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2. Unsuitable Format
3. Unreadable text, figure, or table
4. *1. Graphical abstract is very bad, just photo of abstract. 2. This is too short paper, minimum for publication is 8 pages. Please read guide of authors. 3. Authors have to add the significant finding and discussion*

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COVER LETTER

04th of March 2022
Dear Dr. Nyoman Suwartha

We wish to submit revision-1 an original research article entitled "Biosorption of Hexavalent Chromium Cr (VI) using Microalgae *Scenedesmus sp* as Environmental Bioindicator" for consideration by the IJTech. We confirm that this work is original and has not been published elsewhere.

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.

We believe that this manuscript is suitable for environmental chemistry and its sustainability for publication. We guarantee that the manuscripts submitted to the journal for review are original written directly by the authors mentioned and have not been published elsewhere. The manuscript is also not currently under consideration for publication by other journals and will not be submitted while it is being reviewed by this journal. This manuscript does not contain defamatory or other unlawful statements and does not contain any material that violates the personal rights or property rights of other people or entities.

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Sincerely

Dr. Rudi Kartika


A
B
C
D
E
Cr (IV)
0.9 ppm
Cr (IV)
0.1 ppm
Cr (IV)
0.3 ppm
Cr (IV)
0.5 ppm
Cr (IV)
0.7 ppm
MICROALGAE GROWTH EXPOSED TO BIOREACTORS

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

Abstract. *Scenedesmus* is freshwater green algae that function as a metal absorbent and can also be a bio-indicator for polluted water with Cr (VI) metal. The maximum biosorption with exposure to Cr (VI) at a concentration of 1.0 ppm on day 12 was 99.93%. In contrast, concentrations (5.0 and 7.0) ppm showed a toxic condition where the growth of microalgae was very poor so that it could be used as a bio-indicator of metal contamination.

Keywords: Chromium; Metal; *Scenedesmus*; Toxicity.

1. Introduction

Microalgae are biomass that can be used to treat aquatic waste. Abundant microalgae can be used to absorb heavy metal biomaterials. However, the most significant biomass in its ability to absorb metals is microalgae which have functional groups to bind metal ions, especially carboxyl, amine, sulfate, and sulfonate groups present in the cell wall cytoplasm. The advantage of using microalgae is environmentally friendly biological materials that can be recycled and have low maintenance costs (Wilan *et al.*, 2020).

Scenedesmus sp is microalgae with cosmopolitan properties that live in brackish water and soil with humid climates. *Scenedesmus* cells are cylindrical and usually live in colonies, with each colony consisting of 2, 4, 8, or 12 arranged sideways. *Scenedesmus* sp has a simple cell structure with 8-20 m length and 3-9 m width. The arrangement of the *Scenedesmus* sp cells is covered by three layers consisting of an inner, middle, and outer layer. The inner layer is cellulose, the middle layer is a membrane-like structure, and the outer layer is a net consisting of pectin and fine hairs (Prihantini *et al.*, 2007).

Scenedesmus sp contains 55% protein, 13% carbohydrates, amino acids, fibers, vitamins B1, B2, B12, and C (Prihantini *et al.*, 2007). *Scenedesmus* sp. is used as a bio-resource to be applied as a supplement and fish feed. *Scenedesmus* sp also works for wastewater treatment as an additional pollutant removal agent such as nitrogen and phosphorus. The fatty acid composition of the *Scenedesmus* sp culture can be used as a source of biofuel production (Makareviciene *et al.*, 2011).

The use of fossil fuels can cause a global crisis and hinder economic growth. However, biofuels are a promising renewable energy source because they are abundant (Sudibandriyo and Putri, 2020). Also, *Scenedesmus* sp works fine as a bio-indicator against water pollution using herbicides as a determinant (Fodorpataki *et al.*, 2009).

