1.0 INTRODUCTION

Water is an indispensable commodity for the existence and sustenance of life on planet earth. All the flora, fauna, microbes and human beings on the earth require water for their survival and growth. Saving water to save the planet and to make the future of mankind safe is what we need (Simpi *et al*., 2011 and Ahlawat and Kumar, 2009).

Rapid urbanisation and technological development has polluted the natural ecosystem by the introduction of sewage, domestic waste, industrial effluent, agriculture and land drainage which impends public health. Industrialization has also brought in water pollution which has become a threatening menace to the survival of life in the planet and also a major hazard which the environment faces today. Textile industry occupies the major sector in Tamil Nadu and plays a pivotal role in the consumption of large amount of water. It has tremendously increased the application or influx of dyes which when released into aquatic bodies may result in biomagnification (Kumar and Jaabir, 2013 and Kant, 2012).

Azo dye occupies the largest and most versatile class of synthetic dyes used in textile processing industries because of its high wet fastness profile, high stability to temperature, detergent and microbial attack and cost effectiveness when compared with natural dyes (Ong *et al*., 2010). However, release of these dyes into the water bodies and terrestrial ecosystem creates escalating problems such as diminishing the photosynthesis by impeding light penetration, inhibition of germination rate in plants, chronic and acute toxicity, allergenic, carcinogenic and mutagenic effects on animals including human beings (Murugesan and Kalichelvan, 2003 and Brookstein, 2009,).

Methyl orange is an anionic azo dye which is widely used in textile, pharmaceutical, printing, paper manufacturing and food industries and also in research laboratories. Inhalation of methyl orange leads to allergies, hypersensitivity and dermatitis. It inadvertently enters the body through ingestion and metabolizes into aromatic amines by intestinal microorganisms which can even leads to intestinal cancer. It is a toxic, carcinogenic and mutagenic azo dye (Chen *et al*., 2011). Methyl orange is a stable, water soluble azo dye with low biodegrading ability.

Hence, it is difficult to remove methyl orange from aqueous solutions by common wastewater treatment methods (Mittal *et al*., 2007 and Chen *et al*., 2010).

The textile dyeing industry is under substantial stress to develop appropriate treatment techniques to efficiently and effectively treat the industrial effluents discharged into the environment and also to comply with environmental regulations limiting the expulsion of wastewater and to prevent deterioration of ecosystem (Chavan, 2001).

Treatment of azo dye containing wastewater and industrial effluents has been adopted to eliminate the dyes in an efficient manner by numerous conventional physicochemical methods such as adsorption, ozonation, photochemical oxidation, membrane filtration, electrochemical oxidation, precipitation, coagulation, flocculation, ion-exchange, evaporation, neutralization, electrolytes, osmosis, oxidation and sedimentation. However, these methods remain unattractive for industrial practise because of related restrictions such as limited versatility, highly expensive, requires intensive energy, intrusion by other wastewater constituents and secondary pollution problems that may arise due to extreme use of chemicals and generation of enormous amount of sludge that may be difficult to dispose. Hence the situation have spurred renewed search for the development of new treatment technology and have shifted attention to biological methods which are cost effective, ease to operate, eco-friendly and amenable to scale up in the field (Nsami and Mbadcam, 2013, Sriram *et al*., 2013, Almutairi *et al*., 2012 and Zhang *et al*., 2012).

In recent decades, bioremediation proves to be a cost effective, attractive and alternative physicochemical phenomenon involving less energy intense to treat wastewater. It also possess numerous inherent benefits such as simplicity of design, cheap and efficient biosorbents in nature, reuse ability of biosorbents, short process time and wide range of operation conditions. It is also an eco-friendly technology which has gained attention among the environmentalist to remove the dyes from industrial effluents using microbial biomass (Senturk *et al*., 2017, Ju *et al*., 2008 and Vijayaraghavan *et al*., 2008).

Recently, biosorption using microbial biomass as biosorbent has emerged as a potential alternative technique to wastewater treatment process which was found to be economically feasible to the prevailing physicochemical methods. Various biological materials such as fungi, yeast, bacteria, moss, aquatic plants and algae have been investigated with the objective of finding the efficient or cost effective biosorbent for dye removal (Al-Masri *et al*., 2010, Lin *et al*., 2010 and Parshetti *et al*., 2010).

