

Review Article

Microbial degradation of reactive dyes- A Review

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ABSTRACT

Keywords

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Nowadays globalization, urbanization and industrialization leads to various environmental concerns. The usage of synthetic dyes increases in many areas. Among the synthetic dyes, reactive dyes are most commonly used in all industries. Reactive dye is one of the prominent and widely used types of azo dye with different reactive groups. Reactive dyes that emanate from dyeing industries increases Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), change the pH of water bodies and it causes serious problems in plant, animal and human beings. Conventional waste water treatment are too expensive since they produce large amount of perilous byproducts, muck production and disposal problem, so biological treatment is relatively inexpensive way to remove dyes from waste water. The successful removal of dyes from effluent is depends on the deployment of microorganisms such as bacteria, fungi, microalgae etc to convert the pollutants into non- toxic substances.

Introduction

Various chemical substances discharged from the industries become a persistent environmental contaminant. Due to rapid industrialization and urbanization, a lot of chemicals including dyes, pigments, and aromatic molecular structural compounds were widely used in several industrial applications such as textiles, printing, pharmaceuticals, food, toys, paper, plastic and cosmetics (Mohana *et al* ., 2008). Textile processing industries were found in most of the countries and their numbers

have been increased. These industries have shown a significant increase in the use of synthetic dyes as a coloring material. The annual world production of textiles is about 30 million tones requiring 700,000 tonnes of different dyes (Zollinger., 1987). The dyes includes such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone dyes which causes a considerable environmental pollution problems. The effluent from the dyeing and desizing processes contributes to the high colorant

content and chemical oxygen demand of the total drainage. The dyes present in textile effluent give persistent color to the receiving streams and interfere with photosynthesis in aquatic flora (Cunningham and Saigo, 2001). Effluent that release from the production process of textiles is not properly disposed, can cause grave environmental pollution, sometimes to levels that can threaten human health, livestock, wildlife, aquatic lives and collapse the entire ecosystem. Presence of dyes in the effluent causes an unpleasant appearance by imparting the color and its breakdown products are toxic, carcinogenic and mutagenic (Conneely *et al.*, 1999; Xu *et al.*, 2005).

Dyes

Unlike most organic compounds, dyes have color because they a) absorb light in the visible spectrum; b) have atleast one chromophore; c) have a conjugated system, (i.e.) a structure with alternating double and single bonds and d) exhibit resonance of electrons, which is a stabilizing force in organic compounds (Abrahart, 1977). Dyes contain one or more azo groups (*i.e.*, azo dyes) include by far the largest family of organic dyes. Distinguished types of dyes are, 1) Acid dyes for protein and polyamide substance such as nylon, wool and silk; 2) Disperse dyes for polyesters and acetate and 3) Direct and reactive dyes for cellulosic materials such as cotton, rayon, linen and paper.

Reactive Dyes

Reactive dyes have intricate chemical structures which form covalent bonds between the reactive groups of cellulose and agiled functional groups of dye molecules. Reactive dyes are the most common dyes because of many advantages such as operating at mild conditions, give

bright colors and stable structures (Wang and Lewis, 2002 and Xie *et al.*, 2008). Reactive dyes are nitrogen – containing heterocyclic rings carrying halogen substituents, therefore undergo nucleophilic substitution reaction with the cellulose fiber. The heteroatom activates the system for nucleophilic attack due to its electro-negativity. The attacking nucleophile can be either a cellulose anion or a hydroxyl ion. This conducts fixation on the fabric, after hydrolysis occurs on the reactive dye and it is also important for a dye molecule to have a high dye – fabric covalent fixation value (Al – Degs *et al.*, 2000).

There are different types of reactive dyes with various reactive groups (i.e) with various reactive systems that react with substrate to form covalent bonds (Table 1). The chromophore of their molecules are very similar *i.e.*, either azo, anthraquinone, phthalocyanine chromophore or others. The general formula for the reactive dyes structure is as follows.



Where

S: Solubilizing group; F: Chromophore T: Briding group X: Reactive system

Reactive dyes composed of two portions

The chromogen and b) The reactive system (Waring and Hallas., 1990).

