, Acid Blue 264, Acid Yellow 42, Direct Black 22, Direct Orange 39, Direct Red 224, Direct Red 243, Direct Yellow 86, Reactive Black NR, Reactive Black 5, Reactive Blue 160, Reactive Blue 171, Reactive Blue 198, Reactive Blue 222, Reactive Green 19, Reactive Red 120, Reactive Red 141, Reactive Red 198 and Reactive Yellow 84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Everzol Red RBN (Reactive Red 198a; R-r198b), Everzol Yellow 3RS (Reactive Yellow 176a; R-y176b), Everzol Black B (Reactive Black 5a; R-BK5b), Everzol Brilliant Red 3BS (Reactive Red 239a; R-r239b), Everdirect Supra Red BWS (Direct Red 243a; D-r243b), Everdirect Supra Yellow RL (Direct Yellow 86a; D-y86b), Everdirect Supra Orange 2GL (Direct Orange 39a; D-o39b), Sigma Orange (acid orange 7a; A-o7b)</td>
<td>&gt;95</td>
<td>Chen et al., (2003b)</td>
</tr>
</tbody>
</table>
Remazol Orange, Remazol Black Magenta, crystal violet, pararosaniline, brilliant green, malachite green, ethyl violet Cibacron Red (reactive dye), Remazol Golden Yellow (azo dye), Remazol Red (diazo dye); Remazol Navy Blue (diazo dye) and Cibacron Orange (reactive dye) and Remazol Blue Diazoe Evans blue (EB) and triphenylmethane brilliant green (BG).


2.5.3.2. Role of fungi in textile dye degradation

The most widely researched fungi in regard to dye degradation are the ligninolytic fungi. White-rot fungi in particular produced enzymes as lignin peroxidase, manganese peroxidase and laccase that degrade many aromatic compounds due to their non-specific activity (Stolz, 2001; Hatakka, 2001; McMullan, et al., 2001; Hofrichter, 2002, Wesenberg et al., 2003; Forgacs et al., 2004; Ehlers and Rose 2005; Srebotnik and Boisson 2005; Harazono and Nakamura 2005; Pazarlioglu et al., 2005b; Toh et al., 2003). Many reports highlighted the potential of these fungi to oxidize phenolic, non-phenolic, soluble and non-soluble dyes (Field et al., 1993, Pasti-Grigsby et al., 1992, Chao and Lee, 1994, Bumpus, 1995, Conneely et al., 1999, Kapdan et al., 2000, Borchert and Libra 2001, Heinfling-Weidtmann et al., 2001, Tekere et al., 2001, Kapdan and Kargi 2002, Martins et al., 2002b, Libra et al., 2003). Laccase from Pleurotus ostreatus, Schizophyllum commune, Sclerotium rolfsii and Neurospora crassa, decolorized (25%) individual commercial triarylmethane, anthraquinonic, and indigoid textile dyes (Abadulla et al., 2000). Other fungi also reported for the decolourization of textile dyes (Kim et al., 1995;
Kim and Shoda 1999; Cha et al., 2001; Abd El-Rahim et al., 2003; Ambrósio and Campos-Takaki 2004; Tetsch et al., 2005). Many organisms such as *Thermomucor indicae seudaticae*, *Pleurotus streatus*, *Gloeophyllum odoratum*, *Fusarium oxysporum*, *Phanerochaete chrysosporium*, *Galactomyces geotrichum*, *Aspergillus foetidus*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Trametes versicolor* are widely used for the decolourization of textile dyes (Kirby et al., 2000; Ambrosio and Campos-Takaki 2004; Machado et al., 2006; Nordstrom et al., 2008; Pakshirajan et al., 2010; Waghmode et al., 2011; Przystas et al., 2013; Idris et al., 2014; Taha et al., 2014). The degradation of different dyes by fungi reported by different authors were listed in Table 3.

