



# Biosorption and desorption of chromium using hybrid microalgae-activated sludge treatment system

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## Abstract

This study utilised a mixed culture of *Chlorella vulgaris* and bacteria from sludge to treat synthetic tannery wastewater (STWW) in modified stirred-tank photobioreactors (MSTPBRs). The MSTPBRs were fabricated locally and operated at irradiance value of 580  $\mu\text{mol}/\text{m}^2\text{s}$  supplied by red light-emitting diodes at 12:12 light–dark cycles and  $100 \pm 1$  rpm continuous stirring. In each case, 50, 100 and 150 mg/L concentrations of STWW were inoculated with mixed culture of microalgae and bacteria in three MSTPBRs, with the control MSTPBR operating at 50 mg/L of STWW. Chromium concentrations were measured using colorimeter whilst Fourier transform infrared spectroscopy (FTIR) indicated possible Cr biosorption. Maximum Cr (VI) and total Cr removal efficiencies of 93 and 94% were achieved, with more than 78% total Cr recovery. Results from FTIR suggested involvement of *Chlorella vulgaris* in the Cr biosorption. The hybrid microalgae-bacteria system efficiently treated tannery wastewater with considerable Cr removal efficiencies. The potentials of the system in treating tannery wastewater in larger scale may require further investigation.

**Keywords** Adsorption · Hexavalent chromium · *Chlorella vulgaris* · Illumination · Chromium recovery

## Introduction

Huge amount of wastewater is produced during tanning operations and this generates about 85% of the initial water used as wastewater (Lofrano et al. 2013; Sawalha et al. 2019). The wastewater which is usually discharged into the environment causes serious environmental pollution which affects the entire ecosystem. The wastewater produced in tanning cycles usually contains considerable amounts of chemical oxygen demand (COD) or biochemical oxygen demand (BOD), tannins, inorganic and organic compounds such as sulphides, chlorides as well as heavy metals such as lead (Pb), zinc (Zn), copper (Cu), nickel (Ni) and chromium (Cr) especially in tanning operations utilising chrome salts (Genawi et al. 2020; Subashini & Rajiv 2018).

The use of chrome tanning processes over vegetable tanning approach is due to its advantages which include

production of thinner and softer leather resisting darkening over time, formation of livid colours, automatability of the process and shorter time of operation. In view of these advantages, about 90% of tanneries prefer chrome over vegetable tanning (Dabai and Mohammed 2020; Dixit et al. 2015). Despite these advantages, however, chrome tanning is associated with the major setback of producing wastewater containing high amount of Cr in the effluent (Durai and Rajasimman 2011; Eris et al. 2020).

Although Cr occurs in a number of oxidation states, trivalent chromium (Cr III) and hexavalent chromium (Cr VI) are more stable. Cr (III) is the most stable, relatively immobile and important for its effects on metabolic activities at trace concentrations (Belay 2010; Dixit et al. 2015). However, Cr (VI) is highly mobile, about 100 times more toxic than Cr (III), and was found to be teratogenic, mutagenic with its toxicity having adverse effects to all forms of life, and persistent with detrimental effects to the environment (Dai et al. 2012; Aththanayake et al. 2022).

The toxicity of chrome tanned wastewater coupled with increasing stringent wastewater discharge regulations makes the handling of tannery effluents very challenging. In developing countries, some of the industries situated near rivers and streams discharge their heavily polluted effluents

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into neighbouring water courses prior to the required level of treatment, disregarding guideline limits for effluent discharge (Bernard & Ogunleye 2015; Hammouda et al. 2015). This may be due to high cost of effluent treatment approaches. Therefore, there is need for developing efficient, cost effective and sustainable tannery wastewater treatment approaches.

Numerous tannery wastewater treatment approaches are available including chemical precipitation (Dai et al. 2012), carbon adsorption (Eris et al. 2020; Mustapha et al. 2020), chemical coagulation (Ahmad et al. 2021; Urbina-Suarez et al. 2022) and electrochemical processes (Al-jabri et al. 2021; Mustapha et al. 2019). These treatment approaches were found to perform well, especially in terms of high metal ion handling capacity and can be achieved within short period of time. Despite these advances, however, these methods are associated with excessive use of chemicals, huge sludge production and high energy requirement.