Reactive dyes are characterized by azo bonds (N=N), and used to dye cellulose fibres. The color of the azo dyes is due to the presence of azo bond with associated chromophores (Moreira *et al.*, 1998.). Reactive dyes are azo compounds that are linked by an azo bridge. (Raymond and Dunald, 1984). Reactive dyes are typically

azo based chromophores with various reactive groups, some examples for reactive dyes are Procion yellow MX- 5B, Cibacron brilliant red B, Drimaren red Z – 2B, reactive black 5 (fig. 1), Reactive yellow 2, Procion Blue MX 2G, Remazol red RB, Remazol Golden Yellow RNL, Remazol Blue B, Remazol Turquoise Blue G133, Remazol blue and Remazol orange etc., which shows different reactivity and it has different absorbance (Table 2).

Impacts of reactive dyes

Effluent from the industries containing reactive dyes causes serious environment pollution because, the presence of dyes in water is highly visible and affects their transparency and aesthetic even if the concentration of the dyes is low (Hao *et al.*, 2000). Reactive dyes cause respiratory and nasal symptoms (Docker *et al.*, 1987); asthma rhinitis and dermatitis (Nilsson *et al.*, 1993); allergic contact dermatitis (Estlander, 1988; Wilkinson and MCGeachan.,1996), mutagenicity (Mathur *et al.*, 2005; Przybojewska *et al.*, 1989), genotoxicity (Dogan *et al.*, 2005; Przybojewska *et al.*, 1989), carcinogenicity (De- Roos *et al.*, 2005; Gonzales *et al.* , 1988) and teratogenicity (Birhanli and Ozmen, 2005). Adverse effects have also been detected from aquatic environment (Richardson, 1983; Michaels and Lewis, 1985). Dyes have a very low rate of removal ratio for BOD to COD (less than 0.1) (De and Radrigues, 1987). Therefore, industrial effluents containing dyes should be processed before their discharge into the environment (Wong *et al.*, 2003).

Microbial discoloration of reactive dyes

Effluents from the textile industries contain reactive dye in a concentration

range of 5-1500mg/L. Processing of dye contaminated effluents is currently a primary environmental problem (Lata *et al.*, 2007). Conventional treatment methods such as activated sludge process, chemical coagulation, carbon absorption, chemical oxidation, photo decomposition, electro- chemical treatment, reverse osmosis, hydrogen peroxide catalysis etc are difficult, ineffective (or) economic disadvantage(Gong *et al.*, 2005; Viyaraghavan and Yun, 2008). Some of these techniques are even effective, although they have some shortcomings, they are: excess amount of chemical usage with obvious disposal problem; costly plant requirements (or) operating expenses; lack of effective color reduction; particularly for sulfonated azo dyes; sensitivity to a waste water input. Techniques by chemical oxidation using sodium hypochlorite to remove the color release a lot of aromatic amines which are carcinogenic or toxic compounds (Anliker, 1979). Physical and chemical methods are effective for color removal but need of more energy and chemicals than biological processes and sometimes it causes pollution into solid or liquid side streams and it requires additional treatment or disposal (Table 3). So, the treatment of dyes focus on some microorganisms which are to biodegrade and biosorb dye in waste water. Number of microorganisms possesses dye decolorizing ability like bacteria, fungi and yeast

Biosorption

Biosorption defined as the property of certain biomolecules to bind and concentrate selected ions/ other molecules from aqueous solution. Biosorption with microorganism, especially fungus as latent sorbent for removal of dyes from industrial effluents has gained

considerable attention. Decoloring of synthetic dyes and dye effluents (Mehna *et al.*, 1995; Revankar and Lele, 2007; Binupriya *et al.*, 2007) have been reported by using various fungi. Bacteria and fungi have been used for decolorization of effluent containing dye (Benito *et al.*, 1997). *Trametes versicolor* mycelium adsorbs dye of 5-10% and *Aspergillus niger* adsorb 10-25%; (Miranda *et al.*, 1996). Most of the work was focused on the biosorption of dyes by bacterial biomass. With respect to bacterial dye biosorption, Won *et al.* (2005) observed *Corynebacterium glutamicum* as a latent biosorbent of Reactive red 4. *Aeromonas sp.*, *Pseudomonas luteola*, *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* are used as biosorbent for the removal of reactive dyes like reactive blue, reactive red, reactive violet and reactive yellow (Hu, 1996).

