Summary
Enzymologists are searching for new advances in environmental fields, from enzymatic bioremediation to the development of renewable methods for the biochemical cleaning of 'wastewater'. Peroxidases in particular have been underlined for this purpose due to their low energy requirements, operation over a wide range of conditions and having minimal environmental impact. A tailoring tool for this achievement is enzyme immobilization with two main benefits, enhancement of storage/operational stability and reusability. Meeting the demand for “green biotechnology”, turnip and bitter gourd peroxidase have enormous potential for replacing conventional wastewater treatment processes.

The present study aimed to work out an inexpensive, simple and high yield procedure for the immobilization of turnip peroxidase, which would be of extreme interest in the remediation of several types of aromatic compounds present in polluted water. Turnip peroxidase was fractionated from the buffer extract by 10-90% ammonium sulphate and was insolubilized by using jack bean extract, a source of concanavalin A. Soluble and concanavalin A complex of turnip peroxidase were entrapped into calcium alginate-pectin beads and these entrapped enzyme preparations retained 52% and 63% of the original activity, respectively. The stability against various denaturing agents is an important factor when selecting an appropriate enzymatic system for any application. Calcium alginate-pectin entrapped concanavalin A-turnip peroxidase showed impressive gains in resistance to inactivation induced by pH, heat, urea, detergents and organic solvents. The exposure of soluble and immobilized turnip peroxidase to low concentrations of detergents; Sodium dodecyl sulphate and Surf Excel caused activation in enzyme activity. However, the immobilized enzyme preparations exhibited further activation in turnip peroxidase activity at higher concentrations of detergent as compared to soluble counterpart.

Another promising plant peroxidase involved in environmental application is bitter gourd peroxidase. In order to increase its potential use, calcium alginate-starch hybrid gel was employed as an enzyme carrier both for surface immobilization and entrapment of bitter gourd peroxidase. The insoluble concanavalin A-bitter gourd peroxidase complex retained 70% of the initial activity. In order to prevent the dissociation of concanavalin A-bitter gourd peroxidase complex, this preparation was crosslinked by 0.5% glutaraldehyde. Entrapped crosslinked concanavalin A-bitter gourd peroxidase retained 52% of the initial activity while surface immobilized and
glutaraldehyde crosslinked enzyme showed 63% activity. Crosslinking of bitter gourd peroxidase resulted in small loss of enzyme activity but glutaraldehyde based chemistry is an effective method for increasing mechanical and operational support of enzymes.

A comparative stability of both forms of immobilized bitter gourd peroxidase was investigated against pH, temperature, chaotropic agent; like urea, heavy metals, water-miscible organic solvents, detergent and inhibitors. The pH and temperature-optima for both immobilized preparations was the same as for soluble counterpart at pH 5.0 and 40 °C. Entrapped bitter gourd peroxidase was remarkably more stable than surface immobilized and soluble peroxidase when incubated for different times at 60 °C. Entrapped peroxidase was significantly more stable as compared to surface immobilized enzyme followed by soluble form of enzyme under various physical and chemical denaturing conditions. Enzyme reuse provides a number of cost effective advantages that are often an essential pre-requisite for establishing an economically viable enzyme catalyzed process. Entrapped crosslinked concanavalin A-bitter gourd peroxidase showed 75% of the initial activity while the surface immobilized and crosslinked bitter gourd peroxidase retained 69% activity after their seventh repeated uses.

The ability of partially purified peroxidases to treat direct dyes used in textile industries was also reviewed. Dye solutions (50-100 mg L⁻¹) were treated with 0.094 U mL⁻¹ of turnip peroxidase under various experimental parameters. Direct dyes were recalcitrant to the action of turnip peroxidase, however the rate and extent of decolorization of direct dyes by turnip peroxidase was significantly enhanced in the presence of different kinds of redox mediators. Six out of ten investigated compounds showed their potential in enhancing the decolorization of direct dyes. Various parameters such as pH, temperature, enzyme and redox mediator concentrations were standardized in order to obtain maximum rate of decolorization of direct dyes. Maximum decolorization of dyes over 60% occurred in the presence of 0.6 mM 1-hydroxybenzotriazole/violuric acid in sodium acetate buffer, pH 5.5 at 30 °C. In order to prove the capability of plant peroxidase in the treatment of industrial effluents, the treatment of mixtures of direct dyes was also investigated. Complex mixtures of dyes were also maximally decolorized in the presence of 0.6 mM redox mediator (1-hydroxybenzotriazole/violuric acid). In order to examine the operational stability of the enzyme, the enzyme was exploited for the decolorization of mixtures of dyes for
different times in a stirred batch process. More than 80% of the dye mixtures were decolorized within first hour of incubation with turnip peroxidase. However, the treatment of direct dyes/mixtures in the presence of redox mediators by turnip peroxidase caused the formation of insoluble precipitate, which could be removed by the process of centrifugation, filtration or adsorption. Thus indicating the removal of end products of the enzyme mediated dye decolorization. Total organic carbon analysis of treated dyes or their mixtures showed that these results were quite comparable to the loss of color from solutions. The results suggested that catalyzed oxidative coupling reactions might be important for natural transformation pathways for dyes and indicated their potential use as an efficient means for removal of dyes color from waters and wastewaters.

