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Adsorption of Acid Red 66 Dye from Aqueous Solution by Green Microalgae *Acutodesmus obliquus* Strain PSV2 Isolated from an Industrial Polluted Site

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Abstract

In the present study, *Acutodesmus obliquus* strain PSV2 was isolated from a textile and dyeing industrial site and investigated as a cost effective and potential adsorbent for Acid red 66 dye. Batch kinetic experiments were carried out as a function of pH (1.0 - 6.0), contact time (0 - 180 min) and initial dye concentration (10 - 50 mg/L) to determine the decolorization efficiency of microalgae. The maximum adsorption of dye was observed at pH 2.0 during the initial 60 min of contact time. The Langmuir and Freundlich adsorption isotherms were applied to experimental data to investigate the efficiency of adsorbent and mechanism of adsorption. It was observed that Langmuir and Freundlich isotherm fitted well with Acid Red 66 dye data. Langmuir isotherm, described maximum adsorption of dye (44.24 mg/g) with good correlation coefficient (r^2 = 0.980) while Freundlich isotherm showed a high correlation coefficient (r^2 = 0.994) with value of n greater than unity (n = 1.27). The present study showed that *Acutodesmus obliquus* strain PSV2 is an eco friendly and highly efficient adsorbent for removal of acid red 66 dye from dyeing and textile industrial wastewater.

Keywords

Acid red 66 Dye, Microalgae, *Acutodesmus obliquus* Strain PSV2, Adsorption, Isotherms

Subject Areas: **Biotechnology, Environmental Sciences**

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1. Introduction

Azo dyes constitute the most widely used dye stuffs in the textile and dyeing industries. They contribute approximately 60% of the total 10,000 different dyes produced worldwide. It is reported that approximately 15% of these dyes are released directly into wastewater during manufacturing process [1]. Discharge of these azo dyes in the environment without any proper treatment has become a major area of concern worldwide. Most of the dyes are synthetic in origin and known to be xenobiotic and recalcitrant to biodegradation phenomenon [2]. A synthetic dye adversely affects aquatic life by reducing light penetration and obstructing photosynthesis. Dyes and there byproducts are reported to be toxic to animals and human beings due to their mutagenic and carcinogenic effects [3].

Some physicochemical methods such as fenton's reagent (H_2O_2 + Fe^{2+}), ozonation, coagulation-flocculation using lime, alum, etc. are used for removal of dyes from wastewater but due to some limitations such as high operating cost, generation of toxic sludge, not eco friendly nature hampers the application of these methods at large scale [4] [5]. Adsorption of dye molecules by activated carbon is also used for removal of dyes but it is very costly and regeneration of adsorbent is not possible [3] [6]. In order to overcome these problems of physicochemical methods, low cost adsorbents are always preferred and are the major area of research in the last few decades.

The biological technique of adsorption by micro organisms is a cost effective, and efficient alternative for treatment of textile and dyeing industrial wastewater. Various micro organisms such as algae [7], fungi [8], bacteria [9], etc. act as efficient adsorbents for removal of different types of dye molecules from wastewater. Amongst the microbial biomass, microalgae preferred as a better candidate for adsorption of dye molecules due to its abundance occurrence in all habitats of nature and high surface area to volume ratio. Microalgae can be easily cultivated in a pond without much cost and used for decolorization of dye containing wastewater [10] [11]. Microalgae consist of several functional groups on cell wall such as carboxyl, hydroxyl, amine, sulphate etc. which act as binding sites for dye molecules and helps in adsorption process [12] [13]. Although, wild type microbes isolated from natural habitat are efficient in adsorption of dye molecules but sometimes their adsorption efficiency adversely affected by the toxicity of dyes. The microalgae growing in the close vicinity of industrial contaminated site possess some inherent defense mechanisms to cope up with excessive dye molecules along with high adsorption [12].

With this background, microalgae was isolated from a textile and dye contaminated industrial site and was investigated for the removal of Acid red 66 dye from aqueous solution.

2. Materials and Methods

2.1. Microalgae Isolation and Cultivation

Microalgae was isolated from soil samples collected from textile and dyeing industrial site of Sanganer town situated at 26˚49'N - 26˚59'N and 75˚46'E - 75˚50'E near Jaipur district of Rajasthan, India. Sanganer is worldwide famous for its colorful and printed clothes. In Sanganer, more than 500 small and large scale dyeing and textile industries are involved in manufacturing and dyeing of different types of clothes. The colored wastewater generated by these industries is directly discharged in to the Amani Shah Nallah drainage situated in the close vicinity of these industries [14]. A microalga was isolated using standard microbiological techniques and axenic cultures were maintained in BG-11 medium under cool-white fluorescent, 1000 lux at 25˚C ± 2˚C [15].