**Table 3. Fungal degradation of mixture of dyes**

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Decolourization (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azo–Anthraquinone dye mixture Azure B, Congo Red, Trypan Blue and Remazol</td>
<td>74.93</td>
<td>Taha et al., (2014)</td>
</tr>
<tr>
<td>Brilliant Blue R</td>
<td>80</td>
<td>Przystas et al., (2013)</td>
</tr>
<tr>
<td>Yellow FG, Red 3BS, Orange 3R, Blue RSP, Black B, and remazol turquoise blue</td>
<td>82</td>
<td>Idris et al., (2014)</td>
</tr>
<tr>
<td>Reactive azo dyes (red, black and orange II)</td>
<td>88</td>
<td>Ambrósio et al., (2012)</td>
</tr>
<tr>
<td>Reactive blue 21, Reactive black 5 and Reactive orange 13</td>
<td>60–66</td>
<td>Pakshirajan and Singh (2010)</td>
</tr>
<tr>
<td>Remazol Brilliant Orange 1, Levafix Gold Yellow 10, Procion Yellow 14, Drimaren Brilliant Blue 17, Remazol Brilliant Blue 18, Cibacron Black 55, Procion Black 59,</td>
<td>80-90</td>
<td>Machado et al., (2006)</td>
</tr>
<tr>
<td>Dyes</td>
<td>Sources</td>
<td>References</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Drimaren Turquoise Blue 62, Drimaren Brilliant Red 67, and Remazol Red 75.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixtures of 4 reactive textile dyes, azo and anthraquinone dyes</td>
<td></td>
<td>Harazono and Nakamura (2005)</td>
</tr>
<tr>
<td>Meta or para aminobenzoic or aminosulfonic acids as diazo components and two fungal bioaccessible groups present in lignin structure, guaiacol or syringol, as coupling components</td>
<td></td>
<td>Martins et al., (2003)</td>
</tr>
<tr>
<td>Drimarene dyes</td>
<td></td>
<td>Sumathi and Manju, (2000)</td>
</tr>
</tbody>
</table>

2.5.3.3. Enzymatic treatment of textile dyes and effluents

The degradation of dyes by bacteria and fungi is mainly because of their enzyme systems. In bacterial dye degradation azo reductases play a vital role where as in fungi it is lignolytic enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase. Azo reductases catalyze the reaction only in presence of reducing equivalents like FADH and NADH (Abadulla et al., 2000; Soares et al., 2001; Joshni and Subramaniam 2011). Production of lignolytic enzymes and the role of oxidative enzymes such as LiP,
MnP and laccase in the bioremediation and biodegradation of pesticides and dyes by white rot fungi were well explained (Shin et al., 1997; Robinson et al., 2001; Mielgo et al., 2003, Lopez et al., 2004; Tišmal et al., 2010; Gao et al., 2010; Singh et al., 2013).

Hydrogen peroxide is essential for catalytic process of Lip and MnP, whereas oxygen is needed for laccases to degrade azo dyes through a non-specific free radical mechanism which prevents the formation of aromatic amines as degradation products (Joshni and Subramaniam 2011; Vishwakarma et al., 2012; Forootanfar et al., 2012; González-Riopedre et al., 2013). Recently laccases have received much attention from researchers due to their ability to oxidise both phenolic and non-phenolic, lignin related compounds as well as highly recalcitrant environmental pollutants (Nyanhongo et al., 2002; Forootanfar et al., 2012; Tian et al., 2013). Several reports highlighted the decolourization of textile dyes such as Reactive Black 5, blue HFRL Reactive Red 158 and Reactive Yellow 27 by laccase (Soares et al., 2001; Abrahão et al., 2008; Krishnaveni and Kowsalya 2011; Malini Devi et al., 2012; Moreira-Neto et al., 2013).