Actinomycetes as absorbent for decolorization of effluent containing anthraquinone, phalocyanine and azo dyes (Zhou and Zimmerman, 1993). Algae are a potential biosorbent because of their availability in both fresh and salt water. Algae have a capacity for biosorption due to their relatively high surface area and high binding affinity (Donmez and Aksu, 2002). During algal biosorption, electrostatic attraction and complexation occur in the cell wall of algae (Satiroglu *et al.*, 2002). Algae biosorbed some reactive dyes such as reactive yellow 22 by *Spirogyra sp.* (Mohan *et al.*, 2002). Based on the dye and the organisms used different binding rates and capacities were observed. Certain dyes have a specific affinity for binding with microbial species. Use of biomass has advantages, especially if the dye-containing effluent is very toxic (Table 4).

Adsorption by dead microbial cells

Killed bacteria, yeast and fungi are used for the decolorization of dye – containing effluents. The dyes from textile industries are varied from their chemistries and so their interactions with microorganisms depend on the chemistry of particular dye and microbial biomass (Polman and Brekenridge, 1996). Adsorption method is used during unfavourable conditions for the growth and preserves microbial population (Modak and Natarajan, 1995). Adsorption process by microorganism is carried out by ion exchange method. Bacterial cells adsorb reactive dyes (Hu, 1992). The use of dead organisms instead live biomass overcomes the problem such as waste toxicity and requirement of nutrients. The dried form of *R. arrhizus* decolorize Remazol black B in aqueous solution (Asku and Tezer, 2000). Dead cells resulted in an effective adsorption than live cells due to increased surface area in gram negative cells and high lipid content in cell wall of Gram positive cells. Functional groups such as carboxyl, phosphonate, amine, hydroxyl group present in the cell wall of bacteria plays an important role in adsorption of dyes (Vander *et al.*, 1997).

Biodegradation of reactive dyes **Fungal Biodegradation**

A group of fungal organisms have an ability to decolorize wide range of dyes (Fu and Viraraghavan, 2001 a,b). Fungus is capable of degrading dioxins, polychlorinated, biphenyls (PCBS) and chloro-organics (Reddy, 1995). White-rot fungi is capable of decolorizing dyes e.g., *Coriolus versicolor* (Sumathi and Manju, 2001), *Trametes versicolor* (Wong and Yu, 1999 and Libra *et al.*, 2003), *Funalia trogii* (Yesilada *et al.*, 1995), *Umbelopsis*

isabellina and *Penicillium geastrivous* (Yang *et al.*, 2003), *Aspergillus foetidus*, *Rhizopus oryzae* (Polman and Breckenridge, 1996) can decolorize or biosorb various dyes.

Major group of fungus produce lignin modifying enzymes like laccase, manganese peroxidase (MnP) and lignin peroxidase (Lip) to mineralize/ degrade synthetic lignin or dyes (Raghukumar *et al.*, 1996; Fu and Viraraghavan, 2001). The decolorization was a secondary metabolic activity linked to the fungus ligninolytic – degradation activity. The degradation of some xenobiotic by other white- rot fungi is known to occur under non- ligninolytic conditions and would mainly be through the laccase enzyme activity (Dhawale *et al.*, 1992)

Basidiomycetes fungi not only decolorize but also degrade and mineralize a broad spectrum of different dye structure (Azo, anthroquinone, heterocyclic, triphenylmethane, and polymeric dyes) and it also degrade numerous toxic organic and recalcitrant compounds. The enzyme system of basidiomycetes fungi is non-specific in the degradation of pollutants and even acts on mixtures of pollutants. (Machado *et al.*, 2005; Shah and Nerud, 2002; Wesenberg *et al.*, 2003).

Bacterial degradation

Work on bacterial degradation of dyes were started in the 1970s with report on *Bacillus subtilis* (Horitsu *et al.*, 1977), then degradation was followed by numerous bacteria such as *Aeromonas hydrophilia* (Idaka and Ogawa, 1978), *Bacillus cereus* (Wuhrmann *et al.*, 1980), *Pseudomonas sp* (Kulla, 1981), *E. Coli* (Chang *et al.* , 2004). Under aerobic conditions azo dyes are not readily

metabolized, intermediates formed during degradation resulted in disruption of metabolic pathways and the dyes were not mineralized. Whereas in anaerobic conditions, bacteria reduce azo dyes gratuitously by the activity of unspecific, soluble, cytoplasmic reductase called azo reductase. Azo reductase results in the production of colorless aromatic amines which may be toxic, mutagenic and carcinogenic to animals (Mcmulan *et al.*, 2001). Anaerobic to aerobic condition should be retaining to achieve a complete degradation. Different members of the consortium needed different conditions for optimum reaction and azo- based cleavage requires azo reductase, which function under anaerobic conditions (Haug *et al.*, 1991). *Pseudomonas luteola* have an ability to remove the color of reactive azo dyes (Hu, 1994).

