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Biosorption of Hexavalent Chromium Cr(VI) using Microalgae *Scenedesmus sp* as Environmental Bioindicator

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Abstract. *Scenedesmus sp.* is a freshwater green alga that functions as an ionic biosorbent and can also be a bioindicator for water contaminated with hexavalent chromium Cr(VI) ion. This study aimed to observe the growth of *Scenedesmus sp.* exposed to Cr(VI) ion at various concentrations and analyze the remaining Cr(VI) ion that did not undergo biosorption by microalgae. This research was conducted on *Scenedesmus sp.* microalgae growth media using five bioreactors, each with a different Cr(VI) ion exposure concentration. The remaining ion in the growth media was analyzed for its concentration with an ultraviolet-visible spectrophotometer at time variations with an interval of two days. Maximum biosorption with exposure to Cr(VI) occurred at a concentration of 1.0 ppm on day 12 of 99.93%. At concentrations of 5.0 ppm and 7.0 ppm, microalgae growth was very poor, indicating the medium was toxic.

Keywords: Biosorption; Hexavalent Chromium; *Scenedesmus sp*; Toxicity

1. Introduction

The microalga *Scenedesmus* sp. is highly competent at binding inorganic ions such as carboxyl, amine, sulfate, and sulfonate, which lends itself viable to treat aquatic waste. Microalgae have the advantage of being environmentally friendly, recyclable, and low maintenance costs (Wilan *et al.*, 2020). *Scenedesmus* sp. is a cosmopolitan microalga that lives in colonies within brackish water and soil with a humid climate. Their cells are cylindrical (8-20 m in length and 3-9 m in width) and are surrounded by three layers consisting of an inner layer (cellulose), a middle layer (membrane structure), and an outer layer net of pectin and fine hairs (Prihantini, Damayanti, and Yuniati, 2007).

Scenedesmus sp. is widely utilized as a supplement, fish feed, pollutant removal agent for wastewater treatment, a source of biofuel, and a bio-indicator of water pollution using herbicides as a determinant (Fodorpataki, Bartha, and Keresztes,, 2009; Makareviciene *et al.*, 2011; Sudibandriyo and Putri, 2020).

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Industrial activities often pollute their surrounding with various classes of contaminants, of which heavy metals are particularly concerning since they persist in the environment and do not decompose or degrade into benign compounds as most organic pollutants do. Heavy metal ions are toxic to aquatic ecosystems and human health above a certain concentration level (Suprapto *et al.*, 2020).

Heavy metal ions can be removed from water through several methods, such as physical adsorption, chemical sedimentation, mechanical filtration, and ion exchange. However, these processes have their drawbacks, such as secondary pollution due to the chemicals used and high cost. An environmentally friendly alternative is using microorganisms to adsorb the ions out of the water, a technique known as biosorption. This method is highly efficient in wastewater detoxification, and it has a simple implementation and a low cost. Microorganisms' adsorption of heavy metal ions is a rapid and reversible process in which the cell wall serves as a binding site, which means that the microorganism does not even need to be alive for this purpose. Using dead microbial cells could be more cost-efficient because they do not require a supply of nutrients during the process. Several factors affect biosorption: characteristics of biomass, temperature, pH, biosorbent concentration, contact time, and biomass surface area. The biomass must be immobilized to avoid blockage of the reaction (Wilan *et al.*, 2020).

Many techniques have been applied to improve the performance of a biosorbent. The chemical composition of the adsorbing surface may be modified by adding or removing certain functional groups to improve specificity and binding energy. The binding surface area may be expanded by increasing porosity (Anuar *et al.*, 2019). Several researchers have used the biosorption method to remove heavy metals in solution using dead biomass to bind pollutants through simultaneous adsorption, complex formation, micro-surface deposition, and ion exchange (Kusrini *et al.*, 2019; Fomina and Gadd, 2014; Ekmekyapar *et al.*, 2012). Certain bacteria can absorb Pb ions, such as micrococcus sp. and flavobacterium sp., by up to 100% at an initial concentration varying from 2.0 ppm to 10 ppm after an exposure of 3 to 30 days (Susanto, Kartika, and Koesnarpadi, 2019).

Chromium is a very toxic and dangerous heavy metal. Among the valence range of chromium from -2 to +6, only hexavalent chromium (Cr VI) and trivalent chromium (Cr III) have environmental significance due to their stability in the form of oxidation in water and poor absorption by soil and organic matter, making them slow to sediment out of the solution (Mnif *et al.*, 2017).

