

## Role of microalgae in dye degradation – a comparative report

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### Abstract

The textile and other dye based industries are important consumers of water and they produce an equal or more amount of contaminated water. These contaminated water stands as the reservoir of dyes and as toxic effluent. In the recent years microbe based remediation of textile wastewater has received a great attention as the physical and chemical based technologies have proved that they are not eco-friendly and economically feasible. Microorganisms isolated from the contaminated sites act as promising candidates for the treatment of waste waters. Among the different groups of microorganisms like microalgae bacteria, fungi, yeast, etc, microalgae proved to be supreme candidates due to their certain special capabilities. Being a photoautotroph, mass cultivation of microalgae is very simple and cost effective. They are also able to fix atmospheric nitrogen and CO<sub>2</sub> in a high level. The mechanism of microalgal remediation involves the enzymatic degradation, adsorption, bioconversion or a combination of these. The present article deals with the role of microalgae in the degradation of dye present in the dye effluents of the textile industry.

**Keywords:** Adsorption, dyes, Dye degradation, Microalgae, remediation

### INTRODUCTION

Dyes and dyestuffs found wide application in many industries, among which textile manufacturing being the first one. Being very recalcitrant xenobiotic compounds they are generally found to be irresponsive to biodegradation processes. Over 1,00,000 commercially available dyes exist and more than  $7 \times 10^5$  tonnes of dyestuff are produced annually (Meyer, 1981; Zollinger, 1987). Inefficient dyeing in textile processing results in about 70% of dyestuffs being lost to wastewater, which directly enter into the environment. The effluents of most of the industries are highly coloured and the disposal of these wastes into receiving waters causes damage to the environment (Baldev *et al.*, 2013). Coloured effluents may cause serious problem in human biological activity and cause water borne disorders such as nausea, hemorrhage, ulceration of skin and mucous membrane, dermatitis, perforation of nasal septum, severe irritation of respiratory tract and cancer (Shah *et al.*, 1999). Dyes may significantly affect photosynthetic activity in aquatic life because of reduced light penetration and may also be toxic to some aquatic life due to the presence of aromatics and metals, chlorides, etc (Hamdaoui, 2006). Dyes usually have a synthetic origin and the complex aromatic molecular structures make them more stable and the biodegradation becomes difficult (Asku and Tezer, 2005). Due to their chemical structure, dyes are resistant to fading on exposure to light, water and many chemicals (Poots and McKay, 1976; McKay, 1979).

The ability of microorganisms to decolourize and degrade dyestuffs has been tested since long time. The treatment of dyes and textile waste water using

bioremediation based technologies has been found to have increased interest nowadays. Microalgal diversity ranges from unicellular to multicellular, coccoid to branched filaments, nearly colourless to intensely pigmented, autotrophic to heterotrophic, psychrophilic to thermophilic, acidophilic to alkylphilic, planktonic to barophilic, freshwater to marine including hypersaline (Thajuddin and Subramanian, 2005). They are considered to be one of the potential organisms which can be useful to mankind in various ways. In this review, an attempt has been made to specifically focus on the role of microalgae in the field of dye decolorization/ degradation and also the mechanism involved in it.

### Dyes

Any coloured substance that has an affinity towards the substrate to which it is being applied can be generally defined as dyes. Dyes and pigments appear to be coloured as they absorb some wavelengths of light more than others. In general textile and other dyestuff industries are found to be the major sources of dye release into the environment. In 1856 William Henry Perkin accidentally discovered the world's first commercially successful synthetic dye. Such dyes are defined as coloured substances that when applied to fibers give them a permanent colour which are able to resist fading upon exposure to sweat, light, water and many chemicals, including oxidizing agents and microbial attack (Rai *et al.*, 2005). Most of the dyes in wastewater are generally harmful and non-biodegradable due to the high concentrations of organic dyestuffs, surfactants and additives used (Al-Momani *et al.*, 2002; Anliker, 1979). The dyes which are toxic in nature, gets accumulated in the environment and stands as a reservoir for various environmental problems.

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Most of these dyes now in use are synthetic in nature and are classified based on their chemical structures into 6 different classes as azo, anthraquinone, sulfur, indigoid, triphenylmethane and phthalocyanine derivatives. Azo and anthraquinone based dyes are the most important classes of synthetic organic dyes used in the textile industry, and are therefore considered as common industrial pollutants (Abdou *et al.*, 2011). It is estimated that 280,000 t of textile dyes are discharged in industrial effluents every year worldwide (Jin *et al.*, 2007). Azo dyes make up approximately 70% of all dyestuffs used worldwide by weight (Zollinger, 1987), making them the largest group of synthetic colourants and the most common synthetic dyes released into the environment (Chang *et al.*, 2001; Saratale *et al.*, 2009; Zhao and Hardin, 2007).

