Abstract

The presence of dyes, including azo functional group (-N=N-) containing dyes, in industrial wastewater is one of the major causes of water pollution. This report showcases the functional role of azoreductase from *Chromobacterium violaceum* (MTCC No: 2656) as a valuable enzyme for the degradation of azo dyes. The enzyme was cloned, expressed, purified and biochemically characterized and further tested for degradation efficiency of an azo group containing dyes like methyl red, amaranth, and methyl orange. Further azoreductase enzyme was characterized by biophysically using experimental and computational tools. The in-silico docking and cross-linking experiments using glutaraldehyde suggest the dimeric nature of the enzyme. The enzyme structure was modelled and also studied using circular dichroism (CD) spectroscopy which suggests 40% α- helix, 30% β- sheet and 30% random coils. In the modelled structure of the azoreductase, the cofactor flavin mononucleotide (FMN) binding energy was -3.8 kJ/mol. The binding of FMN affects azoreductase-cofactor complex stability. The stability-folding studies indicate that the cofactor, FMN is required for folding, stability, and activity. Further, purified azoreductase enzyme was immobilized on amberlite beads and the degradation efficiency of various azo dyes (methyl red, methyl orange, and amaranth dye) have been studied. The toxicity and phytotoxicity of degraded azo dyes were verified in fibroblast cell lines (L929) and *Cicer arietinum*, respectively. The reusability of the immobilized azoreductase enzyme makes the process cheaper and can be utilized by various industries for the degradation of dye waste before releasing it into the environment.