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## DECOLOURIZATION OF ORANGE G DYE BY MICROALGAE *ACUTODESMUS OBLIQUUS* STRAIN PSV2 ISOLATED FROM TEXTILE INDUSTRIAL SITE

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### Abstract

**Introduction-**Release of different types of synthetic dyes into the aquatic environment by various industrial sources is a major area of concern worldwide due to their low biodegradable nature. Conventional methods of dye removal are effective but high operating cost and energy requirement, generation of toxic sludge and regeneration problem of adsorbent limits their use at large scale. Biosorption of dye molecules by microorganisms is an economic and eco friendly technology for the treatment of textile wastewater. **Aims & objective-**The study was carried out to isolate microalgae from industrial site and to evaluate its efficiency for adsorption of Orange G dye from aqueous solution. **Materials and methods-** A unicellular green micro alga was isolated from textile and dye contaminated site of Sanganer, Jaipur (India). Biosorption studies were conducted in a batch system for the removal of orange G dye from aqueous solution. Different parameters of pH, time, and initial dye concentration were investigated to optimize the process. Kinetic and equilibrium isotherms were implemented to experimental data in order to investigate the mechanism of biosorption. **Results-** Microalgae isolated from industrial site was found to be highly efficient for absorption of Orange G dye from aqueous solution. Batch studies depicts that *Acutodesmus obliquus* strain PSV 2 can absorb orange G dye at optimum pH 2.0 and maximum absorption (56.49 mg/g) occurred within initial 60 min of contact time. Equilibrium studies showed that Freundlich isotherm fitted well with experimental data with high correlation coefficient ( $r^2=0.995$ ) and n value ( $n=1.192$ ). Langmuir isotherm showed  $r^2=0.96$  and value of  $b=0.008$ , which represents favorable adsorption of dye on algal surface. FTIR analysis of adsorbent before and after dye adsorption showed the involvement of some functional groups in dye absorption.

**Key words:** Microalgae, *Acutodesmus obliquus* strain PSV 2, Orange G dye, Adsorption, Isotherms

### Introduction

In recent decades due to industrialization and urbanization contamination of environment by various pollutants is a major area of concern worldwide. Synthetic dyes are one of the toxic pollutants released by various industrial sources such as textile and dyeing industries, paper, paint, plastics, petroleum, electroplating and cosmetic industries. It is estimated that total colorant production in world is 800,000 tons per year and at least 15% of the dyestuff is released into the environment through wastes (Hai et al., 2007). Dyes are the synthetic chemical compounds having aromatic structure and recalcitrant to biodegradation due to xenobiotic nature (Pandey et al., 2007). Azo dyes are amongst the most widely used dyes in textile industries and therefore released in larger amount in the wastewater. Dyes are toxic to aquatic flora and fauna as they reduce the light penetration and obstruct photosynthesis process in aquatic system. Azo dyes and their transformed products are hazardous to animals and human beings and severely affect liver, kidney, brain, nervous system and reproductive system when ingested (Kumar et al., 2008;

Gao et al., 2010). Orange G is a type of azo dye and generally used by textile and dyeing industries. It exhibit toxic effects to animals and human beings and aquatic life (El-Sheekh et al., 2009).

The majority of technologies used for color removal from wastewater are based on physicochemical processes such as flocculation, sedimentation, precipitation, coagulation, fenton oxidation, reverse osmosis, but due to their high operating cost and energy requirement, and generation of toxic sludge limits the use of these methods (Maurya et al., 2006). Activated carbon is one of the most widely used process for removal of dye molecules from wastewater but its high price and problem with regeneration hampered its application at large scale (Çoruh et al., 2011).

Over the past few decades, biosorption of dye molecules by micro organisms emerged as a cost effective and eco friendly technique. The term 'biosorption' refers to the passive absorption of organic and inorganic species such as metal ions and dye molecules by the microbial biomass (Banat et al., 1996). Many microbial biomass such as microalgae (Clark and Anliker, 1980; Wang et al., 2007), fungi (Fu and Viraraghavan, 2002), bacteria (Kalme et al.,

2010), etc. acts as effective biosorbents for removal of toxic dyes from industrial effluents.

Amongst the various microbes, microalgae have been proved to be a better candidate for the removal of dye molecules due to their abundant occurrence in all habitats and because of high surface area. Algal cell wall possesses different types of functional groups such as carboxyl, amide, hydroxyl, sulphurhydryl, etc which are responsible for absorption of dye molecules (Jinqi and Houtian, 1992; Acuner and Dilek, 2004; Ertugrul et al., 2008). Although, wild type microbes existing in normal habitat are efficient in absorbing dye molecules but their absorption capability is sometimes affected by the toxicity of dye molecules. The microalgae growing in the contaminated site possess some inherent defense mechanisms of tolerance for excessive metal and dye concentration (Sarwa and Verma, 2013).

