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**Review Article**

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# Enzymatic treatment of effluents from textile industries

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**ABSTRACT**

*Environmental Pollution is becoming a major threat around the world due to the release of toxic and hazardous substances. Anthropogenic influence of water resource is a global problem. The major pollutants such as dye stuffs from the textile industries affect the aquatic ecosystem. Due its toxicity it increases the Biological Oxygen Demand (BOD) and also depletes the oxygen in water. The conventional methods such as extraction, steam distillation, absorption, filtration etc., will have drawbacks of incomplete removal of dye stuffs. This article describes the use of enzymes as an alternative method for the treatment of such recalcitrant completely. It is the most effective method compared to conventional methods. Enzymes involved in this technique can be regenerated and is available for next catalytic cycle. This review concludes the current research based on the removal of dyes from the waste streams by enzymes such as lignin, peroxidise, manganese peroxidise and laccase. Enzymes reduces their adverse impact on the environment thereby making enzymatic wastewater treatment an ecologically sustainable technique.*

**Keywords:** Effluents, Microbial treatment, enzymes, consortium culture.

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## INTRODUCTION

The limited availability of fresh water is a global crisis. The growing consumption of fresh water by anthropogenic activities has taken its toll on available water resources. Unfortunately, water bodies are still used as sinks for wastewater from domestic and industrial sources. The textile industries plays a major role in discharging untreated effluents in the form of wastewater into public drains that eventually empty into rivers. The dyes are released into the environment, in the form of coloured wastewater. This can lead to acute effects on exposed organisms due to the toxicity of the dyes, phytoplankton form abnormal colouration and reduction in photosynthesis because of the absorbance of light that enters the water *(Duran and Esposito 2000; Mester and Tien 2000).* These recalcitrant alters the pH, increases biological oxygen demand (BOD) and chemical oxygen demand (COD) and it decreases the water quality. The presence of colorants in wastewater and eventually in receiving waters poses a threat to aquatic life forms. More than 8000 chemical products are found associated with the dyeing process and over 100,000 commercially available dyes exist with over $7\times10^5$ metric tons of dyestuff produced annually from Industries as untreated effluents which is released into water which contaminates the available water source. It is important to treat these effluents in water which are pathogenic to living sources. The possible long – term effects of few dyes and dye degradation are becoming of increasing concern. The possible mutagenic, carcinogenic and/or allergic effects of dyes tested in ETAD survey had LD 50 (Lethal Dose at 50% survival) values greater than $2x10^3$mg/kg. The highest rates of toxicity were found amongst basic and diazo dyes.

