ABSTRACT


The microbial screening program to obtain potential dye degrader from textile dye contaminated effluent treatment plant led to isolation of nine isolates having potential in decolorizing and degrading mixture of 10 different dyes called as simulated effluent. The isolates were further identified using phenotypic, biochemical and genetic (16S rRNA) test, for knowing their taxonomic status and discovered as Bacillus circulans, Providencia alcalifaciens, Enterobacter aerogenes, Ochrobactrum anthropi, Pseudomonas aeruginosa, Acinetobacter sp., Bacillus sp., Curvularia lunata and Aspergillus niger. The two isolates Bacillus circulans NPP1 and Providencia alcalifaciens VNB exhibited greater suitability and decolorization potential, hence selected and 16S rRNA sequence were further deposited in as Genebank accession number GQ478243 and GQ478244 respectively. Both the isolates demonstrated complete or substantial decolorization of various groups of dyes viz. Acid dyes (Acid orange 7, Acid black 210, Amaranth, Methyl red and Methyl orange), Reactive dyes (Reactive black 5, Reactive red 195 and Reactive yellow 145), Direct dye (Chrysohenine G or Direct yellow 12), Triphenyl methane dye (Malachite green) and Indigoide dye (Indigocarmine) and simulated effluent (mixture of 10 dyes).

In order to study physiological and metabolic aspect of decolorization process methyl red was selected as model azo dye. The Bacillus circulans completely decolorized (50 mg/l) Methyl red within 4.5 hour under static conditions. The maximum Methyl red decolorization rate obtained was 13.66 mg/l/h (initial concentration 150 mg/l) and 6.25 mg/l/h ((initial dye concentration 250 mg/l) for Bacillus circulans and Providencia alcalifaciens respectively. Both the strain possessed broad decolorization range under various physicochemical conditions of growth without affecting significant decolorization efficiency. The requirement of co-substrate was must for decolorization and can be substituted using cheap source-s like whey and starch containing effluent.
The *Bacillus circulans* was successfully employed and reused in immobilized (alginate beads) form for decolorization of Methyl red and textile dyes up to 10 repeat cycles.

The investigation of the biodegradation mechanism was carried out. The metabolites formed after decolorization of Methyl red were analysed by TLC, UV/VIS, GCMS and FTIR. Comparative UV/VIS Spectroscopy revealed azo reduction of various textile dyes used in study. Further isolates exhibited mineralization/utilization of azo reduction product like 2-amino benzoic acid. TLC detected 2-amino benzoic acid and GCMS detected 2-amino benzoic acid, NN Dimethyl amino aniline and N-methyl aniline as product of decolorization. Comparative FTIR spectrum of Methyl red and degraded dye indicated azo bond cleavage and absence of aromatic metabolites (mineralization).

The induction of various oxidative (lignin peroxidases, laccases and tyrosinases) and reductive enzyme (azoreductase) indicate involvement of these enzymes in decolorization and biodegradation process.

The collective results of phytotoxicity test (on seeds of *Sorghum bicolor* and *Pennisteum americanum*), antimicrobial activity against beneficial microorganisms (*Bacillus megaterium*, *Azotobacter vinelandii* and *Rhizobium japonicum*) revealed less toxic nature of degraded product as compare to original dye. The antibiotic susceptibility profile pointed out the wild type nature of isolates.

This study emphasizes the potential of *Bacillus circulans*, *Providencia alcalifaciens* and microorganisms undiscovered for the bioremediation of dye contaminated sites.