INTERNATIONAL JOURNAL OF ADVANCED RESEARCH IN ENGINEERING AND TECHNOLOGY (IJARET)

ISSN 0976 - 6480 (Print)
ISSN 0976 - 6499 (Online)
Volume 4, Issue 4, May – June 2013, pp. 74-83
© IAEME: www.iaeme.com/ijaret.asp
Journal Impact Factor (2013): 5.8376 (Calculated by GISI)
www.jifactor.com

IJARET

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BIODEGRADATION OF ANTHRAQUINONE BASED COMPOUNDS: REVIEW

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ABSTRACT

Anthraquinone dyes, the second largest class of dyes used extensively in the textile industries. But majority of these recalcitrant dyes are resistant to degradation due to their fused aromatic structure. This necessitates the need to explore effective treatment systems for the degradation of anthraquinone dyes. Though several physico-chemical decolorization techniques have been reported only few have been accepted by the textile industries. Among the current remediation technologies, biodegradation of these synthetic dyes by different microbes is emerging as an effective and promising approach. This review paper focuses on the science of various microbial strains and the enzyme systems involved in the bio degradation of anthraquinone dye.

1. INTRODUCTION

Dyestuff sector is one of the most important sectors exposed to great developments in the field of textile industries. Around 10^6 tons of dyes are produced annually, of which 1–$1.5*10^5$ tons are released to the environment in wastewater (Stolz 2001). These discarded dyes remain long-term in the environment and accumulate (Anliker 1979; McMullan *et al.* 2001) due to their stability, recalcitrant nature leading to toxicity, and blocking sunlight for photosynthetic processes. The structural diversity of dyes comes from the use of different chromophore groups (e.g. azo, anthraquinone, triarylmethane and phthalocyanine groups) and different application technologies (e.g. reactive, direct, disperse and vat dyeing) (Heinfling *et al.*,1998). Azo and anthraquinone are two of the most common groups of dyes used in coloring different textiles. Reactive anthraquinone dyes represent the second largest class of textile dyes, after azo dyes and are used extensively in the textile industry due to their wide

array of color shades, ease of application and minimal energy consumption (Aspland, 1997). Anthraquinone dyes are resistant to degradation and are toxic, carcinogenic and mutagenic (Itoh *et al*., 1996).The frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies an important priority (O'Neill *et al*. 1999). The major disadvantages of physicochemical methods like adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis, etc for wastewater treatment include the high cost, low efficiency, limited versatility and handling of the waste generated (Van der Zee and Villaverde *et al.,* 2005). Biological methods are generally considered environment friendly as they can lead to complete mineralization of organic pollutants at low cost (Pandey *et al*., 2007). Biodegradation is a promising approach for the remediation of synthetic dyes wastewater because of its cost effectiveness, efficiency, and environment friendly nature (Gopinath *et al*. 2009, Jirasripongpun *et al*. 2007, Shedbalkar *et al*., 2008,Verma and Madamwar 2003).It is now known that several microorganisms, including fungi, bacteria, yeasts, and algae, can completely decolorize many anthraquinone dyes. (Pandey *et al*. 2007).

1.1 Anthraquinone dye

Anthraquinone dyes represent the second largest class of textile dyes, having the chromophore group, =C=O, forming an anthraquinone complex. During its manufacture and usage of an estimated amount of 10–15% is released into the environment (Chung and Stevens 1993). Many of these dyes have been reported to convert to harmful compounds such as benzidine. Additionally, most of these dyes are toxic, carcinogenic and mutagenic (Itoh *et al., 1996*) due to their stability and resistance towards light or oxidizing agents (Lee *et al.,* 2005). These dyes in water strongly absorb sunlight, which decreases the intensity of its assimilation by aquatic plants and phytoplankton thereby reducing the self purification capacity of water reservoirs. The existing physicochemical technologies for textile dye removal are expensive, ineffective and commercially unattractive due to low biodegradability of the dyes. Therefore, researchers are currently seeking to develop more effective treatment strategies for the treatment of dye wastewater. A significant amount of research has already been done on the decolorization/degradation of azo dyes and their related products (Perey *et al.,* 2002), however, limited information exists in case of anthraquinone dyes and only a few published data regarding their degradation pathways. Therefore, effective treatment systems of microbial stains for the degradation of anthraquinone dyes are to be explored.

