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Decolorization, Degradation, And Toxicological Analysis Of Textile Dye Effluent By Using Novel Techniques – Review

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ABSTRACT

In textile industry during the dyeing process roughly 10% of synthetic dyes were used and let into the wastewater. Among all dyestuff Azo dyes occupies in majority, because they are extensively used in the textile, paper, food, leather, cosmetics and pharmaceutical industries. They represent chief polluting components ranging from inorganic compounds to polymers and organic elements. However prevailing effluent treatment techniques are unable to remove recalcitrant azo dyes completely from effluents because of their color fastness, solidity and highly resistance to degradation. To ensure the safety of the effluents, proper technologies need to be used for the absolute degradation of dyes. Various kinds of physico-chemical methods are used for treating textile effluent. But these methods lack in environment friendly and cost-effective and hence become commercially unattractive. On other hand nature boon with many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolourize azo dyes. Thus biodegradation technique approach will be of eco friendly with no secondary hazard. Nowadays, in advance, enzymes can be utilized to develop remediation processes that are ecofriendly than conventional techniques. Their versatility and efficiency even in mild reaction conditions gives them an advantage over other methods. The biological origin of enzymes reduces their adverse impact on the environment, thereby making enzymatic wastewater treatment an ecologically sustainable technique. We focused on the enzymatic mechanisms involved in the bacterial degradation of azo dyes, and also investigated the toxicity level. Thus overview of this review deals with the bacterial decolorization/degradation of azo dyes and enumerate the role of these processes for the treatment of textile dye effluent. In the present review the decolorization and degradation of azo dyes by fungi, yeast and bacteria have been cited along with the toxicological, cytotoxicity, genotoxicity studies were discussed and also the role of enzymes involved in the microbial decolorization of azo dyes have been discussed.

1. INTRODUCTION:

Past few decades, there have been a startling increase in the pollution of various water bodies primarily due to industrialization. Textile industry requires large volume of water during process, thereby it release huge volume of

effluent. It is estimated that 2, 80,000 tons of textile dyes are discharged in such industrial effluents every year worldwide (Jin *et al*., 2007; Kalyani *et al*., 2009). The color produced by minute amount of organic dyes in water is considered very important because, besides having possible harmful effects, the color in the water is aesthetically unpleasant. Colored water eventually affects the entire ecosystem. Sometimes dyes used in the coloration process are also toxic and even carcinogenic in nature, ultimately affecting the living system badly, including plants, animals and humans. Overall conditions dictate the necessity of dye containing water to undergo treatment before disposal to the environment. Approximately 10-15% of the dyes are released into the environment. Commonly used dyes are the azo, anthraquinone, nitro, methine and quinoline dyes Tsuboy *et al.* (2007). Most of them are recalcitrant in nature, especially azo dyes. The involvement of extracellular oxidative enzymes such as, tyrosinase, lignin and manganese peroxidases and lakes in the degradation of azo dyes by bacteria and fungi (Fu and Viraraghavan 2001; Shanmugam et al. 2005; Zille et al. 2005; Ulson et al. Ulson de Souza et al. 2007; Kaushik and Malik 2009; Joshi et al. 2010; Kurade et al. 2011). The total degradation by conventional wastewater treatment processes so that the stability and their xenobiotic nature of reactive azo dyes make them recalcitrant. O'Neill *et al.,* (2000); (Hu 2001); Wang *et al.* (2008). The biological effects of azo dyes after biotransformation have been shown to be toxic, and in some cases these compounds are carcinogenic and mutagenic. Therefore, alternative to chemical decomposition processes the microbial decolorization and degradation have appeared as an ecofriendly and cost-competitive.

2. DECOLOURIZATION OF TEXTILE DYES:

Dye decolorization has been a primary goal of dye wastewater treatment processes because in textile effluent the high color content of dye, let out in aquatic system, it inhibits photosynthetic aquatic plants and algae by absorption of light (Banat et al. 1996). However, beyond color, the presence of these dyes in aqueous ecosystems presents serious environmental and health concerns as a result of the toxicity of the free dyes themselves and their transformation into toxic, mutagenic and carcinogenic amines, primarily as a result of anaerobic microbial reductive cleavage of the azo bond (Chung and Cerniglia 1992; Weisburger 2002; Asad et al. 2007).