Cr (VI) compounds are generated by various industries such as metallurgy, leather tanning, paint, textile, pulp, ore and petroleum refining, metal corrosion, and electroplating. Those compounds may be released into the environment due to leakage, poor storage, or improper disposal. Chromium ions are toxic in the human body because they can irritate the respiratory tract, blood vessels, kidneys, and skin at high levels. According to the World Health Organization (WHO) drinking water guidelines, the maximum recommended limit for total chromium is 0.05 ppm (Rahman and Singh, 2019; Khatoon and Rai, 2016; Khatoon *et al.*, 2013).

This study aims to observe the growth of *Scenedesmus sp.* exposed to Cr(VI) ion at various concentrations in the growth medium, during which the alga should adsorb the ions, and then analyze the remaining Cr(VI) ion in the growth medium at an interval of two days. The extent of absorption of Cr(VI) ion can be a bioindicator for the environment by providing information about the growth of the microalgae *Scenedesmus sp.*, which is disturbed at a certain concentration and is characterized by a colorless growth media (not growing or dying). However, if the growth medium is green, the growth is normal (not disturbed by Cr(VI) ion).

2. Methods

2.1. Microalga Cultivation Process and Exposure to Bioreactors

2.13 g of the growth media for algae or bold basal medium (BBM) was dissolved in 3 L of distilled water to obtain a BBM solution (Figure 1a). Five photobioreactors were prepared and charged with potassium dichromate ($K_2Cr_2O_7$) solution to obtain a Cr(VI) solution with a concentration of 0 ppm, 1 ppm, 3 ppm, 5 ppm, and 7 ppm. The *Scenedesmus sp.* culture was inoculated into the five photobioreactors that had been filled with BBM solution and aerated. The microalgae were cyclically illuminated with fluorescent lighting (1500 lux), receiving 12 hours of light and 12 hours of darkness at 25 °C. The Cr(VI) with variation concentration (0 ppm, 1 ppm, 3 ppm, 5 ppm, and 7 ppm) was measured in each bioreactor every two days for a total of 12 days (Figure 1b).

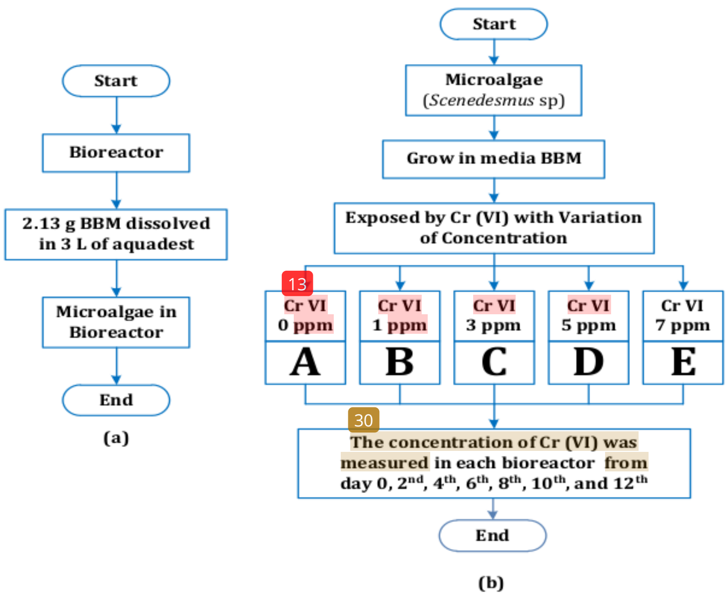

Figure 1 Scheme of (a) Microalgae Cultivation, (b) Exposed to Bioreactors

2.2. Preparation of Cr(VI) Standard Solution

A mass of $K_2Cr_2O_7$ weighing 0.1414 g was dried in an oven and dissolved in 100 mL distilled water in a volumetric flask to yield a Cr(VI) 500 ppm solution. 10 mL of the Cr(VI) 500 ppm solution was diluted with 100 mL distilled water in a volumetric flask to obtain a Cr(VI) 50 ppm solution. 10 mL of Cr(VI) 50 ppm solution was diluted with 100 mL distilled water in a volumetric flask to obtain a standard Cr(VI) 5 ppm solution.