2.6. Importance of laccases for the treatment of textile dyes and effluents

Enzymes are biodegradable and exhibits high level of catalytic activity, the high degree of specificity and without side reactions. Removal of enzymes from contaminated streams, standardization in commercial preparations can be performed easily. However, the unstable nature of enzymes and the high cost of enzyme isolation and purification still discourage their extensive use. In spite of these disadvantages, the research on enzyme applications is in steady development and the technological problems are constantly being overcome (Chaplin and Bucke 1990; Silva et al., 2013). In contrast to the generally high
specificity of enzymes, laccases are rather unspecific. Laccase was first discovered by Yoshida in plants (Yoshida, 1883). The enzyme was named laccase about 10 years later after its isolation and purification (Michael et al., 2004). Laccases (EC 1.10.3.2) are multi–copper oxidases, which catalyze one electron oxidation of a wide range of inorganic and organic substances coupled with one four-electron reduction of oxygen to water (Robles et al., 2000; Alexandre and Zhulin 2000, Claus, 2003). Laccases can catalyze the polymerization of various phenols and halogen, alkyl- and alkoxy-substituted anilines (Kobayashi et al., 2001; Kobayashi and Higashimura 2003). In higher plants, laccase involves in the lignification process. (Bao et al., 1993, Wallace and Fry, 1999, Boudet, 2000; Richardson et al., 2000). Among other roles, laccase can protect the fungal pathogen from the toxic phytoalexins and tannins in the host environment (Pipe et al., 2000; Brasier and Kirk, 2001; Schouten et al., 2002). In insects, laccase is involved in cuticle sclerotization (Kramer et al., 2001; Mayer and Staples, 2002). Laccase genes have been found in gram-negative and gram-positive bacteria (Freeman et al., 1993; Givaudan et al., 1993; Claus and Filip, 1997; Diamantidis et al., 2000; Sanchez-Amat et al., 2001; Endo et al., 2002; Suzuki et al., 2003).

2.7. Culture conditions and laccase enzyme production

Laccases are the enzymes which are secreted out in the medium extracellularly by several fungi during the secondary metabolism (Scherer and Fischer 1998; Abdel-Raheem and Shearer 2002; Morozova et al., 2007).

Laccases are produced by several organisms such as Bacillus sp., Saccharomyces cerevisiae, Aspergillus sp., Phanerochaete chrysosporium, Theiophora terrestris and
Lenzites, Betulina, Phlebia radiate, Pleurotus ostreatus and Trametes versicolor, Gaeumannomyces graminis, Magnaporthe grisea, Ophiostoma novo-ulmi, Marginella, Melanocarpus albomyces, Monocillium indicum, Neurospora crassa, Botryosphaeria and Podospora anserina reported by Vasconcelos et al., 2000; Kiiskinen et al., 2002; Iyer and Chattoo 2003; Lyons et al., 2003; Palonen et al., 2003; Kiiskinen et al., 2004; Viswanath et al., 2008; Sheikhi et al., 2012. Hatakka (2001) reported that highest laccase enzyme producers are basidiomycetes and saprophytic fungi. Laccase enzyme production is influenced by many factors such as medium composition, Carbon and Nitrogen ratio, pH and temperature etc.

2.8. Influence of carbon and nitrogen source for laccase production

Carbon takes an essential part of all living organisms. Upon breakdown, carbon sources liberate energy, which is utilized by the organism for growth and development. The most readily usable carbon source is glucose (Levin et al., 2002). Several reports highlighted that glucose is the best carbon source for the maximum production of laccase from different organisms such as Trametes versicolor, Ganoderma lucidum, Lentinula edodes, Grifola frondosa, Phlebia sp., Trametes gallica, Pleurotus florida, Phlebia floridensis, P. brevispora, P. radiata, P. fascicularia, Botryosphaeria sp. and Pichia pastoris (Collins and Dobson, 1995; Perumal, 1997; Arora and Rampal, 2002; Cavallazzi et al., 2005; Dong et al., 2005; D’Souza-Ticlo et al., 2006; Revankar and Lele, 2006; Minussi et al., 2007; Palvannan and Sathishkumar, 2010; Sun et al., 2012)
Wang et al. (2005) reported that the production of laccase reached maximum when the medium is amended with 0.2 % of maltose as carbon source. Arora and Gill (2000) proved sugarcane bagasse is the best laccase inducer among the various supplements.

