Chapter VII

Ecotoxicological Evaluation of Textile Dye Effluent Treated with

*R. oryzae*

Although several treatments are efficient in decolorization and degradation, it is essential to know if toxic metabolites have been detoxified. Despite the efficiency of biological treatments, in some cases microorganism can transform the effluents into compounds more toxic than the original compounds. Consequently there is a need to evaluate the toxicity of the end product after the biological treatment. To evaluate the toxicity status of effluent treated with *R. oryzae*, ecotoxicological tests as phytotoxicity by observing seed germination and root elongation, fish bioassays and microbial toxicity using *E. coli* were carried out.

Materials and Methods

**Phytotoxicity or the seed germination – root elongation test (Wang, 1987)**

For seed germination – root elongation test, uniform healthy grains of wheat (*Triticum aestivaum* L.) and Mung beans (*Phaseoulus aureus* Roxb.) were used. The seeds were surface sterilized with 0.1 % aqueous solution of mercuric chloride, followed with repeated washings by using sterilized distilled water. Seeds (10) were scattered on three different Whatmann No. 1 filter papers, placed in petridishes. The filter papers were presoaked each in textile dye effluent, distilled water (as control) and *R. oryzae* treated effluent and the plates were kept at 25±2°C in dark. After five days, the number of seeds that germinated was counted and the root length was measured to the closest millimeter. To combine these
endpoints (seed germination and root elongation), results were expressed as Germination Index in percent of the control (%GI), according to the equation:

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% \text{GI} = 100 \times \frac{(G_s \times L_s)}{(G_c \times L_c)}
\]

Where, Gs and Gc are the number of germinated seeds in the sample and control, respectively, Ls and Lc are the lengths (mm) of the roots in the treated sample and control.

**Fish Toxicity / Bioassay Test**

Static bioassay test was carried out at Department of Zoology, Maharaja Sayajirao University of Baroda, using *Poecilia reticulate* as fresh water test fish. The test fishes were kept in an acclimatization tank and fed for 3 days and subsequently starved for a day. In this acclimatization period, dissolved oxygen was maintained above 4 ppm. 3 jars (tanks) of 4 L capacity were filled each with 3 L of water (control), treated effluent and untreated effluent. Fishes from the acclimatization tank were transferred to these jars which were aerated to maintain the dissolved oxygen level to 4 ppm. Fishes were not fed for the test period of 96h. Results were noted for any mortality or behavioral changes in fishes in the control and test jars.

**Microbial Toxicity**

Actively growing culture of *E. coli* (0.1 ml) was inoculated in 3 Nutrient agar plates. A well was prepared in each petri dish with help of a sterile borer; each filled with sterile textile dye effluent, sterile distilled water (control) and treated textile dye effluent. Plates were incubated at 37°C for 24h and the plates were checked for inhibition zone in tests as compared to the control.
Results and Discussion

Seed germination and plant growth bioassays are the most common techniques used to evaluate phytotoxicity (Kapanen and Itavaara, 2001).

For seed germination – root elongation test using wheat, in test (textile dye effluent treated with R. oryzae), 10 (out of 10) i.e. all the seeds germinated with mean root length of 5 mm as shown in Fig. 7.1. Whereas in control (distilled water) also, all the seeds germinated with root length of 5.5mm. Using textile dye effluent as such, none of the seeds were found to be germinated. With mung beans also in both tests (treated) and control (untreated), all the seeds germinated as shown in Fig. 7.2. Whereas with textile dye effluent the case was same as in wheat i.e none of the seed germinated. Cumulative root length values were slightly lower in test (treated 3.3mm) than in control (distilled water 3.5mm).

Fig. 7.1 Phytotoxicity test with Triticum aestivum L. (Wheat seeds) with A - first stage effluent (untreated) and B - effluent treated with R. oryzae
Hence, with wheat and mung seeds, % GI values for test (treated) were 91 and 94 respectively. Whereas, with untreated textile dye effluent, these values were found to be “0” for both the seed type tested. Thus, untreated textile dye effluent was found to be highly phytotoxic. In treated samples, germinability and root elongation were markedly increased and found to be almost equal to that observed with distilled water as control proving the fact that the textile dye effluent treated with *R. oryzae* was completely non-toxic.

At the forefront of effluent studies are the bioassays undertaken to identify their potential ecotoxic affects by means of predictive analysis of a sample’s toxicity or by analyzing the impact of these complex mixtures on ecosystems. The major advantage in using bioassays is that biological response to a complex mixture of chemicals integrates the effects of environmental variables such as solubility, pH, antagonism, synergism and time of exposure, all of which affects toxicity of the test organism. Serious concern has been expressed over the discharge into the environment of complex mixtures (industrial effluents) that are potentially persistent or toxic or that accumulate in the biota. Present strategies clearly call for combined biological / chemical approaches to appraise the suspected impact of industrial pollution United States Environmental Protection Agency (USEPA, 1985). In bioassay, sensitivity of a test organism to toxic substances is a complex
issue, as it involves types of toxicants, environmental conditions, test methods, etc. Even when all these variables are held constant, intrinsic organism variables such as genetics, physiology, and toxicological pathways may affect the sensitivity. As expected, the variations in sensitivity increases as the taxonomic distance increases. In the present study, *R. oryzae* treated textile dye effluent sample was found to be completely nontoxic.

The evaluation in toxicity of fish in control as well as in tests revealed no mortality even after 96h of test period. Throughout the test period of 96h the fishes were found to be healthy with no side effects whereas in test jar with the untreated effluents all the fishes died within a period of 8h proving effluent to be highly toxic.

For evaluating microbial toxicity of textile dye effluent, *E. coli* was used as the test organism. No zone of inhibition was observed with treated sample as in control with sterile distilled water. Whereas, there was complete inhibition of growth of *E.coli* with untreated textile dye effluent indicating it to be highly toxic (Fig. 7.3).

![Fig. 7.3 Microbial toxicity test using E. coli with A - first stage effluent (untreated) and B - treated effluent with R. oryzae](image)

![Fig. 7.3 Microbial toxicity test using E. coli with A - first stage effluent (untreated) and B - treated effluent with R. oryzae](image)
The results indicate a positive correlation between decolorization (%), COD reduction and the toxicity assays. Contrary to our reports, Bergsten – Torralba, 2009 have reported contradictory results that suggest that even after the fungal treatment its toxicity increased from minor acutely toxic to moderately acutely toxic. The toxicity increase could be explained by the presence of metabolites produced by the organism after decolorization, which was more toxic than the effluent. The present result suggest the non – toxicity of the treated effluent proving efficacy of *R. oryzae* which could be further exploited for its use in treatment of textile dye effluent. The study confirms that the determination of toxicity of textile dye effluent is an efficient criterion to determine the efficacy of a bioremediation approach.