## Synergistic use of plant-microbe system for pollutant removal Mohanapriya J Registration Number : 1624569138 ABSTRACT

The textile dye industry wastewater is one of the major contributors of pollutant load to the water bodies. The wastewater contains many harmful chemicals, many of which are non-degradable. The seeping of untreated wastewater into the ground or improperly treated water, released to the water bodies lead to water pollution, affecting flora, fauna and disturb the ecosystem. The dye-containing wastewater cause many health hazards (from skin irritation to cancer) to the humans.

There are many conventional treatment methods, such as adsorption, coagulation, filtration, advanced oxidation processes and biological methods, used in the treatment of textile dye wastewater. However, the methods involve high cost and cause sludge production. To overcome these, a sequential anaerobic-aerobic plant-microbe treatment system was attempted for the degradation of dye pollutants in the textile dye wastewater. The reduction (anaerobic) and oxidation (sequential aerobic) conditions and also the plant-microbe synergistic association in the rhizosphere aids the removal of various pollutants in the textile dye industry wastewater containing varied pollutants. The choice of sequential reactions helps in the complete degradation of pollutants

A model dye methyl red is degraded using plant alone, microbe alone and plant-microbe integrated system in the sequential anaerobic-aerobic conditions. The integrated system showed effective dye decolorization (92.1±3.5%), and its intermediates amine degradation (89.79±0.69%). The dye degradation was confirmed using Fourier Transform Infra Red Spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC) and Gas Chromatography Mass Spectrometry (GC-MS) analysis. The intermediates obtained by azo bond reduction were 4-aminobenzoic acid (ABA) and p-dimethyl phenylene diamine (DMPD). The formed intermediates were degraded further to nitrobenzene, benzoic acid and benzoic acid, 3-hydroxy, methyl ester. The treated water was tested for its phytotoxicity (using *Vigna radiata*) and teratogenicity (using *Danio rerio*). The treated water showed the highest seed germination (93±0.57%) and radicle and plumule growth compared to the individual treatment systems. Similarly, zebrafish embryos incubated in plant-microbe treated water showed greater survival (maximum of 50%) and less malformation (maximum of 3.7%) compared to the individual treatment methods. Teratogenic Index (TI) value for zebrafish embryos incubated in the sequential anaerobic-aerobic plant-microbe treated water showed less than 1 (analyzed using GraphPad Prism), which indicates the non-toxic nature of the treated water. The plants released oxygen in the rhizosphere, which affected the maintenance of complete anaerobic conditions, so for the further experiments, the treatment was modified by not having plants for the anaerobic component and retaining plants only in the aerobic set-ups.

The modified integrated treatment strategy was then adopted to treat the real-time textile dye wastewater under shade house condition. In this trial, the mixture of dyes present in the wastewater (namely C.I. Direct Brown 52, Fast Blue B and Carbanthrene Red G 2B), amines and other pollutants' degradation was analyzed using Liquid Chromatography Mass Spectrometry (LC-MS) analysis. The removal of chemicals that are used during various steps of wet processing, in the textile dye industry were evident. Reduction in Electrical Conductivity (EC) - 70.87 $\pm$ 0.42%, Total Dissolved Solids (TDS) - 60.15 $\pm$ 1.33% and Chemical Oxygen Demand (COD) - 79.44 $\pm$ 0.57%, was observed after the sequential anaerobic-aerobic treatment. Besides, the dye adsorption on to the roots of vetiver and microbial attachment were confirmed using Fourier Transform InfraRed Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) analysis.

The treatment was then carried out at an industrial site in pilot-scale mode. The dye decolorization of 92.49±6.57% and TAA removal of 99.2±1.15% was observed. The GC-MS analysis of the treated water showed oxidized products after the treatment. The treated water was then analyzed for phytotoxicity (seed germination of 70±1.0 %, radicle growth of 50.71±26.32% and plumule growth of 98.15 $\pm$ 3.21% and embryotoxicity (Teratogenic Index (TI) < 1). Acclimatized microbial culture was then used in the determination of Hydraulic Retention Time (HRT). During HRT determination, the dye decolorization was 78.8±0.01 % and TAA degradation was 69.2±2.36% after the sequential anaerobic-aerobic plantmicrobe treatment. GC-MS analysis of sequential anaerobic-aerobic plant-microbe treated water showed the formation of aliphatic compounds. In this trial, the HRT of the anaerobic and sequential aerobic process was found to be four hours and 24 h, respectively. Hence, the integrated treatment system using microbes only during the anaerobic treatment followed by aerobic treatment with plant-microbes proved to be an effective treatment system for the removal of pollutants in the textile dye wastewater.