2.3. Curve Calibration

2 mL of the Cr(VI) 5 ppm standard solution was added into a 100 mL volumetric flask, followed by five drops of H_3PO_4. The pH of the mixture was adjusted by adding 0.2 M H_3PO_4 until it reached pH 2. Next, 2 mL of diphenylcarbazide was added, and the flask was filled with distilled water up to the marked line, resulting in a 0.1 ppm standard solution for the calibration curve. The procedure was repeated with the volume of the Cr(VI) 5 ppm standard solution incremented by 2 mL up to 20 mL, resulting in standard solutions with a concentration of 0.2 ppm, 0.3 ppm, 0.4 ppm, 0.5 ppm, 0.6 ppm, 0.7 ppm, 0.8 ppm, 0.9 ppm,

and 1.0 ppm. The solutions were each rested for 10 min before their absorbances were measured at a wavelength of 540 nm.

2.4. Measurement of Chromium Concentration

A 10 mL sample of the culture solution was filtered using a folder membrane at 0.45 microns. It was treated according to a calibration curve standard solution, and the concentration was measured at a wavelength of 540 nm.

2.5. Determination of Remaining Cr(VI) Ion Concentration in Growth Medium with Time Variations

The concentration of Cr(VI) ion in the culture medium was measured by taking a 10 mL sample and running it through a vacuum filter using a millipore membrane (0.4 microns), then determining the concentration of Cr(VI) ion. The measurement was performed on the initial solution, then every other day up to the twelfth day. The Cr(VI) ion which has undergone biosorption is the concentration of Cr(VI) ion obtained (ppm) reduced with the concentration of Cr(VI) ion remaining in the medium.

3. Results and Discussion

Table 1 shows that the Cr(VI) ion concentration decreased with increasing contact time. The longer the exposure time, the larger the possible interactions between the biosorbent material and the metal ions, which allowed more active groups to bind metal ions and increase the number of metal ions absorbed. The biosorption proceeded with increasing contact time until the equilibrium point was reached. The length of contact time affected the metal ion-binding process by the biosorbent surface before the surface reached the saturation point. When the biosorbent has reached the equilibrium point, the biosorbent will not bind any heavier metals because the surface of the cell wall is saturated.

Table 1 Absorption of Cr(VI) with variations in concentration and time

Day	Concentration				
	A (0.0 ppm)	B (1.0 ppm)	C (3.0 ppm)	D (5.0 ppm)	E (7.0 ppm)
0	0.00 ± 0.00	0.97 ± 0.03	2.92 ± 0.07	4.86 ± 0.06	7.08 ± 0.06
2	0.00 ± 0.00	0.83 ± 0.01	2.71 ± 0.02	4.75 ± 0.02	6.89 ± 0.03
4	0.00 ± 0.00	0.71 ± 0.02	2.53 ± 0.02	4.68 ± 0.05	6.75 ± 0.03
6	0.00 ± 0.00	0.63 ± 0.01	2.39 ± 0.02	4.64 ± 0.02	6.66 ± 0.02
8	0.00 ± 0.00	0.41 ± 0.01	1.95 ± 0.02	4.53 ± 0.01	6.53 ± 0.01
10	0.00 ± 0.00	0.23 ± 0.00	1.63 ± 0.01	4.50 ± 0.01	6.54 ± 0.03
12	0.00 ± 0.00	0.00 ± 0.00	1.42 ± 0.03	4.36 ± 0.04	6.51 ± 0.03

Based on the concentration of Cr(VI) ion exposed and remaining in the growth medium, the percentage of biosorption can be determined based on the following equation (Vendruscolo, da Rocha-Ferreira, and Antoniosi-Filho, 2017):

$$\%\ Biosorption\ of\ Cr\ (VI) = \frac{(Ce - Cr)ppm}{Ce\ ppm} \times\ 100\%$$

Note *Ce* = Concentration of Cr(VI) ion exposed in the growth medium (ppm)
Cr = Concentration of Cr(VI) ion remaining in the growth medium (ppm)

Based on Table 1 and the percentage of biosorption equation, the calculation results for the percentage of Cr(VI) ion removal are listed in Table 2 below.

Table 2 Percentage of Cr(VI) ion removal with variations in concentration and time

	Cr(VI) ions removal (%)			
Day	1 ppm	3 ppm	5 ppm	7 ppm
0	0.0000	0.0000	0.0000	0.0000
2	14.7766	7.1836	2.3320	2.6836
4	26.4605	13.5690	3.6351	4.6139
6	35.5670	18.2440	4.4582	5.9322
8	57.6632	33.2953	6.6968	7.7067
10	76.4261	44.2987	7.4273	7.6733
12	99.9313	51.4253	10.2030	8.0410

Figure 2 showed that the growth medium exposed to 1.0 ppm Cr(VI) could absorb almost completely or 99.93%, indicating that the microalgae could grow and multiply in water with this chromium concentration. The growth medium is not yet toxic to the growth of microalgae *Scenedesmus sp.*

