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# To study pretreatment and the impact of key operating factors for the adsorption of anthraquinone dye onto non-viable *Oscillatoria* sp.

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**Abstract**
Water pollution by visible coloured pollutants, such as dyes is a big concern. In this study, a non-viable cyanobacterium *Oscillatoria* sp. is used to adsorb the toxic anthraquinone dye Alizarin from synthetic aqueous solutions in pursuit of an inexpensive and conveniently accessible biomaterial. The primary study examines key adsorption parameters, Initial solution pH, dye concentration, adsorbent dosage, contact time, and temperature. Systematically, alizarin dye adsorption was examined at a range of pH values (1-6), initial dye concentrations (50-200 mg/L), adsorbent doses (1-10 g/l), temperatures (298K–318K), and contact periods (5-160 minutes). Adsorption was optimized at pH 1, 200 mg/l dye concentration, 4 g/L cyanobacterial dosage, 318K, and 120 minute's contact duration. Acid pre-treatment (HCl) enhanced the biomass's ability to absorb Alizarin dye. SEM and FTIR were employed in addition to the determination of cyanobacterial surface area and elemental composition. FT-IR spectra showed few changes after dye sorption, demonstrating the involvement of biomaterial cell wall surface functional groups.

**Keywords:** *Oscillatoria* sp. cyanobacterium, adsorption, alizarin

## 1. Introduction

Dye chemicals are of importance to the industrial sector because of their widespread application in the coloring process across a variety of product categories. However, the discharge of industrial effluents that contain dyes is a major contributor to the problem of water pollution [1]. It is widely known that dyes have poisonous, carcinogenic, and mutagenic effects on human beings [2]. Additionally, dyes can produce discoloration in natural waters, which may have a negative impact on the photosynthesis of algae and other submerged plants [3]. For this, the elimination of dyes from industrial effluents ahead of their release into natural waters is a task that must be accorded the greatest consideration.

Despite the fact that a wide range of treatment approaches, including physical, chemical, and biological methods, have been researched by many researchers to deal with dye-containing effluents, only a few are employed by the relevant industries due to limitations and they are unsuitable in terms of efficiency and economy [4]. Adsorption has been proven to be superior to other technologies for treating wastewater in terms of initial cost, simplicity of design, ease of operation, and insensitivity to harmful compounds [5-6]. The advantages of the adsorption technique have been similarly documented by other research that has used biomass-based adsorbents for the purpose of dye removal [7-8]. The anthraquinone dye like Alizarin, used in many fields, is highly stable and resistant to degradation by most chemical, physical, and biological methods. Therefore, research into methods of removing Alizarin dye is essential. There are reports indicating Alizarin dye removal using mango seeds and mustard husk [9-10].

Recently, there has been a lot of interest in using algae to remove colored effluent. Because of their widespread availability and quick, easy development, algae have been revealed as potential and acceptable adsorbents [11]. Therefore, a locally accessible filamentous Cyanobacterium (algae) named *Oscillatoria* sp. is used as a biosorbent material in this communication. Cyanobacterium cell walls are composed of a variety of biologically active biopolymers, including cellulose and proteins associated with polysaccharides. These abundant biopolymers have surface active functional groups (-COOH, -OH, -$NH_2$) that aid in providing adequate binding sites for the pollutants [12].

Few papers have mentioned using this Cyanobacterium as a possible biosorbent material for eliminating dyes. M. Maqbool *et al*. utilized bio composites of polyaniline and sodium alginate prepared with *Oscillatoria* biomass for the uptake of Basic blue 41 dye from industrial effluents and reported good adsorption efficiency [13]. Similarly, D. Kumar *et al* reported the ability of *Oscillatoria* sp. dominated cyanobacterial mat in sorbing Methylene blue, a cationic dye, from aqueous solutions [14]. Our group recently utilized *Oscillatoria* sp. to eliminate methylene blue and methyl orange dyes from synthetic wastewaters with high sorption capacity [15-16]. Our previous research revealed that *Chara* sp.and *Vaucheria* sp. algae effectively remove Alizarin dye [17-18]. So, out of ongoing interest, the ability of cyanobacterium *Oscillatoria* sp. was examined in this study to remove the anthraquinone dye Alizarin.

The fundamental goal of this research is to identify and optimize the impact of pH, contact time, temperature, and adsorbent dose on the adsorption process. The sorption mechanism was identified with the aid of instrumental methods including Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). In addition, the effect of several chemical pre-treatments on test algae for alizarin dye adsorption from synthetic aqueous solutions is investigated in this communication.

