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# POTENTIAL OF *NOSTOC MUSCORUM* FOR THE DECOLORIZATION OF TEXTILE DYE RGB-RED

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## ABSTRACT

*Nostoc muscorum,* a dominant kind of cyanobacterial bloom was found as a viable biomass for decolorization of synthetic RGB-Red dye. Batch studies revealed the capacity of algal species in dye degradation which was dependent on initial pH (2-12), initial dye concentration (10 mg $L^{-1}$ - 50 mg $L^{-1}$), temperature (20 $^{\circ}C$, 30 $^{\circ}C$, 40 $^{\circ}C$) and contact time (5 min-180 min). Maximum growth of algae was observed at 50 mg $L^{-1}$ dye concentration. Optimal pH for maximum decolorization was determined at pH 3. The data obtained fitted well with Langmuir adsorption isotherm. This study confirmed the potential of blue green algae *Nostoc muscorum* to degrade textile dye, suggesting the possibility of its application in developing an oxidation pond system for textile effluent treatment at pilot scale study.

**KEYWORDS:** ***Nostoc muscorum*, RGB-Red dye, optimization, Langmuir adsorption, Bioremediation**

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# INTRODUCTION

Water contamination with synthetic dyes is a severe environmental issue and represents a threat to human well being. Worldwide over 10,000 different dyes and pigments are used in dyeing and printing industries. The total world colorant production is estimated to be 8, 00,000 tons per year and at least 10% of the used dyestuffs enter the environment through wastes[1]. Dye is used to impart color to textiles, paper, leather, and other materials in such a manner that the coloring is not readily altered by washing, heat, light etc. Synthetic dyes are used especially in the textile and dyeing industries because of their ease and their cost effectiveness in synthesis, firmness, high stability to light, temperature, detergent, microbial attack and variety in color compared with the natural dyes[2]. This has resulted in the discharge of highly colored effluents that affect water transparency and gas solubility in water bodies[3]. In the process of dyeing, about 15–20% of the dyes used for dyeing does not bind to the fibers and are lost in the effluent[4]. The textile industry produces large volumes of colored wastewater due to the use of synthetic dyes in dyeing products and low levels of dye fiber fixation[5]. Synthetic dyes have complex molecular structure that contain variety of functional groups which renders them stable against sunlight, oxidizing agents or microbial attack and also are responsible for the toxicity to some aquatic animals[6]. Azo dyes are the main constituents of such pollution because of their wide applicability and usage and therefore these are present in large quantity in industrial effluents[7]. More than 2000 azo dyes are produced annually constituting 50% of total dyes[8]. Reductive cleavage of azo dyes, which comprises about 70% of all dyes used, also results in the production of amines that are mutagenic to human and are also retained in the anaerobic compartment of the lower intestine by intestinal micro flora after ingestion of azo dyes[9]. Therefore, industrial effluents containing dyes must be treated prior to their discharge into the environment. Many physicochemical methods are used for the treatment of these dyes but these methods are limited by many factors like post treatment process and cost[10]. Also these processes require labors and have disposal problems[11, 12]. Hence, in recent year's interest has been focused on different biological treatment systems which are new, economical and involves the use of easily available micro-organisms like algae, bacteria, yeast and fungi from the removal of dyes from wastewater. These biological treatments are gaining interests due to their efficiency, low cost and environment friendly nature[13, 14, 15, 16, 17, 18]. Algae have been shown to be capable of removing color from various dyes through mechanisms such as biosorption, bioconversion and biocoagulation. Algae is considered as promising biosorbents[19] due to its high sorption capacity and available in unlimited amount[20] both in fresh water and salt water. For instance Vishal shah et al.[21] showed that *Phormidium valderianum,* a marine cyanobacteria can remove up to 90% of textile dyes Acid red 119 and Direct black 115 from solutions in the basic pH range. A marine cyanobacteria *Oscillatoria formosa* NTDM02 was used to decolourize Amido black dye[22]. Also, free and immobilized cyanobacteria *Oscillatoria brevis and Westiellopsis prolifica* were used to remove color and other nutrients from dye industry effluents[10]. Some species of *Oscillatoria*, *C. pyrenoidosa* and *C. vulgaris* have ability to degrade azo dyes and decolorize dye wastewater[23, 24, 25, 26]. *Nostoc muscorum* is a nitrogen fixing, photoautotrophic cyanobacteria. RGB-Red dyes contain a monoazo chromophore which gives color to the dye. This azo group is degraded by *Nostoc muscorum* and also the nitrogen present in the azo group is utilized by the algae for its growth. The aim of the present work is to establish the optimum experimental conditions for the algal growth and the maximum decolorization of RGB-Red dye from aqueous medium.