2.1 *Bacterial decolourization*

By aerobic or anaerobic method bacterial decolorization of azo dyes is takes place Pandey *et al.* (2007). Decolorizing bacteria for azo dye can be isolated from soil, water, human and animal excreta and even from contaminated food materials. However, other potential ecological niches for isolating such bacteria are colored effluents arising from dye manufacturing and textile industries.

At a temperature of 37°C and pH 8.0 the maximum decolorization was observed. Also, it

has been found that bacterization of seeds of *Vigna radiata* with *Castellaniella denitrificans* SA13P increases germination rate. They have reported for the first time that *Castellaniella denitrificans* SA13P may be used as a novel strain for dye decolorization (malachite green) and biological treatment of tannery effluent (Ankita Chawla and Baljeet Singh Saharan, 2014).

Adebiyi *et al.* (2010) work was performed on the degradation and discoloration of an anthraquinone dye, reactive blue 2 (RB 2) to determine the influence of heat treatment in sewage sludge and addition of zero valent iron (ZVI). A consortium of sulphate reducing bacteria (SRB) in a biosulphidogenic batch reactor with biodigester sludge was used. 75% decolorization efficiency was achieved within 24 h of inoculation when 4 g ZVI/l was added in an SRB reactor with unheated sludge as opposed to 59% colour removal after four days in the same reactor without ZVI. *Bacillus subtilis, Bacillus megaterium, Erysipelothrix* and Amphibacillus *xylanus.* These isolates were cultured with three different concentrations of seven different textile dyes viz. cibacron red FN-R, novacron blue, terasil green, novacron navy, novacron orange and novacron yellow. By dye decolourization assay, the degradation ability of dye stuffs by the isolates was observed. Almost all days except novacron red were decolorized up to 99% of bacterial isolates after 3 days of incubation.

Celik *et al.* (2011) reported that as carbon source and energy by the widely spread, eco-friendly, photoheterothrophic strain 51ATA of the sulphonated reactive red 195 dye (RR195) was used that belongs to *Rhodopseudomonas palustris*. This bacterium, which was isolated from Lake Akkaya, (Nigde, Turkey), was able to completely degrade and minerals the day under anaerobic conditions with 100% efficiency.

To decolorize the anthraquinone dye Acid Green (AG) 25 and diazo-dye Acid Red (AR) 18 Engineered *P. putida* cells were applied. The results showed that decolorization of both dyes is Cu2+- and mediator-independent, with an optimum temperature of 35°C and pH of 3.0, and can be stably performed across a temperature range of 15°C to 45°C. A high activity toward AG25 (1 g/l) with relative decolorization values of 91.2% (3 h) and 97.1% (18 h), as well as high activity to AR18 (1 g/l) by 80.5% (3 h) and 89.0% (18 h), was recorded Wang *et al.,* (2012).

General composition of the 96 isolates comprised of *Bacillus sp., Enterobacteriaceae, Pseudomonas sp., Micrococcus sp., Alcaligenes sp., Aeromonas sp., Staphylococcus sp.,* and *Lactobacillus sp*. For the detection of preliminary decolorization these bacterial strains were freshly screened by plate method on solid media containing Remazol golden yellow (RNL), Red (RGB) and Blue (RGB). Among 96 strains tested, 20 exhibited significant decolorization. The liquid culture method was adopted for secondary screening decolorization confirmed that 6 efficient strains decolorize the dye concentration within 24 hours under static condition. The strains utilized Remazol golden yellow dye as a carbon sources for their growth. RNL dye decolorization by 6

strains was attained and maximum of 84% decolorization was recorded at 48 hours in microaerophilic condition. Furthermore the mixed cultures of the potential strains were in the stimulated time period of 24-48 hours, which attributed to effectively decolorize the dye contaminated effluent along with RNL dye Palani Velan *et al.* (2012).

