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Evaluation of toxicity reduction in textile effluent by different treatment protocols involving marine diatom *Odontella aurita* on freshwater fish *Labeo rohita*

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ABSTRACT

Raw textile effluent, collected from Common Effluent Treatment Plant (CETP), Perundurai, Tamil Nadu, was subjected to toxicity reduction employing varied laboratory treatment protocols viz. chemical (Advanced Oxidation Process), physical (Electrocoagulation), bacterial (*Lysinibacillus sphaericus*), phycoremediation (*Odontella aurita*) and their combinations. Aquatic toxicity was tested using freshwater fish *Labeo rohita* and the variations in fish histopathological, biochemical, enzymology, hematological and immunological parameters were analyzed. The intermediatory dye metabolites and secondary amines produced during chemical ($96hLC_{50}$ = 5%) and bacterial remediation ($96hLC_{50}$ = 15%) had enhanced the stress factor and organ damage in the exposed fish. In phycoremediation, though the newly explored *Odontella aurita* could effectively absorb metal ions than other algal species and moderately reduce effluent physical parameters, but was less efficient in the removal of azo dyes from the effluent ($96hLC_{50}$ = 50%). Subsequent physical remediation had removed these dyes and other effluent solid sludge, and had enabled the fish to survive acute toxicity (96h+). However, it was less efficient in removing the secondary metabolites produced during bacterial ($96hLC_{50}$ = 20%) and chemical ($96hLC_{50}$ = 25%) remediation. Secondary phycoremediation had effectively reduced effluent physical parameters, metal ions and the hydroxyl radicals left over after primary chemical remediation. Therefore, this combined chemical and phycoremediation processes had enabled the fish population survive short term chronic toxicity (168h+), with less organ damage and slightly enhanced immunological response. This treatment is certainly better than the methodology adopted by CETP, where the fish could survive only acute toxicity with higher organ damage.

1. Introduction

Raw textile Effluent (RE) contain substances such as azo dyes, carcinogenic amines, detergents, acids, bases, inorganic chemicals etc. are very resistant to degradation by natural processes and when released into water system can lead to intense colouration and variation in physicochemical parameters. The high alkaline pH, salinity and low dissolved oxygen etc. content can lead to respiratory metabolism and can be toxic to aquatic organisms causing high mortality [29,24]. Bacteria can be used to degrade textile dyes but they also develop carcinogenic simpler amines [28] which can enter the food chain affecting the ecological cycle [3]. Still they are employed in many remediation procedures because of their cost efficiency [37]. Bacteria such as *Aeromonas hydrophila* and *Lysinibacillus sphaericus* [38] showed better rate for degradation of textile dyes, among other bacteria species of *Pseudomonas, Bacillus, Comamonas, Enterococcus,* etc. hence increasing the rate of decolourization. *Aeromonas hydrophila* is a fish pathogen [34], hence *Lysinibacillus sphaericus* is chosen in the present study for remediation of RE. The RE treated by electrocoagulation using either aluminum rod (used in this study) or iron rod can only flocculate solid sludge of the effluent [42] forming complex agglomerates causing serious disposal problems. The chemical decolourization of RE in Advanced Oxidation Processes (AOP) produces highly reactive hydroxyl radicals (HO· ions), which is active even after the treatment processes. The oxidized textile dyes and other organic compounds form reactive non-degradable intermediates [36]. This effluent can further be treated

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by biological process to reduce the toxicity of the chemicals used in the primary treatment, thus improving the efficiency of remediation process [13]. Phycoremediation (using diatom *Odontella aurita*; first used in remediation of RE) following chemical treatment has proved to be very efficient in reducing the toxicity of the effluent better than electrocoagulation and absorbent charcoal [11]. The diatom *Odontella aurita* was very efficient in remediation processes compared to the other species of diatom *Chaetoceros* and *Navicula*. Common Effluent Treatment Plant (CETP) installed at the State Industries Promotion Corporation of Tamil Nadu (SIPCOT) in Perundurai, Erode District, Tamil Nadu treats nearly 36,00,000 Liters of RE per day, adopting a six stage treatment processes [35] as detailed below. One of the major problems of chemical techniques adopted by SIPCOT is the enormous production of sludge which is proportional to the amount of chemicals used (around 1.4 g/L) creating sludge storage and disposal problems.

Species of fish *Labeo rohita* are highly sensitive to sub-lethal concentration of pollutants, and hence used as a potential biomarker for assessing aquatic pollution studies [39]. On exposure to RE they exhibit stressed/abnormal behavioral changes such as erratic swimming, gasping and ultimately leading to their death [44]. Fish histopathology is helpful in denoting the toxicity induced in the targeted organs, observed by alteration in the cellular organization and functional changes. The contaminants of the effluent can disturb gill functioning by interrupting respiratory gas exchange, preventing liver/kidney functions in biotransformation and excretion of nitrogenous waste products, and acid-base regulation [12]. Therefore, the freshwater fish *Labeo rohita* is used here as an aquatic marker in evaluating toxicity reduction in the effluents,

