5.0 SUMMARY AND CONCLUSION

The present study entitled "***Bioremediation of methyl orange from aqueous solutions using Oedogonium subplagiostomum AP1***" has been conducted in eight phases and the findings are summarised below:

PHASE I

In phase I, the alga was collected from the natural pond located near Perur, Coimbatore, Tamilnadu and identified based on its morphological and phylogenetic method.

The morphological identification indicated that the alga belongs to *Oedogonium* species. The 5.8S rRNA of *Oedogonium* species was sequenced and compared with 5.8S rDNA database of NCBI for further identification. The strain showed 99% identity with *Oedogonium subplagiostomum*. Hence, the strain was identified as *Oedogonium subplagiostomum* AP1. The sequence of 5.8S rRNA of *Oedogonium subplagiostomum* AP1 was submitted to GenBank with the accession number KY575148.

PHASE II

In phase II, the screening of textile dyes against the selected alga was carried out. The effectiveness of the *Oedogonium subplagiostomum* AP1 for the removal of methyl orange from aqueous solutions was executed in batch mode. Various process parameters like dye concentration, biosorbent concentration, pH, temperature and contact time were optimized and under optimal conditions the decolourisation and uptake of dye by *Oedogonium subplagiostomum* AP1 was assessed. The algal biomass and the process variables were optimised for the maximum dye removal in batch mode using Response Surface Methodology (RSM). The efficiency of different eluents to desorb the methyl orange from the algal biomass and their regeneration efficiency was recorded by adsorption-desorption experiments under optimised conditions. Column study was conducted to check the different flow rate on the removal of dye.

The findings revealed that under optimal conditions (dye concentration: 500mg/L, biosorbent concentration: 400mg/L, pH: 6, temperature: 30^{o}C and contact time: 5 days) 97% of dye was removed and 120mg/g of dye uptake was obtained from the aqueous solutions.

Optimisation carried out using Box-Behnken Design against the process variables showed maximum dye uptake and removal of 97% and 120mg/g respectively (Optimised conditions: 500mg/L dye concentration with 400mg/L biosotbent concentration, pH 6.5 and contact time 5.5 days). The values of the predicted response were in good agreement with experimental values indicating the suitability of the experimental model and the success of RSM. The biosorbent could be regenerated using 0.1N NaOH as a best desorbing agent with 83% recovery on 2nd day. A consistent reduction in the adsorption capacity of the dye was noticed after three cycles of adsorption-desorption. The column studies indicated that at low flow rate (10ml/h) maximum colour removal (97%) was noted and increased flow rate (120ml/h) reduces the decolourisation efficiency (2%). This suggests that the column packed with *Oedogonium subplagiostomum* AP1 can be developed for the removal of methyl orange in large scale level.

PHASE III

The capacity of an adsorbent is described by its equilibrium sorption isotherm, which is characterized by certain constant values that express the surface properties and affinity of the biosorbent. Langmuir and Freundlich isotherm models were performed to analyse the validity of adsorption data. The mechanism of biosorption efficiency was investigated by testing the adsorption kinetic data using pseudo-first and pseudo-second order kinetic models. The nature of adsorption was evaluated by thermodynamic parameters such as Gibbes free energy, enthalpy and entropy.

The results of equilibrium data well suited with the Langmuir isotherm than Freundlich isotherm model thus, proving monolayer adsorption of methyl orange on the algal surface. Analysis of data shows that the process involves pseudo-second order kinetic model and the thermodynamic study portrays the evidence for the

feasibilty, spontaneous and exothermic nature of the adsorption which is controlled by chemisorption process.

PHASE IV

In phase IV, the interaction of the adsorbent-adsorbate was demonstrated using UV-Vis spectral analysis, FT-IR analysis and the surface morphology of the biosorbent was documented by SEM with EDX (determine the chemical components) and XRD respectively.