Few authors have reported that the high nitrogen concentration is required for the maximum production of laccase from *Phanerochaete chrysosporium*, *Pycnoporus sanguineus*, *Lentinus edodes* and *Coprinaceae* sp. (Buswell et al., 1995; Heinzkill et al., 1998; Baldrian et al., 2002; Chen et al., 2003; Eugenio et al., 2009). Whereas few authors have also reported that the low nitrogen concentration required for the maximum production of laccase from white rot fungi (Leatham and Kirk, 1983; Lo et al., 2002; Janusz et al., 2006). Best nitrogen sources for maximum laccase production from *Trametes pubescens* and *Trametes versicolor* are NH$_4$Cl, yeast extract and peptone reported by Revankar and Lele, 2006; Sharma and Arora, 2010; Strong, 2011. D'Agostini et al. (2011) reported that the highest laccase activities were recorded with a C/N ratio of 5.

**2.9. Influence of temperature on laccase production**

The effect of temperature is limited in case of laccase production. The optimal temperature of laccase differs greatly from one strain to another. In general the fungi were cultivated at temperatures between 25 °C and 30 °C for optimal laccase production (Minussi et al., 2007). It has been found that 25 °C is the optimal temperature for laccase production in presence of light, but, in case of dark, the optimal temperature is 30 °C (Thurston, 1994). The wood decaying basidiomycete *Steccherinum ochraceum* isolate 1833 was reported to produce three highly thermostable laccase isoforms with maximum activities in the region 75 - 80 °C (Chernykh et al., 2008; Hildén et al., 2009).
Several reports highlighted that the optimum temperature for maximum laccase activity was 30 - 40 °C (Zadrazil et al., 1999; Pointing et al., 2000; Nyanhongo et al., 2002; Sivakumar, 2010; Sivakami et al., 2012)

2.10. Influence of pH on laccase production

Much information is not available on the influence of pH on laccase production, but when fungi are grown in a medium with pH 5.0 laccase will be produced in excess (Thurston et al., 1994). The optimum value of pH varies according to the substrate because different substrate causes different reaction for laccases (Cordi et al., 2000a). Han et al. (2005) extracted laccase from *Trametes versicolor* which showed optimum activity at pH 3.0 and at 50 °C temperatures. Several reports highlighted that the optimum pH of laccase production as falls between 5.0 and 6.0 in many fungi such as *Fomes sclerodermeus, Trametes versicolor, Pleurotus florida* and *Ganoderma lucidum* (Papinutti et al., 2003; Thiruchelvam et al., 2007; Sivakumar, 2010; Kenkebashvili et al., 2012)

2.11. Influence of inducer on laccase production

The promoter region encoding for laccase contains various recognition sites that are specific for xenobiotics and heavy metals they bind to the recognition sites and induce laccase production. Laccase-producing fungi generally produce laccase in low concentration but with the addition of various supplements such as xenobiotic compound to media higher concentrations of laccases were obtained (Eggert et al., 1996; Mansur et al., 1997; Vasconcelos et al., 2000; Dhawan and Kuhad, 2002). Upon the addition of aromatic compounds such as 2,5- xylidine, lignin, ferulic acid and veratryl alcohol is known to increase and induce laccase activity from *Cerrena unicolor, Armillaria* sp.,
Trametes villosa, P. sajor-caju and Trametes versicolor (Pointing et al., 2000; Dhawan and Kuhad 2002; Cambria et al., 2011; Saraiva et al., 2012).