## 2. Material and Methods

### 2.1. Chemicals and Equipment

All the chemicals used in this study were of analytical grade, obtained from SD Fine chemicals limited, India. These include hydrochloric acid (HCl), nitric acid ($HNO_3$), sodium hydroxide (NaOH), and ethanol ($C_2H_5OH$). Throughout the investigations, all working solutions were prepared in double-distilled water.

A UV-Visible spectrophotometer (model UV-Vis 119 Systronics India Ltd.) was used to determine the dyes spectral properties. The pH measurements were taken with a pH metre (PERFIT, India). KBr pellets were utilized to create infrared spectra in the 4000-400 $cm^{-1}$ range using a Thermo Nicolet FTIR (Germany). SEM was performed with a ZEISS EVO 40 EP (Cambridge, UK) and the Quantax 200 analytical software. An Elementar analyze system Vario MICRO CHNS V3.1.1 (GmbH, Germany) was employed to find the adsorbent's elemental composition, and a Micro metrics ASAP 2010 surface area analyzer (England, UK) was utilized to determine the adsorbent's Brunauer-Emmett-Teller (BET) surface area.

### 2.2. The Test Biosorbent (*Oscillatoria* sp.) and its preparation method

In this work, *Oscillatoria* species was chosen as the test biosorbent. It belongs to the genus of filamentous cyanobacterium. Oscillatoria is the term given to it because of the oscillation that can be seen in its movement. Its filaments are built from a trichome, which has rows of cells (Figure 1). The trichome's apex swings back and forth like a pendulum. The test biomass collected from nearby ponds and rivers, was rinsed in double-distilled water to remove debris and contaminants before being dried on filter paper. After two days of drying in the sun, the biomass was dried at 343K for 24 hours to remove any remaining moisture. A mortar and pestle were used to break them down further, and the resulting powder was sieved through 100 µm screen. In order to preserve the powdered non-viable adsorbent, it was stored in vacuum desiccators.

**Fig 1:** Cyanobacterium *Oscillatoria* sp.

### 2.3. The Test Dye (Alizarin) and its stock preparation

SD Fine Chem. Ltd., India supplied the alizarin dye used as adsorbate in this study. This adsorbate is of commercial purity and can be used without further purification. It has the molecular formula, $C_{14}H_8O_4$, a molecular weight of 240.21 g/mol, the chemical name 1,2-dihydroxyanthraquinone, and a maximum wavelength ($\lambda_{max}$) at 567 nm. In the laboratory, dyes and distilled water were used to produce artificial (synthetic) aqueous solutions for the study. According to standard procedure, a stock dye solution (1g/L) was prepared by dissolving the required amount of dyes (Alizarin) in double-distilled water. Using a digital pH meter calibrated with standard buffer solutions, the initial pH was adjusted with 0.1M HCl and 0.1M NaOH solutions.

### 2.4. Methodology

#### 2.4.1. Biosorbents Characterization Studies

The BET method [19] was used to calculate the adsorbents' surface area, and scanning electron microscopy (SEM) scans were analysed to learn about the adsorbents' surface morphology post gold coating. Element analysis was employed to assess the relative abundance of individual elements such Carbon, Nitrogen, Hydrogen, and Sulphur, while infrared spectroscopy was used to determine the functional groups available on the adsorbent's surface.

#### 2.4.2. Batch Adsorption Procedures

Many researchers have explored different approaches to bring adsorbate and adsorbent together to remove as many pollutants as possible from water. In this work, the batch technique produced simple adsorption isotherms. Adsorption parameters could be optimized to maximise adsorption on a particular adsorbent. The first experiment examined contact time and the shortest time needed for dye-algae systems to establish equilibrium.

100ml of each dye solution (adsorbate) was mixed with a set amount of algae (adsorbent). A thermostat-controlled shaking mechanism held the 100 ml Erlenmeyer flask with the adsorbate. The flasks were withdrawn from the shaker at regular intervals and swirled at a steady temperature. The equilibrium contact time is when adsorption stops. Next experiments use this best time period to test other factors. Under optimized contact time conditions, similar sets with four adsorbate concentrations (50, 100, 150, and 200 mg/L) and adsorbent dosages (1-10mg) were studied. Adsorption tests were performed at various pH values from 1 to 10 by adding 0.1 M HCl or NaOH to change the surface characteristics of the adsorbent and adsorbate. Adsorption also depends on temperature. Three tests at three temperatures (298, 308 and 318 K) are performed to determine the effect of solution temperature on adsorption.