UV-spectral analysis revealed the disappearance of peak in treated dye solution indicating the decolourisation of methyl orange by biodegradation or biosorption. FT-IR spectra revealed the presence of functional groups namely hydroxyl, amino, N-N and C-N on the algal surface which were the main active binding sites responsible for the adsorption of methyl orange. Scanning electron micrograph revealed a smooth texture of the biosorbent surface indicating the entrapment of dyes. The EDX spectrum of *O. subplagiostomum* AP1 shows the major elements such as C, O, Mg2+, Si, Cl-, K+ and Ca2+ respectively. When the algae interacted with methyl orange the elements such as Mg2+, Cl- and K+ were eliminated suggesting the possibility of the dye interaction with O. subplagiostomum AP1. The XRD spectrum of the biosorbent before adsorption of methyl orange showed the crystalline nature and after dye adsorption reveals amorphous nature suggesting the alteration in the structure when the dye molecules were adsorbed.

PHASE V

Bioassay studies such as phytotoxicity, zootoxicity, microbial toxicity and cytogenotoxicity were performed to evaluate the toxicity of the degraded metabolites of dye after decolourisation.

Phytotoxicity studies were conducted to evaluate the impact of the T_1 (tap water), T_2 (untreated dye solution) and T_3 (treated dye solution) on *Tagetes erecta* by determining its biometric (germination percentage, seedling length, vigour index) and yield attributes (Height of the plant, number of flower, size and weight of the flower) on 7^{th} and 60^{th} day respectively. At the end of 60^{th} day, the experimental soil

samples were also analysed for their physicochemical parameters namely pH, electrical conductivity, total nitrogen, total phosphorus, total potassium, sodium, calcium, iron, copper, zinc, nickel and manganese respectively.

Seven days old seedlings of *Tagetes erecta* showed maximum germination percentage, seedling length and vigour index in T_3 plants when compared to T_2 plants indicating the non-toxic nature of the degraded metabolites whereas untreated dye solution showed inhibitory effect on the growth parameters. Similar result was recorded in the yield attributes (height of the plant, number of flower, size and weight of the flower) on 60^{th} day old *Tagetes erecta*.

The treated dye solution might have enriched the soil nutrients which thereby enhance the growth and yield of *Tagetes erecta* whereas the untreated dye solution retarded the plant growth and yield.

Zootoxicity study was analysed to assess the impact of tap water (T_1), untreated dye (T_2) and treated dye (T_3) solutions on the behavioural response, mortality, haematological (RBC, PCV, Hb, WBC, MCV, MCH and MCHC), biochemical (plasma glucose and protein), enzymological (AST and ALT) and histological examination (gills, liver and kidney) of *Labeo rohita*.

The fishes exposed to tap water (T_1) and treated dye solution (T_3) showed normal behavioural response whereas T_2 fishes showed abnormal behaviour. The mortality rate was less in T_1 and T_3 fishes whereas T_2 fishes recorded maximum mortality. Fishes exposed to untreated dye solution showed reduction in RBC, PCV, Hb, MCHC, plasma glucose and plasma protein, increased level of WBC, MCV and MCH and also alteration in aspartate and alanine amino transaminases (AST and ALT) thereby indicating the toxicity of the dye. No such reduction and alteration was observed in haematological, biochemical and enzymological levels of fishes exposed to tap water (T_1) and treated dye solution (T_3) indicating the non-toxic nature of the degraded metabolites of dye.

Histological examinations in T_2 fishes revealed necrosis of secondary lamella, swelling in interlamella and severe haemorrhage in the gills, vacuolar degeneration,

sinusoidal congestion, necrosis and haemorrhage of hepatocytes and degenerative and necrotic changes in the renal tubules, congested and shrunken glomeruli in kidney thereby indicating the toxicity of the dye. The histo architecture of T_1 and T_3 fishes showed no structural changes indicating the non-toxic nature of the degraded metabolites of dye.

Microbial toxicity was assessed against the selected bacterial and fungal isolates by agar well diffusion technique. The toxic effect of the dye and the degraded metabolites of dye were observed by the presence of zone of inhibition against the tested microflora.