Copper, as a micronutrient, plays key role as metal activator in several fungal enzymes like laccase, cytochrome oxidase, tyrosinase, ascorbic acid oxidase and superoxidase of oxidases group (Palmieri et al., 2000).

Several reports highlighted that copper act as a strong laccase inducer in several species like Neurospora crassa, Trametes versicolor, Phanerochaete chrysosporium, Panus osteratus, Pleurotus sajor-caju, Trametestrogii, Volvariella volvacea, Lentinula edodes, Trametes pubescens, Ganoderma lucidum and Grifola frondosa (Huber and Lerch 1987; Dittmer et al., 1997; Palmieri et al., 2000; Chen et al., 2004; Xing et al., 2006; Songulashvili et al., 2007; Liu et al., 2009; Du et al., 2012; Kocyigit et al., 2012; Shankar and Shikha et al., 2012).

2.12. Purification and characterization of laccase

For the enzyme purification, Ammonium sulphate is being commonly used for many years. But researchers have found much more efficient methodologies such as protein precipitation by ammonium sulphate, anion exchange chromatography, desalt/buffer exchange of protein, and gel filtration chromatography. Laccase from LLP13 was first purified with column chromatography and then purified with gel filtration (Kiiskinen et al., 2004). Laccase from T. versicolor is purified with Ion Exchange chromatography followed by gel filtration with specific activity of 101UmlL−1 and 34.8 fold purification (Cordi et al., 2000b). Laccase from Stereum ostrea is purified with
ammonium sulphate followed by Sephadex G-100 column chromatography with 70-fold purification (Viswanath et al., 2008).

Several reports highlighted that laccases are mainly monomeric, dimeric, and tetrameric glycoproteins carrying molecular mass between 50 kDa and 130 kDa purified from several organisms such as *Trametes hirsuta*, *Trametes versicolor* and *Ganoderma lucidum* (Mansur et al., 1997; Morozova et al., 2007). Glycosylation plays an important role in copper retention, thermal stability, susceptibility to proteolytic degradation and secretion. Dhakar and Pandey (2013) reported laccase molecular mass of 45 kDa from *Trametes hirsuta*. D’Souza-Tiico et al. (2006) performed various experiments to determine the effect of inhibitor on the activity of Lac-II in the presence of sodiumazide, SDS and mercaptoethanol. It was recorded that activity of Lac-II was inhibited in the presence of Sn, Ag and Hg by 32 - 37% while 56% and 48% Lac-II activity was inhibited in the presence of Cr and W, respectively.

2.13. Laccase applications

2.13.1. Role of laccase in bioremediation

Due to rapid industrialization and extensive use of pesticides for better agricultural productivity, contamination of soil, water, and air take place which is a serious environmental problem of today (Viswanath et al., 2008). Laccase from fungi remove a wide variety of hazardous chemicals. The use of laccase in the textile industry for decolourization of dyes is growing very fast (Raghukumar, 2000). Laccase purified from the fungus *Trametes hirsuta* was able to degrade triarylmethane, indigoid, azo, and anthraquinonic dyes, Amaranth, Tropaeolin O, Reactive Blue 15, Congo Red, and Reactive
Black 5 used in dyeing textiles as well as 23 industrial dyes (Abadulla et al., 2000; Ying et al., 2002). Laccase of Flavodon flavus, was shown to decolourize the effluent from a Kraft paper mill bleach plant (Soares et al., 2001).

### 2.13.2. Role of laccase in delignification and pulp bleaching

Laccase from Cerrena unicolor has the capability to reduce lignin content from sugarcane bagasse up to 36% within 24 h at 30 °C (D’Souza-Tielo et al., 2009).