2. FUNGAL DEGRADATION

The treatment of recalcitrant and toxic dyes, especially the anthraquinone group of dyes, with traditional technologies is not always effective and environmentally friendly. The use of bacteria in the biological treatment of dye effluents may result in the generation of colorless dead products more toxic than the parent compounds (Banat *et al*., 1996, Kulla *et al*., 1983) and, therefore, may have poor adaptability and limited application to a wide range of dye wastewater (Kulla *et al*., 1983). This has impelled the search for alternative technologies such as biodegradation with fungi which are capable of using the dye molecules as a sole source of carbon, nitrogen, and energy. The use of fungi is becoming a promising alternative to replace or complement the current technologies for dye removal (Susana Rodrguez *et al*.,2009) as it is an economical and feasible alternative to the present treatment

technologies (Singh, 2006). In a recent study on fungal biodegradation, *A.fumigatus XC6* isolated from rice straw was found to be an efficient strain for the decolorization of reactive textile dyes effluents at an optimum pH 3.0. When supplemented with appropriate carbon or nitrogen sources significant effect on colour reduction was observed (Xian-Chun *et al*.,2007). However some results on co- effect of carbon and nitrogen sources on effluent decolorization showed that effluent supplemented with both carbon and nitrogen sources need more time to completely decolorize the effluent than those supplemented with only carbon or nitrogen source. This may be due to catabolite repression which thus, delays the decolorization time. The single class of microorganisms most efficient in breaking down synthetic dyes is the white-rot fungi (Couto *et al*., 2009). They produce efficient enzymes capable of degrading a wide range of dyes under aerobic conditions (Nozaki *et al.* 2008). In addition to their natural substrate, white-rot fungi are capable of mineralizing a diverse range of persistent organic pollutants, which distinguishes them from substrate specific biodegradative bacteria. (Reddy 1995). Several studies have described that anthraquinone dyes are decolorized at higher rates than azo dyes by white rot fungi (Abadulla E *et al*., 2000; Jarosz-Wilkolazka *et al*., 2002). Effective decolorizers among mitosporic moulds have been described by Jarosz- Wilkolazka *et al*., (2002). The decolorization profiles obtained with the most effective aquatic mitosporic isolates indicate comprehensive decolorizing abilities that are not restricted to certain dyes, which proved to be advantageous to the often changing dye compositions in real effluents (Hao *et al*., 2000; Pophali *et al*., 2003; Wesenberg *et al*., 2003). Radha *et al*. (2005) found that *P. chrysosporium* was able to degrade several synthetic dyes of different chromophores such as azo, anthraquinone, thiazine and vat dyes in both free and alginate-immobilised cultures with a decolorization percentage higher than 75%. But many studies reveal that processes using immobilized growing cells seem to be more promising than those with free cells, since immobilization allows using the microbial cells repeatedly and continuously (Susana Rodriguez 2009).The results of these findings provide important insights into the development of effective treatment method for the fungal degradation of anthraquinone dyes.