Of the available treatment approaches employed to treat industrial effluents, hybrid microalgae-activated sludge system was found to be a good and promising alternative due to its efficient carbon capture capability and photosynthetic oxygen production. Some of its other credits include high biomass production, effective nutrient removal, heavy metals bioremediation potential and production of both renewable energy and non-energy source materials (Mohammed et al. 2014; López-rosales et al. 2016; Chen et al. 2019; Solé-bundó et al. 2019; Dabai & Mohammed 2021). Microorganisms utilise nutrients available in wastewater for growth thereby mitigating eutrophication (Donmez et al. 1999; Mohammed and Mota 2019), adsorption of metal ions by organic ligands on microalgal cell walls (Lage et al. 2018; Subashini & Rajiv 2018) and biotransformation of these pollutants to non-toxic or less toxic substances by cellular constituents (Hossan et al. 2020).

Furthermore, due to the huge variability in tannery wastewater quality within the tanning cycles and high selectivity of metal ions by biomass amongst the competing heavy metal ions in biological tannery wastewater treatment and the preference in removing metal ions with lower valencies by negatively charged cell components (Bilal et al. 2018;

Mohammed and Mota 2019), the current study employed the use of synthetic tannery wastewater (STWW) in accordance with Donmez et al. (1999); Sibi (2016) Mahmoud & Mohamed (2017), to assess Cr (VI) bioremediation potentials using microalgal specie *Chlorella vulgaris*, in modified photobioreactors.

## Materials and methods

### Algal culture

*C. vulgaris* was isolated and cultured in 1 L volumetric flask with Bold Basal Medium (BBM) in accordance with Fernández-Linares et al. (2017) in the Herbarium Laboratory of the Department of Plant Biology, Bayero University, Kano. This culture was illuminated under 20 W white fluorescent bulb and agitated using mechanical stirrer at an average speed of  $200 \pm 1$  revolution per minute (rpm) and 25 °C, with initial pH adjusted to about 7. This was then up-scaled in modified stirred-tank photobioreactors (MSTPBRs) to reach 100 mg/L Cr (VI) concentration.

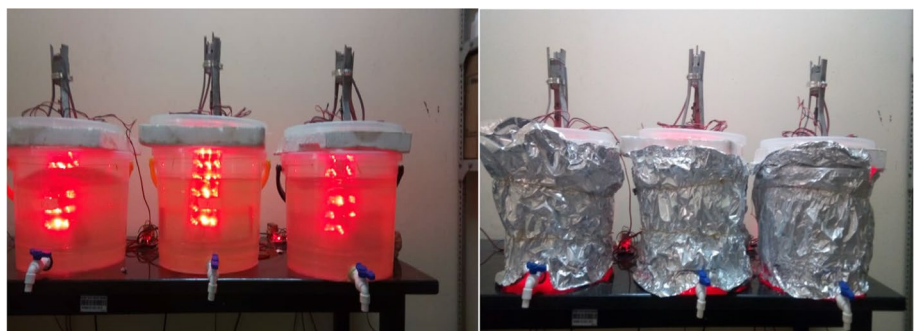
### Synthetic tannery wastewater

The synthetic wastewater was prepared by diluting specific amount of industrial grade potassium dichromate to obtain specific concentrations of Cr (VI) of 50, 100 and 150 mg/L as described by Donmez et al. (1999); Mahmoud & Mohamed (2017) and Sibi (2016).

### Modified stirred-tank photobioreactors (MSTPBRs)

Three MSTPBRs were operated at optimum irradiance of  $583 \mu\text{mol}/\text{m}^2\text{s}$ , at  $100 \pm 1$  rpm for 12:12 light–dark cycles in accordance with Mohammed et al. (2013) and wrapped with aluminium foil to minimise loss of light as shown in Fig. 1. The MSTPBRs were inoculated with a mixture of *C. vulgaris* and activated sludge based on 90:10 (weight/weight) microalgal culture-activated sludge ratio as suggested by Mohammed et al. (2013) and labelled as R<sub>2</sub>, R<sub>3</sub>

**Fig. 1** Image of the Modified Stirred-tank Photobioreactors



and  $R_4$  with the synthetic wastewater containing 50, 100 and 150 mg/L Cr (VI) concentration, respectively, whilst another reactor,  $R_1$ , was supplied with 50 mg/L Cr (VI) concentration contained in the synthetic wastewater but with neither illumination nor aeration, serving as the control.

The central illumination chamber was made up of transparent Plexiglas (Globe Plastic Industries; Malaysia), and photobioreactors had maximum capacity of 21 and a 16 L working volumes (Dabai & Mohammed 2021). Samples were collected every 48 h for analyses.