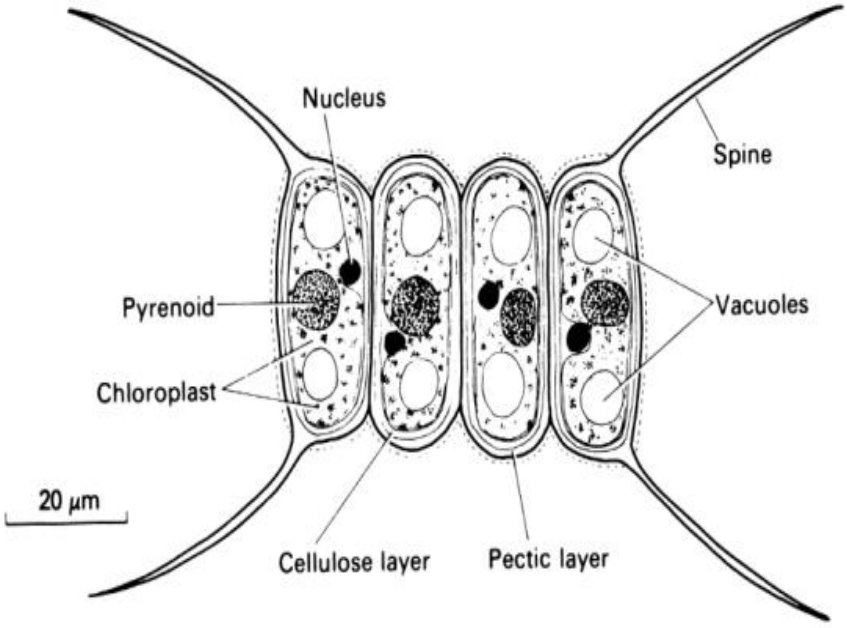

Figure 1 Microalgae *Scenedesmus sp*

Many industries have resulted in an increase in metal concentrations in the environment. It is a serious matter, as heavy metals are non-biodegradable and persistent. When it reaches a certain concentration, it will endanger aquatic ecosystems and human health (Suprapto *et al.*, 2020).

Some methods have separated heavy metals in waters, such as physical adsorption, chemical sedimentation, mechanical filtration, and ion exchange. However, the process has drawbacks, such as causing secondary pollution due to the chemicals used and the cost. One of the environmentally friendly alternative methods to overcome this is biomass or microorganisms, also known as biosorption. The advantage is that this method has high efficiency in detoxifying wastewater and low costs associated with its application. The adsorption of heavy metal ions by microorganisms is rapid and reversible. Biosorption occurs on living or dead biomass because it takes place only on the surface of the microalgae cell wall. However, dead biomass is more profitable because it does not require a supply of nutrients during its growth. Several factors affect biosorption: biomass characteristics, temperature, pH, the concentration of biosorbent, contact time, and surface area of biomass. Biomass must be immobilized to avoid blockage of the reaction. Immobilization is the physical absorption of biomass into cells. Polymers such as chitosan, alginate, cellulose, and gelatine immobilize biomass (Wilan *et al.*, 2020). Many techniques are applied to improve the ability of biosorbent, such as modification of chemical composition to increase absorption capacity. This modification of the biosorption properties was studied due to the expansion of the contact surface of the biosorbent by increasing its porosity and removal ability. In addition, the target for modification is an active functional group on the surface of the cell wall of the biosorbent because it plays an important role in binding contaminants (Anuar *et al.*, 2019).

Recently, researchers have used the simultaneous adsorption method (Kusrini *et al.*, 2019). Biosorption is an alternative technology for removing heavy metals from a solution based on dead/inactive biomass properties to bind and accumulate pollutants such as adsorption, complex formation, and micro-surface deposition (Ekmekyapar *et al.*, 2012). Biosorption is a physicochemical process that occurs without the need for metabolic processes, as for the mechanisms in the biosorption process, such as adsorption and ion exchange (Fomina and Gadd, 2014). Microorganisms like bacteria can absorb Pb metal by *micrococcus* sp and *flavobacterium* sp. Up to 100% at 2.0; 4.0; 6.0; 8.0 and 10.0 ppm exposure at 3; 6; 9; 12; 15; 18; 21; 24; 27 and 30 days (Susanto *et al.*, 2019).