2.2. Identification and Characterization of Microalgae

The axenic cultures of microalga were identified and characterized using microscopy and molecular technique of 18S rRNA sequence analysis (**Figure 1**) [16]-[18]. After 18S rRNA sequences analysis of microalgae, sequences were submitted to NCBI Gene Bank and get the Accession no. as JX 519262.1 (*Acutodesmus obliquus* strain PSV2). The algal strain labeled hereafter as *Acutodesmus obliquus.*

2.3. Preparation of Adsorbent

Microalgae, *Acutodesmus obliquus* was harvested in the late exponential phase and sun dried for 2 - 3 days. Dried powder was prepared with the help of mortar and pestle and used for adsorption of dye molecules without any pretreatment.

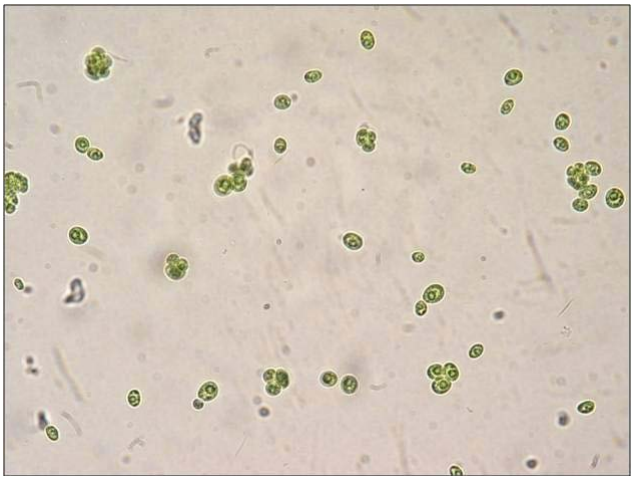

Figure 1. Microscopy picture of *Acutodesmus obliquus* strain PSV2.

2.4. Adsorbate

The Acid Red 66 (AR) dye used in this study was procured from Himedia (Mumbai, India). The properties of AR dye are represented in **Table 1**. Its chemical structure is shown in **Figure 2**. The chemical is used without any further purification.

2.5. Preparation of Dye Solution and Quantification

1000 mg/L stock solution of AR was prepared by dissolving it in distilled water. Dilutions of the stock solution were prepared according to the experiments. pH of the AR solutions were adjusted by adding either 0.1 M NaOH or 0.1 M HCl.

The concentration of AR before and after adsorption was quantified using UV spectrophotometer at a maximum absorbance (λ_{max} = 504 nm). The concentration of dye in the experimental samples was estimated from the previously prepared calibration curves.

2.6. Adsorption

The adsorption experiments were conducted in a batch system to study the effect of pH, contact time and initial dye concentration for the removal of AR from aqueous solution. The batch studies were carried out in 250 mL erlenmeyer flasks containing 100 mL dye solution of desired concentration and kept on shaker at a constant speed of 180 rpm at room temperature.

The effect of pH was determined by preparing different pH solutions (range, 1.0 - 6.0) in separate flasks having dye concentration of 20 mg/L. 0.1 g of dry algal biomass was added in each flask and experiment was carried out for 3 hrs. Kinetic studies were carried out by exposing 0.1 g algal biomass with 20 mg/L dye concentration at pH 2.0. A 5 mL sample was withdrawn from flask at an interval of 5, 15, 30, 60, 120, 150, and 180 min. The dye concentration was analyzed with spectrophotometer as mentioned earlier.

Adsorption equilibrium studies were conducted by adding 0.1 g algal biomass in a series of flasks containing dye concentration in the range of 10, 20, 30, 40 and 50 mg/L at pH 2.0. All flaks were kept on shaker for 3 hrs to reach the equilibrium. Dye concentrations in the flasks after equilibrium were analyzed as mentioned earlier.

2.7. Statistical Analysis

All the experiments were carried out in duplicates and values used in calculations are the arithmetic averages of experimental data. All statistical analysis was done using Sigma plot 8.0 Software.

The amount of dye adsorbed, q_e (mg/g), by the algal biomass was calculated using mass balance equation (1),

$$q_e = \frac{(C_i - C_f)V}{1000\,W} \quad (1)$$

Table 1. The chemical properties of acid red 66 dye.