Algal Biodegradation

Algal culture also has an ability to degrade azo dyes through azoreductase (Jinqi and Houtian, 1992). *Chlorella* and *Oscillatoria* were capable of degrading azo dyes to aromatic amines and further to simple organic compounds. *Synechocystis sp* and *Phormidium sp* have a capacity to remove reactive dyes such as Reactive Red, Remazol Blue, and Reactive Black B (Karacakaya *et al.*, 2009).

Yeast Biodegradation

Limited amount of studies about yeast decolorization were reported. *Kluyveromyces marxianus* IMBS decolorize Remazol Back B dye of about 98% (Meehan *et al.*, 2000). *Pseudozyma rugulosa* Y -48 and *Candida krusei* G-1, are the yeast strains exhibited excellent color removal of reactive azo dyes. *Saccharomyces cerevisiae* MTCC 463

Table.1 Introduction of commercial reactive dyes (Ahmed, 1995)

Commercial Name	Firm	Introduced Year
Procion M	ICI	1956
Procion H	ICI	1957
Cibacron	CIBA	1957
Remazol	Hoechst	1958
Levafix	Bayer	1958
Reactow	Geigy	1959
Drimaren	Sandoz	1959
Levafix E	Bayer	1961
Primazin	BASF	1964
Solidazol	Cassella	1964
Procilan	ICI	1964
Levafix P	Bayer	1966
Lanasol	CIBA	1966
Reactofil	Gergy	1968
Verofix	Bayer	1970
Drimalan	Sandoz	1970
Procion HE	ICI	1970
Procion T	ICI	1977
Procion H – EG	ICI	1979
Kayacelon	Nippon kayaku	1984
Procilence	ICI	1987
Cibacron C	CIBA	1988

Fig.1 Structure of Reactive Black - 5

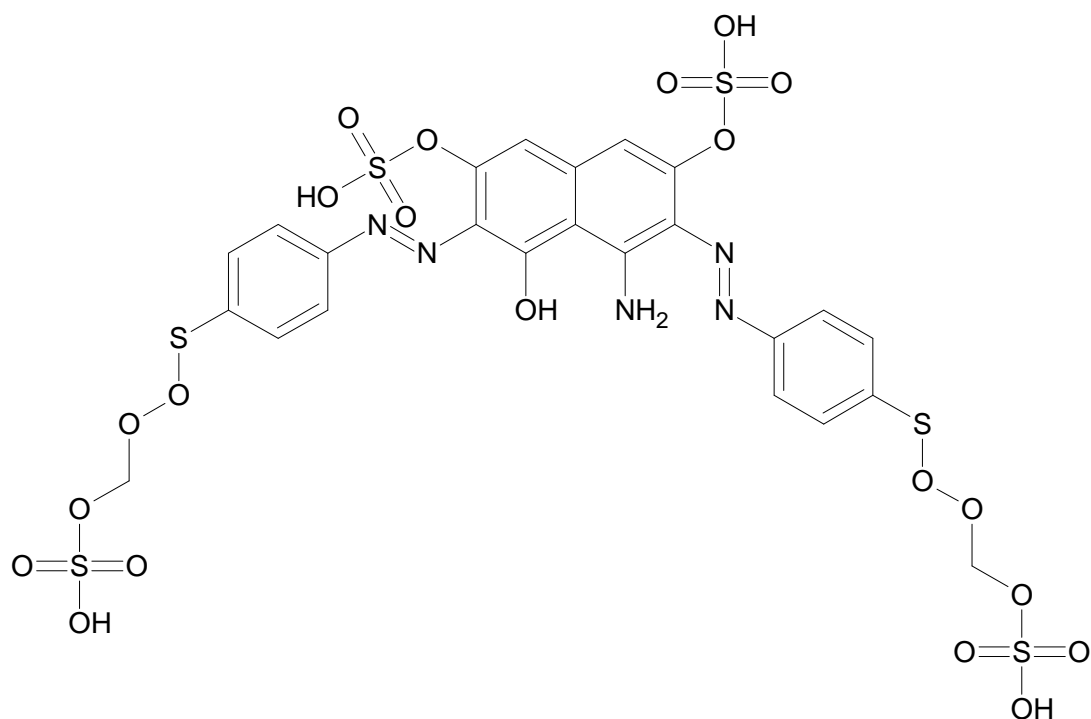


Table.2 Wavelength of light absorbed by the reactive dyes

Dyes	Absorbance _{max} (nm)
Cibacron Red C- 2G	515
Remazol Navy Blue GG	620
Remazol Red RB	525
Cibacron Orange CG	489
Remazol Golden Yellow RNL	410
Remazol Blue B	590
Remazol Turquoise Blue G133	660
Remazol Black B	600

