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# Accumulation of Crystal Violet Using *Chlorella vulgaris*

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**Bioaccumulation of the crystal violet (CV) dye using live alga *Chlorella vulgaris* was established in batch mode. Growth profile of the *Chlorella vulgaris* alga was demonstrated with presence of dye solution and compared with absence of dye in the medium. Bioaccumulation parameters, such as algal cell concentration and dye concentrations were studied. The maximum percentage colour removal was obtained for CV dye at algal cells concentrations of 9 × $10^6$ cells/mL. The maximum percentage colour removal was observed at 100 mg/L of dye concentration. UV-spectral analysis was performed to confirm the bioaccumulation of dye on to the living algal cells.**

**KEYWORD**

Biological treatment, Bioaccumulation, Crystal violet, *Chlorella vulgaris.*

## INTRODUCTION

Pollution control is one of the prime concerns of society in recent year. Delivering untreated or partially treated wastewater and industrial effluents into natural ecosystems poses a serious problem to environment (Aravindhan *et al.,* 2007). Especially organic, inorganic and dye pollutants from industrial effluents disturb human health (Padmesh *et al.,* 2006). Among various industries, the textile industry ranks first in the usage of dye for colourizing fibers. The textile sector alone consumes about 60% of the total dye production for coloration of various fabrics (Mohan *et al.,* 2002). The dye based effluents induce persistent colour coupled with organic load and affects the total ecological symbiotic balance of water and receiving water streams (Ozer *et al.,* 2006; Robinson *et al.,* 2002). Colour affects the nature of water and inhibits the sunlight penetration into stream, thus reducing the photosynthetic activity (Aksu, 2005; Kumar *et al.,* 2006). Furthermore, the effluents from dyeing industry constitute one of the most problematic wastewater to be treated not only for high chemical and biological oxygen demands, suspended solid, contents of toxic and carcinogenic, mutagenic (Heis *et al.,* 1992) or teratogenic compounds, but also for colour, which is the first contaminant to be recognized by human eyes (Kumar *et al.,* 2006; Chang *et al.,* 2004; Daneshvar *et al.,* 2003; Khalaf, 2008). So the removal of dyes from water body has received considerable attention within environmental research (Walker and Weatherley, 2000). Dyes are majorly synthetic in nature which is widely used in textile industries (Michaels and Lewis, 1986; Goronszy and Thomas, 1992).

Various physical and chemical methods are used to achieve the decolourization of the dye, such as chemical-coagulation, ozonation (Vendeviere *et al.,* 1998; Aplin and Waite, 2000) electrochemical destruction (Sheng and Perg, 1994; Jia *et al.,* 1999) and sorption (Hu, 1996; Pagga and Taeger, 1994). However, implementation of these methods may generate a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage or economic unfeasibility (Mohan *et al.,* 2002). On the other hand, biological decolourization of dye remains more cost-effective (Acuner and Dilek, 2004). However, conventional biological treatment systems, such as activated sludge or lagoons fails to remove colour from the textile industrial wastewaters due to the complex structures of the dyes. In recent years, a number of studies have focused on

**Table1.** *Physical and chemical property of dye*

| Property of dye | Crystal violet |
|---|---|
| Common name | Crystal violet |
| C.I. no. | 42535 |
| C.I. name | Crystal violet |
| Molecular formula | $C_{25}N_3H_{30}Cl$ |
| Molecular weight | 407.19 |
| Water solubility | Soluble |
| Ionization | Basic |
| Maximum absorption | 590 nm |
| Colour | Violet |

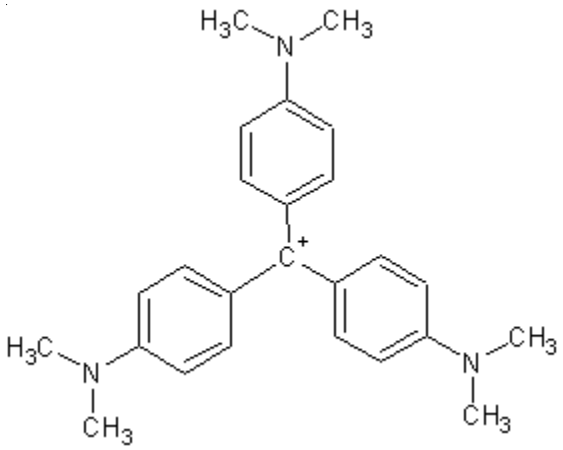

**Figure 1.** *Chemical structure of crystal violet dye*

some microorganisms which are capable to biodegrade or bioaccumulate the dyes in wastewaters.