Among the valence range of chromium from -2 to +6, only hexavalent chromium and trivalent chromium have environmental significance due to their stability in the form of oxidation in water. However, this depends on several factors, such as the pH of Cr (VI) usually exists in two forms, chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). These two oxyanions are highly soluble in water and poorly absorbed by soil and organic matter, making them difficult to mix with soil (Mnif *et al.*, 2017).

One of the most dangerous and toxic heavy metals in wastewater is chromium. Cr (VI) is released from various industries such as metallurgy, leather tanning, paints, textiles, pulp production, ore and petroleum refining, metal corrosion, and electroplating. Cr (VI) released into the environment is usually caused by leakage, poor storage, or improper disposal. According to the World Health Organization (WHO) drinking water guidelines, the maximum recommended limit for total chromium is 0.05 ppm (Khatoon and Rai, 2016; Khatoon *et al.*, 2013).

The negative impact of chromium metal is toxic if it is in the human body. This metal can irritate the respiratory tract, blood vessels, kidneys, and skin at high levels. In the respiratory tract, chromium can cause the risk of lung cancer and chronic ulcers. The impact on the skin surface is chronic ulcers. Chromium can also increase plaque thickening in blood vessels, while the kidney can occur in the form of renal tubular necrosis (Rahman and Singh, 2019).

The research aims to expose the growth media of Scenedesmus in the hope that if the growth of the Scenedesmus microalgae is sensitive to exposure to Cr (VI) metal ions, then in its application it can give a good indication as a bioindicator in wastewater that will be released into water bodies, this is based on the green color of the algae. If it grows normally, the green color will decrease if it is disturbed by Cr (VI) metal ions, which means that the growth rate decreases or do not grow.

2. Methods

2.1. Preparation of Bold Basal Medium

The bold basal medium (BBM) media was prepared by dissolving 2.13 g of BBM in 3 L of aquadest.

2.2. *Cultivation of Microalgae*

Microalgae *Scenedesmus sp* culture was inoculated into five filled photo bio-reactors with BBM and aerated. During cultivation, microalgae were given fluorescent lighting (1500 lux) with 12 h of light and 12 h of dark at a temperature of 25 ^{o}C.

2.3. Preparation of Cr (VI) Standard Solution

$K_2Cr_2O_7$ of 0.1414 g was dried in an oven, then dissolved with 100 mL aquadest in a measuring flask, and achieved Cr (VI) 500 ppm solution. After that, 10 mL of Cr (VI) 500 ppm solution was diluted with 100 mL aquadest in a measuring flask and achieved Cr (VI)

50 ppm solution. Next, 10 mL of Cr (VI) 50 ppm solution was diluted with 100 mL aquadest in a measuring flask and achieved a standard solution of Cr (VI) 5 ppm.

2.4. Curve Calibration

The concentration of 0.0; 1.0; 3.0; 5.0; and 7.0 ppm was made for the calibration curve in a 10 mL measuring flask. A Cr (VI) 5 ppm solution of 2.0; 4.0; 6.0; 8.0; 10.0; 12.0; 14.0; 16.0; 18.0 and 20.0 mL was put into a 100 mL volumetric flask then added five drops of H_3PO_4. The pH is adjusted by adding 0.2 M H_2SO_4 to reach pH 2. Then each added 2 mL of diphenylcarbazide solution and added distilled water until the limit mark. So as in can be a standard solution for the calibration curve concentration of 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 and 1.0 ppm. Be awaited for 10 min and measure the absorbance at a wavelength of 540 nm.

2.5. Measurement of Chromium Concentration

10 mL of the sample solution filtered using a 0.45-micron folder membrane was treated the same as the standard solution of the calibration curve, and the concentration was measured at a wavelength of 540 nm.

