Asian Journal of Environment & Ecology

15(4): 10-19, 2021; Article no.AJEE.70194
ISSN: 2456-690X

Bioremediation of Some Reactive Dyes Commonly used in Fabric Re-dyeing by *Chlorella vulgaris*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ZMS and ASD designed the study and wrote the first draft of the manuscript, author BSA helped with the laboratory analyses. Authors KM and YYM performed the statistical analysis and reviewed the drafted manuscript, while author SI being the team leader supervised the analyses as well as literature searches of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEE/2021/v15i430233
Editor(s):
(1) Dr. Ravi Kant Chaturvedi, Chinese Academy of Sciences, P. R. China.
Reviewers:
(1) A. Doss, RVS College of Arts and Science, India.
(2) Vera Ivanova Kolyovska, Institute of Experimental Morphology, Pathology and Anthropology with Museum (IEMPAM), Bulgaria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/70194

Original Research Article

Received 23 April 2021
Accepted 28 June 2021
Published 06 July 2021

ABSTRACT

Aim: The work was aimed at assessing the potential of *Chlorella vulgaris* in remediation of reactive dyes.
Place and Duration of Study: Department of Biological Sciences, Department of Plant Biology and Department of Biochemistry, Bayero University, Kano, Nigeria, between January 2019 and December 2019.
Methodology: Wastewater containing individual reactive dyes: reactive red 198 (RR198), reactive yellow 176 (RY176), reactive green 19 (RG19), reactive orange 122 (RO122), reactive red 195 (RR195) and reactive violet 1 (RV1) were collected from a local fabric re-dyeing pit at Kofar Na'isa, Kano, Nigeria. The green microalga *C. vulgaris* was cultured in Bold Basal medium (BBM) at 30 ±

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2°C and subjected to adsorption and decolourization assays of the dyes.
Results: The highest dye removal efficiency by enzymatic action was recorded after 48 hours, while that for the biomass adsorption was at day 14, at pH 11.3 and temperature of 30°C. The percentage dye removal by adsorption and decolourization were within the ranges of 68.1-97.8% and 69.8-99.9% respectively. Dye removal decreased with increase in contact time until saturation is attained. Freundlich's isotherm model was best fitted for the adsorption of the dyes with a strong linear correlation coefficient, R^2 ranging from 0.954-0.811. There was a strong linear relationship and high statistical significance among the dyes for both decolourization and adsorption (*P* value; .01).
Conclusion: *Chlorella vulgaris* was found to be effective in the removal of reactive dyes from textile wastewater samples. The results revealed *C. vulgaris* to be a cost-effective and eco-friendly biosorbent that can be used for the treatment of wastewaters containing toxic dyes.

Keywords: Adsorption; Chlorella vulgaris; decolourization; reactive dyes; pollutants; wastewater.

1. INTRODUCTION

Reactive dyes are the most commonly used in textile dyeing due to their binding capacity through covalent bonding with textile fibers and their affirmative individuality such as water-fasting, colour brightness and simple application techniques [1]. About 20-40% of these dyes are lost in the effluent which most of the times is directly discharged untreated into the environment [2,3] .These dyes exhibit a wide variability in chemical structure, primarily based on substituted aromatic and hetero cyclic groups. They possess high solubility in water and thus, their removal from wastewater using conventional coagulation and activated sludge processes becomes very difficult [4].

Chlorella species are spherical/sub-spherical, unicellular, motile and photosynthetic microalgae found in all nutrient-rich aquatic habitats [5]. They are described as freshwater thallophytes rich in chlorophyll content which help in sunlight capturing for energy conversion as such regarded as freshwater pollution microalgae [6,7]. *Chlorella* species grow fast and are able to uptake nutrients and bind to most of the toxic organic pollutants in wastewater [8]. Some organic pollutants in wastewater have been reported to be eliminated by *Chlorella* species [9,10,11]. The removal of dye molecules from textile wastewater by *Chlorella* depends on surface area, toxicity and chemical structure of the dyes [3,12]; they were reported to be capable of removing dyes with very high toxicity [13]. Fazal et al. [14] reported that the species have been effectively used to detoxify dyes and nutrients in textile wastewater, thus, improving wastewater treatment. Acuner and Dilek [15] revealed that several *Chlorella* species such as *C. vulgaris*, *C. pyrenoidosa* and *C. sorokiniana* were capable of detoxifying azo dyes by degrading them to aromatic amines which are subsequently metabolized to simpler organic compounds or carbon dioxide. Previous studies have shown that dye removal by *Chlorella* species is mainly achieved through cellular adsorption or absorption which is dependent on the rate of algal biomass production [16].