All the samples from the aforementioned studies were centrifuged at 200 rpm for 10 minutes at 298K to completely separate the adsorbent particles from the solution. Using a calibration curve and a UV-Vis spectrophotometer, the residual dye concentration was then determined. Three sets of tests were conducted, and the mean and standard deviation were reported. It was determined that differences between standard deviations were within 1.3%. The error bars for the numbers were so narrow that they could not be presented alongside the graphing symbols. Following is the mass balance equation used to calculate the amount of Alizarin dye adsorbed.

$$q_e = (C_o - C_e)\,V/M \qquad (1)$$

Where $C_o$ and $C_e$ are the initial and the equilibrium concentration of Alizarin dye (mg/L) respectively, $q_e$ is the amount adsorbed by Cyanobacterium (mg/g), V is the volume of the reaction mixture (L) and M is the mass of adsorbent used (g).

### 2.4.3. Chemical Pre-treatment Studies

A standard algal concentration suspended in a 1 mol/L pre-treatment solution was used to assess the efficiency of chemical pre-treatment. Chemical pre-treatments included hydrochloric acid, nitric acid, sodium hydroxide, sodium chloride, and ethanol. The mixtures were swirled for 5 hours at 318 K and 110 revolutions per minute, followed by filtering to extract the processed biomass. Adsorption tests were conducted out in an oven at 343 K for 24 hours, after the collected samples had been dried.

## 3. Results and Discussion

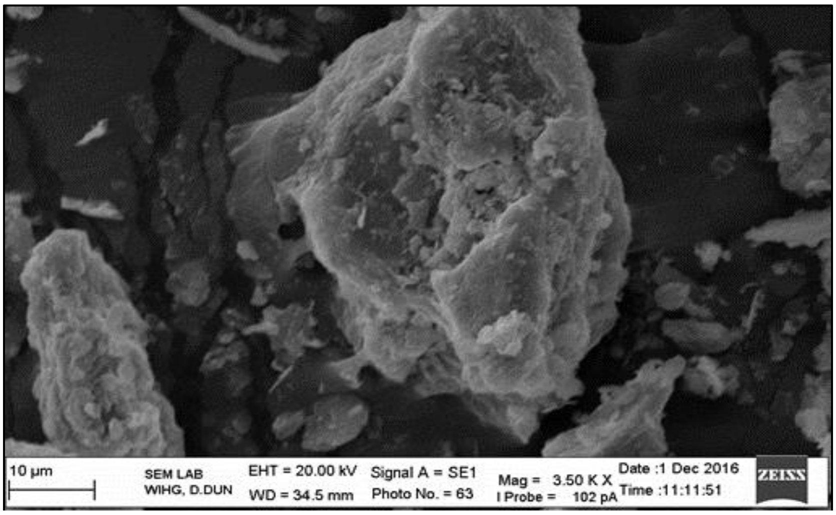
### 3.1. Characterization of *Oscillatoria* Sp.

### 3.1.1. Elemental analysis and Surface area determination

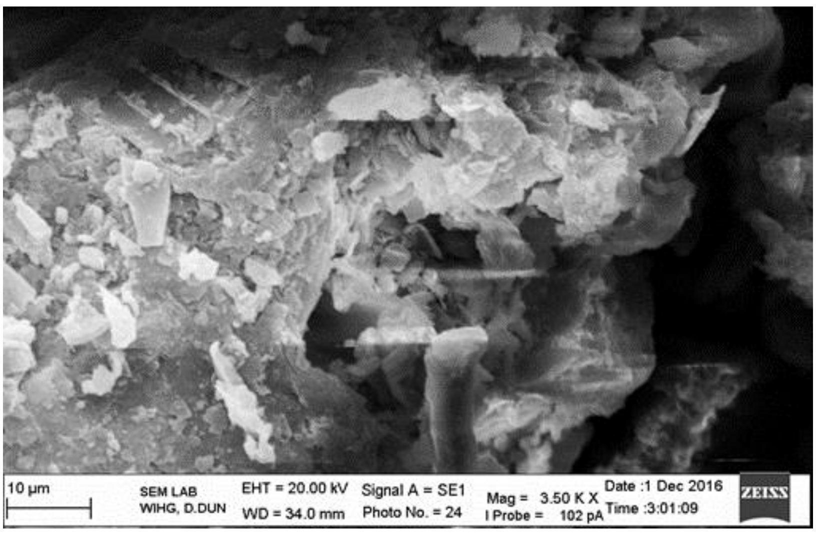
The BET method revealed that the surface area of the non-viable Cyanobacterium *Oscillatoria* sp. to be 0.525 $m^2/g$. On chemically analysing the biomass, it was found to be organic in nature. It contains Carbon (21.55%), Hydrogen (2.949%), Nitrogen (4.15%), and Sulphur (0.312%), with Carbon being the most abundant component of the test Cyanobacterium biomass.