Franciscon et al., 2012 stated that for of a textile company the sequential decolorization and detoxification of the azo dyes by the use of Brevibacterium sp. strain VN-15, isolated from an activated sludge process. Tyrosinase activity was observed during the biotreatment process suggesting the role of this enzyme in the decolorization and degradation process, but no-activity was observed for laccase and peroxidase. Toxicity, measured using Daphnia magna, was completely eliminated.

2.2 *Fungal decolorization*

Responsible for the decolorization and degradation of many different dyes, several fungi are capable of mineralizing pollutant compounds through their highly oxidative and non-specific ligninolytic enzymes. The ability to biodegrade various types of dyes by whole cells of white rot fungi has proven to be effective, with their elimination being mediated through oxidoreduction reactions catalyzed by the lignin degrading enzymes they produce, such as lignin peroxidase, manganese peroxidase (MnP) and laccase by Bergsten-Torralba (2009).

Namdhari *et al.* (2012) reported that under static in vitro condition the decolorization capabilities of the fungal species were evaluated for reactive blue MR dye (100-300mg/L) in carbon limited Czapek Dox broth (0.5%) were carried out. It was found that A. allhabadii and A. sulphureus showed higher decolorization capabilities (95.13±0.11%), (93.01±0.25%) with 200mg/L dye, but A. Niger showed higher decolorization (83.14±0.19%) with 100 mg/L after ten days of incubation.

It was interpreted that by both batch mode and continuous mode the colour removal by the basidiomycetes fungi were mainly due to adsorption of the dyes to the mycelial surface and also due to metabolic breakdown. The results suggested that *Schizophyllum commune* is more efficient than *Lenzites eximia* for the treatment of azo dyes and textile dye industry effluent by Selvam and Shanmuga Priya *et al.* (2012).

3. BIODEGRADATION OF TEXTILE EFFLUENT:

3.1 *Physico Chemical Method*

To treat textile wastewater for decolorization and detoxification, such as coagulation, flocculation, adsorption, membrane filtration and irradiation by employing physicochemical methods. Anaerobic method provides a low-cost and efficient means for the reductive decolorization of washed-out reactive dyes which can then be reused as process water and/or the treatment of textile effluents before their final disposal Asgher *et al.* (2008); Susla and Svobodova (2006). Even though these treatments

achieve high levels of mineralization and decolorization, they have two main constraints: high cost (e.g. photocatalysis and advanced oxidation processes), and the production of significant amounts of sludge that requires a final destination, such as incineration or landfill disposal Niebisch *et al.* (2010).

In advance decolorization, biodegradation was assessed by UV–Vis spectroscopy, FTIR spectroscopy and HPLC. Identification of biodegradation product was carried out by GC–MS Mane *et al.* (2008).

3.2 *Biological Method*

Biological treatment of textile dyes is a best method due to the potential to almost the degrade dye stuff and overcomes the many disadvantages posed by the physical- chemical processes. Many studies have been focused on microorganisms that are able to degrade dyes, suggesting that biodegradation is an environmentally friendly and cost-competitive alternative for wastewater treatment Vitor and Corso (2008); Pajot *et al.* (2010). The ability to decolorize and degrade dyes by several classes of dyes by many microorganisms belonging to the different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported by Asad *et al.* (2007).

3.3 *Bacterial degradation*

Bacterial decolorization is normally more rapid and efficient. For dye degradation under anoxic conditions various studies on bacteria indicated that, bacterial strains like *P. mirabilis*, *P. luteola*, *Pseudomonas* sp. and *K. rosea* have shown very promising results reported by Parshetti *et al.* (2006); Chang *et al.*, (2001); Yu *et al.* (2001); Chen *et al.* (1999).

Nachiyar *et al.* (2012) Reported that the ability to degrade Acid Blue 113, a diazo dye bacterial consortium was constructed using five different bacterial strains isolated from the effluent. The consortium was supplemented with glucose and ammonium nitrate, which found to degrade 90% of the dye by 22 h in 80% diluted textile effluent.