Many reports suggested that most of the azo dyes are mutagenic and carcinogenic xenobiotic pollutants that are ubiquitously discharged into the ecosystem every year (Vanhulle *et al.*, 2008; Pandey *et al.*, 2007; Umbuzeiro *et al.*, 2005). The largest amount of azo dyes is used for the dyeing of textiles, and it has been estimated that about 10% of the dye-stuff used during these dyeing processes does not bind to the fibers and is therefore released into the sewage treatment systems or the environment (Anliker 1979; Chudgar, 1985; Clarke and Anliker 1980; Reisch 1996; Zollinger, 1991). Apart from the azo dyes, the soluble reactive dyes, which are also used in increasing quantities, are known to hydrolyze during application without a complete fixation, which may result in an even larger proportion of these dyes being released into the environment (Carliell *et al.*, 1994; Jeckel, 1997; Weber and Stickney, 1993). Azo dyes absorb light in the visible spectrum due to their chemical structure, which is characterized by one or more azo groups ( $-N=N-$ ) (Chang *et al.*, 2000). The azo group is substituted with benzene or naphthalene groups, that contain many different substituents such as chloro ( $-Cl$ ), methyl ( $-CH_3$ ), nitro ( $-NO_2$ ), amino ( $-NH_2$ ), and hydroxyl ( $-OH$ ), carboxyl ( $-COOH$ ), which give different types of azo dyes (Zollinger, 1991). Moreover, the azo dyes account for majority (more than 3000 different varieties) of all the textile dyestuffs produced because of the ease and cost effectiveness of their synthesis, their stability and the variety of colours available compared to natural dyes (Chang *et al.*, 2004). They are extensively used in the textile, paper, food, leather, cosmetics and pharmaceutical industries (Telke *et al.*, 2008). Improper discharge of textile dye effluents containing azo dyes and their metabolites into the aquatic ecosystems is aesthetically unpleasant and leads to increase in turbidity, which in turn decreases photosynthetic activity, dissolved oxygen concentration and water

quality, and had acute toxic effects on aquatic flora and fauna, causing severe environmental problems throughout the world. (Vandevivere *et al.*, 1998).

### Different methods of dye degradation

Removal of dyes is a very difficult process since they are stable to light, heat and oxidizing agents and are biologically non-degradable. Dye effluent is usually treated either with physical or chemical treatment methods. They generally include chemical coagulation/flocculation, ozonation, oxidation, ion exchange, irradiation, precipitation and adsorption (Ansari and Thakur, 2006). Some of these techniques have been shown to be effective, although they have limitations such as excess amount of chemical usage, accumulation of concentrated sludge with obvious disposal problems, expensive plant requirements or operational costs, lack of effective colour reduction, and sensitivity to a variable wastewater input (Ansari and Thakur, 2006; Rajeshkannan *et al.*, 2010). In general activated carbon has been widely used as an adsorbent for the removal of dyes from the effluents. Activated carbon is quite expensive, as the cost is directly proportional to the quality. This has led many workers to search for a new cheap and efficient alternative biosorbents such as bagasse pith, carbonized bark, peat, soil, tree and eucalyptus barks, chitin, rice husk, wood, fly ash and carbonized sewage sludge (Gregorio Crini, 2005). However, these low-cost adsorbents have generally low adsorption capabilities and hence a relatively large quantity of adsorbents is required to be used. The treatment of textile effluents involves mainly physical and chemical methods, which are often very costly as stated previously (Robinson *et al.*, 2001). It is difficult to treat dye wastewater by chemical and physical processes because of the complex molecular structures. Furthermore, the disposal of the concentrated sludge creates another problem. There has been increased interest in using biological methods for remediation of textile wastewater, especially in colour removal. Most of the previous studies had been centred around the use of fungi and bacteria to treat coloured wastewater (Tastan *et al.*, 2010; McMullan *et al.*, 2001; Senthilkumar *et al.*, 2012). However, additional carbon sources are required for such systems. In the recent years, the use of microalgae in the process of bioremediation of coloured wastewater has attracted great interest due to their central role in carbon dioxide fixation. Besides, the algal biomass generated has great potential to be used as substrate for biofuel production (Huang *et al.*, 2010). Some of the treatment methods generally used for the dye degradation, illustrated in Figure 1.