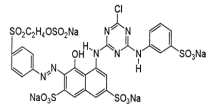
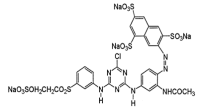
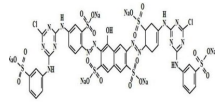
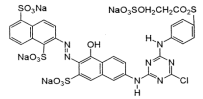
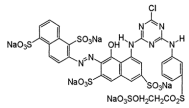
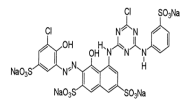
Chlorella vulgaris is a microalga having many structural elements similar to those in higher plants. It is fast growing, survives harsh conditions and resistant to certain invaders. It employs mixotrophic processes to take up organic materials from wastewater during treatment [17]. *C. vulgaris* is regarded as an efficient nutrient remover due to its ability to breakdown and remove toxic contaminants and nutrients in wastewater [18]; it also has the potential to degrade and remove azo compounds and heavy metals from textile wastewater [19]. The treatment of dye wastewater with *C. vulgaris* is an eco-friendly and effective option which recycles nutrients and improves water quality [18].

2. MATERIALS AND METHODS

2.1 Dyes

Wastewater containing individual reactive dyes (reactive red 198 (RR198), reactive yellow 176 (RY176), reactive green 19 (RG19), reactive orange 122 (RO122), reactive red 195 (RR195) and reactive violet 1 (RV1)) were collected in sterilized sampling bottles from a local fabric re-dyeing pit at Kofar Na'isa, Kano, Nigeria, immediately after removing the dyed cloth. The chemical characteristics of the dyes are presented in Table 1.

Table 1. Chemical characteristics of the dyes used in the study

Dye	Chemical Constituent	Molecular Weight (g/mol)	Molecular Formula	Molecular Structure	Wavelength (nm)	CAS Registry Number
RR198	Azo, Sulphonic, Imino, Halogen (Chloride) and Vinyl Sulphone groups	984.21	$C_{27}H_{18}ClN_7Na_4O_{16}S_5$		526	145017-98-7
RY176	Azo, Sulphonic, Imino, Halogen (Chloride) and Amide groups	1025.26	$C_{29}H_{21}ClN_8Na_4O_{16}S_5$		309	140876-15-9
RG19	Azo, Sulphonic, Amino, Imino and Halogen (Chloride) groups	1418.94	$C_{40}H_{23}Cl_2N_{15}Na_6O_{19}S_6$		651	61931-49-5
RO122	Azo, Sulphonic, Imino and Halogen (Chloride) groups	1034.27	$C_{31}H_{20}ClN_7Na_4O_{16}S_5$		542	12220-12-1
RR195	Azo, Sulphonic, Imino and Halogen (Chloride) groups	1136.32	$C_{31}H_{19}ClN_7Na_5O_{19}S_6$		542	93050-79-4
RV1	Azo, Sulphonic, Imino and Halogen (Chloride) groups	926.54	$C_{25}H_{13}Cl_2N_7Na_4O_{14}S_4$		537	12239-45-1

2.2 Preparation of *C. vulgaris* Biomass

The green microalga, *C. vulgaris* was obtained from Plant Biology Department of Bayero University, Kano, Nigeria. It was cultured in Bold Basal medium (BBM) in 250 ml conical flasks at 30 ± 2°C [20]. The pH of culture media was adjusted to 7 before being sterilized by autoclaving (Laboratory autoclave Series 2100) [21]. After cooling, the *Chlorella* isolate was inoculated and incubated at 30°C on a shaker (Innova 4000) at 150 rpm; an air pump (Shining Beach SB660) and fluorescent light with intensity of 2500 lumens (light/dark cycle of 12:12 hours) were also introduced to provide aeration and light respectively. The microalgal cells were harvested after fourteen days (at the log phase of growth) by centrifuging at 1800 rpm for 15 minutes, subsequently; the cells were washed thoroughly with distilled water. A constant number of cells determined by counting using a Neubauer chamber (Marienfeld) [22] were used for the biosorption study.