By another strain for further decomposition the individual strains may attack the dye molecule at different positions or may use decomposition products produced. However, it should be stressed that the composition may change during the decomposition process, which interferes with the control of technologies using mixed cultures.

3.4 *Fungal degradation*

Among the wide range of microorganism fungi also have degrading capacity. In particular white rot fungi a group of species associated with biodegradation of various pollutants, including textile dyes. White rot fungi possess a group of enzymes (phenol oxidases and peroxidases) that participate in lignin degradation by Wesenberg, *et al.* (2003). These enzymes have able to oxidize man-made pollutants such as pesticides, drugs, polycyclic aromatic hydrocarbons (PAHs) and textile dyes, among many others Asgher *et al.* (2008); Cabana *et al.* (2007); Susla and Svobodova (2006).

Laxminarayana *et al.* (2010) Stated that for assessed the decolourisation and biodegradation of three sulphonated azo dyes the fungi were isolated from dye contaminate soils. It showed 41-80% decolourisation of scarlet red and 80-90% of fast greenish blue and 48-89 % of brilliant violet. The protease production ranged from 25-38 U/ml by the majority of the fungi tribe *T. notatum* was found to be efficient in production of catalase, thus fungi play a vital role in decolorization and degradation of textile dyes.

P. simplicissimum reduced efficiently the toxicity of RB21 from moderately acutely toxic to minor acutely toxic and it also reduced the toxicity of RB214 and MXD, which remained minor acutely toxic. However, the fungus increased the toxicity of RR198 despite of the reduction of MXD toxicity, which included this dye. Thus, *P. simplicissimum* INCQS 40211 was efficient to decolorize different textile dyes and the mixture of them with a significant reduction of their toxicity. In addition, this investigation also demonstrated the need of toxicological assays associated with decolorization experiments (Torralba et al., 2009).

By mixed fungal cultures from semi-arid region of Brazilian Northeast the degradation and detoxification of three textile azo dyes (Reactive Red 198, Reactive Red 141 and Reactive Blue 214) was carried out. In order to select the consortia of fungi capable to degrade and detoxify these dyes sediment samples of twenty water reservoirs in the surroundings of Serra da Capivara National Park, area of environmental preservation in the caatinga in the State of Piauí, with semi-arid climate, were evaluated. The mixed fungal culture from Caldeirão Escuridão (CE) reservoir was the most efficient in the degradation and detoxification of the dyes (Nascimento, 2011).

3.5 *Yeast degradation*

Yeasts exhibit attractive features than compared to bacteria and filamentous fungi. In recent years, there has been intensive research on dye removal of yeast species. It is becoming a promising alternative to replace or supplement existing treatment processes. (Das *et al.,* 2012).

To decolorize Remazol Black-B dye, the ability of Kluyveromyces marxianus IMB3 was investigated and maximum colour removal 98% was achieved at 37 degrees C (Meehan et al, 2000).

Several yeast strains display similar decolorizing behavior. The yeast-mediated process requires an alternative carbon and energy source and is independent on previous exposure to the dyes. Ramalho et al., 2005 reported *S. cerevisiae* mutant strains Δ*fre1* and Δ*fre1*Δ*fre2*, but not Δ*fre2*, showed a much reduced decolorizing capability. *FRE1* gene complemented the phenotype of *S. cerevisiae* Δ*fre1* cells recovering the ability to grow in medium without externally added iron and to decolorize the dye, following a pattern similar to the one observed in the wild-type strain. These results recommend that under the conditions tested, Fre1p is a major component of the azo reductase activity.

3.6 *Algae degradation*

The degradation of azo dyes by algae was evaluated and it was found that certain algae can degrade a number of azo dyes to some extent. The reduction rate appears to be related to the molecular structure of the dyes and the species of algae used. The azo reductase of algae is responsible for degrading azo dyes into aromatic amine by breaking the azo linkage. The aromatic amine is then subjected to further metabolism by algae. So in the stabilization of ponds, algae can play a direct role in the degradation of azo dyes, rather than only providing oxygen for bacterial growth. (Jinqi and Houtian, 1992).