### Decolorization/Degradation using microalgae

The photosynthetic organisms, such as cyanobacteria or algae are distributed ubiquitously and are found in

almost all kinds of habitats (Thajuddin and Subramanian, 2005). Algae can degrade number of dyes, postulating that the reduction appears to be related to the molecular structure of dyes and the species of algae used. Jingi and Houtian (1992) were the first to report that microalgae could be used for dye decolorization. They found that *Oscillatoria* sp. and *Chlorella* sp. completely mineralized aniline to CO<sub>2</sub> on extended incubation. It was reported that more than 30 azo compounds were decolorized and biodegraded into simpler aromatic amines by *Chlorella pyrenoidosa*, *Chlorella vulgaris* and *Oscillatoria tenuis* (Yan and Pan, 2004). The potential of *Cosmarium* species, belonged to green algae, was investigated as a viable biomaterial for biological treatment of triphenylmethane dye; malachite green (Daneshvar *et al.*, 2007). *Synechocystis* sp. and *Phormidium* sp. have a capacity to remove reactive dyes such as Reactive Red, Remazol Blue, and Reactive Black B (Karacakaya *et al.*, 2009).

Most of the reports based on azo dyes (Jingi and Houtian, 1992) state that some of the tested azo compounds could be used as sole sources of carbon and nitrogen by the algae. This clearly suggests that the algae could play an important role in the removal of azo dyes and aromatic amines in stabilization ponds (Banat *et al.*, 1996). Algae have been studied in the field of decolorization of industrial effluents (Nagasathya and Thajuddin, 2008; Vijayakumar *et al.*, 2005; 2007a; 2007b; Daneshvar *et al.*, 2007; Vijayaraghavan and Yun, 2008). This is due to the versatile ability of the algae to degrade, partially or completely various dyes. Immobilized microalgae are the other alternative to be used in the process of decoloration of dyes. For example, *C. vulgaris* (Chu *et al.*, 2009) and *S. quadricauda* (Ergene *et al.*, 2009) immobilized with alginate could remove a higher percentage of colour from textile dyes than suspended algae. Potential dye degrading microalgae, *Coelastrella* sp. immobilized with alginate is shown in Figure 3.

### Mechanism of algal dye decolorization and degradation

Though there are many reports on the biodegradation of dyes, the exact the mechanism by which the microalgae decolorize/ degrade the dye effluents has not been elucidated. But there is no doubt that algae have the potential to rapidly, efficiently and effectively remove the reactive dyes or wastewaters to very low concentrations and to prevent the accumulation of large quantity of dyes. The probable mechanism involved in the microalgal mediated removal of dyes from effluents has been hypothesized by many researchers on the basis of experimental results. Most of the studies highlighted algae as the cheaply available, good and efficient absorbent. Algae have been shown to be capable of removing colour from various dyes through

mechanisms such as biosorption, bioconversion and bioagulation. Daneshwar *et al.* (2007) showed that *Cosmarium* sp. is efficient in removing colour from the triphenylmethane dye, Malachite Green through biodegradation. It has been reported that dried biomass of *Spirogyra* can remove up to 85% of the textile dye Synazol by biosorption (Khalaf, 2008). Acuner and Dilek (2004) described that *Chlorella vulgaris* can remove 63–69% of the colour from the mono-azo dye Tectilon yellow 2G by converting it into aniline giving a strong evidence for bioconversion potential of microalgae. Yan and Pan (2004) reported that more than 30 azo compounds were decolorized and biodegraded into simpler aromatic amines by *Chlorella pyrenoidosa*, *C. vulgaris* and *Oscillatoria tenuis*. The degradation of aromatic amines formed after cleavage of azo bonds by *C. vulgaris* was studied by Jingi and Houtian (1992). They reported that *C. vulgaris* can utilize aniline, and no other compounds were detected after treatment by *C. vulgaris*. They claimed that *C. vulgaris* converted aniline into some simple inorganic materials. Mohan *et al.* (2005) observed that 10% of azo dye removal by algae was through the biosorption. But Aziz and Ng (1994) claimed that adsorption onto the surface of the algal cells was the primary mechanism of colour removal from textile waste water. Hence, the main mechanism of TY2G removal by *Chlorella vulgaris* was considered to be bioconversion. Jingi and Houtian (1992) also stated that during the reduction of azo dyes by algae, the azo bridge is degraded by azo reductase and aromatic amine arises as a cleavage product. Colour removal by algae is due to three intrinsically different mechanisms of assimilative utilization of chromophores for the production of algal biomass, CO<sub>2</sub> and H<sub>2</sub>O, transformation of coloured molecules to non-colored ones, and adsorption of chromophores on algal biomass. Several species of *Chlorella* and *Oscillatoria* are capable of degrading azo dyes to their aromatic amines, and can further metabolize the aromatic amines to simpler organic compounds or CO<sub>2</sub> (Acuner and Dilek, 2004). Mohan *et al.* (2002) attributed the decolorization to biosorption followed by bioconversion and bioagulation using algae.

