Summary & Conclusion

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The combined effect of the increasing population growth and industrial development has inevitably led to an increased volume of industrial based pollutants which have a serious impact on the ecosystem. While, the advent of technology has brought about simplified and modern products onto the market, the downstream effects of these products tend to pose hazardous effects on both the consumers and the environment. Textile manufacture results in production of potentially carcinogenic and harmful waste water, which if untreated are discharged into the ecosystem by water bodies. Traditional treatment methods have not only managed to remove the effluent from the water, but have rather aggravated the problem by introducing secondary effluent through chemicals used in the 'effluent treatment'. This has led man to turn back to recover the nature to try, where application of biocatalysts in the treatment of various industrial wastes including dye containing effluent has been investigated for the last two decades.

In light of this on going research, enzymes were found favor in the treatment of textile effluent, mainly due to their broad specificity, stability, decolourization capability and non pathogenic nature can therefore be utilized on a large scale. Azo dyes are the most commonly used coloring compounds and they were thus used in this study to investigate the ability of azoreductase to degrade them under aerobic conditions. It can be concluded that azoreductase can breakdown azo compounds under aerobic conditions.

After identification and characterization of the isolate *Pseudomonas oleovorans PAMD_1* the biochemical characters as well as decolorization activity gives constructive

information with regards to the further applications of the strain. Azoreductase production is the prime mechanism of this strain during dye decolorization process. Therefore the optimization of the catalytic activity was achieved by statistical based experiment Plackett-Burman design followed by Response surface methodology. The optimal percentage degradation of azo bonds of variables for maximum 85.18 % were dye (200 mgl^{-1}), peptone (6.44 gl^{-1}), NADH (1.14 mM) and glucose (2.07 gl^{-1}), respectively.

Analysis of the produced intracellular azoreductase under optimized conditions was purified up to 9 fold with the recovery of 16 % were characterized. The purified azoreductase was monomeric with an apparent molecular mass of 29 KDa with an optimum pH, temperature of 7.0 and 35 °C respectively. Because of the well defined purification procedure and high yield; the azoreductase can be used for the treatment of textile dyeing effluent and other bioremediation processes.

Further the stability assay was carried out with additives provide certain advantages over raw enzymes. 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, showed endothermic transition peak was about 66.27 °C, which is comparatively higher than the control. The regression analysis in Arrhenius plot showed break point temperature (T_{BP}) of 45 °C for free enzyme with activation energy of 5.20 $KJmol^{-1}$. This break point shifted to 68 °C with activation energy of 10.35 $KJmol^{-1}$ for enzyme protected by the additive starch whereas for the additives, agar, and sucrose the break point and activation energy was 59, 54 °C and 8.02, 6.64 $KJmol^{-1}$ respectively. The analysis of Arrhenius plot also proves the involvement of additives in improving the thermostability of the azoreductase. This will

enable proper biochemical analysis of industrial effluent in order to identify and detoxify their toxic constituents.

Finally, the desirable biosensor construction with azoreductase was achieved that respond to azo compounds in the environmental samples. The immobilized azoreductase on a glassy carbon electrode and the reaction catalyzed by the enzyme can be monitored by detection of conductivity variation. Amperometric measurement by cyclic voltammeter of different dyes such as Orange II, Methyl red and Reactive black with the concentration of 10 mM shows a reduction peak of 250 μA, 16 μA and 15 μA respectively. The reproducibility of the fabricated biosensor was good with standard deviation value of 2.1 % (n= 10), shelf life of more than 15 days when stored at 4 °C and the sensitivity for Orange II about 2 mM concentration was achieved. It can be concluded that it is possible to use this azoreductase biosensor for detection of azo dyes that are used in textile industry.

The use of potential azoreductase for the bioremediation of textile effluent offers a cheaper alternative, and was thus investigated in the current study. Successfully optimized decolorization of commercial azo dyes with azoreductase was investigated. This satisfy the overall objective of the present study to degrade of industrial dyes and their effluents by using azoreductase enzymatic treatment could be used as a powerful bioremediation of industrial textile dyes containing effluent.