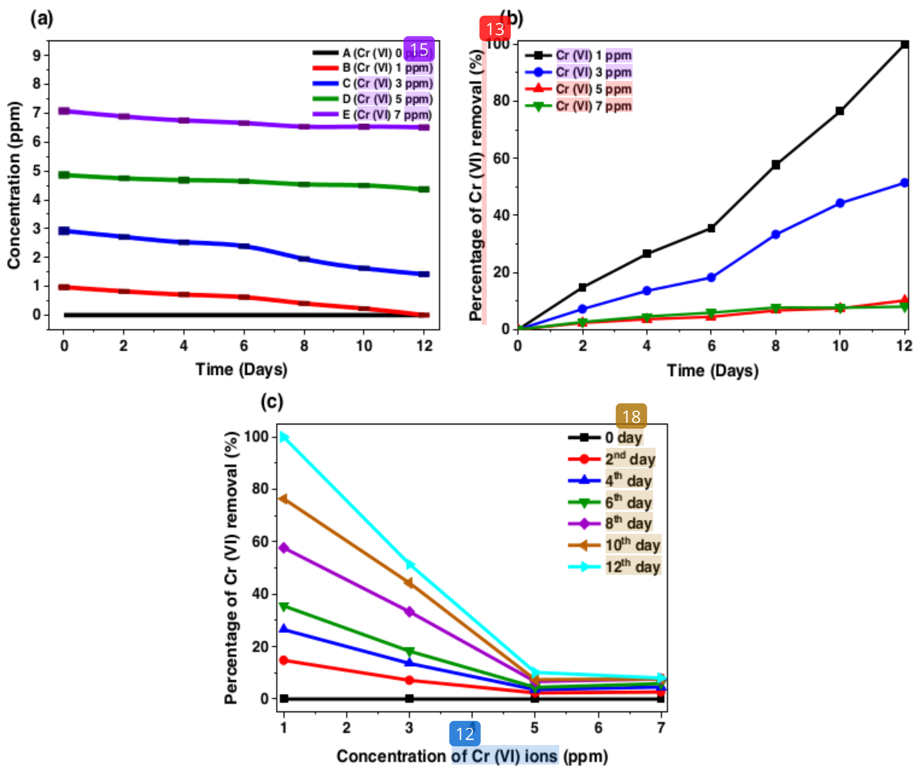

Figure 2 (a) Plots of Cr(VI) concentration as a function of time, (b) plots of the percentage of Cr(VI) removal as a function of time, and (c) the plots of the percentage of Cr(VI) removal as a function of Cr(VI) concentration

At Cr(VI) 3.0 ppm, the a similar trend of increasing ion absorption throughout the study period. However, the amount of chromium ion absorbed was lower than the Cr(VI) 1.0 ppm exposure, which meant that Cr(VI) ion was still absorbed but was toxic. The growth of *Scenedesmus sp.* was disrupted when exposed to this level of chromium because the metal ion cofactor required by its enzymes was non-competitively inhibited, and the complex reagents exchange metal ions from the enzyme exceeded their tolerance limit (Daneshvar *et al.*, 2019; Susanto, Kartika, and Koesnarpadi, 2019).

At Cr(VI) 5.0 ppm, the absorption of Cr(VI) dropped precipitously, indicating that the solution was already highly toxic to the microalga and no microbial growth was occurring. The same result was obtained from the 7.0 ppm medium, and in both media, no green color developed beyond the initial very pale green color. It is a bio-indication that the growth media already contained chromium ions at high concentrations (Susanto, Kartika, and Koesnarpadi, 2019).

The reduction of ion concentration in the growth media was due to (1) the biosorption with bonds between metallothionein thiol groups, namely polypeptides containing about 30% of the amino acid cysteine (Dewi, Yuniastuti, and Ahmed, 2018), and (2) the non-competitive inhibitory effect of Cr(VI) ion to form mercaptide salts with sulfhydryl groups of enzyme proteins :

$$M + R - SH \rightleftarrows MSR + H^+$$

Notes: M = Metal, R = Protein radicals from microalgae, and SH = Sulfhydryl
This condition inhibits the action of the enzyme because it is not similar to the cofactor as an activator of the enzyme (Dewi, Yuniastuti, and Ahmed, 2018).