Baldev *et al.* (2013) previously studied the biodegradation of Rhodmine B dye using microalga, *Coelastrella* sp. and found a complete degradation of the molecular structure of dye. They also reported that the enzyme activities of the algae, also act as the enhancing factor of degradation. It was also found that the absorbance maximum of Rhodamine B at 550nm diminished, which indicated that the dye had been removed by the microalgal interaction (Fig. 4 ). The analysis using GC-MS showed that the residual degraded Rhodamine B formed a number of small molecular weight compounds as a result of biodegradation (Fig. 5 ). This in accordance with the

Table 1: Microalgal mediated dye degradation

Microalgal species	Dye	References
<b>1) Green algae</b>		
<i>Chlorella vulgaris</i>	Azo dyes	Jingi and Houtian, 1992
<i>Chlorella vulgaris</i>	Eriochrome blue SE	Jingi and Houtain, 1992
<i>Chlorella vulgaris</i>	Tectilon Yellow 2G (mono-azo dye)	Acuner and Dilek, 2004
<i>Chlorella vulgaris</i>	Remazol Black B (CI Reactive Black 5) or Remazol Red RR or Remazol Golden Yellow RNL (CI Rective Orange 107)	Asku and Tezer., 2005
<i>Chlorella ellipsoidea</i> , <i>Chlorella kessleri</i> <i>Chlorella vulgaris</i> , <i>Scenedesmus bijuga</i> <i>Scenedesmus bijugatus</i> <i>Scenedesmus obliquus</i>	Tartrazine Ponceau SS	Omar, 2008
<i>Chlorella sp.</i>	Triphenylmethane dye, C.I. Basic Green 4 (BG 4)	Khataee <i>et al.</i> , 2009
<i>Chlorella vulgaris</i> UMACC 001	Supranol Red 3BW (azo acid dye), Lanaset Red 2GA (azo metal complex dye), Levafix Navy Blue EBNA (azo reactive dye).	Chu <i>et al.</i> , 2009
<i>Scenedesmus quadricauda</i>	Remazol Brilliant Blue R	Ergene <i>et al.</i> , 2009
<i>Chlorella vulgaris</i> (Immobilized)	Textile dyes	Chu <i>et al.</i> , 2009
<i>Chlorella vulgaris</i>	Supranol Red 3BW, Lanaset Red 2GA, Levafix Navy Blue EBNA	Lim <i>et al.</i> , 2010
<i>Chlorella vulgaris</i>	Textile dyes	Singh <i>et al.</i> , 2010
<i>Coelastrella sp.</i>	Rhodamine B	Baldev <i>et al.</i> , 2013
<i>Acutodesmus obliquus</i>	Orange G	Sarwa and Verma, 2013
<i>Volvox aureus</i>	Methyl red , Orange II , Basic cationic, G-Red (FN-3G) , Basic Fuschin	El-Sheekh <i>et al.</i> , 2009
<b>2) Cyanaobacteria</b>		
<i>Oscillatoria sp.</i>	Azo dyes	Jingi and Houtian, 1992
<i>Gloeocapsa pleurocapsoides</i> <i>Phormidium ceylanicum</i> <i>Chroococcus minutes</i> <i>Gloethece sp</i> , <i>Oscillatoria splendida</i>	Acid Red 97 and FF Sky Blue, Amido Black 10B	Parikh and Madamwar, 2005
<i>Spirogyra rhizopus</i>	Acid red 247	Ozer <i>et al.</i> , 2006
<i>Cosmarium sp.</i>	Malachite Green., Triphenylmetahane dye	Daneshvar <i>et al.</i> , 2007
<i>Anabaena laxa</i> <i>Anabaena subcylindrica</i> <i>Nostoc muscorum</i> <i>Oscillatoria angusta</i>	Tartrazine Ponceau SS	Omar, 2008
<i>Phormidium</i>	Textile dyes	Ertugrul <i>et al.</i> , 2008
<i>Spirogyra</i>	Reactive dye (Synazol)	Khalaf, 2008
<i>Synechocystis</i> and <i>Phormidium</i>	Reactive Red, Remazol Blue, Reactive Black B	Karacakaya <i>et al.</i> , 2009
<i>Spirogyra sp.</i>	Direct Brown	Sivarajasekar <i>et al.</i> , 2009
<i>Lyngbya lagerlerimi</i> <i>Nostoc linckia</i> <i>Oscillatoria rubescens</i>	Methyl red , Orange II, Basic cationic , G-Red (FN-3G) Basic Fuschin	El-Sheekh <i>et al.</i> , 2009
<i>Spirulina platensis</i> <i>Anacystis nidulanse</i> <i>Oscillatoria agardhii</i>	Methyl Red , Methyl orange , Crystal violet , Eriochrome black Malachite Green	Sharma <i>et al.</i> , 2014
<i>Oscillatoria curviceps</i> BDU92191	Acid Black	Priya <i>et al.</i> , 2011
<i>Oscillatoria formosa</i> NTDM02	Amido Black G200	MubarakAli <i>et al.</i> , 2011
<b>3) Algal cells of mixed culture</b>		
	Azo dye mixture	Aziz and Ng, 1994