During paper processing at industrial level the separation and degradation of lignin carried out by using chlorine and oxygen-based chemical oxidants. But some problems such as recycling, cost, and toxicity remain unsolved. The pre-treatments of wood pulp with laccase can provide milder and cleaner strategies of delignification that also respect the integrity of cellulose (Sigoillot et al., 2005; Gamelas et al., 2005). However, in the existing bleaching process, Laccase mediated system could be easily implemented because it leads to a partial replacement of ClO₂ in pulp mills. More recently, the potential of this enzyme for cross-linking and functionalizing lignaceous compounds was discovered. Laccases can be used for binding fiber-particle and paper-boards (Gübitz and Cavaco-Paulo, 2003).

### 2.13.3. Role of laccase in organic synthesis

Recently, interest is increasing in organic synthesis using laccase as a new biocatalyst (Mayer and Staples, 2002). Laccase provided an environmentally benign process of polymer production in air without the use of hydrogen peroxide (Kobayashi and Higashimura 2003; Mita et al., 2003). Laccase is potential in crosslinking and functionalizing lignaceous compounds (Grönqvist et al., 2003). It is also pointed out that
laccase induced radical polymerization of acrylamide with or without mediator (Ikeda et al., 1998). It has also been used for the chemo-enzymatic synthesis of lignin graft-copolymers (Gübitz and Cavaco-Paulo, 2003). Laccases are also known to polymerize various amino and phenolic compounds (Ikeda et al., 1996; Aktas et al., 2000; Aktas and Tanyolaç 2003; Karamyshev et al., 2003; Güreir et al., 2005). The ability of laccases to generate color “in situ” from originally non-colored low-molecular substances makes their use an alternative to the conventional dyeing processes (Barfoed et al., 2001; Pilz et al., 2003). Surface modifications of the fabrics can be carried by the abilities of laccase for the synthesis of new compounds. The enzymatic modification and dyeing processes can be applied in several natural substrates like cotton, wool, flax and wood (Tzanov et al., 2003a). Recently, to improve the production of fuel ethanol from renewable raw materials, laccase was expressed in Saccharomyces cerevisiae to increase its resistance to phenolic inhibitors in lignocellulose hydrolyzates (Larsson et al., 2001).

2.13.4. Role of laccase in beverage industries

Wine stabilization is one of the main applications of laccase in the food industry as alternative to physical-chemical adsorbents (Minussi et al., 2002). Musts and wines are complex mixtures of different chemical compounds, such as ethanol, organic acids (aroma), salts and phenolic compounds (color and taste). Polyphenol removal must be selective to avoid an undesirable alteration in the wine's organoleptic characteristics. Laccase presents some important requirements when used for the treatment of polyphenol elimination in wines, such as stability in acid medium and reversible inhibition with sulphite (Plank and Zent, 1993; Servili et al., 2000; Tanrıöven and Ekşi, 2005). Laccases are also used to improve storage life of beer. Haze formation in beers is a persistent
problem in the brewing industry. Nucleophilic substitution of phenolic rings by protein sulphydryl groups may lead to a permanent haze that does not re-dissolve when warmed. As an alternative to the traditional treatment to remove the excess of polyphenols, laccase could be added to the wort. (Mathiasen, 1995, Minussi et al., 2002; Ahmad et al., 2011)

2.13.5. Role of laccase in food improvement

The flavor quality of vegetable oils can be improved with laccase by eliminating dissolved oxygen. Laccase can also deoxygenate food items derived partly or entirely from extracts of plant materials. Cacao was soaked in solutions containing laccase, dried and roasted in order to improve the flavor and taste of cacao and its products (Takemori et al., 1992). Laccase controls odour, taste enhancement, or reduction of undesired products in several food products. Laccases are used in fruit juice stabilization (Piacquadio et al., 1998; Minussi et al., 2002; Alper and Acar, 2004). Laccase is supplemented to the dough which is used for producing baked products, to exert an oxidizing effect on the dough constituents and to improve the strength of gluten structures in dough and/or baked products (Figueroa-Espinoza et al., 1999; Labat et al., 2001; Minussi et al., 2002 Couto and Toca Herrera, 2006; Minussi et al., 2007)