Table.3 Merits and Demerits of physical treatment method (Robinson *et al.* , 2001)

Physical and Chemical treatment Methods	Merits	Demerits
Coagulation/ Flocculation	Simple, economic feasible	Large amount sludge production, handling and disposal problems, more amount chemicals required for pH adjustment
Membrane separation	All types of dyes are decolorized	High pressure, expensive, sludge generation incapable for large scale treatment
Ion exchange	Effective with no loss of redevelopment	Renewal is possible economic constraints, not effective for some dyes
Oxidation	Hasty and efficient process	High energy cost, chemical required, secondary metabolite production
Advanced oxidation process	No sludge production, little consumption of chemicals, efficiency for recalcitrant dyes	Economically impracticable
Adsorption on activated carbon	Effective	Expensive loss of absorbent
Fenton's reagent	Proficient decolorization for both soluble and insoluble dyes	Sludge formation
Photochemical	No sludge production	Formation of Secondary metabolic pollutants
Irradiation	Effective only in laboratory level	Need dissolved oxygen

Table.4 Biosorption of reactive dye by microorganisms

Organisms	Dye	Mechanism	Reference
<i>Myrothecium verrucaria</i>	Orange II RS (red)	Adsorption	Brahimi- Horn <i>et al</i> ., 1992
<i>Candida sp</i>	Procion Black, Procion Blue, Procion Blue MX 2G, Procion Red HE7B, Procion orange HER	Adsorption	De Angelis and Rodrigues ., 1987
<i>Streptomyces</i> BW 130	Azo reactive red 147	Adsorption	Zhou and Zimmermann.,1993
<i>Alternaria raphani</i>	Reactive black 5, Reactive orange107	Adsorption	Ramalakshmi <i>et al</i> ., 2011a,b
<i>Corynebacterium glutamicum</i>	Reactive Yellow 2	Adsorption	Won and Yun .,2008
<i>Phanerocheate chrysosporium</i>	Reactive blue 4	Adsorption	Grazso ., 2001
<i>Rhizophus arrhizus</i>	Remazol blue, Remazol orange, Cibacron red	Adsorption	Mahony <i>et al.</i> , 2002

Table.5 Various Microorganisms that decolorize reactive dyes

Organisms	Dyes	Reference
<u>Fungi:</u> <i>Trametes villosa</i> <i>Pycnoporus sanguineus</i> <i>Pleurotus ostreatus</i> <i>Funalia trogii</i> <i>Aspergillus ochraceus</i>	Drimaren Brilliant Blue Remazol Brilliant Blue R Remazol Brilliant Blue R Reactive Blue - 25	Machado <i>et al.</i> , 2006 Palmieri <i>et al.</i> , 2005 Deveci ., 2004 Parshetti <i>et al.</i> , 2007
<u>Bacteria:</u> <i>Rhizobium radiobacter</i> <i>Pseudomonas luteola</i> <i>Citrobacter sp., CK3</i> <i>Pseudomonas sp.</i>	Reactive Red 141 Reactive azo dyes Reactive Red 180 Reactive Red 2	Telke <i>et al.</i> , 2008 Hu ., 1994 Wang <i>et al.</i> , 2009 Kalyani <i>et al.</i> , 2009
<u>Algae</u> <i>Synechocystis sp.</i> <i>Phormidium sp.</i>	Reactive Red Remazol Blue, Reactive Black B	Karacakaya <i>et al.</i> , 2009
<u>Yeast:</u> <i>Kluyveromyces marxianus</i> IMB3	Remazol Black B	Meehan <i>et al.</i> , 2000

Table.6 Enzymatic degradation of reactive dyes by microorganisms

Dyes	Enzymes	Organisms	Reference
Bromophenol Blue, Orange G, Amaranth and Malachite Green	Laccase	<i>Pycnoporus sanuineus</i>	Mayer and Staples ., 2002
Reactive Orange 16, remazol Brilliant Blue R	Manganese peroxidase	Not available	Novonty <i>et al.</i> , 2004
Reactive Blue – 59	Lignin Peroxidase	<i>Streptomyces krainskii</i>	Mane <i>et al.</i> , 2008
Remazol Brilliant Blue R	Peroxidase and Laccase	<i>Pleurotus ostreatus</i>	Machado and Mathews ., 2006

effectively decolorize methyl red at different pH with involvement of azoreductase (Jadhav and Govindwar, 2007) (Table 5).