### Determination of Cr (VI)

Potassium dichromate solution was prepared by dissolving 141.4 mg of potassium dichromate solution in distilled water and diluted up to the 100 mL mark in a volumetric flask. Up to 250 mg of diphenylcarbazide (DPC) was dissolved in 50 mL acetone and stored in a dark brown bottle. Series of reference solutions were prepared at 0, 2, 4, 6, 8 and 10 mL. The stock Cr solution was pipetted into a volumetric flask and diluted with 95 mL of distilled water for calibration. The pH was adjusted with 0.25 mL phosphoric acid and 0.2 N sulphuric acid using colorimeter and then, 2 mL of DPC was added, mixed well, and allowed to stand for 10 min for colour development. The absorbance at 540 nm was read in 10 mm glass cuvette using a Lambda 35 UV/Vis Spectrophotometer (PerkinElmer, USA). The Cr stock solution was then replaced with an experimental sample (Heryanto et al. 2022; Hofmann et al. 2018; Sibi 2016).

### Determination of total chromium ( $Cr_T$ )

To determine the  $Cr_T$ , the sample was oxidised with potassium permanganate prior to the reaction with DPC. The sample was digested with nitric-sulphuric acid solution and then oxidised with potassium permanganate. The excess permanganate was heated in a water bath at 100 °C for 30 min to decolourise it followed by reaction with 2 mL DPC and the above procedure was repeated (Sibi 2016; Hofmann et al. 2018; Heryanto et al. 2022;).

### Cr recovery after biosorption

In desorption and biosorption regeneration process, the Cr was recovered from heavy metal-loaded *C. vulgaris* using solvent elution method. This was carried out by using a stripping agent (i.e. 0.1 M  $NH_3O$ ). A 0.1 g of dried Cr loaded *C. vulgaris* pellets was mixed with 50 mL of the stripping agent and centrifuged at  $4000 \times g$  for 5 min, and the supernatant was decanted and analysed for total Cr concentration in accordance with Dawodu et al. (2020) and Rezaei (2016).

### FTIR spectroscopy

To determine the functional groups of *C. vulgaris*. Involved in Cr removal from the treated wastewater, about 0.1 g of the dried microalgal biomass was studied using Fourier transform infrared (FTIR) spectroscopy from each MSTPBR by Cary 630 FTIR Spectrophotometer (Agilent Technology, USA) with 32 scans each at a range of  $650\text{--}4000\text{ cm}^{-1}$  (Han et al. 2021; Subashini & Rajiv 2018).

## Results and Discussion

### Cr (VI) removal

Results obtained from varying concentrations of the Cr stock solution were plotted against their corresponding optical densities for the calibration of the UV/Vis Spectrophotometer with correlation coefficient of 0.9967. It was observed that a remarkable Cr (VI) reduction was obtained in all of the bioreactors and the biosorption, in the form of percentage Cr (VI) removal, are shown in Fig. 2, with the following ranges: 8–92, 11–93, 6–89 and 7–83% for reactor A, B, C and D, respectively, during the bioremediation period.

Maximum Cr (VI) removal efficiency of 93% was achieved in reactor B, operated at 50 mg/L Cr (VI) concentration, followed by 91, 89 and 83% in reactors A, C and D, respectively. All the samples collected from the MSTPBRs satisfy discharge requirements for Cr (VI) except that from reactor D which operated at 150 mg/L and found to be at 0.8 mg/L concentration after 24 days.

In a related research, 23% of Cr (VI) was removed when 100 mg/L of synthetic tannery wastewater was treated with *C. vulgaris* under the influence of other competing heavy metal ions (Donmez et al. 1999), and 77% Cr (VI) removal efficiency was reported at 200 mg/L by Mahmoud & Mohamed, (2017). A slightly higher Cr (VI) removal efficiency of 81% was reported by Sibi, (2016) when heavy metal-loaded industrial effluent was treated using *C. vulgaris* under extreme conditions of contact time, algal dose, pH, and salinity.