2.3 Adsorption and Decolourization Assays

In this test, 4.47×10^7 cells/ml of the *C. vulgaris* were placed in individual test-tubes containing 1.0 ml of wastewater (separate for each dye – RR198, RY176, RG19, RO122, RR195 and RV1) and 5.0 ml of distilled water. The initial absorbance of the solution was taken after mixing with an auto-vortex mixer and incubated at 30°C. The absorbance of the mixture was recorded using a spectrophotometer at 650 nm within 14 days. The amount of dye adsorbed per gram (Qe) of microalgal biomass and percentage adsorption of the dye by the algal cells were calculated using equations 1 and 2 respectively [23,24].

$$Biosorption\ (\%) = \frac{(A-B)}{A} \times 100 \quad (1)$$

$$Qe = A - B \times \frac{V}{M} \quad (2)$$

Where,

Qe = Concentration of dye at equilibrium

A = Initial concentration of dye in solution

B = Final concentration of dye in solution

V = volume of solution in millilitre, and

M = quantity of biomass.

The Bold Basal medium from which the cells for the adsorption were removed was centrifuged at 10,000 rpm for 10 minutes and 9.0 ml of the supernatant was dispensed into six sterile test-tubes, to each of which 1.0 ml of the respective dye wastewater was added and stirred. The initial absorbance of the test solution was measured at 650 nm and then, incubated at 30°C. Subsequently, absorbance readings were recorded at intervals of 24 hours for 14 days. Percentage biodecolourization of the dye by enzyme activity was calculated according to Ozsoy et al. [23];

$$Biodecolourization\ (\%) = \frac{(A-B)}{A} \times 100$$

Where,

A = Initial concentration of the dye in solution (1.0 ml of dye wastewater and 9.0 ml of supernatant)

B = Final concentration of dye in solution after enzyme activity

Freundlich's isotherm was used to elucidate the adsorption process. A graph of log Qe against log B was plotted [25]. All experiments were performed in triplicates and the numerical values were expressed as mean ± standard error and analyzed by one-way analysis of variance (ANOVA) using Microsoft Excel 2007. Results were considered significant when $P < .05$.

3. RESULTS AND DISCUSSION

The percentage decolourization is presented in the Figs. 1 and 2. The highest percentage decolourization was recorded after 48 hours of inoculation, after which, there was a significant declination in the percentage in subsequent days and after the fourteenth day the percentage became constant.

The alga (*Chlorella vulgaris*) was able to decolourize all the six reactive dyes from the wastewater samples. Similar findings were reported by El-Sheekh et al. [26] who suggested that *C. vulgaris* possess an azoreductase enzyme which may be responsible for the decolourization of a variety of azo dyes within a short period of time. Cheriaa et al. [27] isolated a new *Chlorella* species and cultivated it in different textile dyes, reporting variability in the decolourization with respect to the dye type. Ishchi and Sibi [28] stated that *C. vulgaris* has the potential to effectively degrade and

decolourize a wide variety of azo containing dyes (reactive, direct and disperse dyes) at optimized conditions and as such can be beneficial for treating textile industrial effluents. El-Kassas and Sallam [29] also reported that *C. vulgaris* has the potential to decolourize textile wastewater containing azo dyes to a percentage of 75.68% using moderate wastewater concentration. In this respect, Acuner and Dilek [15] reported that several species of *Chlorella* such as *C. vulgaris*, *C. pyrenoidosa,* etc. were capable of degrading azo dyes to their aromatic amines and to further metabolize the aromatic amines to simpler organic compounds or CO_2, thus, detoxifying them.

