SYNOPSIS
"BIODEGRADATION OF DYES"
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Environmental pollution is a major problem faced by this century. Rapid industrialization and urbanization deteriorates the quality of air, water and land. Almost all processes employed by man for the production of goods and services lead to the production of environmental pollutants. The Blacksmith Institute issues an annual list of the world's worst polluted places and in the 2007 issues the ten top nominees are located in Azerbaijan, China, India, Peru, Russia, Ukraine and Zambia. Various pollutants produced by humans reach the aquatic systems directly and indirectly. Water pollution is mainly due to discharge of sewage or industrial substances into the water.

Coir is a unique natural fiber extracted from the husk of coconut, seed/fruit of a species of palm, *Cocos nucifera*. It is used in diverse applications and Kerala has the largest number of coir industries in India. It is an important sector as far as economy of Kerala state is concerned. The industry provides direct employment to more than 3.5 lakhs workers, majority of whom are female. It is mainly concentrated in coastal districts of the state especially in Alleppy, Kollam and Ernakulam districts. Dyeing of coir fibre is essential for improving the marketability of coir products and satisfying the requirements of consumers. Dyes used in these industries are of great concern due to its toxicity, mutagenicity and carcinogenicity (Myslak and Bolt, 1998). As per the survey conducted by KITCO, 17.8% workers are suffering from some form of allergy due to the nature of the work.

Indian dye industry produces every type of dyes and pigments and is the second largest exporter of dyestuffs and its intermediates after China. In Kerala, coir industries account for the largest consumption of dyestuffs and 89.7% of units are in small scale sector and does not have a proper effluent treatment plant. The coir and textile industry produces effluents that contain several types of chemicals such as dispersant leveling agents, acid, alkalies and various dyes. These industries daily discharge millions of liters of untreated effluents in the form of waste water into public drains that eventually empty into rivers. This alters the pH, increases the bio-chemical oxygen demand (BOD), chemical oxygen demand (COD) and gives the rivers intense coloration (Olukanni *et al.*, 2006).

Azodyes make up approximately 70% of all dyestuffs used worldwide by weight (Zollinger, 1987), making them the largest group of synthetic colorants and the most common synthetic dyes released into the environment (Chang *et al*., 2001b). Improper discharge of these dyes to aqueous ecosystem leads to reduction in sunlight penetration, which in turn decreases photosynthetic activity, dissolved oxygen concentration and water quality causing severe environmental problems worldwide (Vandevivere *et al*, 1998). In addition, azo dyes also have an adverse impact in terms of Dissolved Oxygen, Total Organic Carbon (TOC), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Moreover, numerous reports indicate that these dyes and effluents have toxic effects on the germination rates and biomass of several plant species which have important ecological functions, such as providing a habitat for wildlife, protecting soil from erosion and providing the organic matter that is so significant to soil fertility (Ghodake *et al*, 2009). Therefore treatment of dye wastewater from industries is necessary prior to their discharge to the environment.

Various physical and chemical methods, such as adsorption, chemical precipitation, photolysis, chemical oxidation and reduction, electrochemical treatment, sonication etc have been used for the removal of dyes from waste water. However, implementation of physicochemical methods have the inherent drawbacks of being economically unfeasible (as they require more energy and chemicals), being unable to completely remove the recalcitrant azodyes and their organic metabolites, generating a significant amount of sludge that may cause secondary pollution problems, and involving complicated procedures (Forgacs *et al*, 2004; Zhang *et al*, 2001).

Environmental biotechnology relies upon the pollutant degrading capacities of naturally occurring microbial consortium in which bacteria plays central role. Decolorization of azodyes by bacteria is typically initiated by oxygen-sensitive azoreductase catalysed reduction (Zimmermann *et al*, 1982) as a consequence; conventional aerobic waste water treatment processes usually are inefficient in removing the colour of azodyes (Chung and Stevens, 1992). But if the aerobic microorganisms are subjected to micro-aerophilic condition they are decolorizing at a faster rate (Hu, 1998). Apparently, there exists a need to develop novel biological decolorization processes leading to more effective clean up of azodyes (Chung and Stevens, 1992). In the present study two azo dyes, Acid Orange 7, a sulphonated monoazo dye and Direct Blue 6, a benzidine based diazo sulphonated dye were selected as model dyes for the degradation analysis and efforts have been made to decolorize dyes especially the most recalcitrant sulphonated azodyes using microbes isolated from soil and water under microaerophilic conditions.

Eventhough a number of coir industries are present in Kerala and large amount of toxic dyes are discharged into the water bodies, no work is available so far either on the toxicological effects of these coir dyes or on the biodegradation of these toxic dyes. So an attempt has been made to address the above aspects in this thesis.