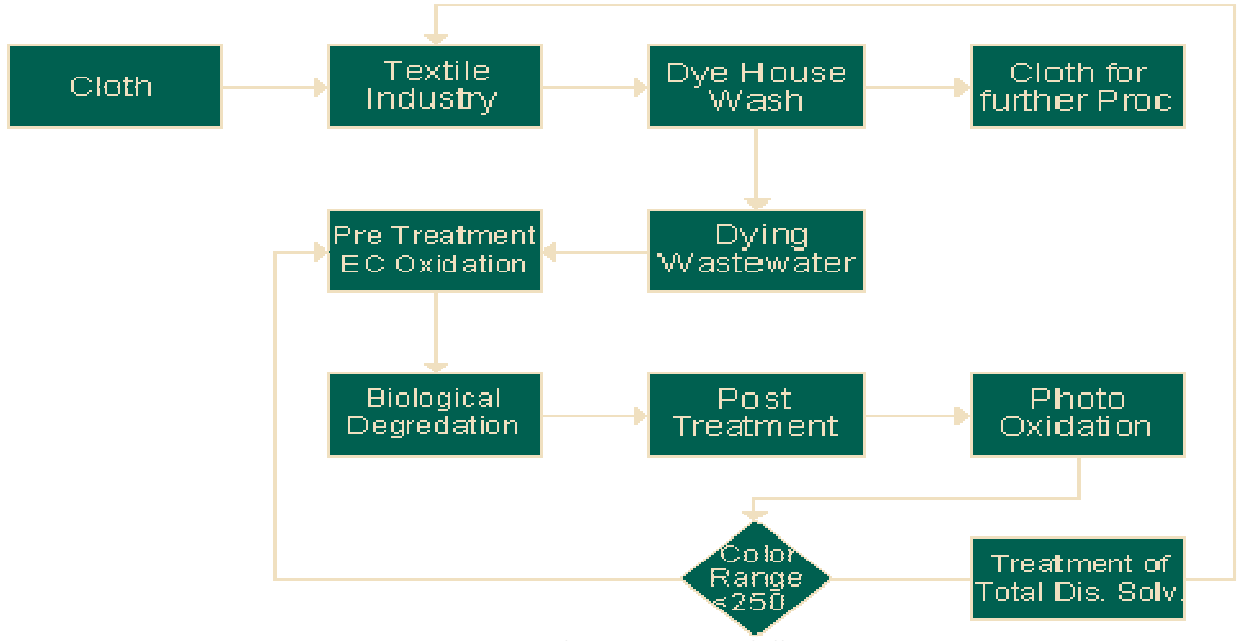


**Fig: 1 Flowchart for biodegradation of effluents**

Several primary, secondary and tertiary treatment processes have been used to treat these effluents. These included flocculation, chemical coagulation , simple sedimentation, aerated lagoons, aerobic activated sludge, trickling filters, reverse osmosis and electrodialysis. However , these treatments are not found effective against the removal of all dyes and chemicals used in the industry. These effluents do not only contain high concentration of dyes, but also contain the chemicals used in the various processing stages. Some trace elements such as Cr, As, Cu and Zn are present in these effluents and capable of causing several health problems including haemorrhage, ulceration of skin, nausea, severe irritation of skin and dermatitis (*Ghaly et al*.,2014,5:1).

**Table 1: Classification of dyes used in textiles**

| S. No. | Classification | Types of Fibres | Dyes Used |
|---|---|---|---|
| 1 | Cellulose fibres | cotton, rayon, linen, ramie, hemp, lyocell | Reactive dyes (remazol, procion MX, cibacron F) direct dyes (congo red, direct yellow 50,direct brown 116) napthol dyes (fast yellow GC, fast scarlet R, fast blue B) indigo dyes (indigo white, tyrian purple indigo carmine) |
| 2 | Protein fibres | wool, angora, mohair, cashmere, silk | Acid dyes (azo dyes, triarylmethane dyes, anthraquinone dyes) lanaset dyes (blue 5G , bordeaux B) |
| 3 | Synthetic fibres | polyester, nylon, spandex, acetate, acrylic, ingeo, polypropylene | Dispersed dyes (disperse yellow 218 , disperse navy 35) basic dyes(basic orange 37,basic red 1) direct dyes |

Bioremediation, either as a spontaneous or as a managed strategy, is usually considered a softer and cleaner methodology than the traditional techniques for the clean-up of polluted systems. The main agents involved in bioremediation processes are plants, microorganisms, enzymes and plant microorganisms associations *(Bumpus, 1993; Dec and Bollag, 1994; Durán et al., 2002; Harvey et al., 2002; Hood, 2002; Karam and Nicell, 1997, Korda et al., 1997; Liu andSulfita 1993; Lynch, 2002; Nannipieri and Bolag, 1991; Nicell, 2001; Pointing, 2001; Reddy, 1995; Roper et al., 1996; Siciliano and Germida, 1998; Smith and Mason, 1999; Sutherland et al., 2002; Walton et al., 1994).* All are effective agents in the transformation of organic pollutants because their enzymatic components are powerful catalysts, able to extensively modify structure and toxicological properties of contaminants or to completely mineralize the organic molecule into innocuous inorganic end products.