2.6. Cr(VI) Exposure

Five photobioreactors were added with potassium dichromate ($K_2Cr_2O_7$) solution to achieve a Cr (VI) solution with a concentration of 0, 1, 3, 5, and 7 ppm.

2.7. Determination of Cr(VI) Concentration

In each treatment of 0; 2; 4; 6; 8; 10, and 12 days, the sample of 10 mL was taken and filtered with a vacuum filter using a millipore membrane, 0.4 microns, then determined the concentration of Cr (VI)

3. Results and Discussion

Based on table 1, it is shown that the concentration of Cr(VI) metal decreased with increasing contact time. The longer the exposure time, the longer the collision time, and the interaction between the biosorbent and metal ions, more active groups will bind to metal ions and increase the amount of metal absorbed. Biosorption will increase significantly with increasing contact time until it reaches the equilibrium point or optimum condition. The length of contact time affects the metal-binding process by the biosorbent surface before its surface reaches the saturation point. When the biosorbent has reached the optimum condition, the biosorbent abilities that can bind heavy metals will decrease because the surface capacity of the cell wall is already saturated.

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

Day	Concentration (ppm)				
	0	**1**	**3**	**5**	**7**
0	0.0000	0.9700	2.9233	4.8600	7.0800
2	0.0000	0.8267	2.7133	4.7467	6.8900
4	0.0000	0.7133	2.5267	4.6833	6.7533
6	0.0000	0.6250	2.3900	4.6433	6.6600
8	0.0000	0.4107	1.9500	4.5345	6.5344
10	0.0000	0.2287	1.6283	4.4990	6.5367
12	0.0000	0.0007	1.4200	4.3641	6.5107

The analysis results for media without exposure for Cr (VI) in all analysis results did not contain chromium metal in the growth medium.

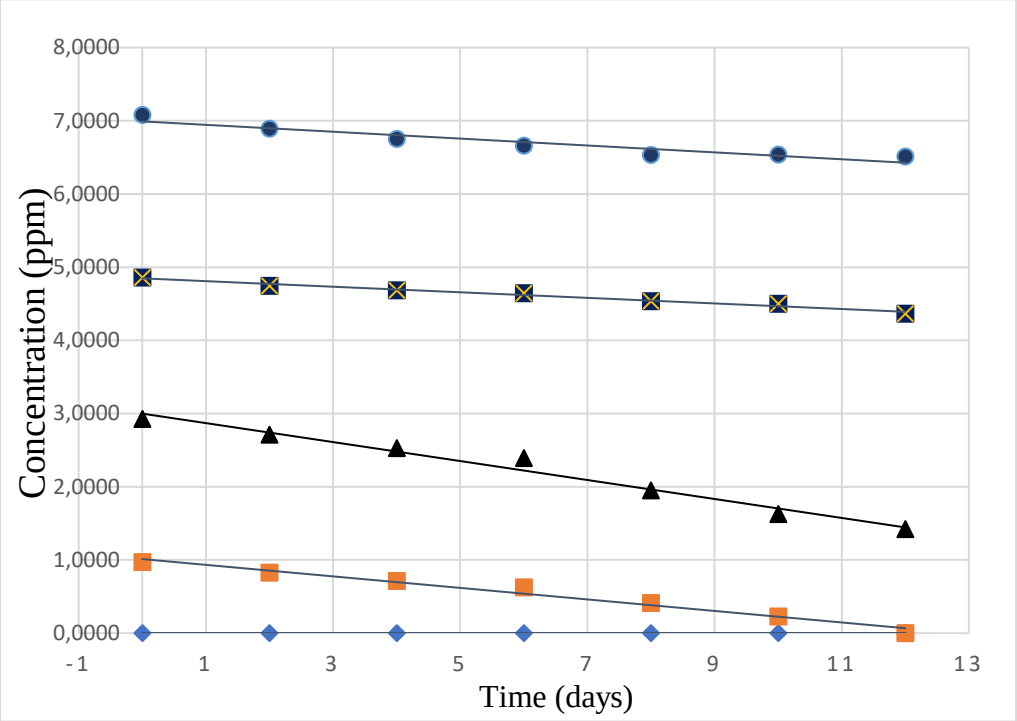

Figure 2 Absorption of Cr (VI) with variations in concentration (ppm) with time (days)