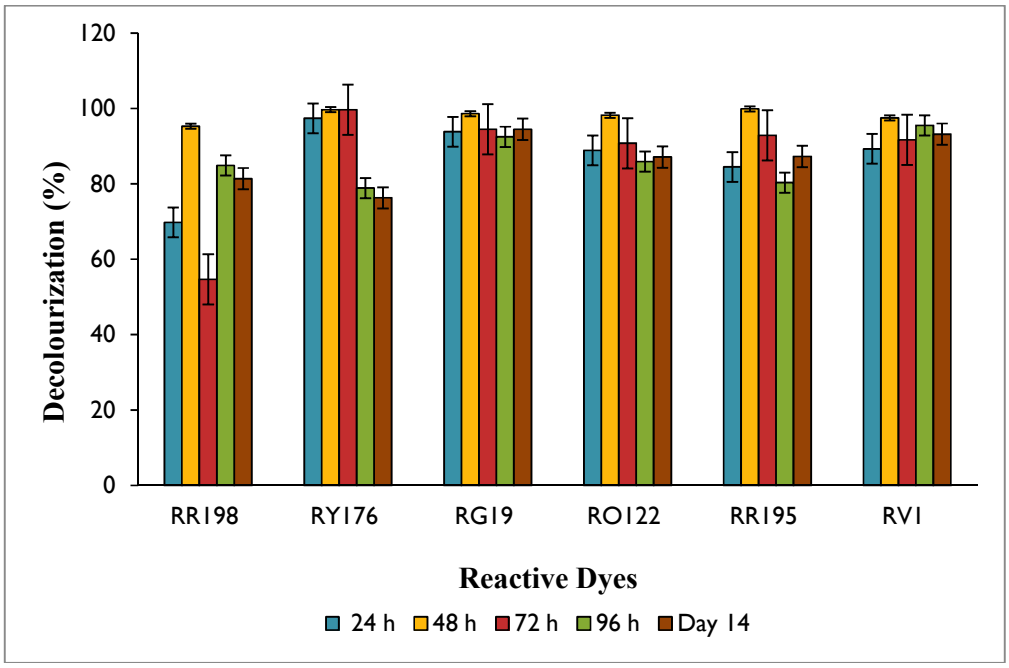


Fig. 1. Percentage decolourization of reactive dyes from wastewater by *Chlorella vulgaris*
There is statistical significant difference in the adsorption of dyes as P < 0.05 (P = 0.01).
Mean ± S.E.M = Mean values ± Standard error of means of six experiments

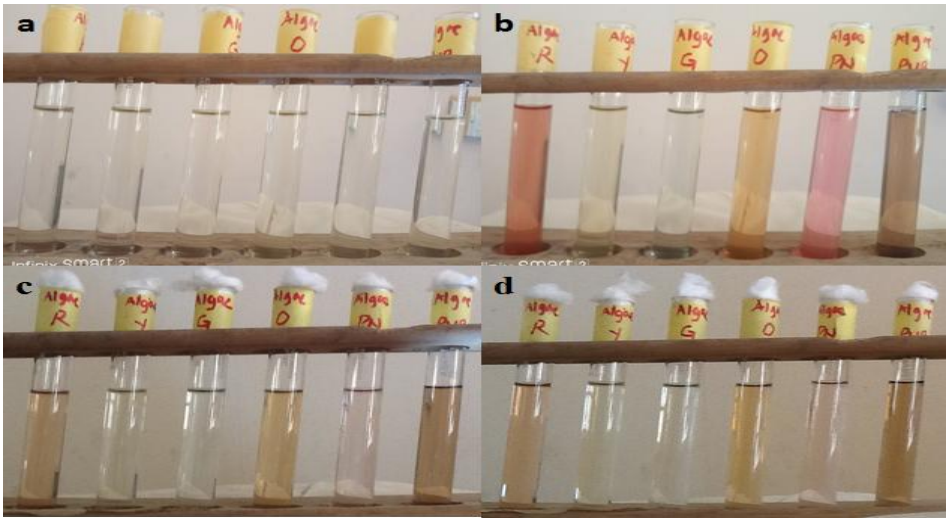


Fig. 2. Algal supernatant a) Before biodecolourization assay; b) Inoculated with dyes; c) 24 hours after inoculation and d) 48 hours after inoculation