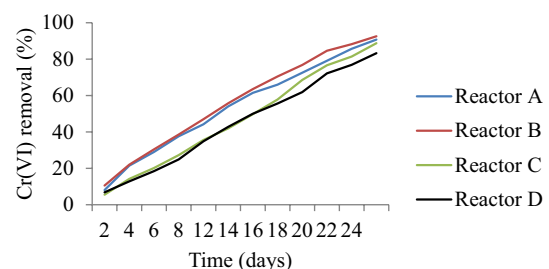


Fig. 2 Cr (VI) biosorption from the bioreactors

Although reactor A was operated with neither illumination nor aeration, an efficient Cr (VI) removal efficiency was achieved at 50 mg/L Cr (VI) concentration reducing it to about 0.5 mg/L within the first 14 days. This satisfies the discharge requirement specified by National Environmental Standards and Regulations Enforcement Agency (NESREA; Mustapha et al. 2019). This higher removal efficiency in the control reactor might be due to the fact that increase in chlorophyll concentration does not always follow increase in light intensity. Some studies have linked variation of chlorophyll content from the surface to the bottom of lakes with nutrients availability (e.g. Desortova 1976; Felip & Catalan 2000).

### Total Cr removal

Total Cr ( $Cr_T$ ) removal efficiencies of 86, 94, 89 and 84% have been achieved in reactor A, B, C and D, respectively, as shown in Fig. 3. The  $Cr_T$  removal efficiency was observed in the control reactor with lowest  $Cr_T$  concentration in the effluent to be 0.26 mg/L.  $Cr_T$  removal efficiencies in all of the reactors satisfy the discharge limits except in reactor D with effluent concentration of 0.86 mg/L after bioremediation from the initial concentration of 150 mg/L which is above the 0.5 mg/L specified by NESREA. This initial total Cr concentration of 150 mg/L appears to be beyond the biosorption capacity of *C. vulgaris*. The highest  $Cr_T$  absorption from the bioreactors was in the order  $B > C > A > D$ .

Several attempts were made to remove Cr from wastewater using different treatment approaches. Pandi et al. (2009) reported 95% Cr removal by *cyanobacteria* in 200 mg/L initial Cr concentration of retan chrome liquor. Another study by Ajayan et al., (2015) reported 81 to 96% Cr removal efficiency using *Scenedesmus sp.* in tannery wastewater and up to 92% Cr was removed by *Scenedesmus sp.* in wet market wastewater. However, only about 40% of Cr was removed by *Chlorella sp.* under the influence of Cu, Zn and Fe and amongst these metals, Cr ions were the least absorbed (Subashini & Rajiv 2018).

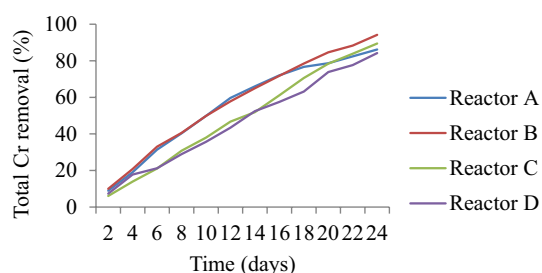


Fig. 3  $Cr_T$  removal from the bioreactors

### Cr recovery

In the desorption and biosorption regeneration process, considerable amount of Cr was recovered after the bioremediation process, and the highest  $Cr_T$  recoveries were found to be 70, 78, 74 and 72% from reactors A, B, C and D, respectively (see Fig. 4).

The highest Cr recovery from microalgal biomass was found to be higher in reactors with medium concentrations of 100 mg/L followed by 150 mg/L. Much higher Cr recoveries of 72, 90 and 95% of Cr desorbed using EDTA, HCl and  $HNO_3$  as stripping agents were reported by Rezaei, (2016). In a similar study, Dawodu et al. (2020) recovered 89–96% Cr in three cycles using nitric acid as stripping agent.

### Fourier Transform Infrared (FTIR) Spectroscopy of *C. vulgaris*

The spectra of *C. vulgaris*. (Fig. 5) revealed the availability of carboxylic, carbonyl, hydroxyl, and amino groups in the Cr bioremediation, as presence of OH together with carbonyl group confirmed the involvement of carboxylic acid group in *C. vulgaris* biosorption.

The presence of NH, OH and carboxylic groups in the biosorbent might be connected with the presence of amines as shown in Table 1. The shift in the stretching vibrations of NH, OH and  $C=O$  groups to a certain extent after biosorption reaction indicates the possibility of involvement of these groups in Cr uptake.

The assignment of *C. vulgaris* functional groups before and after the Cr bioremediation was done with the help of FTIR chart, and the differences in peaks were more pronounced in the functional group region between 1200 and 4000  $cm^{-1}$  than in the finger print region of 650 to 1200  $cm^{-1}$ . It was also observed that carboxyls, aldehydes, alkynes, amides, sulphonamides, alcohols, ethers, esters, and anhydrites might be involved in the Cr bioremediation process.