# MATERIALS AND METHODS

### *Algal biomass and growth conditions*

The algal strain *Nostoc muscorum* was cultured in 500 mL CHU-10 media[27] contained in cotton stopped 1000 mL Erlenmeyer flasks at temperature $28^0$ C, providing 24 hrs fluorescent illumination (40watt, white tube light). The basal culture medium and

glassware were sterilized at 1.0546 kg/cm$^2$ pressure and 121$^0$ C for 15 min before inoculation. The algal cells at late exponential growth phase (after 14 days) were harvested by centrifugation at 5,000 rpm for 15 min. In order to remove any algal exudates present in the initial alga pellet, the collected cells were washed two times with sterile deionized water through centrifugation and finally resuspended in CHU-10 media to obtain a concentrated algal suspension. The cell growth was monitored at 665 nm by UV-Visible spectrophotometer (Shimadzu UV-1650).

### *Dye and Chemicals*

All the chemicals used were of highest purity available and of analytical grade. The dye used in this study was RGB-Red dye collected from the textile dyeing industry located at Faridabad, India. The chemical structure and general data of the dye is given in Table 1. A stock solution (200 mg L$^{-1}$) of RGB-Red dye was prepared in distilled water. The dye concentration in supernatant solution was determined by measuring the absorbance with UV- Visible Spectrophotometer (Shimadzu UV-1650) at maximum absorption wavelength ($\lambda_{max}$ = 520 nm).

**Table 1**
***Commercial name and the functional group of the dye used in the study***

| Dye | Commercial name | Type | Chromophore | Chemical structure | Functional group |
|---|---|---|---|---|---|
| RGB-Red | RGB-Red | Reactive | Monoazo | Vinyl sulfone | Monochloro triazine bifunctional dye |

### *Decolorization Assay*

The dye concentration in supernatant solution was determined at characteristic wavelength ($\lambda_{max}$=520) before and after treatment by double beam UV-VIS spectrophotometer (Shimadzu UV-1650). The efficiency of dye removal was expressed as the percentage ratio of decolorized dye concentration to that of initial one.

Color removal (%) = $C_i$-C/$C_i$ x100

where, $C_i$ is the initial concentration of dye and C is the concentration of dye at time t.

The amount of dye bound to the algal biomass was determined using the following formulae

$q_{eq} = (C_o - C_{eq})$ x V/W1000

where, $q_{eq}$ is the amount of dye adsorbed (mg g$^{-1}$), $C_o$ is the initial concentration of dye (mg L$^{-1}$), $C_{eq}$ is the equilibrium concentration of dye (mg L$^{-1}$), V is the volume of the solution (mL) and W is the dry weight of the cells (g).

### *Optimization of parameters*

To evaluate the decolorization efficiency of algal biomass, batch experiments were performed at various initial dye concentration (10 mg L$^{-1}$-50 mg L$^{-1}$), temperature (20 $^{\circ}$C, 30 $^{\circ}$C, and 40 $^{\circ}$C), contact time (5 min-180 min) and pH values (2-11). The pH was adjusted using 0.1N NaOH and 0.1N HCl solutions. The experiments were conducted in 250 mL Erlenmeyer flasks containing 50 mL media, inoculated with homogenized algal inoculums having 0.4 O.D. at 665 nm and required amount of RGB-Red dye.