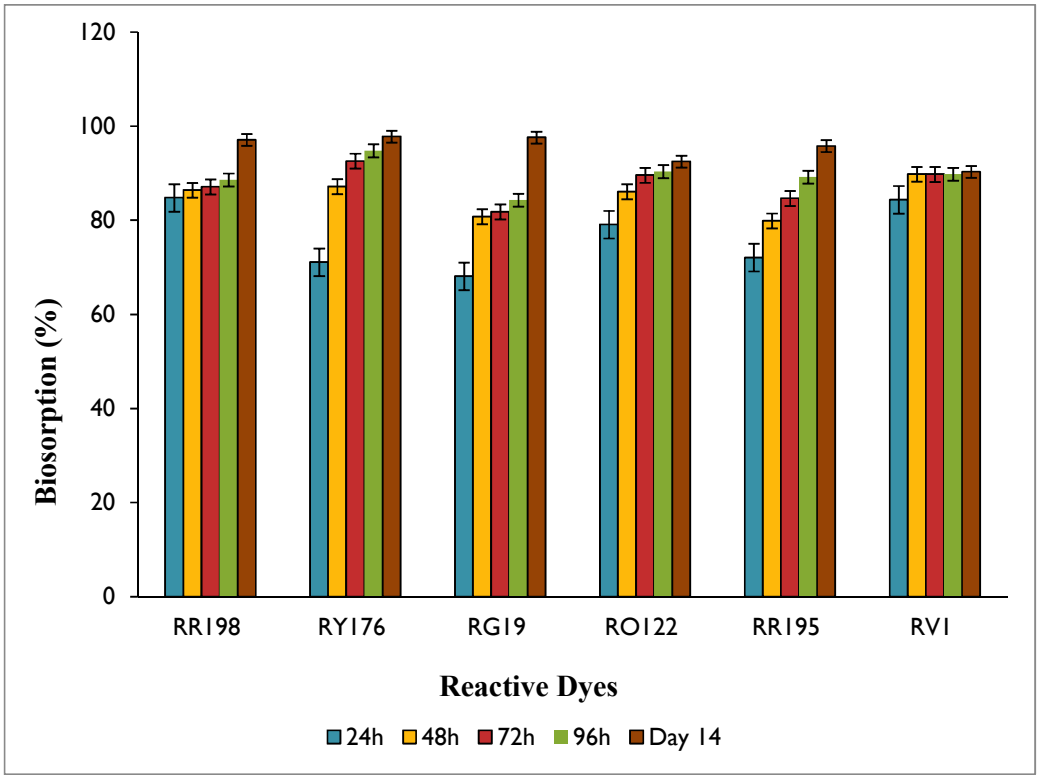


Fig. 3. Percentage adsorption of reactive dyes from wastewater by *Chlorella vulgaris*
There is statistical significant difference in the adsorption of dyes as $P < 0.05$ ($P = 2.54 \times 10^{-5}$).
Mean ± S.E.M = Mean values ± Standard error of means of six experiments

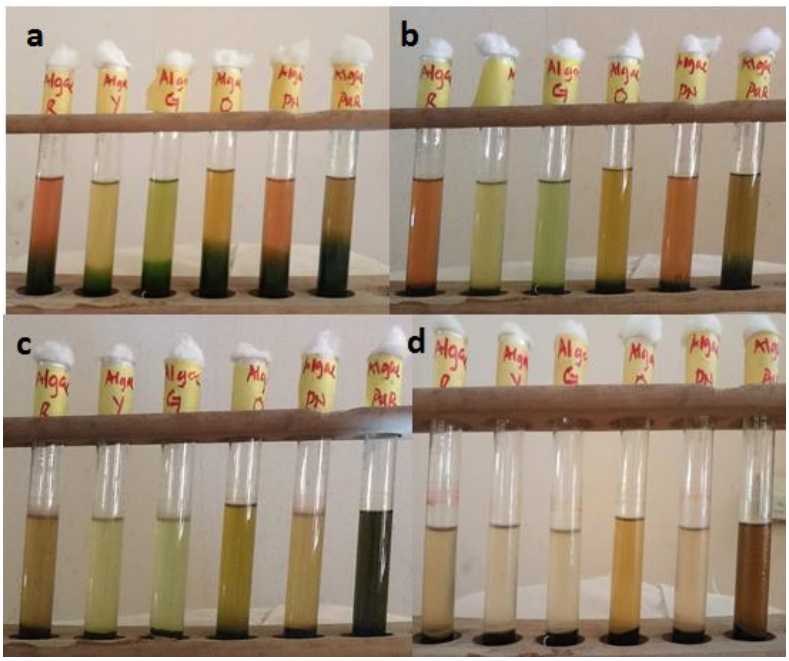


Fig. 4. a) *Chlorella* biomass inoculation in dyes (0 hours) b) Adsorption after 24 hours c) Adsorption after 48 hours d) Adsorption after reaching saturation level (at day 14)