2.13.6. Role of laccase in textile finishing

Laccase from a newly isolated strain of T. hirsuta was responsible for improvement of whiteness in cotton most likely due to oxidation of flavonoids (Tzanov et al., 2003a). More recently, Basto et al. (2007) proposed a combined ultrasound-laccase treatment for cotton bleaching. Cellulases were used to partially replace the load of pumice stones and laccase was an effective agent for stone-washing effects of denim fabric with and without
using a mediator and also bleach indigo dyed denim fabrics to lighter shades (Xu et al., 1999; Campos et al., 2001; Shi and Clemmons 2003; Pazarlioglu et al., 2005a).

2.13.7. Role of laccase in medical applications

For the synthesis of complex medical compounds such as anesthetics, anti-inflammatory, sedatives, etc. laccase can be used (Nicotra et al., 2004). Laccase has been used to distinguish morphine from codeine simultaneously in drug samples injected into a flow detection system (Bauer et al., 1999).

2.13.8. Role of laccase in biosensors

A biosensor is an integrated biological-component probe with an electronic transducer, which converts a biochemical signal into a quantifiable electrical response that detects, transmits and records information regarding a physiological or biochemical change (D'Souza, 2001).

Due to its broad substrate specificity, laccase is used in biosensor which mainly allows this enzyme for the detection of a broad range of phenolics (Palmore and Kim 1999; Fogel and Limson 2013).

Several authors detected phenolic compounds in wine, food, tea, lignins and wastewaters using electrode laccase biosensor (Giovanelli and Ravasini, 1993; Palmore and Kim, 1999; Ahmad et al., 2011; Chawla et al., 2011; Shimomura et al., 2011; Fogel and Limson, 2013). Several researchers reported for the immobilization of laccase on different solid supports such as glassy carbon, carbon paste, platinum, gold by different immobilization strategies such as direct adsorption, covalent binding, entrapment in polymeric membranes or gels and cross-linking procedures in order to design biosensors
for various applications (Durán, et al., 2002; Vianello et al., 2004; Gutiérrez-Sánchez et al., 2012).

For immunoassays, glucose determination, aromatic amines and phenolic compound determinations laccase biosensors have been developed (Simkus et al., 1996, Bauer et al., 1999, Huang et al., 1999, Ghindilis 2000, Freire et al., 2002, Gomes et al., 2004; Fogel and Limson, 2013). Laccase based and tyrosine based paper biosensors were developed and detected phenols (Oktem et al., 2012).

2.14. Mycofilter for bioremediation

Mycoremediation is a process in which the fungal mycelium involved in the treatment of wastes, soils, and other environmental contamination to reduce toxicity, carcinogenicity, pathogenicity, or other undesired effects of compounds. The use of bioremediation/mycoremediation rather than conventional treatment offers an inexpensive and a simple process by introducing an organism to a pollutant (Tim Rogers, 2012).

Mycoremediation is an innovative best management practice to reduce contaminants from non-point source pollution. The effectiveness of mycofilters has not been widely tested. The efficiency of a mycofilter at decreasing biological oxygen demand and removing ammonia, phosphorous and suspended solids from agricultural runoff was tested by Stamets, 2012. The effectiveness of the mycofilter can be improved if the design of the filter allowed longer water retention time and exposure to mycelium.

Mycofiltration is the process of using mushroom mycelium mats as biological filters. The term was coined by mycologist Paul Stamets. Stamets controled Eschericia coli in the water outflow from his property using mycofilter. After planting a mushroom bed in
the gulch where the water was leaving, within a year the coliform count had decreased to nearly undetectable levels. Water filters made of biodegradable materials inoculated with fungi to have a positive effect in reducing biological and chemical pollutants, sediment flow and siltation. Mycosorption or mycoaccumulation is the remediation of sites contaminated with heavy metals using fungi is termed (also termed bioabsorption or bioaccumulation, which includes all organisms used in the absorption process, such as bacteria).