In the present study, we report the decolorization of Orange G dye by a unicellular microalga isolated from a textile and dyeing industrial site.

## Materials and Methods

### Microalgae isolation and cultivation

A unicellular green microalga was isolated from soil samples collected from Amani Shah Nallah drainage of Sanganer town situated at 26° 49' -26° 59' N and 75° 46' -75° 50' E near Jaipur, Rajasthan (India). Approximately 500 small and large scale dyeing and textile industries are situated in the close vicinity of this drainage and discharging their huge amount of colored wastewater directly in to the drainage (Mathur et al., 2006; Sarwa and Verma, 2013). Pure cultures of algae were developed using standard microbial isolation and culturing techniques and axenic cultures were maintained in BG-11 medium (R. Rippka, et al., 1979) under cool-white fluorescent, 1000 lux at 25±2 °C.

### Identification and characterization of microalgae

The axenic cultures of algae were identified and characterized microscopically and by 18s rRNA sequence analysis using standard methods (Desikachary, 1959; Prescott, 1962; Liu, 2006). The algae was identified as *Acutodesmus obliquus* and 18s rRNA sequences were deposited at NCBI Gene Bank and get the Accession no. as JX 519262.1 (*Acutodesmus obliquus* strain PSV2).

### Preparation of adsorbent

Microalga, *Acutodesmus obliquus* was grown in BG-11 medium under controlled conditions of light and temperature (1000lux at 25±2°C). In the exponential phase, alga was harvested and air dried for some days. Powder of the dried biomass was made with the help of mortar and pestle and used for dye biosorption study.

### Dye

The Orange G (OG) dye used in this study was procured from Himedia (Mumbai, India). The chemical properties of the OG dye are given in Table 1. Its chemical structure is shown in Fig. 1.

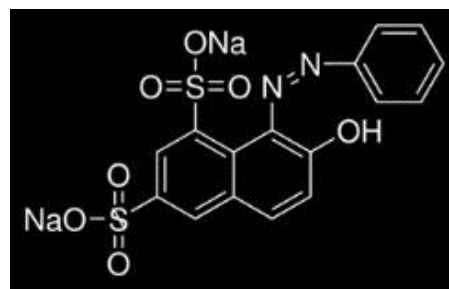


Fig.1: Chemical structure of Orange G dye

### Preparation of dye solution and quantification

Stock solution (1000 mg/L) of OG dye was prepared using de ionized water. Different dilutions of the stock solution were prepared according to the requirement of experiment. pH of the solutions were adjusted by adding either 0.1 M NaOH or 0.1M HCl .

The concentration of the dye was quantified before and after absorption using UV spectrophotometer at a maximum absorbance ( $\lambda_{max}=477\text{nm}$ ). The concentration of dye in the experimental samples was estimated from the calibration curves.

The amount of dye uptake,  $q_e$  (mg/g), by algal biosorbent was calculated using mass balance equation (1),

$$q_e = (C_i - C_f) V / 1000W \quad (1)$$

Where,  $C_i$  (mg/L) is the initial dye concentration,  $C_f$  (mg/L) is the dye concentration after adsorption,  $W$  (g) is the amount of biosorbent and  $V$  (ml) is the volume of the solution.

### Batch biosorption studies

The biosorption experiments were conducted in a batch system to study the effect of pH, contact time and initial dye concentration for the removal of OG dye from aqueous solution. The effect of pH was determined by preparing different pH solutions (range, 1.0-7.0) in separate flasks having dye concentration of 20 mg/L. 0.1g of dry algal biomass was added in each flask and experiment was carried out for 180min. Kinetic studies were carried out by exposing 0.1g algal biomass with 20 mg/L dye concentration at pH 2.0. 5 ml of samples were withdrawn from flask at an interval of 5, 15, 30, 60, 120, 150 and 180 min. Equilibrium studies were carried out by taking different dye concentrations in the range of 10, 20, 30, 40 and 50 mg/L at pH 2.0 and by using 0.1 g algal biomass. All flasks were kept on shaker at 180 rpm for 3 hrs to reach the equilibrium.