3. BACTERIAL DEGRADATION

Microbial degradation of anthraquinone dyes is considered to be a subject of great interest in environmental chemistry of which constitute several bacterial species. (Fontenot, *et al.,* 2001). Decolorization of dyes may take place either by biosorption on the microbial biomass or biodegradation by cells (Zhou and Zimmermann 1993). In case of biosorption, the original structure of the dyes remains intact, and not degraded into fragments thereby not being a practical approach for treating large volumes of dye-contaminated industrial effluents due to disposal problems (Kuhad *et al.* 2004). In biodegradation, the original dye structure is destroyed, and the pollutant is split into fragments by the microbial cells. In a research conducted by Suizhou Ren, Jun Guo *et al(*2006*)*, the cells of *Aeromonas hydrophila* strain DN322 remained colorless during the process of decolorization which attributed to biodegradation. This strain isolated from activated sludge of a textile wastewater treatment plant is a highly promising one both for application in the treatment of industrial wastewater and bioremediation of triphenylmethane, azo, and anthraquinone dye pollutants from contaminated environment. For color removal, the suitable pH and temperature range were pH 5.0–10.0 and 25–37°C, respectively. For azo and anthraquinone dyes Great Red, Reactive Red KE-3B, and Reactive Brilliant Blue K-GR (50 mgl^{-1}) decolorization of more than 85% within 36h under anoxic condition was observed. However, microbial strains capable of

utilizing the dye as sole source of carbon and energy for their growth are of special interest as they eliminate the pollutant in a real sense and convert the undesirable chemicals into harmless and useful product (Ali *et al*., 2009).

4. ALGAL DEGRADATION

In recent years, the use of algae in bioremediation of colored waste water has attracted great interest (Caparkaya & Cavas, 2008; Kumar *et al*., 2005). Being a cheap source of biosorbent, readily available in large quantities, they also offer a large surface area and have been proven to be an effective biosorbent in the treatment of wastewater (Aravindham *et al*., 2006; Daneshvar *et al.,*2007, Mohan *et al*., 2002; Ozer *et al.,* 2006;, Schiewer and Wong, 2000). Biosorption by using algae has mainly been attributed to the cell wall properties where both electrostatic attraction and complexation can play a role (Satiroglu *et al*., 2002). Most of the reported studies proved that algae species possess impressive sorption capacities for a range of dyes, however, little attention has been paid to anthraquinone dye biosorption by algal biomass.

5. ENZYMATIC DEGRADATION

The degradative ability of micro organisms has opened up new prospects for the development of biotechnological processes aimed at the degradation of complex polymers such as xenobiotics for effluent decolorization.

5.1 Fungal laccases

The ability of fungi to rapidly adapt their metabolism to varying carbon and nitrogen sources is an integrated aspect for their survival which is achieved through the production of a large set of intra and extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase that are capable to degrade complex kinds of organic pollutants (Saratale *et al.,* 2007) and thus appear to be the most appropriate in the treatment of colored and metallic effluents (Ezeronye and Okerentugba, 1999). Wong and Yu (1999) decolorized three synthetic dyes namely anthraquinone, azo and indigo by the action of laccases produced from white-rot fungus *Trametes versicolor,* which were not degraded by conventional treatment due to their unique and stable chemical structures. Ashutosh Kumar Verma and Chandralata Raghukumar (2012) reported a rapid two-step technique for bioremediation of the anthraquinone dye, Reactive Blue 4 (RB4) which resulted in a decrease of 29% in total carbon accompanied by two-fold decrease in toxicity from an initial dye concentration of 1000 mg L^{-1}. This is the first report on decolorization, detoxification and mineralization of RB4 by laccase from a marine-derived fungus. Susla *et al*. (2007) have focused their study on the production of ligninolytic enzymes and dye degradation capacity of *Dichomitus squalens* immobilized on polyurethane foam or pine wood PW in a fixed bed reactor at a laboratory scale. According to them, immobilization of fungal cultures on pine wood improved laccase production eminently in comparison to the liquid cultures and immobilized *D. squalens* was able to decolorize the anthraquinone dye Remazol Brilliant Blue R and an azo dye Reactive Orange 16. Treatment of dyes with this immobilized laccase reduced the toxicity of dyes up to 80% (anthraquinonic dyes).