A wide variety of microorganisms including bacteria, fungi and algae are capable of decolourizing the dye via aerobic, anaerobic and sequential anaerobic-aerobic treatment processes (Chang and Kuo, 2000; Gupta *et al.,* 2006; Daneshvar *et al.,* 2007). In recent years, the use of the white-rot fungi in the decolourization of textile dyes has been investigated, however, the requirement for the intrusion of other carbon sources is considered as the major drawback (Swamy and Ramsay, 1999). Therefore, there is a still demand to develop more effective and more economical alternative means of dye decolourization. Although bacteria play a key role in the treatment of dyes, recent studies indicate that in addition to provide oxygen for an aerobic bacterial biodegradation or bioaccumulation of dyes, microalgae can also be used directly. In this respect, there are some recent reports regarding treatment of textile dyes by algae (Mohan *et al.,* 2002; Guolan *et al.,* 2000). Certain algae (*C. reinhardtii, C. pyrenoidosa, Spirogyra* and *Ocillatoria tenuis*) can decolourize a number of textile dyes to some extent by bioaccumulation via the species of algae used. Moreover, the dye removal mechanisms suggested by these researchers show some variation depending upon the dyes and species of algae used. For example Mohan *et al.* (2002) proposed a mechanism of biosorption/ respiration/ photoconversion during the treatment of yellow 22 using *Spirogyra* species where as Jingi and Houtain (1992) reported that eriochrome blue SE was biodegraded completely by *C. vulgaris* and *O. tenuis.*

On the other hand, Aziz and Ng (1994) suggested a mechanism of adsorption onto the surface the algal cells of mixed culture for the treatment of dye mixtures. Furthermore, only limited studies have dealt with the effect of removal dyes using algal species. This study was aimed to investigate the potential of *Chlorella vulgaris* for treatment of wastewater containing a cationic textile dyes, namely crystal violet with both non acclimatized and acclimatized cultures of *C. vulgaris*. The effect of environmental parameters on the removal of textile dyes using *C. vulgaris* was studied. In addition, the UV spectral analysis for the removal of textile dyes was established. Dye toxicity reduction achieved during the dye treatment was also sought.

## MATERIAL AND METHOD

### Dye

Crystal violet used in the study was purchased from CDH (Central Drug House), New Delhi. The stock solution was prepared by dissolving 1g of dye in 100 mL of double distilled water with dye solution concentration of 10,000 mg/L. Experimental solutions were prepared from the original stock solution. The crystal violet dye is basic cationic dye which is widely used in textile indus-

try. These dyes are also called as the triphenylmethane dyes. The chemical structure of the dye is shown in figure 1. Physical properties are given in the table 1.

### Cultivation of *Chlorella vulgaris*

The alga, *C. vulgaris*, obtained from the Botany Department of University of Madras, Chennai was used in this study. The green algae *Chlorella vulgaris* was grown in 1L conical flask containing growth medium BBM (bold basal medium) with pH value of 7.4, in order to get several stock cultures to be used during the experiments. Algal cells were cultivated in shaker at 100 rpm at 25°C for a maximum period of 15 day. The pH of the medium was adjusted to 7.4 with dilute $H_2SO_4$ and NaOH solution. *Chlorella vulgaris* biomass was measured by counting the number of cell by light microscope using Neubauer haemocytometer. The growth profile was established at 640 nm and various parameters, like pH, temperature and initial dye concentration were optimized.

### Preparation of algal biomass

Algal biomass was cultivated for 15 day. The algae was collected from the stock and centrifuged at 16,000 rpm for 15 min. The supernatant was discarded and pellet was washed thrice with double distilled water. The pellet was allowed to dry at 37 °C for 6 to 8 hr and then weighed.

### Calculation for colour removal

The absorbance was measured with spectrophotometer at maximum absorption wavelengths at 590 nm for crystal violet dye. Samples were filtered out through 0.2 nm membranes to remove algae while measuring absorbance. The efficiency of colour removal was expressed as the percentage ratio of decolourized dye solution to that of initial one

$$\text{Colour removal} = \frac{C_0 - C}{C_0} X100 \qquad ...(1)$$

Where, $C_o$ is initial concentration of dye (mg/L) and C is concentration of dye at time t.

### Statistical analysis

The initial dye concentration was employed by 6 replicate. Equilibrium uptake capacity was determined in batch colour removal studies. Standard deviation was calculated for the 6 final dye concentration values using the statistical analysis and the values were observed to be less than 5% of the mean value.