Table 1: The chemical properties of Orange G dye

Dye	Type	$\lambda_{max}$	C.I number	Empirical formulae	Molecular weight
Orange G	Acid	477	16230	$C_{16}H_{10}N_2Na_2O_7S_2$	452.37

**FTIR analysis**

Fourier Transform Infrared Spectroscopy (FTIR) of algal biomass before and after dye adsorption was recorded on Perkin Elmer (Model Frontier FTIR) Fourier transforms infrared (FTIR) spectrophotometer. The analysis was carried out in the range of 400-4000  $cm^{-1}$ .

**Results and Discussion**

**Effect of pH**

The complexation of dye with algal surface is dependent on the aqueous phase pH and the functional groups on the algal cell walls and their ionic states (at particular pH) (Genc et al., 2003; Mohan et al., 2008). The adsorption of OG dye was studied in the pH range of 1.0-7.0 and maximum adsorption (8.44 mg/L) was observed at pH 2.0. Fig. 2, represents the adsorption pattern of OG dye by algal biomass. Adsorption of dye increased from pH 1.0 to 2.0 and then decreased on increasing pH beyond pH 2.0. The maximum adsorption at lower pH may be due to more positive charge on the adsorbent surface. On increasing the pH beyond 2.0, number of negatively charged sites increased and positively charged sites decreased on biosorbent and thus results in decrement in biosorption of dye molecules at high pH. Similar kind of results were also reported for adsorption of Congo red dye (Chowdhary et al., 2009; Ahmad and Kumar, 2010).

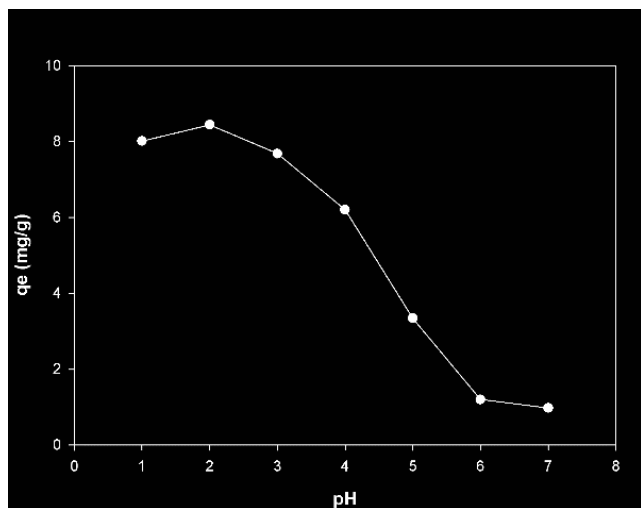


Fig. 2. Effect of pH on biosorption of orange G dye by *Acutodesmus obliquus* strain PSV 2

**Effect of contact time and initial dye concentration**

The dye adsorption at varying dye concentration (10-50 mg/L) and contact time (0-180min) is represented in Fig. 3. The results indicate that adsorption of OG dye occurred in two stages: (i) initial rapid stage where adsorption rapidly increased within first 60 min due to rapid surface

adsorption followed by (ii) a slower stage of adsorption after 60 min. The rapid dye adsorption could be attributed to more functional groups present on algal surface and later slow absorption could be due to intra particle diffusion of dye molecules. Fig. 3, shows that with increase in initial dye concentration, adsorption of dye increased. The increase in dye uptake with increase in dye concentration could be attributed to higher initial dye concentration provides driving force to overcome all resistances of the dye between the aqueous and solid phases, thus increasing the uptake. Ranjusha et al., 2010, reported the similar pattern of results for Remazol Black B dye adsorption on *Aspergillus flavus*.

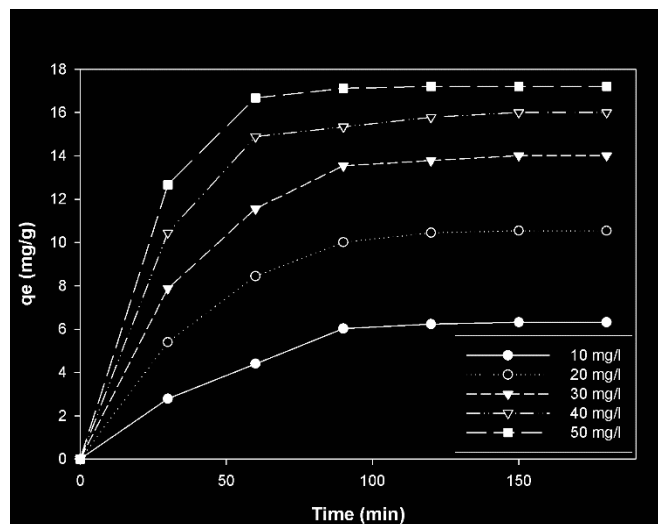


Fig 3. Effect of contact time and initial dye concentration on biosorption of orange G dye by *Acutodesmus obliquus* strain PSV 2.