### 3.1.2. Scanning Electron Micrograph (SEM) Studies

SEM has been the main method for determining the fundamental physical properties of adsorbent surfaces. The SEM of the unloaded biomass is heterogenous in nature with irregular surface having grooves and cavities (Figure 2a) which facilitates the adsorption of dyes on the cell wall of non-viable *Oscillatoria* sp by interacting with surface functional groups. The micrographs of dye loaded *Oscillatoria* sp. (Figure 2b) showed the reduction in number of pores and thus surface area is available for further adsorption.




A.



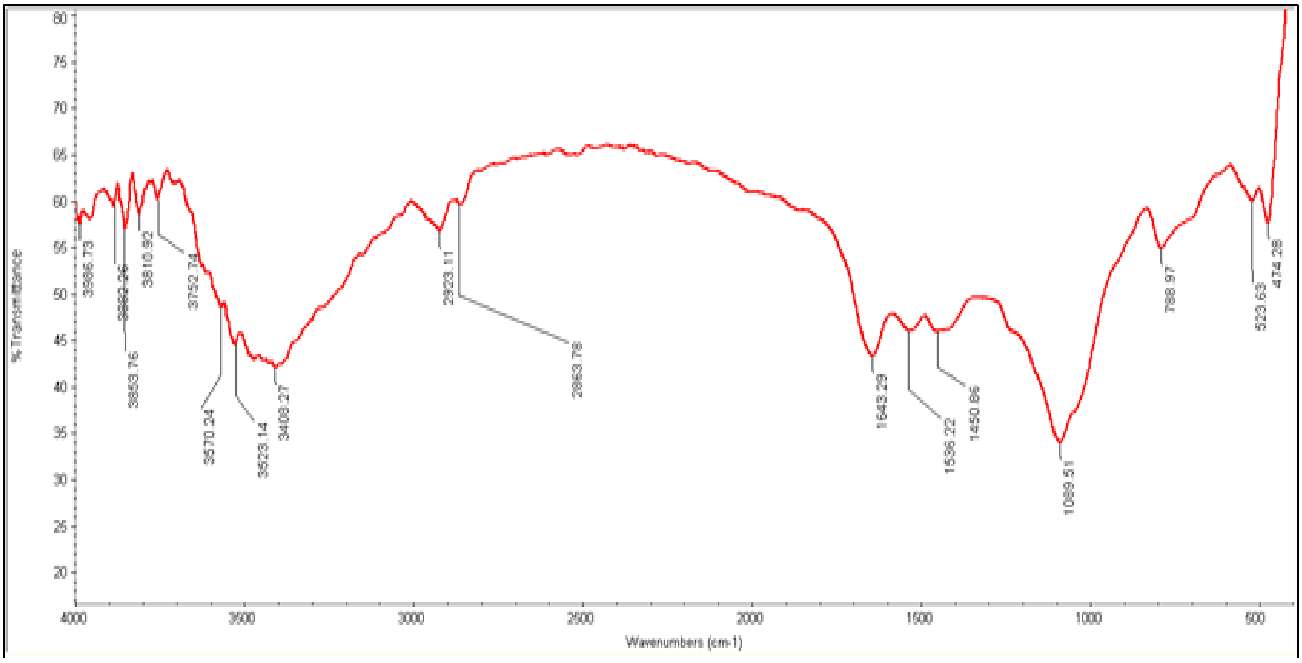

B

**Fig 2:** SEM micrographs for (a) Unloaded *Oscillatoria* sp. and (b) Alizarin loaded *Oscillatoria* sp.

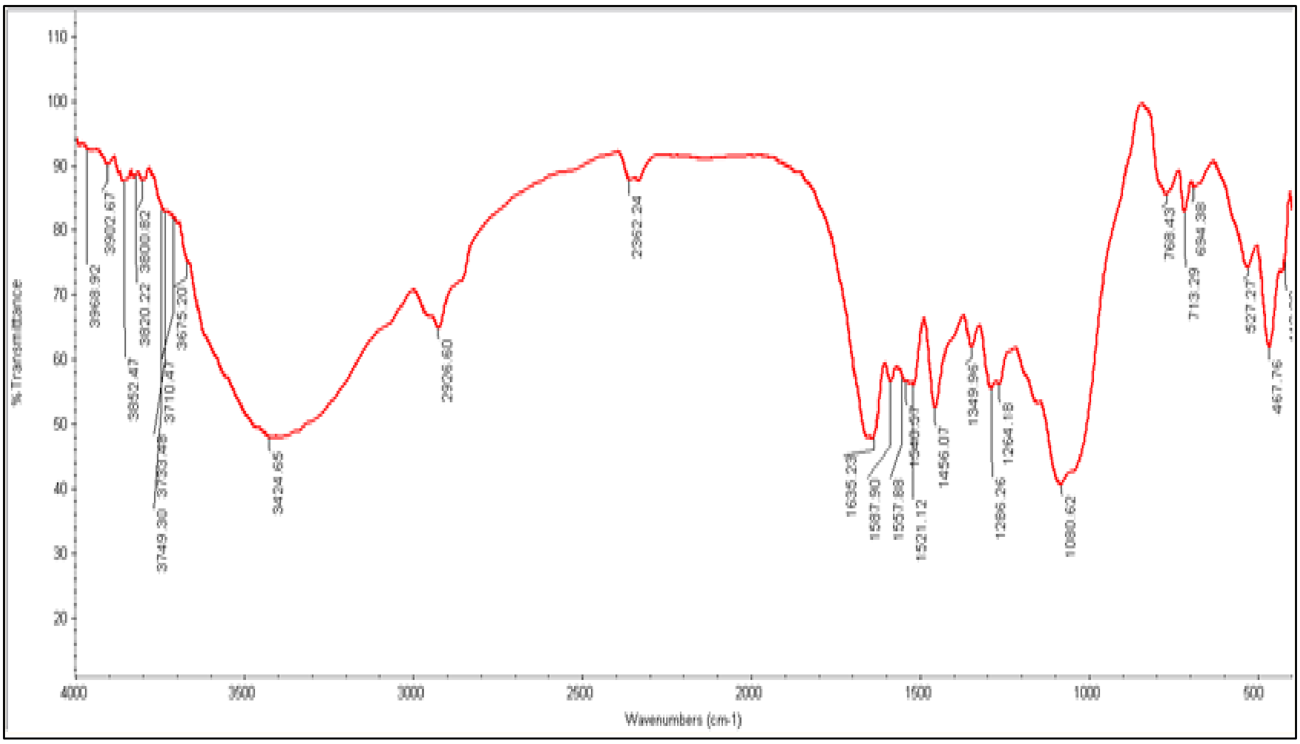
### 3.1.3. Fourier Transform Infrared Spectral Studies

This method was employed to study the surface binding mechanism of non-viable cyanobacterium *Oscillatoria* sp. and to identify the functional groups present on the surface of biomass that are responsible for the adsorption of dyes. The FTIR spectra of the native and the Alizarin dye loaded biomaterial *Oscillatoria* sp. was recorded in the range 4000-400 $cm^{-1}$. In the spectrum of unloaded biomass (shown in Figure 3a), the most prominent bands appeared at 3408.27, 2923.11, 1643.29, 1536.22, 1450, 1089.51, 523.63, and 474 $cm^{-1}$. The adsorption peak at 2923.11 $cm^{-1}$ is created by carboxylic and phenolic stretching bands, but the strong band at 3408.27 $cm^{-1}$ could be assigned to O-H groups of glucose or NH groups of proteins. Other significant bands can be assigned as follows: 1643.29 $cm^{-1}$ (C=O Chelate stretching of carboxylic group), 1089.51 $cm^{-1}$ (-CN stretching), and several bands in the finger print region (523.63 $cm^{-1}$ and 474 $cm^{-1}$) can be assigned to the phosphate groups.

The FTIR results were similar to those reported in the literature for the cyanobacterium *Spirulina* [20] and brown marine macro alga *Sargassum muticum* [21]. The jerking of the band was identified after the dye adsorption on the algal biomass (Figure 3b). This shift in the wavelength showed that there was a dye binding process taking place on the surface of the alga. Thus the major mechanism of dye adsorption is found to be the ionic interactions and complex formation between the dye ions and ligands contained within the structure of the adsorbent.