Figure 2 shows that the growth media with 1.0 ppm Cr(VI) metal exposure can be absorbed completely or 100%, indicating that it can still absorb the exposure concentration of 1 ppm very well, and microalgae can still grow well. It can conclude that the growth media is not toxic yet to the growth of microalgae *Scenedesmus* sp.

In the 3.0 ppm of Cr (VI), exposure growth media saw that metal absorption increased from the 2nd to the 12th day. Still, the increase was smaller than the 1.0 ppm Cr (VI) metal exposure, which indicates that metal Cr (VI) can still be absorbed, but it is toxic. The growth of microalgae *Scenedesmus* sp is disturbed compared to exposure to 1 ppm Cr (VI) metal.

In the exposure of Cr(VI) 5.0 ppm metal, the growth medium showed that its absorption value decreased was due to the increased toxic nature of Cr(VI) metal to the growth of microalgae *Scenedesmus* sp. Likewise, the absorption of Cr(VI) metal on exposure to 7.0 ppm growth media, the absorption value became very small, and almost nothing grew. This *Scenedesmus* sp microalgae showed that the tolerance limit for growth by exposure to Cr(VI) metal was 7.0 ppm.

The growth media that were not exposed to Cr (VI) (reactor A) in Figure 4 had no detectable presence of Cr (VI), and the microalgae growth was quite good, seen from a greener color than other growing media. In media contaminated with Cr (VI) of 1 ppm (reactor B), absorption occurred from day 2 to day 12 were measured that the remaining metal in the concentration medium decreased until on day 12 to 0 ppm (absorbed 99.93%), which is the growth was smaller than the growth on the metal exposed media. It shows a good absorption at the exposure to a concentration of 1 ppm, and only the growth began to be slightly disturbed. It is due to biosorption with bonds between the metallothionein thiol groups (Figure 3), namely polypeptides, containing about 30% cysteine amino acids (Dewi *et al.*, 2018).