To decolorize and remove methyl red, orange II, G-Red (FN-3G), basic cationic, and basic fuchsin was investigated by using the ability of *Chlorella vulgaris*, *Lyngbya lagerlerimi*, *Nostoc lincki*, *Oscillatoria rubescens*, *Elkatothrix viridis* and *Volvox aureus*. These algae showed different efficiency for color removal; varied from ~4 to 95%, according to the algal species, its growth stage and the dye molecular structure. The results also showed that treatment of either *C. vulgaris* or *N. Linckia* with G-Red or methyl red, respectively, induced 72 and 71% the algal azo dye reductase enzyme reported by Mostafa 2009.

Hanan et al., 2008 reported that monoazo dye (Tartrazine) and diazo dye (Ponceau), affects decolorization capabilities of green algae, cyanobacteria and diatoms. The results revealed that the removal of azo dyes was rapid at the initial period of study (3 days) and became slowly by the time (6 days).

4. Degradation Of Dyes By Using Mixed Microbial Cultures:

Three bacteria identified as Acinetobacter sp., Citrobacter freundii and Klebsiella oxytoca were isolated from enrichment cultures of activated sludge in 4-nitroaniline, after which the isolates and the mixed culture was studied to determine optimal conditions for biodegradation. Azo dyes undergo degradation under anaerobic conditions environmentally toxic aromatic amines, including nitroanilines are commonly generated in the dye contaminated wastewater. In HPLC analyses it showed under aerobic conditions, these mixed culture was capable of complete removal of 100micromol/L of 4-nitroaniline within 72h (Khalid et al., 2009).

Shah et al., 2014 stated that a bacterial consortium was constructed using five different bacterial strains isolated from the effluent with the ability to degrade Acid Blue, a diazo dye. These organisms were identified as *Pseudomonas putida* (2 strains-designated as A & B), *Bacillus subtillis*, *Pseudomonas aeruginosa* (2 strains) using 16S rRNA analysis. The consortium was found to degrade 90% of the dye by 22 h in 80% diluted textile effluent supplemented with glucose and ammonium nitrate.

5. Degradation of Dyes Using Immobilized Cells

Whole bacterial cells are widely applied for the reduction of azo dyes present in textile dyeing waste water. During the last few years, different reactor designs have been proposed for an effective continuous anaerobic/aerobic

treatment of azo dyes. They are fixed film bioreactors, packed bed bioreactors, anaerobic/aerobic rotating biological contactors, aerobic suspended-bed activated sludge reactor, an aerobic up-flow fixed bed column together with an aerobic agitated tank and pulse flow bioreactor (Puvaneswari et al., 2006).

Compared to other than suspension cultures Immobilized cultures tend to have a higher level of activity and are more resilient to environmental perturbations, such as pH or exposure to toxic chemical concentrations. The decolorization of the azo dye Orange II by free and alginate-immobilized cells of an unidentified white-rot fungus (Hazrat Ali, 2010).

6. Factors Affecting During Degradation of Dyes

It is very difficult to treat textile industrial effluents by commonly used physical and chemical methods mainly because of their high biological oxygen demand, chemical oxygen demand, heat, color, pH and the presence of metal ions (Kalyani et al., 2008).

6.1 *pH*

In dye decolorization the pH has a major effect on the efficiency. The optimal pH for color removal in bacteria is often between 6.0 and 10.0. The tolerance to high pH is important in particular usually performed under alkaline conditions for industrial processes using reactive azo dyes. (Chen *et al*. 2008.)

Asgher *et al.,* (2008b) reported a fungi consortium of *Schizophyllum spp*. which decolorized 73% of solar golden yellow at a pH of 4.5 after 6 days and the efficiency decreased from 59% to 8% as pH was increased from 5 to 6 while a bacterial consortium of *Acinetobacter spp., Citrobacter freundii* and *Klebsiella oxytoca* decolourised under aerobic conditions with shaking 92% of 4-nitroaniline (and structurally different azo dyes) at a pH of 7.2 within 42 hours which decreased as pH varied below 7 or greater than 7.2 (Azeem et al., 2009).