**PURPOSE OF DYE REMOVALS FROM RECALCITRANCE**

The removal of colour from wastewaters is often more important than the removal of the soluble colourless organic substances, which usually contribute to the major fraction of the biochemical oxygen demand (BOD). Methods for the removal of BOD from most effluents are fairly well established; dyes, however, are more difficult to treat because their synthetic origin are mainly complex aromatic molecular structures, often synthesized to resist fading on exposure to sweat, soap, water, light or oxidizing agents. This renders them more stable and less amenable to biodegradation. Industries involved in dyeing of textile, paper, leather and plastics, release effluents that are highly colored. Azo dyes feature among the most widely used synthetic dyes in industry globally. The fixation of azo dyes (on textile) is quite low and often, up to 50% of the applied dye may be lost in the wash stream. The removal of dyes from wastewater presents a formidable challenge, as most dyes are completely soluble in aqueous solutions.

Although dyes constitute only a small portion of the total volume of waste discharge in textile processing. The chromophores of dyes strongly absorb sunlight. When the effluent reaches the receiving water body, the dyes hinder photosynthesis by the aquatic flora. Several dyes have been found to be potentially toxic. Thus, the presence of synthetic dyes is a serious environmental concern. Evidently, it is necessary to remove colorants from the effluent before it is discharged into a water body.

**REMOVAL OF COLORANTS BY ENZYME ACTION**

The use of enzymatic proteins may represent a good alternative for overcoming most disadvantages related to the use of microorganisms *(Nannipieri and Bollag, 1991; Karam and Nicell, 1997; Nicell, 2001; Gianfreda and Bollag, 2002, Gianfreda and Rao, 2004)*. Enzymes have several beneficial characteristics. They can selectively degrade a target pollutant without affecting the other components in the effluent. Therefore, enzymatic treatment is suitable for effluents that contain relatively large amounts of the recalcitrant target pollutants in comparison to others. They are the main effectors of all the transformations occurring in the biota. They are catalysts with either narrow (chemo-, region- and stereo-selectivity) or broad specificity and, therefore, they can be applied to a large range of different compounds in mixture, as well.

As claimed by *Alcade et al., (2006),* biocatalysis by enzymes (very often known as white biotechnology) fully participates in the "green chemistry" concept introduced in the 90s by *Sheldon and van Rantwijk, (2004),* and its effect on sustainability is now established beyond question". The most representative enzymatic classes in the remediation of polluted environments are: hydrolases, dehalogenases, transferases and oxidoreductases. Recently, very interesting examples of structures and methods for immobilization of biomolecules, including enzymes, were illustrated by *Rodríguez Couto and Toca Herrera (2006)* with specific reference to laccase, an enzyme very often used in decontamination of pollutants *(Gianfreda et al., 1999).*

**Table 2: Enzyme mediated decolorization of some dyes**

| SUBSTRATE | ENZYME | REFERRENCE |
|---|---|---|
| 3-(4 dimethyl amino-1 phenylazo) Benzene sulfonic acid. | Laccase from *Trametes villosa* | *Zille et al., 2004* |
| Acid Orange 6,Acid Orange 7,Methyl Orange and Methyl Red. | Mixture of Bacterial Oxidoreductases from sludge Methanogens. | *Kalyuzhnyi et al., 2006* |
| Direct Yellow | Horseradish peroxidase from *Armoracia rusticana* | *Maddhinni et al., 2006* |
| Acid Blue | Laccase from *Cladosporium cladosporioides.* | *Vijaykumar et al., 2006* |
| Tartrazine and Ponceau | Azoreductase from Green Algae | *Omar, 2008* |
| Reactive Yellow, Reactive Black, Reactive Red and Direct Blue | Azoreductase from *Staphylococcus arlettae* | *Franciscon et al., 2009* |

**Enzymes in dye decadence**

Azo reductases and laccases seem to be the most promising enzymes in the enzymatic remediation of dyes. Low molecular weight compounds like 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) may also be necessary to mediate the actual electron transfer steps of laccases *(Wong and Yu, 1999).* Azo reductases catalyze the reaction only in presence of reducing equivalents like FADH and NADH. Recently ,it was proved that an azo-reductases from a thermoalkalophilic *Bacillus sp.* was able to reduce a large structural variety of systematically substituted azo dyes *(S.Pricelius et al.2006).*It seems that almost all azo compounds tested are biologically reduced under anaerobic conditions. For example: breakage of Azo dye using azo-reductases.