Dye	Other names	Ionization	Class	λ_{max}	C.I number	Empirical formulae	Formula weight
Acid red 66	Ponceau B	Acid	Azo	504	26905	$C_{22}H_{14}N_4Na_2O_7S_2$	556.5

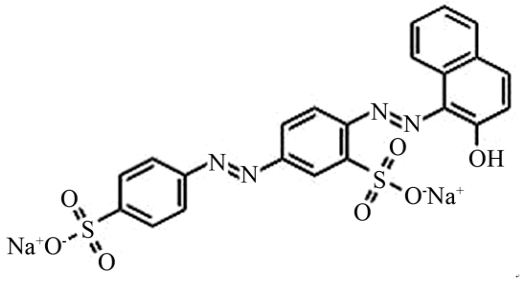

Figure 2. Chemical structure of acid red 66 dye.

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 adsorbent and V (mL) is the volume of the solution.

3. Results and Discussion

3.1. Effect of pH

pH of dye solution is an important factor to be considered in adsorption process as it influences the surface charge of the adsorbent and the ionization of dye present in the solution. In a batch system, the effect of pH (range 1.0 - 6.0) was monitored to find out the optimum pH for maximum adsorption of dye molecules from solution. Removal of AR dye was found to be increased from pH 1.0 to 2.0 and then decreased on further increasing the pH up to 6.0. Maximum adsorption of dye observed at pH 2.0 (8.7 mg/g) with 20 mg/L initial dye concentration (**Figure 3**). This pattern of AR adsorption at different pH could be attributed to electrostatic force of attraction between positively charged functional groups on the algal surface and negatively charged dye anion molecules [19]. On increasing the pH beyond 2.0, number of negatively charged group's increased and positively charged groups decreased on algal surface and thus results in decrement in adsorption at high pH [20]. Similar pattern of adsorption was also observed for reactive red 2 dye [21] and for Congo red dye [22].

3.2. Effect of Contact Time and Initial Dye Concentration

The effect of contact time (0 - 180 min) and initial dye concentration (10 - 50 mg/L) on dye adsorption is represented in **Figure 4**. The results indicate that AR dye adsorption was rapid for initial 60 min followed by a slower rate of adsorption for next 60 min. Saturation in the adsorption of dye was observed after 120 min of contact time. At higher contact time, AR adsorption decreases and gradually attained equilibrium due to saturation and less number of free binding sites on algal surface. **Figure 4**, clearly depicts the increase in adsorption of AR from 4.4 mg/g to 16.49 mg/g with increase in initial dye concentration from 10 mg/L to 50 mg/L. The increase in dye uptake with increase in dye concentration could be attributed to the driving force created by higher initial dye concentration to overcome all resistances of the dye between the aqueous and solid phases, thus increasing the uptake. Remazol black B dye adsorption by *Aspergillus flavus* [23] and Congo red adsorption on organo attapulgite [24] showed similar findings.

3.3. Adsorption Isotherms

Isotherm models are the tools that investigate the adsorption mechanisms and the surface properties of the adsorbent. In the present investigation, the experimental data were fitted into commonly used isotherm models-Langmuir and Freundlich in order to investigate the most suitable one to represent the adsorption of AR dye by algal biomass.

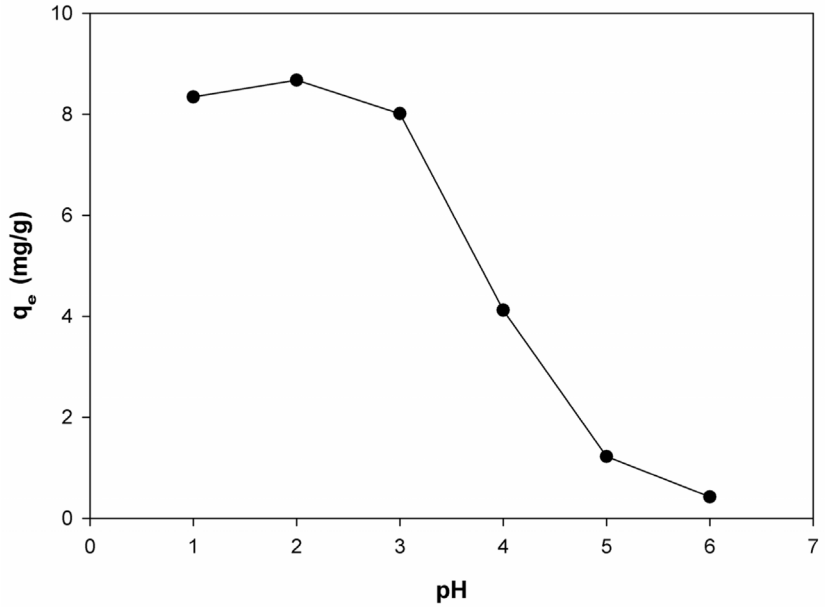

Figure 3. Effect of pH on adsorption of Acid red 66 dye by *Acutodesmus obliquus*.