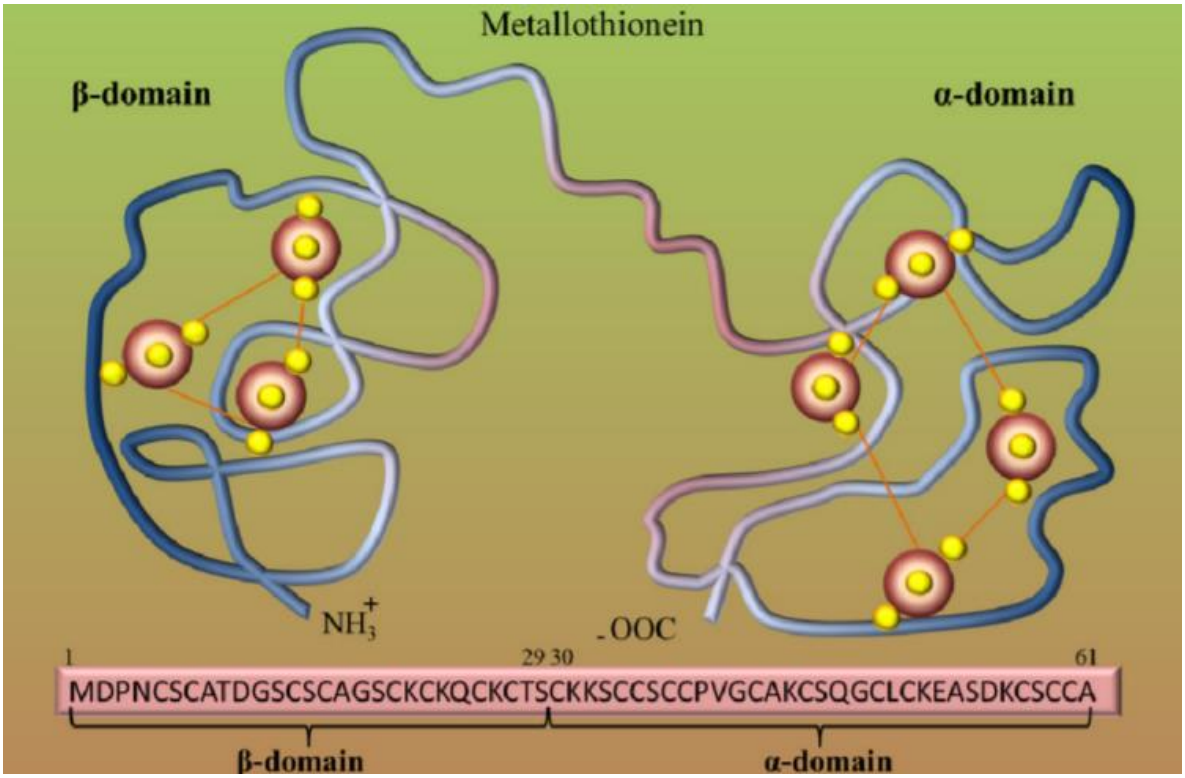

Figure 3 Metallothionein (MT) structure

In media contaminated with Cr (VI) at three ppm (reactor C) in Figure 4, it saw that there was a decrease in the concentration of Cr (VI) in the growing medium also means that there is an increased absorption where until the 12th day there is a decrease of about 50%, the growth in this medium has decreased slightly compared to the media exposed to Cr (VI) of 1 ppm.

Media contaminated with Cr (VI) by five ppm (reactor D) in Figure 4, the decrease in the concentration of Cr (VI) in the growing medium was very low until day 12. It decreased only about 10.29% and showed poor growth, indicating that the growth medium is potentially toxic to microalgae growth.

In the growth medium exposed to 7 ppm Cr (VI) (reactor E) in Figure 4, the decrease in Cr (VI) concentration in the growing medium was also relatively low, or around 8.05%, and also shows that the growth media has the potential to be toxic to microalgae growth where the growth is the least visible compared to the exposure with a lower concentration.

The application of the results of this study can be implemented in a new way as a bio-indicator for companies that produce Cr (VI) metal waste before being released into water bodies or the environment. The growth color, which shows a paler color (least growth), contains high Cr (VI) waste.

Figure 4 Microalgae growth exposed to bioreactors Cr (VI) (0.0; 1.0; 3.0; 5.0, and 7.0) ppm

Application of this technique is where wastewater containing Cr (VI), which has been treated before being released into water bodies, is first flowed through a pond overgrown with *Scenedesmus* sp microalgae. If the growth of *Scenedesmus sp* microalgae is good (green), the waste treatment is good and vice versa if the growth of Scenedesmus sp microalgae is not. Good (pale color) means that the treatment of waste containing Cr (VI) is not good.

4. Conclusions

Microalgae can absorb Cr(VI) well (99.93%) on exposure with a concentration of 1.0 ppm on the 12th day, with a concentration of 3.0 ppm is only able to absorb about 50% on the 12th day, but on with concentrations of 5.0 and 7.0 ppm tend not to absorb and even interfere with the growth of microalgae so this is a new way to be used as an environmental bioindicator for companies that produce Cr(VI) metal waste before being discharged into water bodies or the environment based on the pale color produced. The application of this technique in which wastewater containing Cr(VI) has been treated before being discharged into water bodies flows first through a pond overgrown with microalgae *Scenedesmus sp*. If the growth of *Scenedesmus sp* microalgae is good (green), then the waste treatment is good and vice versa if the growth of *Scenedesmus sp* microalgae is not good. Good (pale color) means that the treatment of waste containing Cr (VI) is not good.