The physiological and biochemical parameters of the fish are affected by the altered protein metabolism (proteolysis), while the carbohydrate and lipid content of the fish are utilized to meet the high energy needed in surviving the polluted environment [23]. Variation of antioxidant (CAT) and detoxifying enzymes (AChE & GST) in the fish population indicates its adaptability to survive the stress induced by free radicals and the other toxicants in the affected environment [20]. Azo dyes and their breakdown products can oxidize aminoacids and lipids in cells at the synaptic junctions inhibiting the activity of Acetylcholine esterase (AChE) thereby affecting nerve impulse transmission of the exposed fish. Glutathione S-Transferase (GST) is an important enzyme involved in detoxification of toxic endogenous substances with glutathione (GSH) for their stabilization and excretion in less toxic form. The enzyme Catalase (CAT) also plays an important role especially in the liver and kidney to overcome the stress caused by lipid hydroperoxides and H_2O_2 produced against dyes/toxicants, in any concentration [20]. The intoxification of the exposed fish to azo dyes even at ppm concentrations can cause anemia, intravascular hemolysis, damage of erythropoietic tissues, necrosis, dilation and congestion of sinusoids. The reduction in White Blood Cells (particularly lymphocytes) in response to sensitive effluent pollutants causes immunosupression/defense mechanism of the fish, causing variation in lysozyme, and free radical production from neutrophils and monocytes (indicated by Nitroblue Tetrazolium Test; [33]. In this study the level of toxicity reduction using single and combined treatment protocols were assessed through fish acute and chronic toxicity tests and the exposed fish were analyzed for biochemical, enzyme, immunological, and haematological changes.

2. Materials and methods

Necessary volume of Raw textile Effluent (RE) was collected from CETP, SIPCOT. The following remediation protocols and their combinations (totally 10) were optimized and carried out on RE in the Laboratory.

2.1. Treatment methodologies

The chemical treatment of RE (Treatment 1) using AOP [26] was optimized using varying dosage of H_2O_2 from 0.1–1 M. Best decolourization results were observed using 0.49 M of H_2O_2 (30% w/w; 50 mL added to one litre of RE) and incubated in direct sunlight for 10 ± 2 h at 1,14,000 ± 15,000 illuminance (Lux). Further increase in the concentration of H_2O_2 played negligible role in improvement in the rate of decolourization of RE. The treated effluent was centrifuged at 3158*g* for 10 min to remove the sludge. The treatment tank was tightly covered with transparent (for photocatalytic reaction) plastic sheets to limit the rate of evaporation. The above effluent was kept under aeration for 48 h at the rate of 8L/min using air pumps to remove the hydroxyl ions left over in the treatment process which could affect the fish population under experiment. The levels of hydroxyl radicals removed were tested by the method adopted by Pouvreau et al. [30].

Physical treatment involving electrocoagulation (Treatment 2) was used to treat RE following Vepsäläinen et al. [42] in plastic cans. Two aluminum electrodes, (positive and negative; 30 × 2 × 0.5 cm; distance between the electrodes – 10 cm) were connected with a laboratory DC for power source (Genei minipack 250). The voltage of 50 V was given for 24 h for primary treatment and 10 h for secondary treatment of effluent. After electro-coagulation the sample was allowed for 10 min for settlement of sludge which was separated later through glass fiber filter (GF/C Whatman, nominal pore size 1.2 µm).

The bacterial strain *Lysinibacillus sphaericus SK* 13 (NCBI Accession No. KF032717) was used for bioremediation (Treatment 3) of RE. This bacterium was isolated from the contaminated soil of textile dye effluent disposal site, screened for its ability to degrade textile dye effluent, and was maintained in Luria Bertani broth under anaerobic condition at 25 °C and pH 8. About 50 mL of 24 h culture with an optical density of 1.0 at 600 nm was added to one litre of RE and maintained in anoxic condition for 48 h [38]. The tanks were covered tightly with plastic sheet for maintenance of anoxic conditions. The decolourized effluent was centrifuged at 12633*g* for 10 min to remove the cell mass from it.

The marine diatom (brown algae) *Odontella aurita* (NCBI Accession No. JN673404) was used for phycoremediation studies (Treatment 4). The Asn-III media (used for growing marine green algae in artificial marine condition; [4] was supplemented with 15 mg/L sodium silicate for better growth of diatom. For phycoremediation (both primary and secondary treatment) of RE, 50 mL of diatom culture consisting of 5×10^5 cells after 5 days of incubation (in ASN- III broth) was inoculated to one litre of RE and incubated at room temperature for 48 h, then centrifuged at 3158*g* for 10 min to remove the sludge. The biomass of the diatom, after treatment process, was separated from the sludge and washed with deionized water. The homogenate of the diatom was tested for the removal of suspended and dissolved solids and metal ions from the effluent [40].

The rest of remediations were pursued in combination with one another (primary and secondary protocol) ie chemical + physical remediation (Treatment 5), bacteria + physical remediation (Treatment 6), phyco + physical remediation (Treatment 7), bacterial + chemical remediation (Treatment 8), chemical + charcoal remediation (Treatment 9; 15 mg sodium silicate/L) and chemical + phycoremediation (Treatment 10). This treated effluent was tested for decreased physicochemical parameters and metal ions.

Similarly a comparative study was also carried out between the present methodologies and that of SIPCOT's pre-treated effluent till reverse osmosis. The preliminary RE treatment in SIPCOT includes adjustment of effluent temperature and flow rate at the equalization tank for a retention time of 24 h (in Primary Clarifier). The effluent is further subjected to a five stage treatment as given below. **Stage I** – Aeration Tank where the RE is biologically treated using aerobic bacteria *Escherichia coli.* The sludge produced in this stage is removed in Secondary Clarifier Tank. **Stage II** – The effluent is then chemically