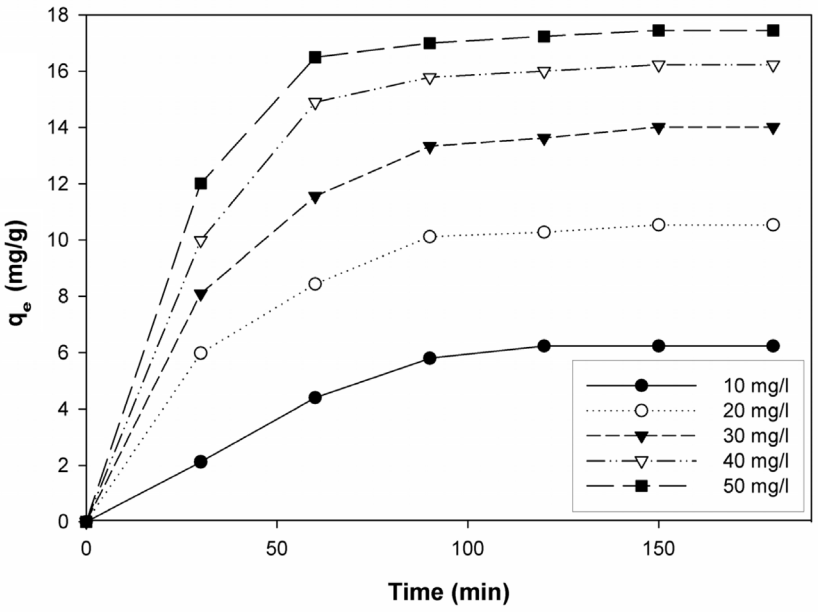

Figure 4. Effect of contact time and initial dye concentration on adsorption of Acid red 66 dye by *Acutodesmus obliquus.*

3.3.1. Langmuir Isotherm

The Langmuir isotherm model represents the homogenous adsorption of adsorbate on adsorbent without any interaction with adjacent sites [25]. Its linear form is represented by equation (2)

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} K_L} + \frac{C_e}{q_{\max}} \tag{2}$$

where C_e is the equilibrium dye concentration in the solution (mg/L), q_e is the equilibrium dye uptake on the adsorbent (mg/g), $q_{\max}$ is the maximum adsorption capacity (mg/g) of the adsorbent, and K_L is the Langmuir constant (L/mg).

Figure 5 shows the Langmuir isotherm plot for AR adsorption by *Acutodesmus obliquus*. The results depicts the relationship between amount of dye adsorbed (mg/g) by algal biomass and the residual dye molecule concentration. The data were found to fit well in Langmuir model with maximum adsorption capacity (q_{max}) 44.24 mg/g and r^2 value 0.980 (**Table 2**). The value of K_L was found to be 0.0122 which represents the favorable monolayer adsorption of dye molecules on algal surface and is not dependent on adjacent site. Similar results are reported for adsorption of Acid Red 57 dye on *Phaseolus vulgaris* waste [26].

3.3.2. Freundlich Isotherm

The Freundlich isotherm demonstrates non uniform adsorption of adsorbate on heterogeneous surface of adsorbent [27] [28]. The linear form of Freundlich isotherm is represented by equation (3)

$$\ln q_e = \ln K_F + \frac{1}{n}\ln C_e \quad (3)$$

where q_e is the amount of dye adsorbed at equilibrium (mg/g), 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 (mg/L). **Figure 6** shows the linear form of Freundlich isotherm for AR adsorption by *Acutodesmus obliquus*.

Freundlich isotherm parameters (**Table 2**) showed that isolated microalgae is efficient in adsorption of AR dye from aqueous solution with good correlation coefficient. The value of n was found to be greater than unity, showing favorable adsorption of AR on algal biomass. The results are in agreement with findings of Ozacar and Ayhan [29] who reported dye adsorption on pine saw dust.

4. Conclusion

From the present investigation, it was observed that *Acutodesmus obliquus* strain PSV2 is a highly efficient ad-

Table 2. Isotherm constants for adsorption of Acid red 66 dye by *Acutodesmus obliquus*.