**Biosorption isotherms**

The surface property, affinity of adsorbent for adsorbate can be characterized by different equilibrium models. The equilibrium data obtained for OG dye adsorption on algal biomass were applied on Langmuir and Freundlich Isotherm models in order to investigate the suitable model to represent the adsorption process.

**Langmuir isotherm**

The Langmuir isotherm states the monolayer adsorption of adsorbate onto adsorbent surface containing finite number of identical sites without any interaction with adjacent sites (Langmuir, 1918; Vannela and Verma, 2006). The linear form of Langmuir isotherm can be represented by equation (2)

$$C_e/q_e = 1/q_{max}K_L + C_e/q_{max} \quad (2)$$

Where  $C_e$  is the equilibrium dye concentration in the solution ( $\text{mg L}^{-1}$ ),  $q_e$  is the equilibrium dye uptake on the biosorbent ( $\text{mg g}^{-1}$ ),  $q_{\text{max}}$  is the maximum biosorption capacity ( $\text{mg g}^{-1}$ ) of the adsorbent, and  $K_L$  is the Langmuir constant ( $\text{L mg}^{-1}$ ).

The results obtained after plotting  $C_e/q_e$  versus  $C_e$  are shown in Fig 4, which depicts the relationship of amount of dye adsorbed ( $\text{mg/g}$ ) by algal biomass and the residual dye molecule concentration. The obtained data were found to fit well with Langmuir model with the maximum biosorption capacity ( $q_{\text{max}}$ ) 56.49  $\text{mg/g}$  and  $r^2$  value 0.961. Langmuir isotherm represents monolayer adsorption of dye molecules on algal surface and was not dependent on adjacent sites.

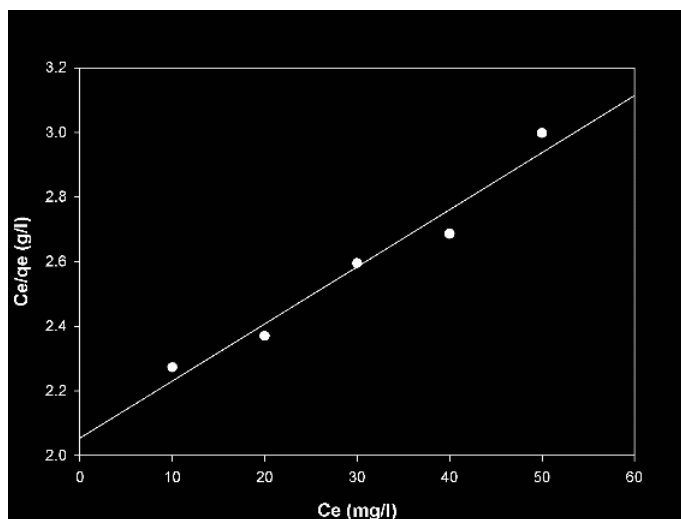


Fig 4. Langmuir isotherm for absorption of orange G dye by *Acutodesmus obliquus* strain PSV 2.

**Freundlich isotherm**

The Freundlich isotherm is an empirical model based on heterogeneous adsorption of adsorbate on adsorbent (Freundlich 1906; Sarwa and Verma, 2013). It is represented by Equation (3)

$$\ln q_e = \ln K_F + 1/n \ln C_e \quad (3)$$

Where  $q_e$  is the amount of dye adsorbed at equilibrium ( $\text{mg g}^{-1}$ ),  $K_F$  is the Freundlich constant,  $1/n$  is the heterogeneity factor which is related to the capacity of the adsorption and  $C_e$  is the equilibrium concentration of dye ( $\text{mgL}^{-1}$ ).

Fig.5 represents the linear form of Freundlich isotherm. The OG dye experimental data were found to fit well in Freundlich isotherm, with value of  $K_F = 0.65$ ,  $n=1.19$  and  $r^2=0.99$ . The value of  $n$  was found to be greater than unity, represents favorable adsorption of OG dye on algal surface. Similar results were mentioned by researchers in the literature for biosorption of Congo red dye on *Azadirachta indica* leaf (Bhattacharyya and Sharma 2004) and methylene blue on marine green algae (Sikaily et al., 2006).