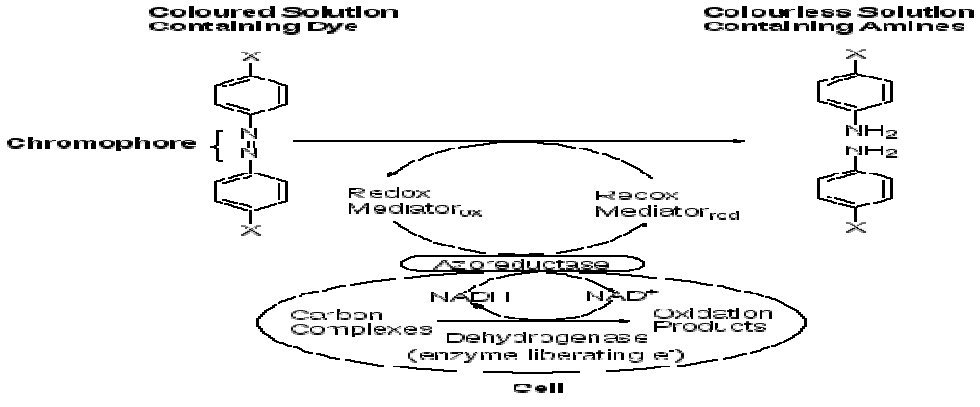


**Fig 2: Mechanism of reduction of azo dyes by azo reductase**

This shows a proposed mechanism for the redox-mediator-dependent reduction of azo dyes using whole bacterial cells, under anaerobic conditions. Although the final reduction of the azo dyes in the cell supernatants is a dominantly chemical redox reaction, the redox mediators depend on cytoplasmic reducing enzymes to supply electrons *(Yoo et al., 2001).*

Laccases have been extensively studied for their degradation of azo dyes *(Chivukula et al., 1995; Kirby et al., 2000; Peralta et al., 2003; Blanquez et al., 2004; Novotny et al., 2004).* Laccase, a cuproprotein belongs to a small group of enzymes denominated as 'blue oxidases'. These enzymes are multicopper phenol oxidases that decolourize azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines *(Chivukula et al.,1995; Wong and Yu, 1999).* For example: breakage of azo dyes using Laccase.