6.2 *Temperature*

Temperature is also a very important factor for the remediation of water and soil in all processes associated with microbial vitality, including. It was also observed that the decolorization rate of azo dyes increases upto the optimal temperature, and afterwards there is a marginal reduction in the decolorization activity.

Generally, photocatalysis is not a temperature dependent. However, an increase in temperature can affect the amount of adsorption and helped the reaction to complete more efficiently with e^-_h^+ recombination (Daneshvar *et al.,* 2004).

Pearce *et al.,* 2003 reported that the differences observed were not significant by the effect of temperature at 20ºC, and at 55ºC. At 20ºC the percentage of colour removal was 88.2±1.2 whereas for 55ºC it was of 87.9±2.0. In many systems within a defined range that depends on the system the rate of colour removal increases with increasing temperature.

6.3 *Concentration of the dye*

Earlier reports show that by dye molecules with different structures increasing the dye concentration gradually decreases the

decolorization rate, probably due to the toxic effect of dyes with regard to the individual bacteria and/or inadequate biomass concentration, as well as blockage of active sites of azo reductase. (Lavanya1 *et al.*, 2014).

Different concentrations of azo dye Reactive Red BL, ranges from 50,100,200,300,400,500mg/lit has been taken for the determination of the effect of dye concentration on the decolorization. In 48 hrs it has been found that with increase in dye concentration the dye decolourizing efficiency of the bacterial strain decreases. The maximum decolorization was found on 50mg/lit concentration and minimum decolorization was found on 500mg/lit concentration (Pandey 2012).

7. Other Factors

The effect of incubation conditions namely shaking and stationary condition on decolorization of Reactive Red BL by 006/a/PP/I*, revealed that static condition was more suitable for decolorization, where the activity was found to be 95% and at shaking condition it was 84.83%. The data suggest that in static condition is more appropriate for the decolorization of the dye by the bacterium (Pandey 2012).

7.1 *Aerobic/Anaerobic Culture Conditions*

A reduction of the bond in the molecules is observed during anaerobic degradation. Then, aerobic conditions are required for the complete mineralization of the reactive dye molecule. The aromatic compounds produced by the initial reduction are degraded via hydroxylation and opening in the process is necessary in which oxygen is introduced after the initial anaerobic reduction of the bond has taken place. Bacteria usually degrade azo dyes under anaerobic conditions to colorless toxic aromatic amines, of which some are readily metabolized under aerobic conditions (Steffan *et al.* 2005). Except for a few, the aromatic amines formed from decolorization of azo dyes are recalcitrant to biodegradation under anaerobic conditions
(Pandey *et al.* 2007).

7.2 *Enzymatic method of textile dye degradation – an alternative approach*

Enzyme-based processes using crude or purified extracellular enzyme preparations constitute another alternative approaches that has been less explored, although it may be an interesting path for biodegradation of textile dyes. In particular, decolorization by crude enzyme filtrates has many advantages. A further advantage is that the organisms or enzymes utilized in these processes can be grown using cheap and abundant carbon and nitrogen wastes derived from agro-industry. It is not a costly process and allows a separate dye decolorization and also eliminate the problem of any fungal growth inhibition by the dye molecules Papinutti *et al.* (2008); Zeng *et al.* (2011). Due to the susceptibility of enzymes to inactivation in the presence of the other chemicals the oxidoreductive enzymes are responsible for generating highly reactive free radicals that undergo complex series of spontaneous cleavage reactions. In particular, laccases (ben-zenediol: oxygen oxidoreductase) that catalyze the four-

electron reduction of O2 to water coupled with the oxidation of phenolic compounds have been shown to be efficient in degrading various dye molecules Lu *et al.* (2007); Yang *et al.* (2009). The enzymatic extract enriched in laccase produced by *T. versicolor* strain DSM11269 can efficiently decolorize Alizarin Red S, RBBR and Direct Blue 71 without any addition of redox mediators, demonstrating the potential interest in such crude enzyme extracts for the removal of dyes issued from industrial effluents Theerachat *et al.* (2012).