5.2 Bacterial laccases

Compared to fungal laccases, bacteria-derived ones have some unique characteristics, such as activity and stability at high pH values (Sharma *et al*. 2007), which make bacterial laccases alternatives for some special fields where fungal laccases are inactive. Studies in recent years have suggested that laccases are widespread in the bacterial kingdom (Alexandre and Zhulin 2000; Sharma *et al.* 2007), but only a few bacterial laccases have been characterized so far. In a bacterial test system, Abadulla *et al*. (2000) found that the toxicity of several dyes, was reduced by laccase treatment, although there was no strict correlation between decolorization and detoxification. Engineered *Pseudomonas putida* cells with a bacterial laccase (WlacD) were applied to decolorize the anthraquinone dye Acid Green (AG) 25 and diazo-dye Acid Red (AR) 18 in and the results showed that decolorization of both dyes are Cu^{2+} and mediator-independent, with an optimum temperature of 35°C and pH of 3.0. 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) was recorded. The engineered system exhibited a comparably high activity compared with those of separate dyes (Wei Wang *et al*.,2012) .This study demonstrates, for the first time, the methodology by which the engineered *P.putida* with surface-immobilized laccase was successfully used as regenerable biocatalyst for biodegrading synthetic dyes, thereby opening new perspectives in the use of biocatalysts in industrial dye biotreatment.

5.3 Peroxidase enzyme

Peroxidase is a heme-containing enzyme that is widely distributed in plants, microorganisms and animals (Duarte-Vazquez *et al*., 2003). These can catalyze degradation/transformation of aromatic dyes either by precipitation or by opening the aromatic ring structure. This degrading ability has opened new prospects for the development of biotechnological processes aimed at the degradation of xenobiotic compounds(Field *et al*., 1993), effluent decolorization (Banat *et al*.,1996, Palma *et al*., 1996) and biobleaching of Kraft pulp(Moreira *et al.,* 1997). Concerted action of two peroxidases TcVP1, versatile peroxidase (VP) and the dye-decolorizing peroxidase (DyP) from *T. cucumeris Dec 1* resulted in complete decolorization of Reactive Blue5(Yasushi Sugano *et al*., 2006). This decolorization proceeded sequentially; DyP decolorized Reactive blue 5 to light red-brown compounds, and then TcVP1 decolorized these colored intermediates to colorless DyP. This is the first description of complete decolorization of an anthraquinone dye in vitro, and the first report of using a dual-enzyme system for such a purpose thus strongly supporting the notion that DyP and TcVP1 are good candidates for development as a novel strategy for the treatment of dye wastewater. From the bacterial kingdom, a novel dye-decolorizing strain *Serratia marcescens* efficiently decolorized two chemically different dyes; Ranocid Fast Blue and Procion Brilliant Blue-H-GR belonging to the azo and anthraquinone groups, respectively by the action of MnP(Verma and Madamwar 2003). Thus, peroxidase based dye treatment will provide a reasonable basis for the development of biotechnological processes for continuous color and aromatic compounds removal from various industrial effluents at large scale.

6. CONCLUSION

The degradation of dyes in textile industrial effluents presents an arduous task. Among the most economically viable choices for anthraquinone dye treatment, microbial systems suits to be the most practical in terms of manpower requirements and running expenses. Although decolorization is a challenging process to both the textile industry and the waste water treatment facilities, the literature suggests a great potential for microbial treatment systems color removal with only hours of exposure. Dye decolorization may take place either by biosorption on the microbial biomass or biodegradation by cells. In case of biosorption, the original structure of the dyes remains intact, and not degraded into fragments whereas in biodegradation, the original dye structure is destroyed, and the pollutant is split into fragments by the microbial cells. Regarding the various carbon sources used, two opinions have been argued for many years: one deems that dyes are not a sole carbon source since the anaerobic bacteria obtain energy from the glucose instead of the dyes and glucose inhibits the decolorizing activity while the other deems that glucose can enhance the decolorizing activity. The variability may be due to the different microbial characteristics involved. Microorganisms capable of using the dye molecules as a sole source of carbon, nitrogen, and energy are of special interest and significance because they consume the dye for their growth and activities while at the same time eliminate the pollutant in a real sense. Their biodegradative potentials can be exploited to deal with the problem of synthetic dyes' pollution and explore new horizons for further research.

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