### Batch studies

The experiments were conducted in 250 mL Erlenmeyer flasks, containing 100 mL of dye synthetic solution and algal biomass. To evaluate the effects of the operation and environmental factors on the efficiency of colour removal, the batch experiments were carried out at different initial dye concentrations (100-400 ppm), algal concentrations (0-7.5 × $10^6$ cells/mL), temperature (5-45 °C) and pH values (3-11). The pH was adjusted using diluted NaOH and HCl solutions. The batch experiments were performed under static incubation condition. The experiments were operated at 25 °C, pH 7, with initial dye concentration of 100 ppm and algal concentration of 4.5 × $10^6$ cells/mL.

## RESULT AND DISCUSSION

### Growth profile

The influence of textile dyes on the growth of algae and the bioaccumulation properties were studied. The effect on the growth of the culture in the presence and absence of dye is shown in figure 2. The decolourization and growth of the algae were presented against number of days in figure 3. Growth profile of the alga was found to be similar as studied by Khataee *et al.* (2009).

### UV- visible spectral analysis

Figure 4 shows a typical time dependent UV-visible spectrum of CV during bioaccumulation. The diminishing of absorption peaks corresponding to the dye with algae indicates that the dye has been bioaccumulated. The spectrum of CV in the visible region exhibits a main peak with a maximum at 590 nm. The decrease in the absorption spectrum indicates

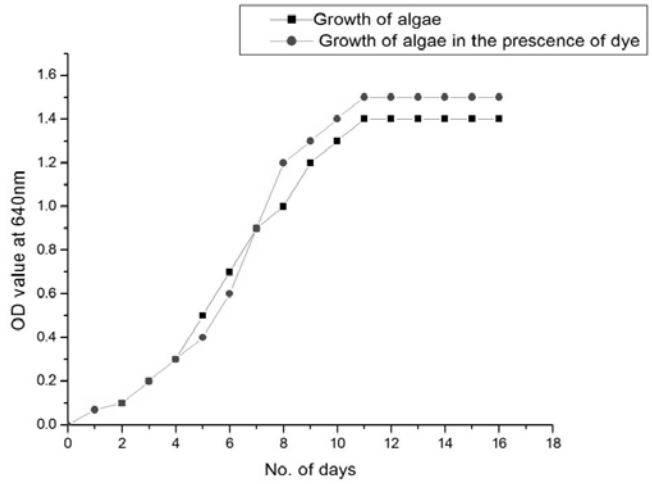


**Figure 2.** *The growth of algae in the presence and absence of the dye*

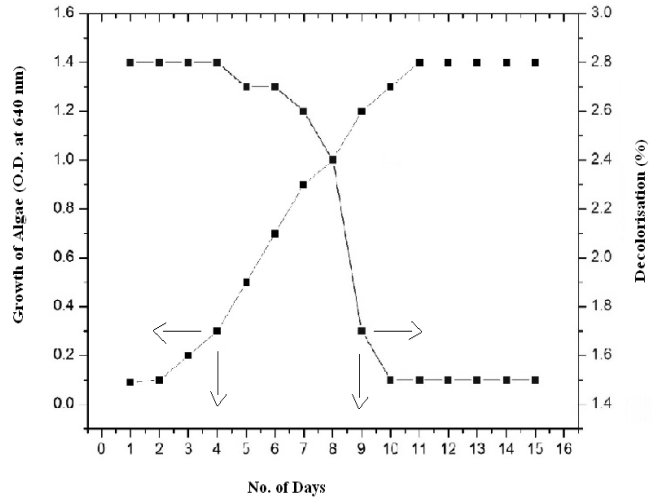


**Figure 3.** *Growth curve of algae in presence and absence of the dye*

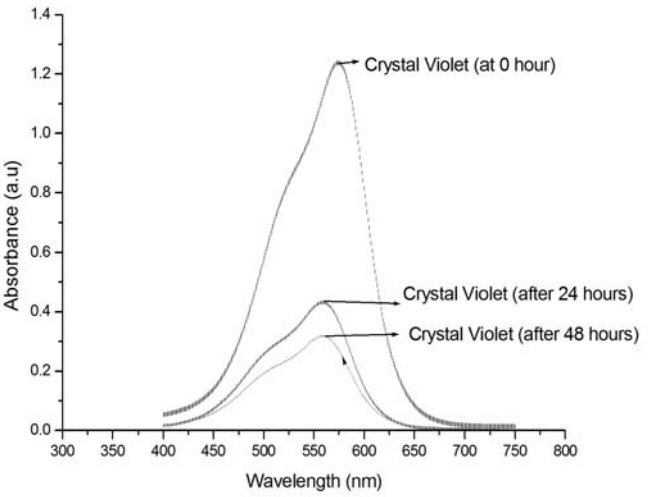


**Figure 4**. *UV-spectrometric analysis of crystal violet dye with the concentration of 100 mg/L in the presence of the algae*

the bioaccumulation of the dye. This can be due to the adsorption of the dye on the surface of the biomass. In the adsorption process, all peaks of the dye, in UV-Vis region decreases approximately in proportion to each other as shown in figure 4. Therefore, this process seems to be the bioaccumulation. The similar results were seen when the *Chlorella* sp. was used for the removal of textile dye by Khataee *et al*. (2009).