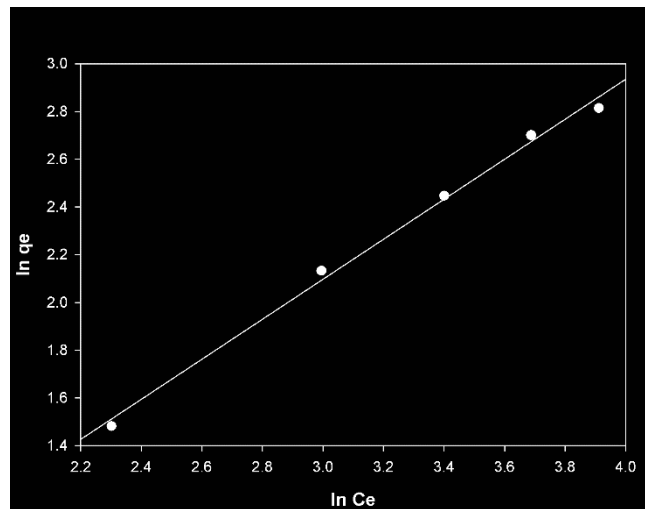


Fig 5. Freundlich isotherm for absorption of orange G dye by *Acutodesmus obliquus* strain PSV 2

**FTIR analysis**

The FTIR spectrum of *Acutodesmus obliquus* strain PSV 2 before and after dye absorption revealed the involvement of different functional groups of algae in dye absorption. Fig. 6. Represents the FTIR spectrum of algae before dye absorption. It showed one or two prominent peaks before dye absorption. Fig. 7 shows the FTIR spectrum of algae after dye absorption and represents that some peaks are shifted or disappeared and some new peaks emerged after dye absorption.

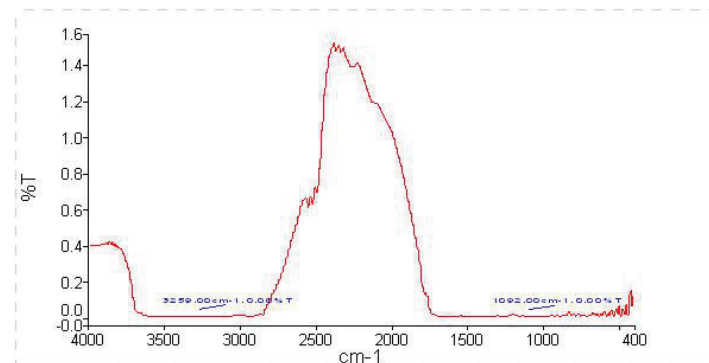


Fig.6. FTIR spectrum of *Acutodesmus obliquus* (before dye adsorption)

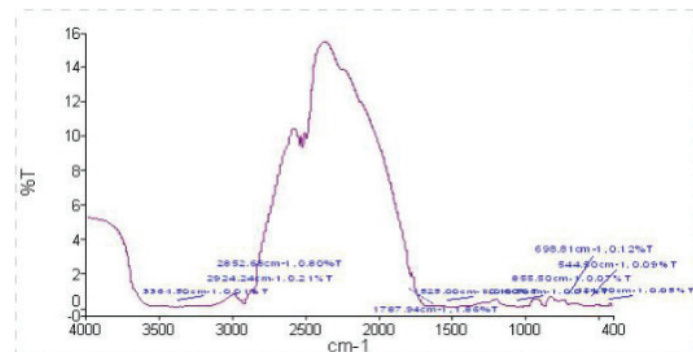


Fig.7. FTIR spectrum of *Acutodesmus obliquus* (after dye adsorption)

The changes observed in the spectrum indicates the possible involvement of different functional groups of algae in dye adsorption process. Before dye adsorption peaks were detected at 1092 cm<sup>-1</sup> and 3259 cm<sup>-1</sup> (Fig 6.) while after dye biosorption some new peaks at 2924.68 cm<sup>-1</sup>, 3364.54 cm<sup>-1</sup>, 1787.94 cm<sup>-1</sup> and 698.81 cm<sup>-1</sup> were observed which indicates the involvement of Hydroxyl and amine groups for dye adsorption.

## Conclusion

From the present investigation, it is concluded that *Acutodesmus obliquus* strain PSV2 is a highly efficient adsorbent for the removal of orange G dye from aqueous solution. Batch experiments showed that at pH 2.0, 1 gram of isolated microalgae can effectively adsorb 56.49 mg of orange G dye. Time course studies revealed the rapid adsorption of dye within 60 min. of contact time. Adsorption isotherms showed the high correlation coefficient (r<sup>2</sup>=0.99) of freundlich isotherm which indicates heterogenous adsorption of dye. The present study showed that microalgae isolated from an industrial site is highly efficient for treatment of textile and dyeing industrial wastewater.

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