TITLE OF THE THESIS: A Study on the Potential of *Stenotrophomonas koreensis* and *Bacillus rigiliprofundi* to Ameliorate the Toxicity of Industrial Dyes Malachite Green and Remazol Brilliant Blue R

INDEX No: 248/16/Life Sc./25

ABSTRACT

Malachite green is a textile dye that has long been used in aquaculture as an antiprotozoal and antifungal agent. It is basic in nature and its chloride and oxalate forms are readily soluble in water. The dye is mutagenic, carcinogenic, teratogenic, and impedes plant growth severely. *Stenotrophomonas koreensis* was isolated from a textile effluent and employed to biologically degrade the recalcitrant toxic dye malachite green. Chromatographic and Spectroscopic analysis confirmed the degradation of the dye. In the absence of any supplements or media, *S.koreensis* decolorized 98% malachite green in 4h. The highest degradation of up to 98.78 % was obtained at pH 6, temperature 45°C and inoculum size 6.36 ml using RSM within one hour. 98.6% decolorization was achieved within 2.5 h of the addition of soymeal extract or peptone. The strain also decolorized the azo dye methyl orange up to 96%. *S.koreensis* was immobilized into reusable PVA-sodium alginate beads to facilitate easier handling, which showed 95.49% decolorization. One of the most promising features of this strain is that even in the presence of heavy metals, *S.koreensis* actively degraded the dye. *S.koreensis* biodegraded malachite green following the first-order reaction kinetics. The enzymes responsible for the malachite green degradation were tyrosinase, malachite green reductase, and NADH-DCIP reductase. Several and detailed ecotoxicological studies were performed in compliance with Organisation for Economic Co-operation and Development (OECD) guidelines to ensure that the products obtained after the bio-degradation were safe for release in the ecosystem. Aquatic toxicity was estimated by exposing photosynthetic cyanobacteria *Anabaena flos-aque*, small crustacean zooplankton *Daphnia magna*, floating aquatic macrophyte *Lemna minor* and zebrafish *Danio rerio* to the dye and the degradation product. Since malachite green can also potentially contaminate land through aquaculture run-offs, textile and tannery effluents; some important land organisms were also tested for toxicity. Beneficial soil bacteria *Bacillus subtilis* and *Bacillus pumilus*, earthworm *Eisenia fetida*, important crop plants *Triticum aestivum* and *Lens culinaris* and model vertebrate *Mus musculus* were also exposed to the dye and the degradation product to evaluate the reduction of toxicity after the bacterial dye degradation process. Genotoxicity of dye and degradation product was evaluated using root tip cells of *Allium cepa*. MTT assays of malachite green and its biodegraded product were conducted using Human cell lines HaCat and HepG2. Results indicated that the degradation product was significantly safer than malachite green. Remazol Brilliant Blue R (RBBR), a common precursor of other dyes and a toxic organo-pollutant, is an extensively used anthraquinone dye in the textile industry. In this study, bioadsorption of RBBR by the bacterial strain *Bacillus rigiliprofundi* was investigated. The effects of various physical parameters on dye adsorption were studied. The kinetics and isotherm of adsorption were analysed. The bacterial adsorbent was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Phytotoxicity and cytotoxicity was evaluated before and after the adsorptive removal of the dye. Upto 80% RBBR was adsorbed and removed by the bacteria in ten days. The optimum conditions for biosorption were pH 5.5, adsorbent dosage 30mg, initial dye concentration 25 mg/L and temperature 40°C. The adsorption followed pseudo-first order kinetics and fitted appropriately to the Langmuir model. This is the first report on adsorptive decolorisation of the highly recalcitrant RBBR by the strain *Bacillus rigiliprofundi*.

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