Langmuir isotherm			Freundlich isotherm		
q_{max} (mg/g)	K_L	r^2	K_F	n	r^2
44.24	0.012	0.980	0.811	1.276	0.994

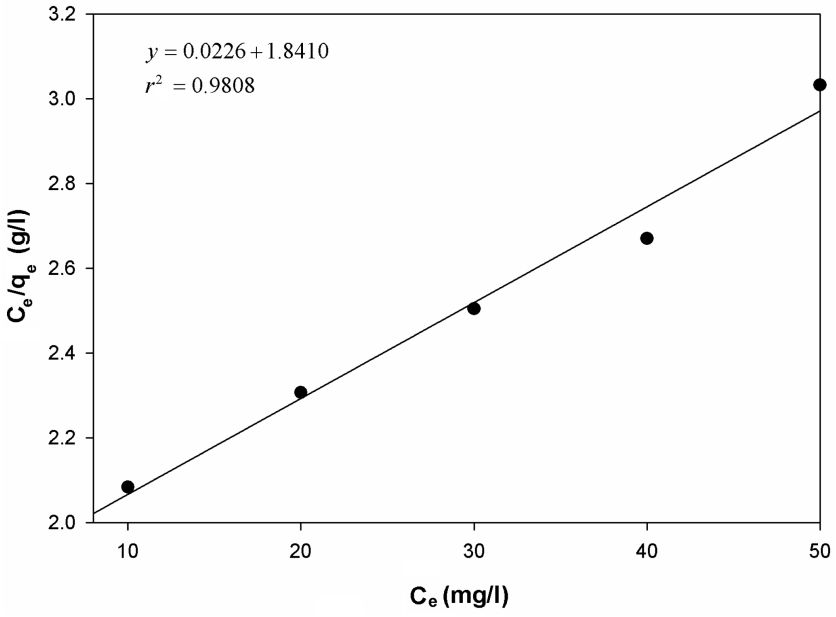

Figure 5. Langmuir isotherm for adsorption of Acid Red 66 dye by *Acutodesmus obliquus*.

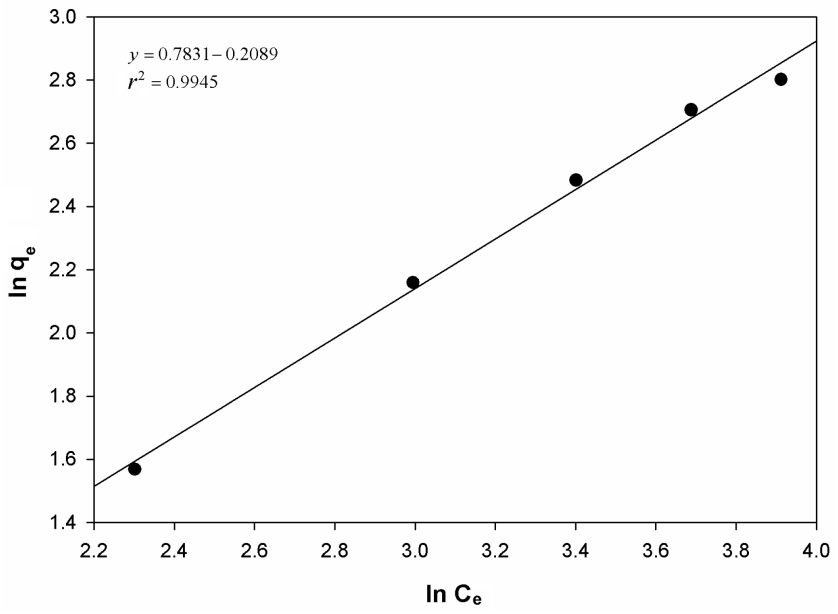

Figure 6. Freundlich isotherm for adsorption of Acid Red 66 dye by *Acutodesmus obliquus*.

sorbent for removal of acid red 66 dye from aqueous solution. Batch experiments showed that 1 gram of isolated microalgae can effectively adsorb 44.24 mg of acid red dye at pH 2.0 within 60 min of contact time. Isotherm models depicts the best fit with Freundlich isotherm and represents the heterogeneous adsorption of dye molecule on algal surface with high correlation coefficient (r^2 = 0.99). The present study concluded that microalgae isolated from an industrial contaminated site are highly efficient in adsorbing dye molecules and can be successfully employed for treatment of textile and dyeing industrial wastewaters.

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