The percentage adsorption of the dyes from the wastewater samples by *C. vulgaris* is presented in Fig. 3 and Fig. 4. The adsorption was observed to increase gradually but after 14 days, a significant declination in the adsorption was observed. It was also observed that adsorption efficiency increases with increase in contact time, after which saturation is attained. This is also in line with findings of Pratiwi et al. [30] who used microalga *Ulva lactuca* to adsorp methylene blue dye. Kumar et al. [31] stated that adsorption rate increases with time and on reaching complete saturation adsorption rate becomes constant. *C. vulgaris* was reported by Lim et al. [32] to decolourize textile wastewater within a percentage range of 4-50% within 10 days. The dye removal may be attributed to the accumulation of dye ions on the surface of algal biopolymers and diffusion of the dye molecules from aqueous phase onto solid phase of the biopolymer [29,33]. Daneshvar et al. [34] stated that the ability of algae to decolourize substances is due to three mechanisms which involve assimilation and utilization of chromophores for the production of algal biomass, carbondioxide and water, transformation of coloured molecules to non-coloured molecules and adsorption of chromophores on algal biomass. In another study by Pathak et al. [35] *C. pyrenoidosa* decolourized methylene blue at a percentage above 90, while Seo et al. [36] reported 99.9% using a different *Chlorella* species on the same dye. El-Kassas and Sallam [29] cultivated *C. vulgaris* in textile wastewater, and observed that it could reduce its chemical oxygen demand (COD) level as well as decolourize it. The decolourization is usually achieved through biosorption followed by bioconversion and biocoagulation [37]. The potential of *Chlorella* species as biosorbents is because of their availability in both fresh and salt water, with their relatively high surface area and high binding affinity [38]. Algal biosorption of dye substituent occur as a result of electrostatic attraction and complexation of the cell wall [39]. The result of this study agrees with the findings of previous studies on dye remediation with *Chlorella* species, even though variation occurs in accordance with the nature and type of dyes. Previous studies have also revealed that the mechanism of colour removal by *Chlorella* sp. is mainly via biosorption which depends on the rate of algal biomass production [16]. *C. vulgaris* have the potential to rapidly, efficiently and effectively remove reactive dyes (such as Remazol Red and Remazol Golden Yellow) and other dye types to very low concentrations by accumulating and degrading them through adsorption [40,41]. *C. vulgaris* was reported to have high tolerance to azo dyes, having the potential to grow in the presence mono-azo dyes [15]. The ability of *C. vulgaris* to break down the azo bond which resulted in about 50.0% colour removal has also been observed by Lim et al. [32] in their study for treatment of textile wastewater with the microalgal strain. *C. vulgaris* can be effectively employed to bioremediate dyes and nutrients in textile wastewater in order to potentially improve wastewater treatment. [14] The results indicate that enzymatic removal of dyes occur within short periods of time while, the use of biomass may take long periods, even though it revealed high effectiveness.

The numerical values of the Freundlich's model constants K_f and N are presented in Table 2. RV1 had the highest K_f value while RY176 had the least.

The Freundlich's constants (K_f and N) explained the adsorption capacity as well as intensity exhibited by the algal biomass [42,43]. The values obtained, show the adsorption to be a multilayer type which happens at numerous sites on the algal surface occurring gradually until complete saturation is attained [43,44,45].

Table 2. Freundlich's isotherm constants for adsorption of reactive dyes by *Chlorella vulgaris*

Dye	N	K_f (Lmg^{-1})	R^2
RR198	0.075	14.626	0.880
RY176	0.075	14.459	0.828
RG19	0.099	17.143	0.811
RO122	0.176	17.523	0.954
RR195	0.130	16.409	0.897
RV1	0.105	20.799	0.908

4. CONCLUSION

Chlorella vulgaris was found to be effective in the removal of reactive dyes from textile wastewater samples. Maximum dye removal in all the dyes with respect to enzymatic action was observed at 48 hours at temperature and pH of 30°C and 11.3 respectively while the adsorption equilibrium was attained at the fourteenth day. Experimental data fitted well to the Freundlich's isotherm model, signifying that a multilayer adsorption occurred due to infinite sites of adsorption on the surface of the C. vulgaris. At the optimal operating conditions and dye concentration of 0.1 g/ml, 69.8-99.9% dye decolourization was achieved, while the adsorption was achieved at a percentage range of 68.1-97.8%. There was a strong linear relationship and statistically significant difference ($P < 0.05$) among the dyes for both decolourization and adsorption. The results revealed *C. vulgaris* to be a cost-effective and eco-friendly biosorbent that can be used for the treatment of wastewaters containing toxic dyes.

ACKNOWLEDGEMENTS

The Authors acknowledge the assistance and dedication of laboratory technologists of the Departments of Biological Sciences, Microbiology, Plant Biology and Biochemistry, Bayero University, Kano, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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