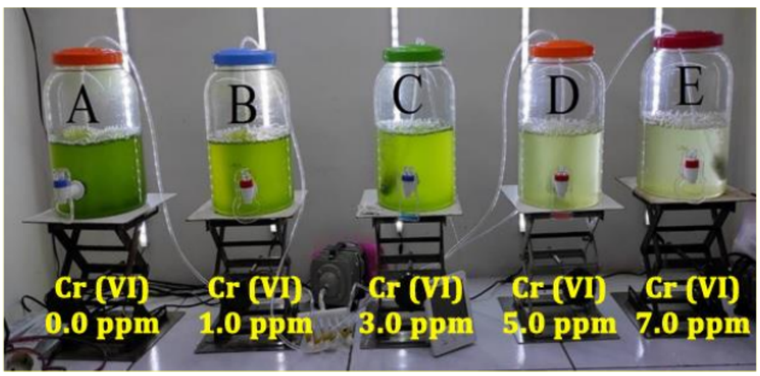

Figure 3 Microalgae growth exposed to Cr(VI) bioreactors of 0; 1; 3; 5, and 7 ppm

The growth medium without any Cr(VI) (reactor A) did not manifest the presence of Cr(VI), and the growth of microalgae was vigorous, as shown in Figure 3, in which the 0.0 ppm medium was deep green. Meanwhile, in the growth media contaminated with Cr(VI) 1.0 ppm (reactor B), the absorption process occurred from day second to twelfth, and the concentration of remaining ions in the growth medium was reduced to 0 ppm on day twelfth (99.93% absorbed). It showed good absorption at exposure to a concentration of 1.0 ppm, and only the growth was slightly disturbed.

The medium exposed to Cr(VI) 3.0 ppm (reactor C) had its Cr(VI) concentration reduced by 50% after twelve days of incubation. The medium exposed to Cr(VI) at 5.0 ppm (reactor D) had its Cr(VI) concentration reduced by only about 10.29% in the growth medium after twelve days. Likewise, the growth medium exposed to Cr(VI) at 7.0 ppm (reactor E) had its Cr(VI) concentration reduced by about 8.05% in the growth medium, which indicated poor growth in reactor D and reactor E. Both of these reactors have the potential to be toxic to microalga growth. This is the result of a comparison with several organisms used as bio-sorbents and the mechanism that occurs in the absorption of Cr(VI) ions stated in Table 3.

Table 3 Several types of biosorbents and mechanism of Cr(VI) ion removal

Name of Organism	Isolation Site	Mechanism of Cr Removal	Initial Cr (VI) Concentration (mg/L)	Remediation (%)
Acinetobacter junii	Chromite mine site	Reduction	54	99.95
Cellulosimicro-bium funkei strain AR6	Leather industry effluent contaminated soil	Biosorption, Reduction	250	80.43
Pseudomonas stutzeri L1	Crude oil	Biosorption, Reduction	100-1000	97
Acinetobacter baumannii L2	Crude oil	Biosorption, Reduction	1000	99.58
Pleurotus ostreatus	Mushroom farms	Biosorption	500	80
Acremonium sp.	Tannery effluent contaminated soil	Biosorption	100	90
Penicillium griseofulvum MSR1	Tannery effluent	Biosorption	67.8	79.9
A. niger	Contaminated soil	Biosorption	125	96.3
Saccharomyces cerevisiae	Culture collection bank	Biosorption	200	85
Opuntia cladodes	Aqueous solution	Biosorption	18.5	83

Source : (Jobby *et al.*, 2018; Fernández-López, Angosto, and Avilés, 2014)

The results of this research can pave the way for a novel bioindicator device to be used by premises that produce a waste stream containing Cr(VI) ions. The growth color, which shows a paler color (slowest growth), indicated high Cr(VI) waste. The wastewater treatment system that would process the stream containing Cr(VI) generated by an industrial activity can be augmented with a pond overgrown with Scenedesmus sp. microalgae. If the growth of Scenedesmus sp. microalgae is vigorous, exhibiting a deep green color in the water, then the waste quality is suitable for discharge. Otherwise, if the growth of Scenedesmus sp. microalgae is inhibited, exhibiting a pale green color or no color, then the water needs more treatment before discharge.

4. Conclusions

The microalga absorbed Cr(VI) well (99.93%) after twelve days of incubation in a medium containing 1.0 ppm chromium. Incubating for twelve days in a medium with 3.0 ppm chromium resulted in only 50% absorption. The mediums with 5.0 ppm and 7.0 ppm chromium were toxic to the microalga, with very little chromium absorbed. This technique may be utilized as an environmental bioindicator for companies that generate Cr(VI) ion waste in their process to test their wastewater before discharging it into water bodies or the environment.

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