**Effect of algal biomass**

The results obtained from the present investigation revealed the ability of *Chlorella vulgaris* in the bioaccumulation of crystal violet. To estimate the desired biomass needed by *Chlorella vulgaris*, removal of 100 mg/L of dye was monitored by different algal biomass cell during various constant time (Figure 5). From the results, it was observed that the percentage colour removal was found to be decreased with increase in the dye concentration. Maximum colour removal was found to be 92.2% for CV dye at biomass content of $9 \times 10^6$ cells/mL (Figure 5). This seems to indicate that $9 \times 10^6$ cells/mL would be the optimal biomass in our study. Khataee *et al.* (2009) reported that *Chlorella* sp. shows maximum colour removal at the biomass of 9 x $10^6$ cells/mL which was found to be similar with this study. In this present study, removal of crystal violet using *Chlorella* sp. was found to be more when compared with the other research work established by Chen *et al.* (2007) using *P. putida* for the removal of crystal violet (78.5%).

**Effect of initial dye concentration**

Dye concentration also affected the efficiency of colour removal. The dye removal was examined at different concentrations of the dye solution by $9 \times 10^6$ cells/mL of algal biomass (Khataee *et al.*,2009). Figure 6 shows the extent of colour removal varied with initial dye concentrations and decreases with increasing the dye concentration. Initial concentration provided an important driving force to overcome all mass transfer resistances of the dye between the aqueous and solid phases.

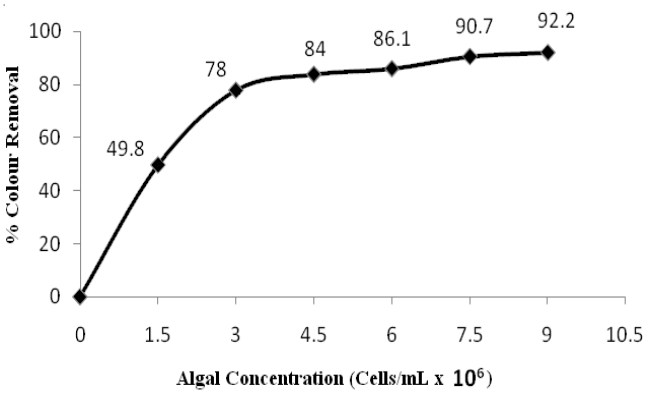


**Figure 5**. *Effect of different algal concentration on biological colour removal of crystal violet*

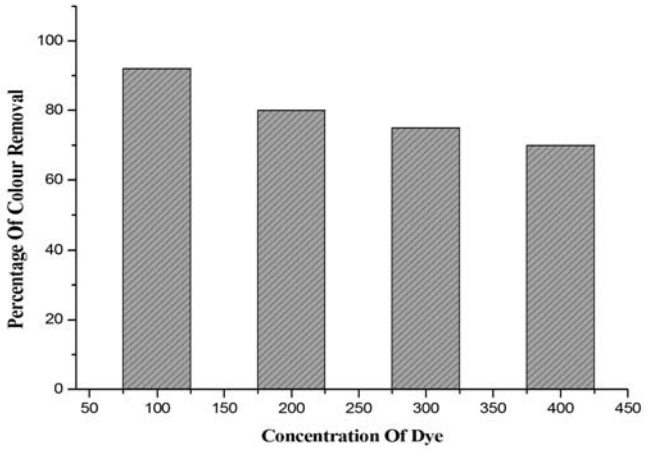


**Figure 6.** *Percentage colour removal of crystal violet dye at different concentration, in mg/L*

## CONCLUSION

The present study revealed that *Chlorella vulgaris* possessed the ability to bioaccumulate the dye crystal violet. Maximum colour removal was found to be 92.2% for CV dye, at biomass content of 9 × $10^6$ cells/mL. Percentage colour removal was found to be more at initial dye concentration of 100 mg/L. The results obtained from this work indicated that the algal species had high efficiency in the process of the treatment of the textile dyes which was dependent on algal cells and dye concentration.

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