Mainly because of their enzyme system that enables the degradation of various toxic compounds, including those that contain aromatic amines in its structure, for not producing material that has to be discarded. Furthermore, the method has generally public acceptance, which makes the treatment using a fungi promising alternative to replace or complement the conventional treatments (Fu and Viraraghavan, 2001). However, more studies are needed on the ability of fungi regarding decolorization and detoxification of dyes used by the textile industry. Many works relate the potential for decolorization of fungi from already impacted areas (Yang et al., 2005). However, results obtained with fungi from non-impacted areas have also shown to be promising (Junghanns et al., 2007; Pajot et al., 2007).

Elbanna et al., 2010 A totals of 120 lactic acid bacteria (LAB) were screened for decolorization of the textile azo dyes. The screening results showed that a total of 80 out of 120 LAB isolates were able to decolorize the dyes, in 4 h ranging from 75 to 100%. Based on API 50 CHL and 16S rDNA sequences, *Lactobacillus casei* and *L.paracasei* were the nearest phylogenetic neighbour for both strains with an identity of 99 %. The biodegradation products of RLB (as a model of textile azo dyes) formed during anaerobic and sequential anarobic/aerobic treatments. Plasmid profiles of wild-type strains and their cured derivatives indicates that the loss of the ability to decolorize azo dyes correlated to loss of a 3 kb plasmid, suggesting that the genes required for textile azo dye degradation were located on this plasmid.

In recent years researchers depends on PCR based method for the analysis of16S rRNA sequences directly from environmental samples. Eschenhagen *et al.* (2003) reported that by culture method, 16S rRNA gene-based surveys clearly demonstrate the broader scope of microbial diversity has solved the problem to a great extent than implied. At present metagenomics is the culture-independent analysis of a mixture of microbial genomes (termed the metagenome) used to analyze the various dye degrading microbial diversity either by expression or on sequencing the genome.

8. Toxicological Analysis of Dye

Toxicological analysis is used for the discharge of treated industrial wastewater before discharged into water bodies. They can be used to estimate the function capability of aquatic biotops and as an ‘early warning’ system for the

monitoring and screening of surface water [Asgher et al., 2008]. Toxicity test is fast and sensitive method. It is used to monitor effects of dye effluents. Toxicity assays are slowly being incorporated into environmental monitoring of remediation sites, with further chemical characterization using generally gas chromatographic-mass spectrometric methods (GC-MS) (Dominici *et al.*, 2010; Hu *et al.*, 2001).

8.1 *Phytotoxicity*

The toxicity studies with respect to generation of oxidative stress in plants are yet to get much significant attention. Some of the plant bioassays, over few decades for genotoxicity assessment carried out by using Allium cepa, Vicia faba and Tradescantia paludosa. Genotoxic, cytotoxic effects of various chemicals, industrial effluents on the root cells of *A. cepa* have been demonstrated previously Jadhav *et al.* (2010); Parshetti *et al.* (2006) Yu *et al.* (2001); Axelsson *et al.* (2006). Many reports have shown detoxification efficiency by performing phytotoxicity bioassays like seed germination tests and root elongation tests Jadhav *et al.* (2010).

The toxicological studies along with genotoxicity studies using A. cepa roots and phytotoxicity studies using *Phaseolus mungo* (P. mungo) and *Sorghum vulgare* (S. vulgare) conclusively designated the toxicity of Remazol red (RR) and comparatively less toxic nature of metabolites formed after dye degradation by P. aeruginosa BCH. Jadhav *et al.* (2011).

Phytotoxicity study exposed the toxic nature of RO 13 to the O. sativa, V. radiate, S. bicolor and T. aestivum plants. The RO 13 was significantly reducing the length of shoot and root than metabolites obtained after its decolorization, indicates the less toxicity of the metabolites obtained after decolorization of RO 13 (Shah et al., 2012).

8.2 *Cell toxicity*

For toxicological analysis animal models were replaced by cells as alternatives. There have several desirable features in Cell Viability tests in microwell plates using fluorescent dyes. It is used to monitor the effects of dye effluents.