(a)




(b)

**Fig 3:** Fourier Transform Infrared spectra of (a) Unloaded *Oscillatoria sp.* (b) Alizarin dye loaded *Oscillatoria sp.*

**Table 1:** IR adsorption bands and corresponding possible functional groups present on non-viable cyanobacterium *Oscillatoria* sp.

| Native *Oscillatoria* sp. (wavenumber in $cm^{-1}$) | Alizarin dye loaded *Oscillatoria* sp. (wavenumber in $cm^{-1}$) | Bonds associated with Functional groupings |
|---|---|---|
| 3408.27 | 3424.65 | Carboxylic/OH stretch and N-H stretch |
| 2923.11 | 2926.60 | Phenolic/ carboxylic |
| 1643.29 | 1635.23 | =C=O stretch, >C=C, >C=N, Amide I band |
| 1536.22 | 1587.20 | Amide II band |
| 1450.86 | 1456.07 | Symmetric bending of $CH_3$ of the acetyl moiety |
| 1089.51 | 1050.02 | ≡C-N< |
| 523.63 | 527.27 | C-N-S scissoring |

### 3.2. Factors impacting Alizarin dye adsorption

The batch experiments were run under different conditions to observe the impact of various operational parameters, *viz*., Contact time, adsorbent dosage, pH of the solution, and initial dye concentration, for the adsorption of Alizarin dye onto non-viable *Oscillatoria* sp., The optimization of these variables can be extrapolated to a large-scale dye removal treatment technique from industrial wastewater.

#### 3.2.1. Contact time Impact

In adsorption-based wastewater treatment, the contact period between the dye and the adsorbent is crucially important. The effectiveness of the adsorbent is indicated by the quick elimination of contaminants and establishment of equilibrium in a short period of time. As a function of contact time, the adsorption of Alizarin at four concentrations (50, 100, 150, and 200 mg/L) on non-viable *Oscillatoria* sp. Was investigated in order to identify the equilibration period for maximal absorption. Figure 4 depicts the adsorption seen with the test dye after a consistent interval of time. It was found that the dye adsorption was quick, reaching its maximum absorption in 120 minutes. After 120 minutes, the adsorption rate slowed dramatically to a plateau, indicating equilibrium in the system. The decrease in adsorption rate as a function of time indicates a gradual approach to equilibrium, most likely via internal diffusion [22]. Consequently, the adsorption of dye occurred in two discrete stages, the first of which was rather rapid and the second of which was slower [23]. Jiang *et al*. [24] obtained a similar outcome for the elimination of Basic violet 14 dye from aqueous solution using bentonite.

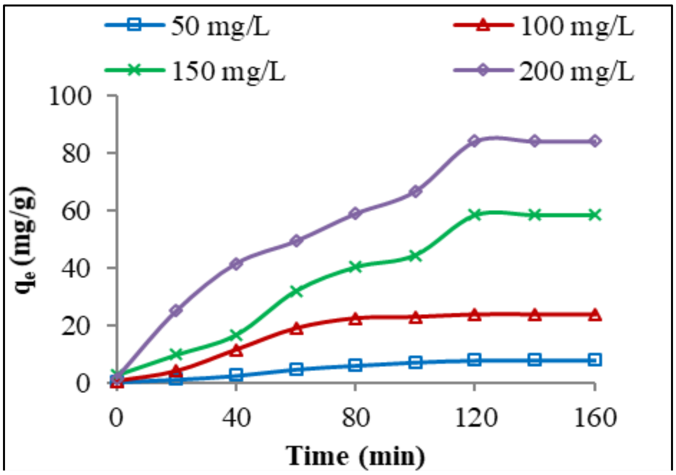



**Fig 4:** Contact time impact on the adsorption of Alizarin dye on non-viable *Oscillatoria* sp. from synthetic aqueous solutions.

#### 3.2.2. Solution pH Impact

One of the key parameter having an impact on the adsorption process is the pH of the dye solution, which affects the ionization state of the adsorptive molecule and the adsorbent's surface characteristics [25]. Alizarin, a test dye, was used in four different concentrations (50, 100, 150, and 200 mg/L) and the solution's pH was optimized by varying the range from 1 to 6. Figure 5 shows that the equilibrium adsorption of Alizarin dye on alga steadily reduced between pH 1 and pH 6, implying that the optimal pH for the removal of alizarin dye onto non-viable cyanobacterium *Oscillatoria* sp. is pH 1. This may be because Alizarin preferentially adsorbed [26] when $H^+$ concentration and its mobility were both high. When the system's pH is raised, more negatively charged sites become available while fewer positively charged ones go. Negatively charged spots on the adsorbent's surface repelled the dye electrostatically, therefore they were less effective at absorbing the colorant [27].