Aerobic/anaerobic degradation of reactive dyes

Under aerobic conditions, bacteria produce an enzyme which helps to break down the organic compounds in waste water. *Rhizopus oryzae*, *Cyathus bulleri*, *Coriolus versicolor*, *Funalia trogii*, *Laetiporus sulphureus*, *Streptomyces sp.*, *Trametes versicolor* and other microorganisms decolorize dyes in aerobic conditions (Nigam *et al.*, 2000; Salony *et al.*, 2006; Zhang *et al.*, 1999). Most of the dyes are recalcitrant for the biological degradation or nontransformable under aerobic conditions (Pagga and Brown, 1986; Rai *et al.*, 2005). Under anaerobic condition, the reactive dyes are decolorized effectively by using glucose as a carbon source (Carliell *et al.*, 1996). Conventional sewage method under anaerobic condition decolorized reactive red 141. The reactive red 141 was decomposed under anaerobic condition by the cleavage of azo bond by the microbial community resulted in the formation of 2 – aminonaphthalene-1, 5 disulfonic acid (Carliell *et al.*, 1995). The addition of salts such as nitrate and sulfate decolorized reactive red 141 under anaerobic condition. An anaerobic – aerobic treatment process by mixed culture of bacterial isolated from textile dye effluent was used to decolorize the reactive azo dyes such as remazol brilliant orange 3R, remazol black B and remazol brilliant violet 5R (Supaka *et al.*, 2004). Treatment of synthetic dye waste water at the combination of anaerobic and aerobic conditions showed that the majority of

colors are removed by the anaerobic process, whereas the chemical oxygen demand(COD) is removed by the aerobic process (Rajaguru *et al.*, 2000 and Supaka *et al.*, 2004). Mixture of bacterial isolates from domestic sewage treatment plant has been reported to be effective in decolorization of reactive azo dyes, red RB, blue M2B and yellow. The mixed cultures decolorize 95% of red RB and blue M2B. Decolorization remarkably enhance when peptone is used in the medium for growing the mixed culture (Vijaya *et al.*, 2003).

Enzymatic degradation of reactive dyes

Enzymes were used for decades in the textile industry as detergents, recently extracellular enzymes tested for their ability to decolor and degrade dyes (Gianfreda and Rao, 2004). To degrade dyes in a waste water plant, the chromophore in the dyes should be oxidized and cleaved. Laccase and manganese peroxidase are quite effective in dye degradation. Laccase from various fungi such as *Trametes versicolor*, *Trametes hirsute*, *Pleurotus ostreatus* and *Phlebia tremellosa* are found to be effective decolorizer for a wide variety of structurally different dye (Kandelbauer *et al.*, 2004). Manganese peroxidase decolors Reactive orange 16, Remazol Brilliant Blue R, Drimaren Blue, Acid Black and Drimaren Red (Novonty *et al.*, 2004) (Table 6).

Manganese peroxidase from *Phanerocheate chrysosporium* decolorized reactive azo dyes such as reactive blue38, reactive violet 5, reactive black 5, reactive orange 96, reactive red 198 and reactive blue 15, whereas *Bjerkandara adjusta* decolorizes all these types of reactive dyes

in presence of Mn²⁺(Heinfling *et al.*, 1998).Lacasse enzyme from *Trametes versicolor* decolorize reactive blue 19 (Peralta-Zamora *et al.*, 2003).

Discharge of effluent into water bodies are serious environmental problem. Reactive dyes are azo based dyes and they are recalcitrant to degrade by conventional treatment method. The biological treatment is an effective and alternate method to decolorize and mineralize the dyes in effluent without leaving harmful byproducts. In biological treatment the microorganisms biosorb/degrade the dyes with the help of some enzymes such as laccase, lignin peroxidase, manganese peroxidase etc. Both anaerobic and aerobic condition is required for complete degradation of reactive dyes.

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