The results revealed that the degraded metabolites of dye did not show growth inhibition zone against the tested microflora suggesting its non-toxic nature. The microbial toxicity of methyl orange against the bacterial and fungal isolates revealed the zone of inhibition or stimulatory effect suggesting its toxicity.

The cytogenotoxicity studies were performed using *Allium cepa* to assess the effect of tap water (T_1), untreated (T_2) and treated dye (T_3) solutions on root growth, root length, mitotic index, mitotic inhibition/depression and chromosomal aberrations.

Allium cepa grown with tap water (T_1) exhibited maximum root growth, root length and mitotic index followed by treated dye solution (T_3) whereas minimum root growth, length and mitotic index were noticed in untreated dye solution. The mitotic inhibition and chromosomal aberrations were high in untreated dye solution (T_2) and meagre in treated dye solution and negligible in tap water. The chromosomal aberrations such as disoriented and abnormal grouping of chromosomes, vagrant and laggard chromosomes, chromosomal loss, sticky chain and disturbed metaphase, pulverized and disturbed anaphase, chromosomal displacement in anaphase, abnormal telophase, and chromosomal bridge at telophase, spindle disturbances and binucleate cells were observed in *Allium cepa* grown with untreated dye solution. No such aberrations were recorded in *Allium cepa* treated with tap water and meagre changes were noticed in *Allium cepa* exposed to treated dye solution (T_3) indicating the non-toxic nature of the degraded metabolites of dye.

PHASE VI

The reuse efficiency of the dye desorbed algae and treated dye solution was subjected to composting and dyeing fabric. The composting of desorbed algae was carried out using *Eudrilus eugeniae* for 60 days. At the termination of the experiment, the algal compost was analysed for its pH, electrical conductivity, organic carbon, moisture content and NPK levels. The values obtained in the physicochemical analysis of algal compost can helps to improve the soil fertility and plant growth. Thus, the algal compost can be used as an alternative for chemical fertilizer and thereby reduces the level of pollution.

In order to save water, recycling of wastewater for dyeing fabrics reduces the wastage of water. The fabrics was dyed with tap water and treated dye solution and evaluated for the physical properties namely fabric weight, thickness, strength, elongation, stiffness (warp and weft) and colour fastness (sunlight, pressing and crocking) respectively. The results revealed that the physical properties of the fabrics dyed with treated dye solution are comparable with that of tap water dyed fabric.

PHASE VII

The untreated and treated textile dyeing effluent was analysed for its physical parameters viz., colour, odour, pH, electrical conductivity, turbidity and chemical parameters such as alkalinity, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), total hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), total alkalinity, chloride, sulphate and nitrate. The levels of physicochemical parameters in untreated dyeing effluent does not fall within the tolerance limits prescribed by BIS for the discharge into inland surface whereas the treated effluent falls within the prescribed limits suggesting the potential of *Oedogonium subplagiostomum* AP1 in the treatment of textile dyeing effluent.

PHASE VIII

The methyl orange was subjected to *in silico* studies for their binding against the target protein (azoreductase) involved in bioremediation using the commercially available tool Glide. The docking study showed that the binding mode of methyl orange was stabilized by hydrogen bonds which might be involved in the effective

interaction of the ligand (methyl orange) with the target protein (azoreductase) leading to undergo degradation. The hydrogen bond interaction from the docking studies indicates that the amino acid such as SER10, ARG12, SER18, SER18 and SER16 play an important role as catalytic residues in azoreductase. A good dock score obtained indicates the higher binding affinity towards the target binding protein.

Thus to conclude, the present study revealed the ability of *Oedogonium subplagiostomum* AP1 in the decolourisation of methyl orange from aqueous solutions. The physicochemical monitoring together with bioassay studies demonstrated the success of algae in wastewater treatment and can be explored for commercial purpose.

RECOMMENDATIONS

- The biosorbent can be tailored upon by physicochemical modification and immobilization techniques to boost their efficiency in dye removal.
- Employment of genetically engineered strains as biosorbent for decolourisation studies.
- Studies on the combined use of algal biomass and agricultural products for dye decolourisation.