The study focuses on the physicochemical characteristics of dye effluents and its impact on aquatic fauna by taking *Anabus testudineus* as a model fish. It was found that the effluents contain a mixture of dyes and other chemicals which increase the BOD, COD, TOC and TSS of the water bodies nearby. The toxicity of two industrially important coir dyes Acid Orange 7 (AO7) and Direct Blue 6 (DB6) was investigated with emphasis on histopathological effects using *Anabus testudineus* with the mean weight 11.7 ± 2.2gm and standard length of 8.3 ± 0.3 cm. After series of range finding test, the fishes were exposed to lethal concentrations of 1.25-6.25 g/L AO7 and 0.75- 3.75 g/L DB6 and as well as sub-lethal concentrations of 0.75 g/L AO7 and 0.5 g/L DB6 dyes for 30 and 90 days in a renewal bioassay procedure. The median lethal concentration (LC_{50}) values for lethal and sublethal tests were 2.765 g/L and 1.524 g/L respectively. Respiratory disturbance, erratic swimming, loss of equilibrium, lethargies and sudden fish death were observed when fishes were exposed to 5.0 g/L and these varied greatly with differences in concentration of the toxicant and this shows that mortality increases with an increase in concentration. The differences observed in the mortalities of *A. testudineus* at varying concentrations were significant ($p < 0.05$), an indication that mortality could be a factor of concentration and time of exposure. The liver of the control fish showed normal parenchyma appearance of hepatocyte with normal staining patterns of the cell. In the treated fishes, there was congestion of central vein, focal hemorrhages, vacoulation of hepatocyte, cellular infiltration and cellular necrosis. The RBC of normal fish appear oval shaped with an oval shaped central nucleus in each cell whereas enlargement of RBC with open chromatin and moderate anisokaryosis was observed in treated fishes. Also alterations in various biochemical parameters in liver, gill and muscle were observed and reveal that dye industry effluent is highly toxic to *A. testudineus*, a hardy fish seen in fresh water. The study infers that aquatic organisms are susceptible to the effluent; therefore, an indiscriminate discharge of this effluent without proper treatment to the surrounding should be discouraged.

The study further focused to find a suitable treatment method and observed from various reports that physical and chemical methods have drawbacks in the removal of dyes. Biological method is suitable since nature provides the remedy for the problems that man create. In the present study an attempt was made to isolate various bacteria and to screen the potential bacteria for the

decolorization of two azo dyes, AO7 (C.I.15510) and DB6 (C.I.22610). Soil and water samples collected from dye contaminated sites of Alleppy, Kerala, India were used for the isolation of organisms capable of decolourizing the selected dyes. Out of 25 isolates, eight bacterial strains were selected for further analysis based on their decolourizing ability and identified by biochemical tests. Out of these, three most active decolorizers were selected and identified as *Staphylococcus gallinarum*, *Bacillus subtilis* and *Burkholderia multivorans* by 16s rDNA sequencing. Based on the available information, this is the first report discussing the ability of *S. gallinarum* and *Burkholderia multivorans* in decolorizing sulphonated azo dyes.

From the screened bacteria, *S. gallinarum* was selected for further studies since it can degrade six out of eight selected structurally different dyes used in coir industry. The various parameters such as pH, temperature, agitation, concentration of dye and effect of different media, carbon source, nitrogen source etc were studied inorder to attain maximum decolorization. The species exhibited 89% and 92% decolorization of 200 ppm AO7 and DB6 respectively within 24 h over a wide pH range from 6.0-9.0 and temperature ranging from 30-50 ^{0}C under static conditions.

No decolorization was observed under shaking conditions suggesting the role of oxygen sensitive azoreductase. Also the presence of laccase was identified by plate assay method using guaiacol as substrate.

The main goal of the work was to develop a better bacterial consortium to degrade azo dyes. Different parameters of the culture conditions were optimized for the better degradation by the consortium. Degradation analysis was carried out by wavelength scan between 250- 700 nm of individual dyes using UV-VIS Spectrophotometer. The products of degradation were determined by HPLC analysis and FTIR spectrum analysis. The consortium includes *S.gallinarum*, *Bacillus subtilis* and *Burkholderia multivorans*. The decolorizing ability of this consortium on different classes of dyes was studied by taking eight structurally different dyes.

The results of the above work have been discussed in this thesis in seven chapters. The consortium proved to be very effective in the decolourisation of selected dyes and degradation of Acid Orange 7 under alkaline conditions and high temperatures. Further studies on metabolic understanding and application of these bacterial isolates as bioremediation agents could be of much interest in future.