Some of the parameters used to evaluate the level of cytotoxicity in textile effluent before and after treatment are neutral red (NR) assay measures the activity of lysosomes, after accumulating the dye with the principle being that binds only the lysosomes of viable cells will fluoresce (Essig-Marcello and van Buskirk, 1990). Propidium iodide (PI) stains the double-stranded nucleic acids and is excluded by living cells; as result cell fluorescence indicates an impairment of plasma membrane function (Wrobel et al., 1996). Alkaline single cell gel electrophoresis (comet assay) with yeast cells has been used in some studies to demonstrate bioremediation and detoxification efficiency Mishra and Thakur (2010); Singhal and Thakur (2009).The MTT assay is an overall indicator of cytotoxicity and is based on the ability of living cells to reduce dissolved MTT (yellow) into insoluble formazan (blue) in the presence of mitochondrial succinate

dehydrogenase Mosmann (1983). The EROD assay has long been used for testing dioxin-like behaviours of environmental contaminants like textile effluents Kinani *et al.* (2010); Laville *et al.* (2004); Louiz *et al.* (2008).

In vitro models using human cancer cell lines have become entrenched tools for rapid and accurate evaluation of toxicity at acute, chronic and sub chronic levels with fair reproducibility (Chang *et al.,* 2007; Tai *et al.,* 1994). Hepatocytes express many nuclear receptor proteins that regulate the expression of xenobiotic metabolizing enzymes, including cytochromeP450 1A1 (CYP1A1) responsible for the metabolism of multiple endogenous and exogenous chemicals making these cells ideal in vitro models for toxicological studies (Tai *et al.,* 1994). In this regard, the human hepato-carcinoma HuH7 cell line has been shown to be promising for toxicity evaluation under in vitro condition.

8.3 *Genotoxicity*

To selected azo dyes the genotoxic effects of textile dyes are most often discussed. Some of these dyes, which contain an azo group (-N=N-), are able to split off genotoxic and carcinogenic amines (e.g. Acid Red 85, which releases benzidine). If ingested orally an azo compound can be reduced by anaerobic intestinal micro flora and possibly by mammalian azo reductases in the intestinal wall or in the liver, to free aromatic amines. Reduction of orally ingested azo compounds to aromatic amines occurs in a wide variety of mammalian species, including mice Tsuda *et al.* (2000), Rhesus monkey and humans. Since many aromatic amines are known mutagens, a complete evaluation of the safety of these dyes in the human environment must include an evaluation of their genotoxicity or mutagenicity.

Dominici (2010) reported that the genotoxicity of *indigo naturalis* was assessed using micronucleus test. This study estimated the genotoxicity of water and DMSO solutions of indigo naturalis (prepared from *Indigofera tinctoria* leaves) using the cytokinesis blocked micronucleus (CBMN) assay in the human metabolically active HepG2 cell line.

The effluents from textile industries even after the treatment can remain toxic and mutagenic; nonetheless they are released into the environment any way. Consequently, the inefficiency of this process leads to the need of toxicological assays after effluent treatment.

9. CONCLUSION

Increase in the pollution of an assortment of water bodies due to industrialization during the past decades. Many treatments can be efficient in decolorization, however, it is necessary to evaluate whether there is the configuration of toxic products during the treatment process. One valuable technique to evaluate the efficiency of a degradation process is the use of bioindicators. Microbial decolorization of dyes has recently received much attention as it is a cost-effective method for dye removal. Recently, trend is shifting towards use of mixed

bacterial culture compared to individual strain. It is well established that pollution lowers the quality of life in various aspects, and affects health and life span; therefore bioremediation of pollutants for reduction of their toxic effects is of prime importance. Further, to ensure the safety of the decolorized wastewater, studies should be conducted on the phyotoxicity and cytotoxicity analysis coupled with the advances in genomics and proteomics revolutionizing various aspects offer a wide range of possibilities for enhancing the performance of bacterial treated effluent/dye solution. In future studies we can introduce bacteria or catabolic genes to monitor the process of optimization during operation in full-scale treatment systems.

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