### *Statistical Analysis*

All the experiments were performed in triplicates and the values are represented as mean ± SD. Repeated measures one way ANNOVA was used to determine the statistical significance during time course studies with RGB-Red dye at $p < 0.05$ using SPSS 20. The adsorption isotherms were tested using regression analysis. The performance of *Nostoc muscorum* for dye removal was also statistically evaluated.

## RESULTS AND DISCUSSION

### *UV-Visible spectra*

Fig. 1 shows a UV-Visible spectrum of RGB-Red dye solution before and after treatment with algae. The spectrum of the dye in the visible region displays main peak at 520 nm. The decrease of the absorbance peak in this figure indicated that there was almost complete removal of dye. This decolorization of dye may be due to the adsorption as well as bioaccumulation of it by the algal cells[28].

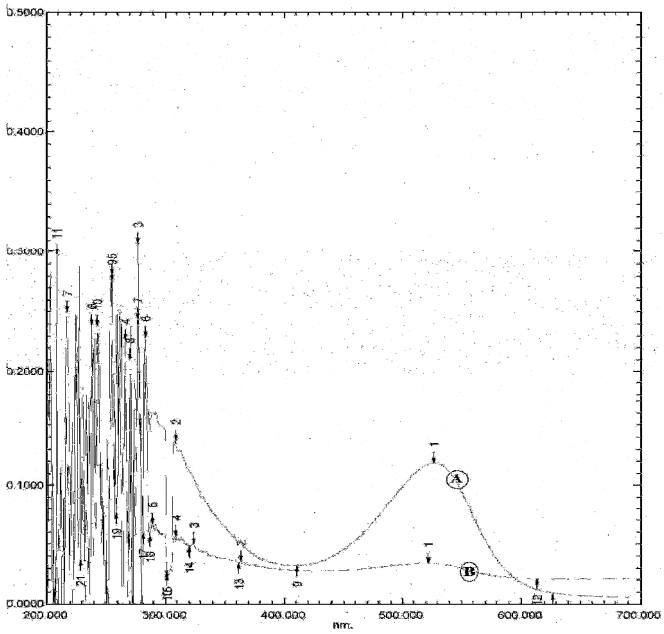


**Figure 1**
***UV-Visible spectrum of RGB-Red dye. A depicts dye solution before treatment and B depicts dye solution treated with algae***

***Effect of Dye Concentration on growth of algae***

The experiment was carried out to evaluate the growth of *Nostoc muscorum* in different concentration of RGB-Red dye (10 mg $L^{-1}$ to 90 mg $L^{-1}$). Fig. 2 depicts that the maximum growth of cells was found at 50 ppm on 6$^{th}$ day which is 1.03 times more than that of control.

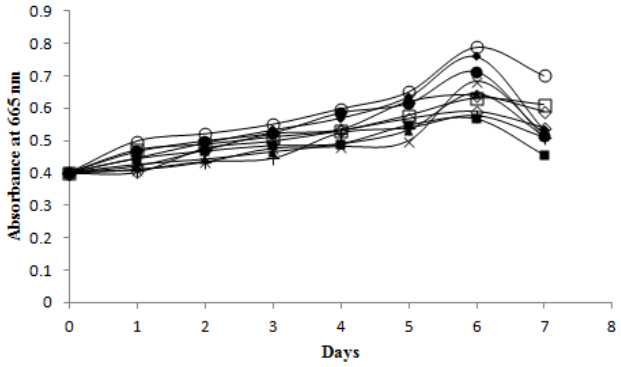


**Figure 2**
***Effect of RGB-Red concentrations on growth. [Algae] = 0.4 OD (♦ control, ■ 10 mg $L^{-1}$, ▲ 20 mg $L^{-1}$, × 30 mg $L^{-1}$, ● 40 mg $L^{-1}$, ○ 50 mg $L^{-1}$, ∆ 60 mg $L^{-1}$, □ 70 mg $L^{-1}$, ◊ 80 mg $L^{-1}$, + 90 mg $L^{-1}$***

