Cloning and Expression of Ferredoxin Reductase in *E.coli*

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Abstract—*Carbazole 1,9a-dioxygenase (CARDO) belongs to the IIB class of Rieske non-haeme iron oxygenases (ROs). ROs are the enzymes that act as catalyst for the degradation of various nitrogen,sulfur and other aromatic heterocyclic compounds present in diesel oil. Its combustion leads to formation of nitrogen (NOx) and Sulfur oxides (SOx). These oxides cause formation of acid rain, depletion of ozone layer etc. Carbazole and Dibenzothiophene (DBT) is one of the most abundant nitrogen and sulfur heteroaromatic in diesel fuel. Carbazole in the refining of diesel inactivate the catalyst leading to the chemical instability of diesel fuel. Its release in the environment is a serious health and environmental concern as it has mutagenic and toxic activities. Degradation of these heterocyclic compounds enhances the quality of diesel. Carbazole 1,9a-dioxygenase (CARDO) is the enzyme that has the ability to degrade various aromatic compounds through the mechanism of dioxygenation. Its ferredoxin reductase contains only Flavin adenine dinucleotide (FAD) and chloroplast type cluster [2Fe-2S]. It consist of three subunits: the terminal oxygenase (encoded by carAa gene), the ferredoxin (encoded by carAc gene) and the ferredoxin reductase (encoded by carAd gene).The present study deals with the cloning of Ferredoxin reductase (carAd) subunit in pGEX-4T3 vector and optimization of expression conditions viz IPTG conc, temperature etc to promote solubilization of catalytic subunit (CarAd).*

Keywords*: ROs; Ferredoxin reductase*