Figure 1: Common methods used for dye decolorization

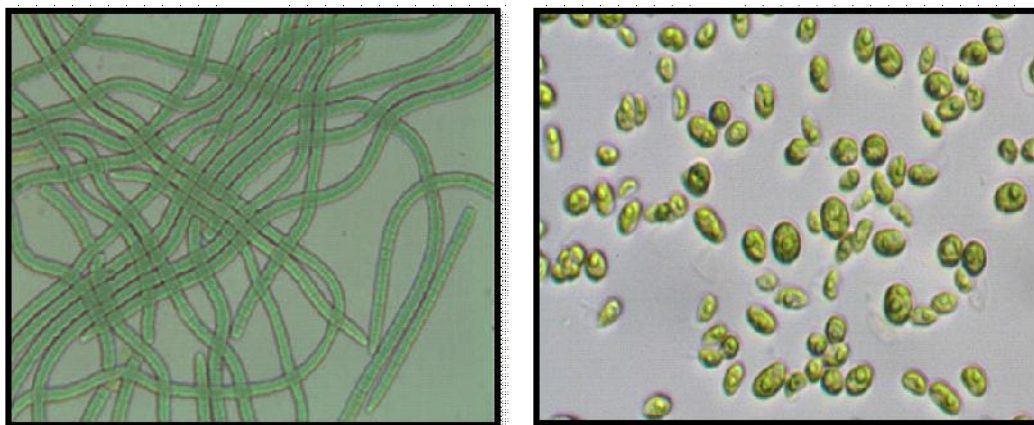
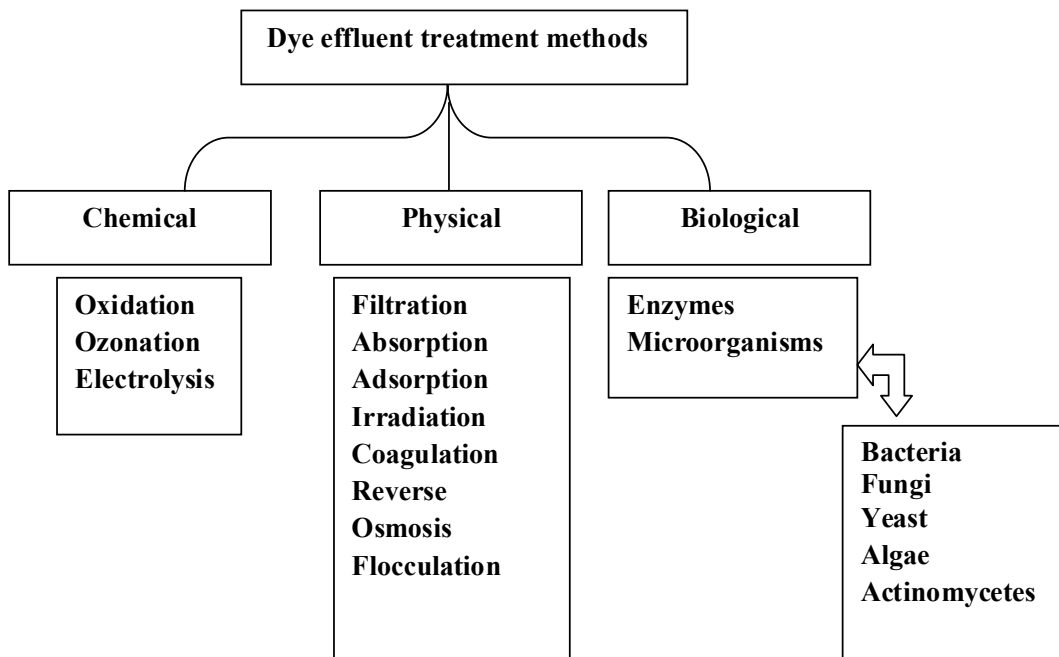