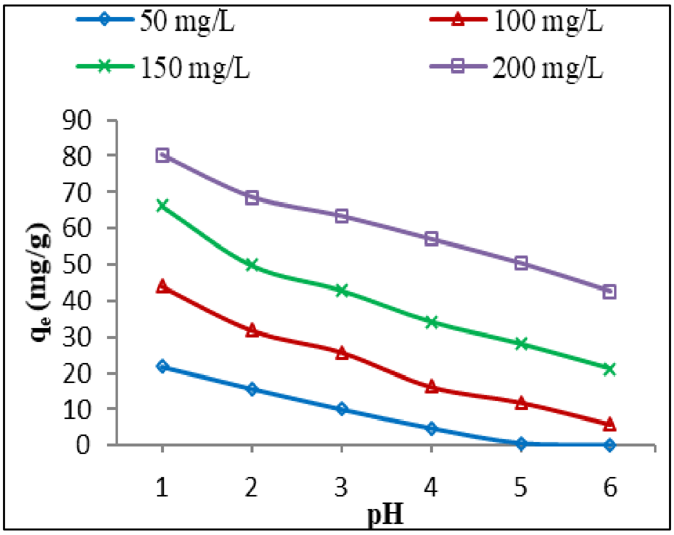



**Fig 5:** Impact of pH on adsorption of Alizarin dye on non-viable *Oscillatoria* sp. from synthetic aqueous solutions.

#### 3.2.3. Adsorbent dose Impact

The dose was optimized by adjusting the adsorbent quantity (1 to 10g/L) at four distinct concentrations, while holding other parameters, such as pH, contact time, and temperature, constant. As shown in Figure 6, the influence of the adsorbent dose on the extent of Alizarin removal at optimal pH 1 suggests that there was a slight change in the extent of Alizarin dye adsorption when the adsorbent dose exceeded 4g/L. At a low dose, all types of sites are completely exposed, and the adsorption on the surface becomes saturated more quickly, resulting in a greater qe value. At increasing doses, however, the availability of higher-energy sites declines as a greater proportion of lower-energy sites get occupied, resulting in a lower $q_e$ value. Therefore, the optimal dose of 4g/L is used for all trials. Similar results have been obtained by other researchers and they have linked the reduction in sorption capacity with increased adsorbent dose at constant dye concentration, to the saturation of adsorption sites caused by particle interactions such as aggregation or partial overlapping [28-29].

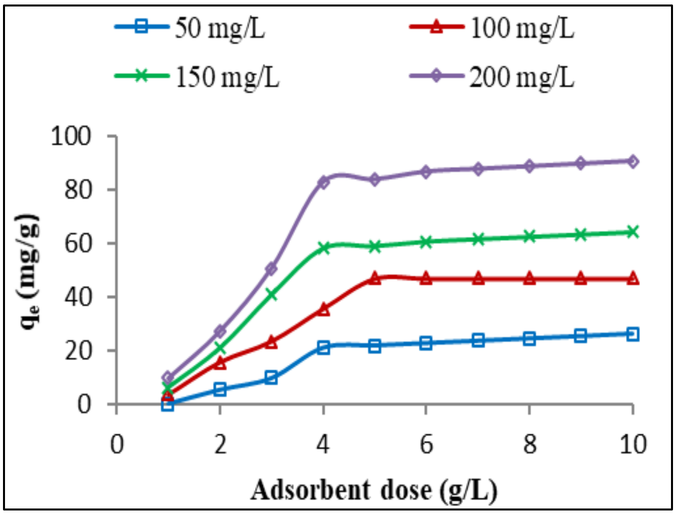



**Fig 6:** Adsorbent dose impact on the adsorption of Alizarin dye on non-viable *Oscillatoria* sp. from synthetic aqueous solutions.

### 3.2.4. Solution Temperature Impact

At three temperatures, 298 K, 308 K, and 318 K, the influence of temperature on the adsorption of Alizarin dye was investigated (Figure 7). At 318K, the highest levels of dye uptake were achieved, indicating that adsorption is endothermic. Figure 7 shows that a rise in Alizarin dye's maximum adsorption from 298K to 318K raises the dye's adsorption capacity from 60.95 to 89.83 mg/g. This phenomenon is associated with the enhanced reactivity between the dye molecule and the surface functional group, which results from the increased mobility of the dye molecule. It has been shown that *Trichoderma viride's* maximum adsorption capacity for MB removal occurs at higher temperatures [30]. Since the adsorbent loses its property due to denaturation at higher temperatures, the adsorption diminishes as the temperature is raised further. *Spirulina platensis* micro and nanoparticles were also found to be effective at removing synthetic colours from water similarly [31]. The information gleaned from these plots was then applied to the Adsorption isotherms and thermodynamic analyses of adsorption.

