Study of the Bacterial Degradation and Detoxification Mechanism of Crystal Violet from Textile Wastewater

SUMMARY OF

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Summary

The textile effluents/wastewaters and mire produced by effluent treatment plants is a well-off foundation of bacterial populations with high possibility of decolorizing different groups of dye eluting. The pH of collected textile wastewater was noticeably below neutral level and temperature was above the standard limits, which is harmful for aquatic life affecting the dissolved oxygen concentration and also can persuade the activity of bacteria in water resources. The collected sample also showed higher EC, TDS, TSS, COD, BOD, carbonates and residual chlorine but lower rates of nitrites, sulphates and phosphates.

Total 10 bacterial strains (SJ1-SJ10) were isolated on the basis of their colony characteristics, Gram's reaction, cell morphology and growth pattern. The samples enclosed jointly with both types of bacteria with the dominance of Gram-negative affiliates. Initially, all ten isolates were weathered for their potentiality in decolorizing Crystal violet dye and finally 4 bacterial strains (SJ4, SJ5, SJ7 & SJ10) were selected on the basis of their CV dye decolorizing ability to higher concentration. The all four strains have shown compatibility to each other. Since strains SJ4, SJ5 and SJ10 have shown much decolorization potential than SJ7, therefore these three strains were further selected for the development of bacterial consortium. These three bacterial strains were well thought-out for the characterization, based on the Gram's reaction, cell morphology, colony morphology, growth pattern in MSM-CV amended broth, decolorization and biochemical tests. These species were identified as *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas media* by 16S rDNA sequencing. The phylogenetic relationship between the identified isolated bacteria and other intimately related bacteria found in the GenBank database was performed.

Various traits of environmental and nutritional parameters were studied for the efficient and enhanced decolorization of Crystal violet dye by the developed bacterial consortium (*Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas media*). The results showed that the bacterial consortium has the high capacity for rapid and efficient decolorization of CV dye. The bacterial consortium showed increased biomass concentration as well as maximum decolorization under shaking condition as compared with static condition which showed sharply reduced decolorization percentage. The most suitable pH for decolorization of CV dye was 7 under shaking condition and optimum temperature was 35 °C. Further, the increase in inoculum concentration has shown continues increase in decolorization percentage with decrease in incubation time. Nutritional parameters were also observed for their consequence on decolorization of CV dye by bacterial consortium. Results revealed that maximum decolorization was obtained with 0.1 % concentration of glucose followed by sucrose and lactose as carbon source. Most applicable nitrogen source was found to be 0.5 % concentration of yeast extract followed by ammonium sulphate and sodium nitrate. Decolorization of CV dye was effected as the concentration of CV dye was increased. The lower concentration of CV has shown maximum decolorization which got reduced with the increase in concentration. The efficiency of bacterial consortium in decolorizing repeated addition of 100 ppm of CV dye solution under the optimized conditions was also evaluated. Results showed that the successive addition of dye resulted in faster rate of decolorization process till 6th cycle and decolorization efficiency of organism decreased and required more time for decolorization of CV dye from 7th cycle to last cycle. The newly developed bacterial consortium (*Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas media*) efficiently decolorized CV dye under various cultural, environmental, and nutritional parameters, stating the importance of this bacterial consortium in treatment of dye wastewater.

Textile industry uses broad variety of structurally dissimilar but extremely variable compositions of dyes which are eluted in the effluent, therefore, the treatment of these dye containing effluents are difficult. The treatment becomes more difficult with the added heterogeneous hazardous chemicals, wide array of pH and different salt concentrations. The developed bacterial consortium also decolorized and degraded CV dye under optimized cultural, environmental, and nutritional conditions displaying the ability/efficiency of the developed bacterial consortium to decolorize and degrade the various dyes and chemical containing textile effluents as well as synthetic CV dye under optimized conditions. The GC-MS/MS analysis of the textile wastewater and bacterially treated textile wastewater was also performed. In GC-MS/MS analysis of textile effluent, we have seen many peaks of harmful compounds but in GC-MS/MS analysis of bacterially treated textile effluent, we have observed that the peaks have either disappeared or breakdown into other smaller peaks as compared to the peaks of control. In GC-MS/MS analysis of metabolites, shown structural changes after CV dye degradation. Three degraded products were formed namely as Phenol, 2,6-bis(1,1-dimethylethyl) (a), 2’,6’-Dihydroxyacetophenone (b) and Benzene (c) after degradation which revealed the significant use of developed bacterial consortium in large scale treatment of textile effluent.

A noteworthy amplification in the production of ligninolytic enzymes (Laccase and Lignin peroxidase) was found during the decolorization and degradation process of CV dye indicating the participation of enzymes during the degradation of CV dye. Lignin peroxidase enzyme was observed to be produced more than laccase enzyme. The metabolites obtained after the degradation of CV dye was found to be abridged in toxicity as compared to the CV dye which was clearly displayed by the phytotoxicity test performed on seeds of *Phaseolus mungo.* Only few articles have been reported on the bacterial decolorization and degradation of CV dye through bacterial consortium. Previously, many articles have been reported on treatment of industrial dyestuffs with single cultures and on a small scale. The only way for eventually controlling the pollutants releasing from textile and dye stuff industries is considered to be biological treatment methods. However, additional research would be required to develop a feasible unconventional process for the treatment of dye wastewaters/effluents.

The continuous dumping of wide variety of dye containing textile wastewater/effluents full of hazardous chemicals has created environmental pollutions as well as medical and visual problems linked with human health and agriculture. Thus, the bioremediation of dumping and contaminated area of textile effluents should be of primary importance. An effluent has wide diversity of Gram-positive as well as Gram-negative bacterial species due to adaptation and thus, has the capability of decolorizing different types of dyes here in it.

Gram-negative bacteria dominated in the samples serene from textile handloom outlet. For rapid decolorization and degradation of CV dye, the bacterial consortium of three strains (*Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas media*) isolated from collected sample was developed, which was found to be the most valuable treatment of CV dye as well as textile effluent than in anoxic conditions. The best decolorization of CV dye and growth pattern of bacterial consortium was recorded to be pH 7 at 35 °C with 0.1 % concentration of glucose and 0.5 % of yeast extract as carbon and nitrogen sources, respectively under shaking culture conditions. During the degradation of CV dye, an increase in the production of ligninolytic enzymes was found indicating its involvement in the degradation process of CV dye. The bacterial consortium has shown much decolorization in addition to degradation potential in treatment of synthetic CV dye but the probability of bacterial consortium was found less in the degradation of textile effluent as investigated through GC-MS/MS analysis of consortium treated textile wastewater. The degradation of CV dye was further indicated by the diminution in toxicity of extracted metabolites investigated through phytotoxicity test on *Phaseolus mungo* plant. GC-MS/MS analysis supported the breakdown of native dye structure into three byproducts, Phenol, 2,6-bis(1,1-dimethylethyl) (a), 2’,6’-Dihydroxyacetophenone (b) and Benzene (c). The extracted metabolites of degraded CV dye were found to be safer not only for plant germination but also in the reduction of phytotoxicity percentage but also for animal toxicity as tested on *Caenorhabditis elegans*.

Thus, the whole work concludes that the developed bacterial consortium from *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas media*, could effectively be used as an alternative to physical in addition chemical process employed for the handling of dye containing textile wastewater.