ABSTRACT

Environmental pollution by various dangerous xenobiotic compounds all over the world is increasing due to wide urbanization and industrial as well as technological development. Since textile sewage, containing organic and inorganic dyes are azo dyes as well as synthetic dyes, is thrown directly into nearby natural water sources and contaminate water. These pollutants pose serious threat to human and animal life, and their removal is a major concern. Now a days, bio-remediation techniques are emerging and suitable methods as these are economically reasonable, environment friendly, and don't produce toxic by-products. In current study, bacterial strains were isolated from the dye polluted areas and screened for their biofilm producing ability followed by their biofilm mediated degradation of Congo red and malachite green dyes. For this study, the soil and water samples were collected from industrial waste effluents from factory area, Sheikhupura. Isolation and identification of dye resistant bacteria was carried out using nutrient agar, minimal salt medium (MSM) plates and plates were supplemented with different concentrations (200, 400, 600 mg/L) of azo dyes. The stock solutions of azo dyes were prepared in 1000 mL of alcohol at the concentration of 1 g/L. Selected isolates were further characterized by using selective and differential media including EMB agar and MacConkey agar. After that, biofilm formation assay was performed using Congo red agar to evaluate the biofilm forming ability of isolates. Time kinetics of biofilm or biofilm-forming potential of bacterial strains was evaluated over a period of different time intervals and specific temperature, *i.e.,* 3, 5, and 7 days for 37 C in test tubes. Biofilm mediated remediation of dyes was performed by placing sample dye solutions into test tubes having preformed mature biofilm on walls, and monitoring by using spectrophotometer at 502nm wavelength. Further remediation of MG and CR was assessed by biofilm forming bacterial strains either individually or in combination by designing seven groups as (G1-G7). These groups were designed as per G1 = *L. sphaericus*, G2 = *Bacillus* sp. (CF3), G3 = *Bacillus* sp. (DF4), G4 = *L. sphaericus* + *Bacillus* sp. (CF3), G5 = *L. sphaericus* +*Bacillus* sp. (DF4), G6 = *Bacillus* sp. (CF3) + *Bacillus* sp. (DF4), and G7 = *L. sphaericus* + *Bacillus* sp. (CF3) + *Bacillus* sp. (DF4). Fourier transform infrared spectrometer (FT-IR) was also used to investigate the involvement of various functional groups in dyes before and after bioremediation by biofilm formers. FT-IR scanning was done within 450 to 4000 cm^{-1}. Results revealed that, three biofilm forming strains (AF1, CF3, and DF4) were reported out of six isolates. After biofilm

formation assay, three isolated strains (AF1, CF3, and DF4) showed strong biofilm formation on 7 day. Isolated strains also showed no growth on MacConkey agar and EMB agar means these strains were Gram positive. After morphological, biochemical testing and 16S rRNA gene sequence analysis, the isolates (AF1, CF3, and DF4) were confirmed belonging to genus *Bacillus* sp. After individual and combine application of biofilm forming strains, a decrease in the concentration of malachite green (MG) and congo red (CR) was observed. Further results revealed that more decrease in the concentration of CR than MG was observed after treating with same groups (G1- G7) of biofilm formers. Group, G1= *L. sphaericus* showed highest degradation (71.2 ± 0.8%) against MG while highest degradation of CR was observed using the combine application of two different groups G6 = *Bacillus* sp. (CF3) + *Bacillus* sp. (DF4), and G7 = *L. sphaericus* + *Bacillus* sp. (CF3) + *Bacillus* sp. (DF4) as 82.5 ± 1.1%, and 82.4 ± 0.9% respectively. FT-IR examination results depicted that in the FT-IR spectra obtained after treating MG treated with *L. sphaericus*, many peaks were disappeared such as peak at 1002cm^{-1}. FTIR analysis of the combination of biofilm by all three strains (AF1, CF3, and DF4) against MG showed the peak at 1508 cm^{-1}. In biofilm of *Bacillus* sp. (DF4), peaks were observed at 1132, 1456, 1506, 1633, 2308 and 3309cm^{-1}. When treated with the combination of biofilm of all strains, many new peaks were appeared such as at 949, 1031 and 1178cm^{-1}. While treatment with *L. sphaericus* against CR dye, the absorption peaks at 3316, 2166, 1633 and 1506cm^{-1} indicated the presence of different bonds as N-H, C=-H, C=N and C–H bonds. From this study, it was concluded that biofilm forming *Bacillus* sp. strains (AF1, CF3, and DF4) isolated from dye effluents discharge have significant potential to degrade malachite green (MG) and congo red (CR) azo dyes in polluted water.