***Effect of initial pH on decolorization***

Removal of dye by the blue-green algae is also dependent on the pH of the dye solution[29, 30]. Fig. 3 shows the ability of *Nostoc muscorum* in removal of RGB-Red dye at different pH ranging from 2-11. Algal cells were found dead at pH 2. Highest percentage of dye removal (95%) was attained at pH 3 with an initial dye concentration of 40 mg $L^{-1}$ after 3 h. It showed that an increase in pH from 4 to 11, leads to decrease in decolorization rate. Maximum percent removal of dye at lower pH was because of the electrostatic interaction between negatively charged dye and positively charged protonated functional groups (e.g. carboxyl and amino groups) present on algal cell wall. During the study it was also found that the pH of the solutions below 7 and above 9 was

adjusted in the range of pH 7.5-8.5 after addition of algal inoculums within few hours. This increase and decrease in the pH was may be due to production of metabolites and enzymes by the algae in the medium during the degradation process of dye[22].

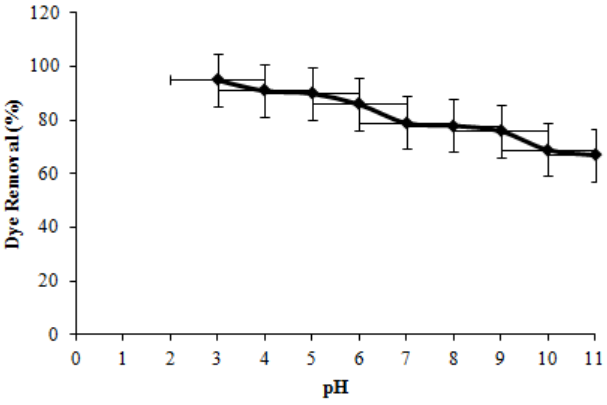


**Figure 3**
***Effect of pH on the decolourization of RGB-Red***
***[Algae]=0.4 OD, [RGB-Red] = 40 mg $L^{-1}$***

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

The effect of contact time on decolorization was carried out to evaluate how quickly the sorption equilibrium was achieved between the algae and the solution they were immersed in. The equilibrium time required to attain maximum sorption (Fig. 4) under static condition was found 30 min due to the rapid adsorption of dye molecule on the surface of algae followed by slower rate of dye decolorization which may be because of further diffusion of the dye molecules from aqueous phase to the solid phase of the algal cells[31]. Results illustrate that the amount of dye bound to the algal biomass ($q_{eq}$) increased with increasing dye concentration and within 30 min equilibrium was achieved. The $q_{eq}$ value increased from 5.97 mg $g^{-1}$ to 31.53 mg $g^{-1}$ of biomass with increase in initial concentration of dye from 10 mg $L^{-1}$ to 50 mg $L^{-1}$. It was also proved by using one way repeated measure analysis of variance that there was a statistical difference ($p > 0.05$) in the uptake of dye by algae at different dye concentration with respect to time.

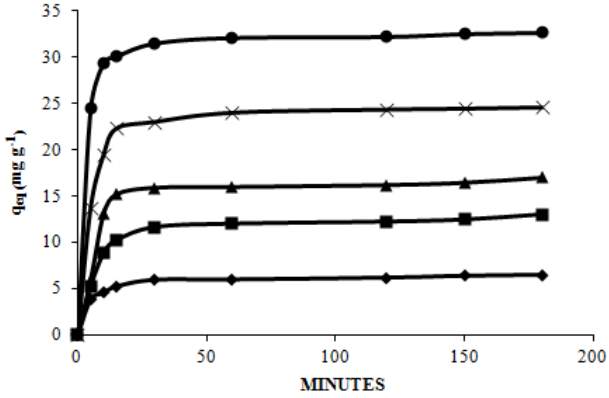


**Figure 4**
***Effect of contact time and initial dye concentration on decolorization of RGB-Red. [Algae]=0.4 OD, pH= 3.0 (♦ 10 mg $L^{-1}$, ■ 20 mg L-1, ▲ 30 mg $L^{-1}$, × 40 mg $L^{-1}$, ● 50 mg $L^{-1}$)***