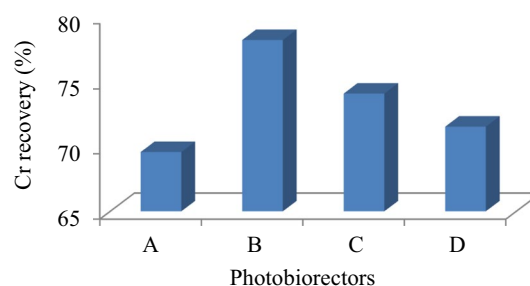
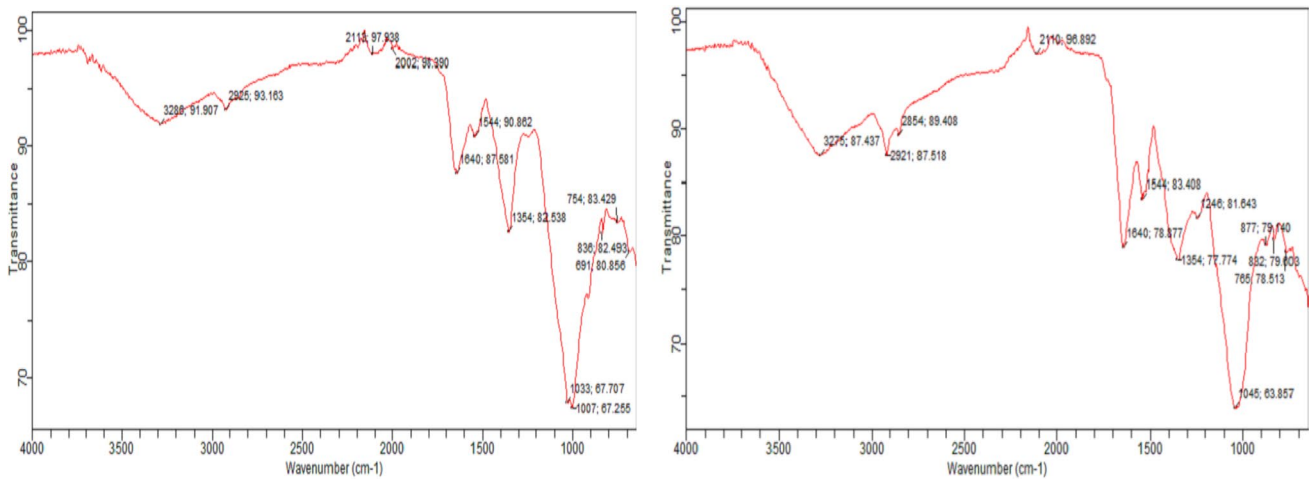


Fig. 4 Cr recovery after bioremediation





**Fig. 5** FTIR Spectra for Reactor D and the Control Reactor

**Table 1** Bands assignment for *C. vulgaris* functional group from FTIR spectroscopy

Wave number (cm <sup>-1</sup> )		Type of vibration	References
Control	Reactor D		
3275	3288	O–H stretching (carboxylic acid)	(Mahmoud & Mohamed 2017)
2921	2925	C–H stretching (aldehyde)	(Chhikara et al. 2010)
2110	2113	C≡C (alkyne)	(El-sheekh et al. 2021)
1640	1640	C=O stretching (amide)	(Roca et al. 2023)
1544	1544	N=H stretching (amide)	(Hossan et al. 2020)
1354	1354	S=O (sulphonamides)	(Almomani & Bhosale 2021)
1045	1033	C–O (alcohols, ethers, esters, carboxylic acids, and anhydrides)	(Tegegn et al. 2022)

### Conclusion

This study exploited the capability of *Chlorella vulgaris* for chromium bioremediation in synthetic tannery wastewater using a hybrid microalgae-activated sludge system. Maximum Cr (VI) removal efficiencies of 91, 93, 89 and 83% were achieved in reactors A, B, C and D, respectively, and 86, 94, 89 and 84% total Cr removal efficiencies were achieved in reactors A, B, C and D, respectively. Highest Cr recovery of 78% was achieved in reactor B. It was also found out that carboxyl, carbonyl, amino and hydroxyl groups of *C. vulgaris* were involved in Cr bioremediation. Although considerable removal efficiencies were obtained in this study, different microalgae–bacteria compositions should be tested in order to exploit the full potentials of the modified hybrid microalgae-bacteria system for biosorption and desorption of chromium from tannery effluents.

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### Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Ethical approval** The compliance of ethical standard was ensured. The ethical conduct was observed.

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