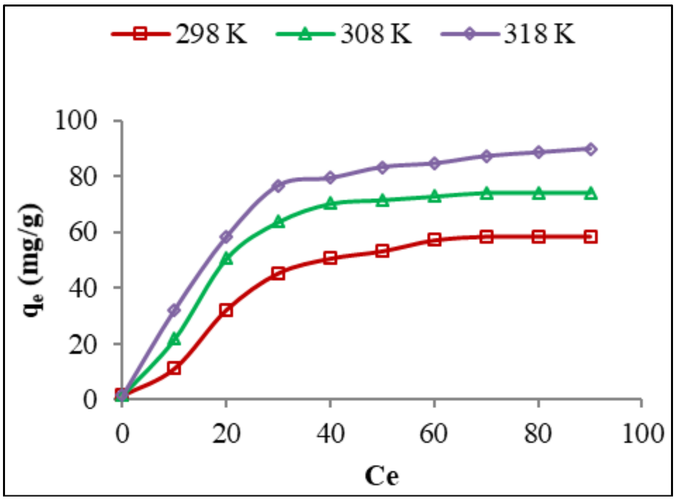



**Fig 7:** Temperature impact on the adsorption of Alizarin dye on non-viable *Oscillatoria* sp. from synthetic aqueous solutions.

### 3.3. Chemical Pretreatment Studies

Physical and chemical surface modification techniques can be utilized to enhance adsorption capacity and efficiency. Chemical pre-treatments, such as washing biomaterial with acid, alkali, or organic solvents, are a simple and effective procedure. In this communication, the effect of different chemical (HCl, $HNO_3$, NaOH and Ethanol) pretreatments is investigated to develop a modified form of cyanobacterial biomaterial and their potential is examined for Alizarin dye adsorption. Figure 8 depicts that the hydrochloric acid (HCl) pretreatment increased the adsorption capacity of alizarin dye on cyanobacterial biomass significantly. Pretreatment with strong mineral acids like Hydrochloric and nitric acid results in the protonation of the amino group in the algal biomass [32] and the excess of $H^+$ ions in the algae cell wall may compete with the dye for surface active sites of the adsorbent.

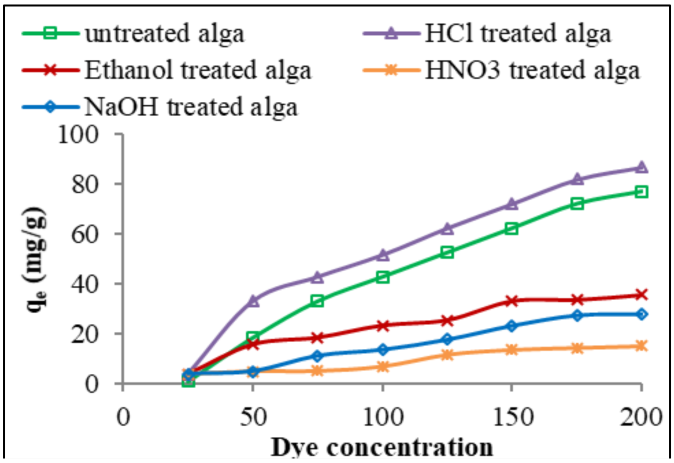



**Fig 8:** Chemical pretreatment impact on the adsorption of Alizarin dye on non-viable *Oscillatoria* sp. from synthetic aqueous solutions.

### 4. Conclusions

The outcomes obtained suggest that the non-viable cyanobacterium *Oscillatoria* sp. could be a useful, cost-effective adsorbent. Contact time, pH, initial adsorbate concentration, temperature, and adsorbent dose all play a role in the adsorbent's ability to remove the dye. Within 120 minutes of interaction between the biomass and dye system, the maximum removal occurred due to the adsorption process. Results were best at 318 K with an initial dye concentration of 200 mg/L, an adsorbent dosage of 4 g/L, and a pH value of 1. Scanning electron micrographs were taken for the unloaded and Alizarin dye-loaded biomaterial showed indications of an adsorption process on the adsorbent's surface. Alizarin dye was shown to be bound to the adsorbent surface via -COOH, -OH, and $-NH_2$ functional groups, as determined by Fourier transform infrared spectroscopy (FTIR). Studies on the chemical alteration of algal biomass found that algal biomass that had been treated with acid had a greater potential to adsorb dye than untreated algal biomass.

### 5. Acknowledgement

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### 6. Conflict of Interest

The author declares no conflict of interest.

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