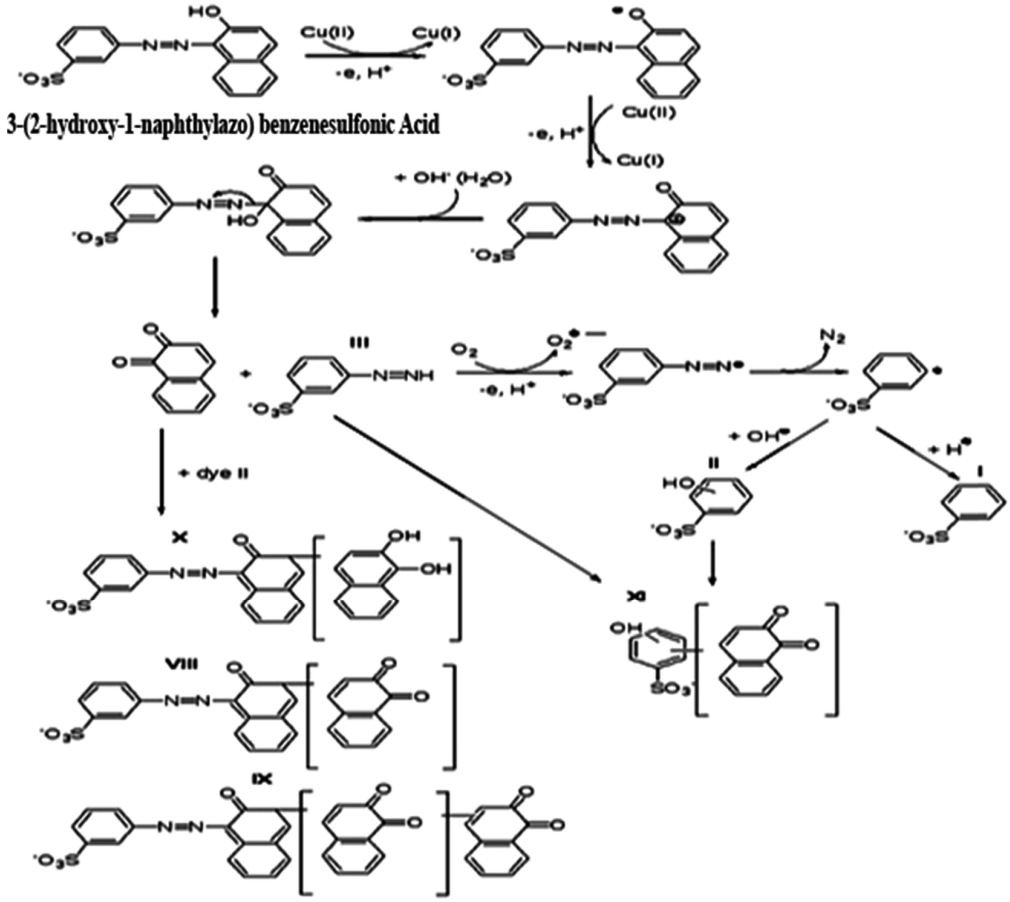


**Fig 3: Mechanism of reduction of azo dyes by laccase**

The above fig. represents the suggested model for the activity of laccase on one of the azo dyes, 3-(2hydroxy-1-naphthylazo) benzenesulfonic acid.

**Microbial degradation of dyes**

Biodegradation is a promising approach for the remediation of synthetic dyes wastewater because of its cost effectiveness, efficiency, and environment friendly nature. The role of some bacterial and algal species for the decolourization and degradation of textile dyes has also been reported *(Jumarkar et al., 2006; Olukanni et al., 2006; Pourbabaee et al., 2006; Togo et al., 2008; Cheriaa et al., 2009).*

**Table 3: Microbial method of dye degradation**

| Microbial type(s) | Degrading dye(s) | Reference(s) |
|---|---|---|
| (I)Bacteria | | |
| Citrobacter sp.CK3 | Reactive Red 180 | *Hui Wang et al., 2009* |
| Listeria sp | Red B5 and Black HFGR | *Kuberan et al., 2011* |
| Bacillus subtilis | Acid Blue113 | *Gurulakshmi et al.,2008* |
| Klebsiella sp. | Orange 3R | *Ponraj1 et al., 2011* |
| Salmonella sp. | | |
| Pseudomonas sp. | | |
| Enterococcus faecalis strain YZ66 | C.I. reactive yellow 145 | *Sahasrabudhe et al., 2011* |
| (II)Fungi | | |
| Penicillium chrysogenum, Aspergillus niger | | |
| Cladosporium sp. | Azo dye-Red 3BN | *Kumar Praveen 2012* |
| P.ostreatus (IE8) | | |
| P.ostreatus (IE8) | | |
| T.hispida (8260) | Acid Black 194, | *Elizabeth Rodri` guez* |
| Bjerkandera sp. | Orisol Blue BH | *et al.,1999* |
| BOS55 P. | | |
| Chrysosporium | Amarnath, Remazol | *Swamy and* |
| P.ostreatus | Black B, Reactive | *Ramsay 1999* |
| T.hirsuta | Blue 15 | |
| T.versicolor | Remazol Orange, Tropaeolin O | |