Among them, algae have proved to be one of the most promising and potential biosorbent in the removal of dyes from aqueous solutions and industrial wastewater. Algae proved to be economic, eco-friendly and easily available material with high regeneration and recovery potential, lesser volume of chemicals or biological sludge to be disposed off, high surface area to volume ratio, high binding affinity and possess high removal efficiency in dilute effluents. Moreover, algae possess high dye binding affinity, because polysaccharides, lipids and proteins present on the surface of their cell wall has various functional groups such as hydroxyl, carboxylate, amino and phosphate which are considered to be responsible for the sequestration of dyes from wastewater through electrostatic attraction, adsorption, chelation, ion-exchange and complexation process. Algae also offer a cost effective solution for industrial wastewater management. The removal of dyes by algae may be ascribed to the accumulation of dye ions onto the surface of algal cells and further to the dissemination of the dye molecules from aqueous phase onto the solid phase of the algal biopolymers (Ozer *et al*., 2006 and Lodeiro *et al*., 2005).

Although there are studies reported on the biosorption of dyes by fresh water algae showing varying removal efficiencies, maximum adsorption capacities and binding constants but there are meagre reports on the removal of methyl orange from aqueous solution using the green algae *Oedogonium subplagiostomum*.

Oedogonium is an unbranched filamentous yellowish green alga with cylindrical cells, which can form dense mats of coiled filaments. *Oedogonium* acts as a target for various applications since it possess high productivity, abundant and renewable non-toxic algae obtained economically on a large scale from the nearby water resources, favourable biochemical composition, cosmopolitan distribution and competitive dominance over other algal species (Gupta and Rastogi, 2008).

Environmental factors are known to play a vital role in affecting the decolourization activity of algae. The sorption of dyes by algae depends upon the chemical nature of the pollutant and the optimization parameters such as dye concentration, biosorbent dose, carbon and nitrogen source, agitation, temperature, pH and incubation time. Optimization process plays an important role in dye decolourisation (Alalewi and Jiang, 2012, Ponraj *et al*., 2011 and Bhatti *et al*., 2010).

Process optimization in batch mode biosorption might be carried by empirical or statistical approaches. The empirical approach is time consuming and does not inevitably facilitate an effective optimization. A statistical based approach, response surface methodology (RSM) is a potent experimental tool used to recognize the performance of composite systems. The RSM embodies an assemblage of experimental design and multiple regression based methods that can be applied where numerous variables or factors might influence a response. It is a consecutive process with an initial aim to lead the experiments quickly and ably along a path of enhancement towards the general vicinity of the optimum. Optimization of the process can be done by carefully studying the response surface plot through different amalgamation of factors for the best response (Kiran *et al*., 2007, Mona *et al*., 2011 and Sharma *et al*., 2009).

However, in recent years due to the use of several biomaterials for sorption purpose, the term adsorption has been substituted by a more ubiquitous term biosorption (Reddy and Lee, 2013a). Adsorption using biomass is considered as an alternative and potential technique for the treatment of dyes present in the wastewater and industrial effluents. The study of adsorption kinetics illustrates the interaction of solute molecules with the active surface of the algae, fixing them in the free sites owing to the action of physisorption (physical process) and chemisorption (chemical bonds). The controlling mechanisms of adsorption process such as diffusion, mass transfer, equilibrium, kinetics and thermodynamics are of great importance to study the mechanism of biosorption. Thus the kinetics of dye adsorption against biosorbent is prerequisite for selecting the best operating conditions for the full-scale batch process (Yagub *et al*., 2014).

In view of ever-increasing urban population and economic activities, there is a demand for the reuse and recycling of wastewater in large scale to minimize the rate of pollution. Consequently, there is a need to assess the toxicity of the degraded metabolites of the pollutants prior to their discharge into aquatic bodies (Mahmood *et al*., 2013 and Phugare *et al*., 2011).

Phytotoxicity studies were assessed to gain the impact on the inhibitory or stimulatory effect of degraded metabolites of pollutants/dyes for the growth of plants these studies also explore the reuse of the treated wastewater for agricultural purpose which not only solve the problem of disposal but also serve as an additional source of liquid fertilizer providing all the macro and micro nutrient need for the growing plants and also improves the fertility of soil (Nachiyar *et al*., 2014, Telke *et al*., 2010 and Tigni *et al*., 2011).

