ABSTRACT

Azo dyes are used in various industries because of their mode of synthesis which is relatively easy and their stable chemical structure. Such dyes are considered as most hazardous pollutants and much attention has been paid to develop efficient biological methods for their degradation. In present study, Azoreductase gene of a potent bacterial strain of *Klebsiella pneumoniae* was heterologously expressed in *E. coli* BL21 C^{+} cells by using pID blue expression vector. The recombinant strain shows 88% decolorization within three hours after 0.5mM IPTG induction. The two responses percentage decolorization and production of protein were optimized by using a statistical approach named as Response Surface Methodology. The Optimum condition for expression was obtained with 1mM IPTG 2g/l yeast extract and 1 hour of induction which shows 98 % decolorization and 0.7778 mg/ml. Azoreductase is utilized as a molecular weapon for the reductive cleavage of N=N bonds in azo dyes. For enzyme reaction Reactive black-05 was used as substrate in phosphate buffer (pH 7) with suitable cell lysate concentration. The reaction was dependent on NADH presence. It shows the best results 90% within 4 hrs.

Key Words: *Klebsiella pneumoniae*, Azoreductase, Heterologous gene expression, Azo dyes, Response Surface Methodology