Cultures of the bacterial strain *Staphylococcus arlettae* were shown to decolorize solutions of four azo dyes (CI Reactive Yellow 107, CI Reactive Red 198, CI Reactive Black 5 and CI Direct Blue 71) in a microaerophilic /aerated sequential process. The average decolorization obtained was 97% *(Ambatkar Mugdha and Mukundan Usha 2011).* Decolourisation of dyes may take place in two ways: either adsorption on the microbial biomass (biosorption) or biodegradation of the dyes by the cells *(BizunehAdinew, 2012).*

The bacterial reduction of the dye is usually nonspecific and bacterial decolourisation is normally faster *(McMullan G et. al., 2001).* A wide range of aerobic and anaerobic bacteria such as *Pseudomonas putida (Tripathi A. et. al., 2011), Bacillus* sp. *(Abraham C.I et. al.,2014), Pseudomonous putida (Wei Wang et. al., 2012) Bacillussubtilis (Milikli G et. al., 2012), Pseudomonas* sp. *( Shah MP et. al., 2013), Bacillus subtilisSPR42 (Baljeet Singh Saharan et. al., 2011), Tsukamurella sp. J8025 (Wen-Tung Wu et. al., 2012), Geobacillus stearothermophilus UCP 986 (Norma S. et. al., 2010), P. fluorescens* and *Corynebac (Saleh M Al- Garni et. al., 2013), Georgenia sp. CC-NMPT-T3 (MadhuriSahasrabudhe et. al., 2013), Bacillus cereus (Vidhyakalarani R et. al., 2013)* have been extensively reported as degraders of dyes . In a review, *Groff and Kim (1989)* described a host of bacterial cultures with capabilities to carry out decolorization, including a *Rhodococcus sp., Bacillus cereus*, a *Ple- siomonas sp.* and *Achromobacter sp.*

A variety of pollutants biodegraded by fungi in which the lignin-degrading system (LDS) is present. The efficiency of pollutant biodegradation depended on both the type of pollutant and the fungus involved in the process. Some of the fungi enzymatic constituents played the primary role in the treatment of enzymes as summarized in the table 4.

White-rot fungi produces lignin peroxidase, manganese peroxidase and laccase that degrades many aromatic compounds due to their nonspecific enzyme systems *(Toh, Y C et. al., 2013).* 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. Lignin peroxidase act a key role in the degradation of azo dyes using *P. Chrysosporium (Ollikka P et. al.,1993).*

Algae have been found to be potential biosorbents because of their availability in both fresh and saltwater *(Wen-Tung Wu et. al., 2012).* The biosorption capacity of algae is attributed to their relatively high surface area and high binding affinity. Several species of Chlorella and Oscillatoria were capable of degrading azo dyes to their aromatic amines and to further metabolize the aromatic amines to simpler organic compounds or $CO_2$. Functional groups such as hydroxyl, carboxylate, amino and phosphate found on the algal cell surface are considered to be responsible for sequestration of contaminants from wastewater *(Asha Srinivasan, 2010).*

**Table 4: Biodegradation of colorants by fungi**

| POLLUTANT | ENZYME | SOURCE | REFERENCE |
|---|---|---|---|
| Azo dyes | Laccase | Pycnoporus sanguineis | *Pointing and vrijmoed 2000* |
| Bio polymers (Kraft, Lignin) | Lip, Mnp | White rot fungi. | *Cameron et al., 2000; Pointing, 2001; Reddy, 1995;* |
| Bleach plant effluents | Laccase | P.sanguineis | *Archibald et al., 1990; Limura et al., 1996* |
| $CCL_4$, $CHCL_3$ | Lip, Mnp, | P.chrysosporium | *Cameron and Aust, 1999* |
| PAHs | LDSs and Laccase | P.chrysosporium, Trametes versicolor Coriolopsis polyzona, Pleurotus ostreatus, T.versicolor | *Bumpus, 1989; Bogan and Lamar, 1996* *Zeddel et al., 1993; Novotny et al., 1997* |
| PCBs | Lip, Mnp | P.chrysosporium, T.versicolor, Inonatus dryophilus | *Alleman et al., 1995; Lin et al., 1990* |
| PCP | LDSs | White-rot fungi | *Cameron, 2000; Bumpus and tatarko, 1994* |
| TNT, RDX | Lip, Mnp | | |