Figure 2: Microphotograph of *Oscillatoria formosa* NTDM02 (a) and *Coelastrella* sp. NTEB01 (b) adopted from MubarakAli *et al.*, 2011 and Baldev *et al.*, 2013 respectively



Figure 3: Immobilized *Coelastrella* sp. (Adopted from M.Sc. Dissertation, Baldev.E, 2010, Bharathidasan University, Tiruchirappalli, Tamilnadu)

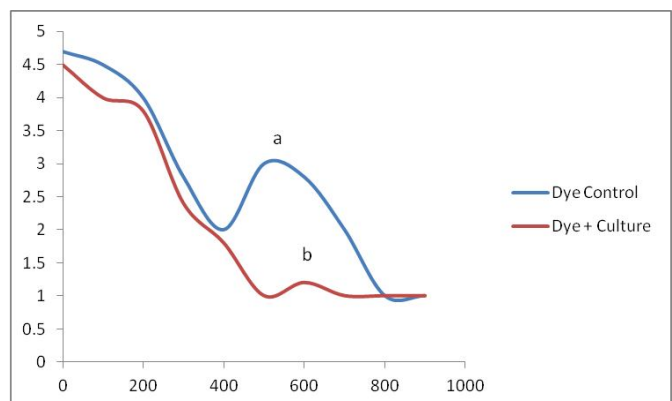


Figure 4: UV-vis spectrum showing the decrease in intensity of Rhodamine B dye absorbance maximum at 550nm after treatment with microalgae (Baldev *et al.*, 2013)

findings of Mubarak Ali *et al.* (2011) who reported a decrease in the intensity of absorbance of dye in the presence of a marine cyanobacterium, *Oscillatoria formosa* NTDM 02, and suggested that the dye removal was due to the action of reductase enzyme.

Bacteria, fungi, yeast, etc. depend on certain carbon sources as their energy, which makes their cultivation expensive. At the same time algae can produce their own energy by photosynthesis and thus make their mass cultivation less expensive. Similar to bacteria, algae are capable of degrading azo dyes through an induced azoreductase to break the azo bond, resulting in the production of aromatic amines (Phugare *et al.*, 2010; Priya *et al.*, 2011; Omar, 2008). The involvement of oxidative enzymes also has been found in the process of decolorization (Omar, 2008). At lower pH, dye degradation was found to be higher in the case of microalgae. At lower pH, the biomass has a net positive charge; and the higher uptake obtained at lower pH values might be due to electrostatic attraction between the negatively charged dye anions and the positively charged cell surface (Srinivasan and Viraraghavan, 2010). On the basis of all these previous reports, it is concluded that the mechanism of algal decoloration involves enzymatic degradation, adsorption, or both.

### Conclusion:

In the present scenario, studies on microbes based dye removal have gained much attention as it is cost effective and eco-friendly in nature. Among the other microorganisms involved in the process, microalgae are considered to play a vital role. The mechanisms involved in the microalgal dye decolorization could be attributed not only to adsorption on to the algal biomass but also to the process of bioconversion and degradation. It is believed that algae have the potential ability to remove the dyes efficiently and effectively in rapid manner. Furthermore, microalgae mediated bioremediation is cost effective and efficient while comparing to the costly activated carbons which are now widely used as biosorbents for dye removal. Dye removal by microalgae was found to be depend on various factors like environment from where the algae was collected, pH, temperature, nature of the effluent, concentration of the dye etc. The perusal of literature proved that there had been only few reports on the potential role of microalgae in dye removal. Hence the objective of this article into provide valuable information regarding the present status and create an impetus in the research in microalgal in general and their processes and treatment in particular.

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