Fish have been used as a bioindicator to assess the stress conditions in an aquatic environment, since it is sensitive to a wide variety of pollutants or toxicants. Hence they serve as a valuable model for assessing the effect of pollutants on the molecular, biochemical, enzymological and histological responses which have been employed as biomarkers of various environmental stresses. The treated wastewater or industrial effluents have been used in aquaculture operations around the world for the production of fish biomass which is the primary goal with marginal concern in wastewater renovation (Gaim *et al*., 2015, Das *et al*.,2012 and Lavanya *et al*., 2011).

Microbial toxicity assays are widely being used to evaluate the effect of degraded metabolites of pollutants/dyes on the microflora contributing to soil fertility, since they take part in the biotransformation of nutrients and organic material in the soil. An assessment on the genetic and ecological impact of the pollutants on plant growth is of great importance since the plant products are utilized by human in a larger extent. Moreover, plants act as a biosensor of genotoxicity assay since they possess good correlation with mammalian test system (Gomare and Govindwar, 2009 and Saratale *et al*., 2010).

In order to minimize the rate of pollution, the dye desorbed algae can be subjected to composting. The algal compost reduces the formation of waste, helps in the reclamation of soil, maintains soil organic matter, increases aeration and water holding capacity and provides required nutrients for the metabolism and physiological activities of microorganisms. The algal compost seems to be an alternative for the chemical fertilizer and can be used in agricultural purposes to enrich soil fertility (Michalak *et al*., 2016).

Wastewater reclamation and reuse has become an attractive and important issue in sustainable urban development, protects the available water resources and environmental benign aspect. In last few years, there has been a remarkable changes in wastewater reuse practices for green space, crop irrigation, amusing impoundment, toilet flushing, industrial uses and water supply augmentation through groundwater or reservoir. Subsequently, wastewater reclamation and reuse in textile sector for dyeing and printing is a promising alternative which can safeguard and supplement the available water resource and eradicate the ecological pollution (Lazarova *et al*., 2000 and Lopez-Ramirez *et al*., 2006).

Algal azoreductase plays an important role in the breakdown of the azo bond present in the textile dyes under aerobic or anaerobic conditions. Technology is delivering a number of bioremediation strategies till date for treating synthetic organics, but these technologies are unlikely to be implemented due to their technical snags in scaling up or during technology transfer to real time scenario. Computational approaches assist us before technology transfer by predicting the nature and toxicity of the interacting ligand with the target receptor protein. Molecular docking is expansively applied in all corners of applied sciences in optimizing the significance and interaction among protein-ligands (Omar, 2008 and Sridhar and Chandra, 2014).

With this background information, the present study was aimed to explore the potential of *Oedogonium subplagiostomum* AP1 as a biosorbent for the removal of methyl orange from aqueous solutions under optimised conditions and to assess its applicability on biological systems.

1.1 Hypothesis of the study

The present study entitled "*Bioremediation of methyl orange from aqueous solutions using Oedogonium subplagiostomum AP1*" was designed to test the following hypotheses.

Null hypothesis (H_0):

Oedogonium subplagiostomum AP1 is not efficient in the decolourisation of methyl orange from aqueous solutions.

Alternate hypothesis (H_A):

Oedogonium subplagiostomum AP1 has a potential to decolourise the methyl orange from aqueous solutions.

To test the above hypotheses, the present study was formulated with the following objectives:

- To identify the alga for its potential in dye decolourisation.
- To study the kinetic behaviour of the dye using batch mode and Response Surface Methodology (RSM) to optimize the interacting process parameters to achieve maximum decolourisation.
- To assess the dye elution, regeneration and reuse efficiency of the biosorbent.
- To evaluate the biosorption mechanism of the dye onto the alga under controlled system by the computation of isotherm and kinetic parameters.
- To determine the adsorbent-adsorbate interaction using UV- vis spectrophotometer, Fourier transform infrared (FT-IR) spectrophotometry, Scanning electron microscopy (SEM) with Energy-dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD).
- To assess the toxicity reduction potential of treated dye solution by means of phytotoxicity, zootoxicity, microbial toxicity and cytogenotoxicity assays.
- To analyze the reuse potential of dye desorbed algae and treated dye solution for composting and dyeing fabrics.
- To evaluate the physicochemical characterisation of textile dyeing industrial effluent.
- To validate the relationship between protein-dye interaction through *in silico* approach.

The following chapter outlines the review of literature relevant to the present study.