**Table 5: Biodegradation of dyes by algal enzymes**

| Algae | Dye | % of removal | Experimental conditions | Time of contact | Referrence |
|---|---|---|---|---|---|
| Cosmarium sp. | Malachite Green | 92.4% | Temperature 5 to 45$^{o}$C | 24 hours | *Daneshvar,2005* |
| Green Algae | Monazo and diazo dyes | 68% | Temperature 25$^{o}$C | 2 days | *Hanan Hafez Omar, 2008* |
| Lyngbya sp. BDU 9001 with coir pith | Textile dye | 73% | pH 7 and the temperature 29$^{o}$C | 15 days | *Henciya, 2013* |
| Algal biomass | Malachite green | 85% | pH 4 to 6, temperature 50$^{o}$C | 45 min | *Swapnali M Gajare, 2012* |
| Green Algae | Indigo<br>Direct Blue<br>Remazol brilliant orange<br>Crystal violet | 89.3%<br>79%<br>75.3%<br>72.5% | pH 8, temperature 25$^{o}$C and salinity at 15 $gL^{-1}$ | 5 days | *ElisanAngelA F* |

Colour removal by algae was due to three intrinsically different mechanisms of assimilative utilization of chromophores for production of algal biomass, CO2 and H2O transformation of coloured molecules to non-coloured molecules, and adsorption of chromophores on algal biomass.

Only limited amount of studies about yeast decolourisation were reported. The ability of *Kluyveromyces maraxianus IMB3* to decolorize Remazol Black-B was investigated and maximum color removal, 98% was achieved at 37 degrees C *(Meehan et al., 2000). Zissie et al (1997)* showed that *Bacillus subtilis* could be used to break down azo dye.

**RECENT METHODS**

Studies carried out at the authors' laboratories have resulted in the isolation of various fungi and mixed bacterial cultures of growth on several kinds of azo, diazo and reactive dyes, both under aerobic and anaerobic conditions. Obtaining these cultures proved to be a time-consuming and demanding task *(Nigam, Marchant,et al., 1995a; b; 1996a; b).* Two mixed bacterial cultures namely, PDW and PDC are capable of decolorizing textile dyes, were isolated from enrichment cultures that were kept growing in minimal media containing dyes as sole carbon sources and anaerobic conditions for over a year *(Nigam et al., 1996a).* An investigation into the efficiency of growth and for these cultures, PDW and PDC concluded they were facultative, with an ability to grow under both aerobic and anaerobic conditions, but with highest growth rate and decolorization ability under anaerobic conditions.

**CONCLUSION**

Economical removal of colour from effluents remains an important problem although a number of successful systems have evolved employing various physico-chemical and biological processes. Coloured-dye-wastewater treatment and decolorization presents an arduous task. These effluents mostly comprises of chemical or synthetic compounds which can severely affect the biotic life of the environment and cause several health hazards to mankind indirectly. Wide ranges of pH, salt concentrations and chemical structures often add to the complication. Recently, we are undergoing a research on consortium culture of bacteria, fungi and yeast for the complete degradation of the

dye components since it has an enormous strength for the breakage of dye components. This research is on process with the sample collected from the industry located near Chennai, Tamilnadu. Therefore characterisation of consortium culture with broad spectrum action on effluent waste is need of the hour which promises the maximum detoriation of recalcitrant from the textile industries in future.

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