***Effect of temperature on decolorization***

The effect of temperature on the decolorization capacity of *Nostoc muscorum* was studied at 20 °C, 30 °C and 40 °C. It can be seen in Fig. 5 that the decolorization

efficiency increased from 67% to 83% with an increase in temperature from 20 °C to 30 °C while from at 40 °C there was 40% decline in decolorization capacity after three hours of exposure. This decrease in the color removal efficiency can be attributed to the loss of cell viability at higher temperature[32, 33].

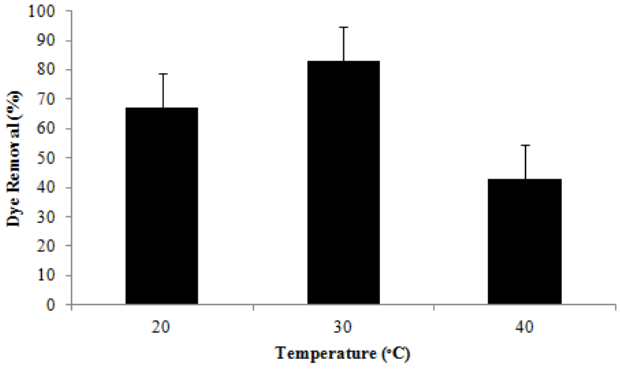


**Figure 5**
***Effect of temperature on decolorization on RGB-Red. [Algae]=0.4 OD, pH=3.0.***

### *Effect of repeated uses*

Repeated batch operations were performed to examine the reusability of *Nostoc muscorum* in the removal of RGB-Red dye. After the four repeated use of *Nostoc muscorum*, its decolorization efficiency was found the same as it showed on the first day (Fig. 6). This may be due to an adaptation effect, since the cells were repeatedly exposed to the dye. The results obtained were similar to that observed in *Cosmarium* species[28].

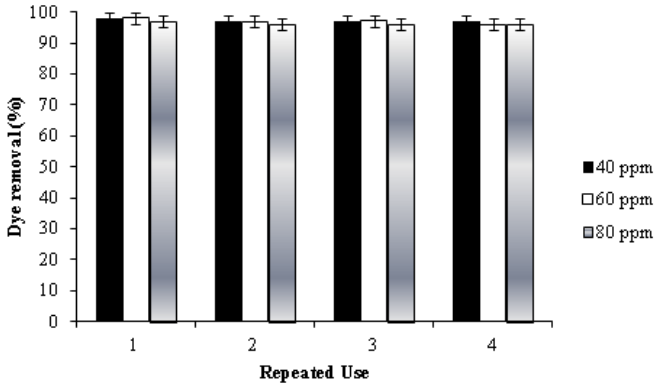


**Figure 6**
***Effect of repeated use of algal biomass on decolorization***

### *Langmuir Adsorption isotherm*

In the present study, the experimental data of RGB-Red dye and algae equilibrium isotherm was established using Langmuir isotherm. Langmuir sorption model projects the maximum uptake values, which could not be attained in the experiments. Langmuir isotherm is expressed by the following equation[34]:

$1/q_{eq} = (1/bq_m)(1/C_{eq}) + 1/q_m$

where, $C_{eq}$ is the equilibrium concentration of dye solution and $q_{eq}$ is the adsorbed dye on the algal cells. $q_m$ and $k_a$ are constants corresponding to the adsorption capacity and rate of adsorption, respectively. Values of $q_m$ and $K_a$ for the adsorption of dye onto the algal cells are determined from the slope and intercept plot of $1/q_{eq}$ vs $1/C_{eq}$.

