Cochineal is a scale insect in the suborder Sternorrhyncha , from which the crimson colored dye carmine is derived. The insect produces carminic acid which occurs as 17 – 24 % of the weight of the dry insects, can be extracted from the insect's body and eggs and mixed with aluminium or calcium salts to make carmine dye. Carmine is today primarily used as a food coloring and for cosmetics. This dye was first used by the Mayan peoples of Central and North America.

Abstract

ABSTRACT

Industrial pollution is one of the problems presently facing throughout world and several efforts are being vigorously pursued to control it. Toxic effluents containing azo dyes are discharged from various industries and they adversely affect the water resources, soil fertility, and ecosystem integrity. Azo dyes are aromatic chromophore moieties linked by (-N=N-), represent the largest class of dyes used in textile processing and other industries. The release of these compounds into the environment is undesirable because of their toxic nature. Removal of azo dyes from wastewater is a challenging task for the researchers. Therefore, this research was aimed to optimize the azo dye reduction by isolating new bacterial species capable of secreting azo reductive enzyme, investigation into the production, characterization and further by consequently applying and obtained insights.

The isolated bacteria *Pseudomonas oleovorans PAMD_1* produced azoreductase as the prime azo reductive enzyme during the dye decolorization process. Azoreductase catalytic activity was optimized by statistical based experiment, Plackett-Burman design followed by Response surface methodology. Dye and NADH were found as potential inducer on azoreductase production. The optimal percentage degradation of azo dyes of variables up to 85.18 % was achieved by dye (200 mgl^{-1}), peptone (6.44 gl^{-1}), NADH (1.14 mM) and glucose (2.07 gl^{-1}), respectively.

The produced intracellular azoreductase under optimized conditions was purified up to 9 fold with a recovery of 16 %. The purified azoreductase was monomeric with an apparent molecular mass of 29 KDa having an optimum pH and temperature of 7.0 and 35 °C respectively. The degradation product of Orange II azo dye sulphanilic acid was analyzed using HPLC with reference to corresponding standards and the FTIR analysis also proves the degradation of the functional group of azo dye in decolorization process.

Toxicity analysis were carried out with the textile effluent and compare the toxic nature of untreated, treated textile effluent with *P. oleovorans PAMD_1* and purified azoreductase textile effluent on Nile Tilapia (*Oreochromis niloticus*). The acute (up to 96 hrs) static bioassay experiment was conducted to determine the median lethal dose (LD_{50}) and changes in the hematological parameters of *Oreochromis niloticus* were also observed. Results showed statistical differences in hematological parameters which were significant when compared to the control groups. The reduction in erythrocyte counts to the 50 % level was similar in sensitivity with fish mortality of 100 % for the maximum dose in control. The textile effluent was analyzed for physicochemical parameters include BOD, COD, TDS, and color intensity prior and after biological treatment with *P. oleovorans PAMD_1* and enzymatic treatment with azoreductase. Hematological profile of *Oreochromis niloticus* was also analyzed with the same manner to assess the toxicity. The results indicates remarkable overall BOD reduction from 371 to 89 mgl^{-1} (77.5 %), COD from 895 to 158 mgl^{-1} (82 %) and TDS from 4400 to 3250 mgl^{-1} and all the hematological parameters levels too greatly elevated when compared with raw untreated and bacterial treated effluent.

Thermostability of azoreductase was studied with various polysaccharide additives. The stability was significantly enhanced with starch (10 %) was proved by its activity assay. Thermostabilization were also assessed by their melting temperature (T_m) using Differential Scanning Calorimetry, which showed an endothermic transition peak of about 66.27 °C, which was comparatively higher than the control. The analysis of Arrhenius plot also proves the involvement of various additives in improving the thermostability of the azoreductase. The break point temperature shift and increase in the activation energy (E_a) proves the increasing thermostability that was achieved using polysaccharide additives.

Finally, the desirable biosensor construction with azoreductase was achieved which respond to azo compounds that are present in the environmental samples. *Pseudomonas oleovorans PAMD_1* azoreductase was immobilized entrapped in sol-gel on an aluminium electrode and the reaction catalyzed by the enzyme was monitored by detection of conductivity variation. Cyclic voltammetric peak of the immobilized azoreductase shows the elevated reduction peak which was attributed to the azo bond reduction catalyzed by the bound enzyme. The successfully developed potentiometric biosensor fabricated with the immobilized azoreductase was used for the detection of azo compounds. The studies carried out will enable proper biochemical analysis of industrial effluents and detection of azo compounds in order to detoxify them.