Surface immobilized bitter gourd peroxidase has been used for the effective decolorization of textile industrial effluent. Effluent was recalcitrant to the action of bitter gourd peroxidase, however in the presence of some redox mediators, it was successfully decolorized. Effluent decolorization was maximum upto 70% in the presence of 1.0 mM 1-hydroxybenzotriazole within 1 h of incubation. However, immobilized bitter gourd peroxidase had optimum decolorization at pH 5.0 and 40 °C. Immobilized bitter gourd peroxidase decolorized more than 90% effluent color after 3 h of incubation in a batch process. In order to evaluate the efficiency of salt fractionated plant peroxidase for the decolorization of textile effluent, it was necessary to decolorize textile effluent in a continuous mode. A two-reactor system, one reactor containing immobilized peroxidase and the other had adsorbent; activated silica was used for the effective decolorization of textile effluent. The system was capable of decolorizing 40% effluent even after 2 months of continuous operation with a flow rate of 16 mL h⁻¹. The absorption spectra of the untreated and treated effluent exhibited a marked difference in absorbance at various wavelengths. Thus immobilized peroxidase/1-hydroxybenzotriazole system has been successfully employed for the treatment of a large volume of effluent in a continuous reactor.

Further an attempt has been made to use a simple, inexpensive concanavalin A-wood shaving bound turnip peroxidase for the decolorization of a direct dye and mixture of direct dyes in batch processes and continuous reactors. The study has shown that wood shaving could be exploited as an inexpensive material for the preparation of bioaffinity support due to its high affinity for concanavalin A; being the natural source of cellulose. Wood shaving (1.0 g) adsorbed nearly 22 mg of
concanavalin A from jack bean extract. Wood shaving adsorbed concanavalin A was used for the immobilization of peroxidase directly from ammonium sulphate fractionated proteins of turnip. Concanavalin A-wood shaving bound turnip peroxidase showed very high effectiveness factor $\eta$ of 0.67. The comparative results between soluble and immobilized turnip peroxidase obtained from the batch process revealed the ability of immobilized turnip peroxidase, to decolorized Direct Red 23 and mixture of dyes up to 92% and 83% after 1 h of incubation in the presence of 0.6 mM 1-hydroxybenzotriazole, respectively. Enzymes used for the treatment of wastewater contaminated with aromatic pollutants could also be affected by the presence of water-miscible organic solvents/salts/heavy metals. Therefore, the decolorization of direct dye and mixture of direct dyes by turnip peroxidase was carried in the presence of water-miscible organic solvent, salt and heavy metals. The immobilized turnip peroxidase could effectively remove more than 70% of color in the presence of metals/salt. The dye decolorizing reusability was gradually decreased up to 8th repeated uses. Immobilized peroxidase could decolorize 47% and 30% of the initial color from Direct Red 23 and mixture of direct dyes even after 8th repeated use, respectively. Decolorization of Direct Red 23/mixture of direct dyes carried in a continuous reactor containing a cheap biocatalyst, immobilized enzyme with a flow rate of 7.2 mL h$^{-1}$ was highly efficient even till 4 and 3 months of operation, removing 64% and 50% of color from the solution of Direct Red 23 and mixture of direct dyes, respectively. Total organic carbon analysis of treated dye or mixture of dyes exhibited that these results were quite comparable to the loss of color from solutions. Thus, this work may provide a reasonable basis for development of biotechnological processes for continuous color and aromatic compounds removal from various industrial effluents at large scale.