**Table 2**
***Langmuir isotherm constant for the decolorization of RGB-Red dye by Nostoc muscorum***

| Algae | $q_{max}$ (mg $g^{-1}$) | $K_a$ | $R^2$ |
|---|---|---|---|
| *Nostoc muscorum* | 454.54 | .007 | .9959 |

The adsorption isotherm of the RGB-Red was found to be linear over the entire concentration range and the correlation coefficient ($R^2$) was less than one and greater than zero illustrating that the algae is supportive for the adsorption of RGB-Red dye.

***Statistical assessment of the performance of Nostoc muscorum for RGB-Red dye removal***

Since *Nostoc muscorum* were cultured in different batches at different time, it is necessary to have a statistical assessment of the performance of the *Nostoc muscorum* in terms of precision between batch to batch, repeated use of algae and harvested algal cells for different batches (Table 3). A set of five different batches (A-1, A-2, B-3, B-4, Z-5) were studied to obtain the maximum dye removal capacity for each batch after 5 days of incubation of 50 mg $L^{-1}$ dye concentration with the algae. In precision between repeated use of algae, the dye removal capacity of *Nostoc muscorum* were found to be 470.66 mg $g^{-1}$ and 402.16 mg $g^{-1}$ with maximum relative standard deviation (R.S.D) of 6.21% confirming the reproducibility in repeated uses. However, the precision significantly varied with the third batch of algae which was harvested for different batches at different time as was evident from the high value of R.S.D (20.40%). While comparing the precision between the batch to batch variability, repeated use of algae and harvested algal cells for different batches, the chance of imprecision is possible as the cells were cultured at different time intervals.

**Table 3**
***Precision of experimentally determined dye removal capacity of Nostoc muscorum***

| Name *Nostoc muscorum* | *Nostoc muscorum* | *Nostoc muscorum* | *Nostoc muscorum* | *Nostoc muscorum* |
|---|---|---|---|---|
| mg $g^{-1}$ | mg $g^{-1}$ | mg $g^{-1}$ | mg $g^{-1}$ | mg $g^{-1}$ |
| Batch to batch variability | | | | |
| A-1 447 | A-2 490 | B-3 390 | B-4 410 | Z-5 315 |
| 450 | 495 | 393 | 418 | 309 |
| 453 | 489 | 387 | 415 | 307 |
| Mean 450 | 491.33 | 390 | 414.33 | 310.33 |
| S.D 3 | 3.21 | 3 | 4.04 | 4.16 |
| R.S.D (%) 0.67% | 0.65% | 0.77% | 0.98% | 1.34% |
| Precision between repeated use of algae | | | | |
| A-1 450 | B-3 390 | | | |
| A-2 491.33 | B-4 414.33 | | | |
| Mean 470.66 | 402.16 | | | |
| S.D 29.22 | 17.20 | | | |
| R.S.D (%) 6.21% | 4.28% | | | |
| Precision between the harvested algal cells for different batches | | | | |
| A 470.66 | | | | |
| B 402.16 | | | | |
| Z 310.33 | | | | |
| Mean 394.38 | | | | |
| S.D 80.44 | | | | |
| R.S.D (%) 20.40% | | | | |

## CONCLUSION

In conclusion, the present study showed that the blue green alga *Nostoc muscorum* was able to grow in RGB-Red dye, although its growth was less in control. The amount of dye bound to the algal biomass increased with the dye concentration. Maximum adsorption capacity was found to be 31.53 mg $g^{-1}$ algae at an initial dye concentration of 50 mg $L^{-1}$ in 30 min. About 95% dye was removed at pH 3 after 3 h. Therefore it may be suggested that *Nostoc muscorum* is an efficient source for removal of synthetic dyes from effluents and could be adopted as a cost effective approach for decolorization of dyes by developing oxidative pond at pilot scale.

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