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Tanggal: Jumat, 21 Januari 2022 pukul 09.44 WIB

International Journal of Technology

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Decision Result : Revise

Dear **Dr. Rudi Kartika**

We have finished the review and made decision on your manuscript entitled **[Biosorption of Hexavalent Chromium Cr (VI) Metal by Microalgae Scenedesmus sp as Environmental Bioindicator]** which was submitted to International Journal of Technology.

We have decided that your manuscript **Need to be Revised**

We also send you the review result from the reviewers. Here is the detail review result:

Notes from Editor:
1. Please revise according to the reviewer's comment, and highlights in different colors that changed 2. It is suggested to include at least 3 relevant IJTech articles as references 3. For your information, the maximum paper must be within 10 pages, the overlength pages will be charged US$ 50 per page.

Reviewer (1)
Introduction:
1. remove figure 1
2. introduction is too much and too long
3. Revise the objective
4. improve the english.
5. the last paragraph is not clear

Methodology:
1. revise and arrange again for writing the experimental section
2. add the scheme for cultivation and scheme of bioreactor used

Results and Discussion:
1. add error bars for Figure 2.
2. remove Figure 3.
3. add the scheme and or illustration, how microalga can eliminate the Cr(VI) ions
4. add mechanism reaction, what is the active component from microalgae can reduce/adsorp the Cr(VI) ions?
5. Microalgae can absorb Cr(VI) well (99.93%) on exposure with a concentration of 1.0 ppm on the 12th day. This value is so high? are you sure? add formula to calculate this value in experimental section.
6. This statement is confusing" with a concentration of
3.0 ppm is only able to absorb about 50% on the 12th day, but on with concentrations of 5.0 and 7.0 ppm tend not to absorb"
how can be this happen?

References:
ok
Other:
1. authors have to revise and improve the abstract. currently the abstract is too short and no objective and method

2. improve and revise the conclusion
-add future work

Originality	2 *(fair)*
Technical	1 *(poor)*
Methodology	1 *(poor)*
Readability	1 *(poor)*
Practicability	2 *(fair)*
Organization	1 *(poor)*
Importance	2 *(fair)*

Attachment from reviewer:
-

Reviewer (2)
Introduction:
I must request that further attention is paid to the use of English within the manuscript. It needs language editing.
Methodology:
As the authors did not discuss any significant limitations of the current work, it may be worthwhile to mention a few.
3- Abbreviations should probably be in the manuscript. Abbreviations that are not repeated must not be a part of the abstract. Perhaps, some abbreviations didn't define clearly in the text.
Results and Discussion:
2- The section of "Results and discussion" is not well-presented. There is insufficient scientific discussion to the obtained results and documenting with the literature, also this section require additional support to strengthen the argument. Furthermore, the manuscript requires some proofreading.
References:
-
Other:
Overall, I believe that the presented paper contains adequate material to be published. I would suggest it to be published after major revision as above.

-

Originality	3 *(average)*
Technical	3 *(average)*
Methodology	3 *(average)*
Readability	4 *(above average)*
Practicability	4 *(above average)*
Organization	4 *(above average)*
Importance	3 *(average)*

Attachment from reviewer:
-

Please login into application https://ijtech.eng.ui.ac.id/login for more detail.

You must respond to this revise and resubmit request before **28 Jan 2022**, after which point we will presume that you have withdrawn your submission from International Journal of Technology (IJTech) Online System.

Yours sincerely,

Dr. Nyoman Suwartha
nsuwartha@eng.ui.ac.id

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COVER LETTER

04th of March 2022
Dear Dr. Nyoman Suwartha

We wish to submit revision-1 an original research article entitled "Biosorption of Hexavalent Chromium Cr (VI) using Microalgae *Scenedesmus sp* as Environmental Bioindicator" for consideration by the IJTech. We confirm that this work is original and has not been published elsewhere.

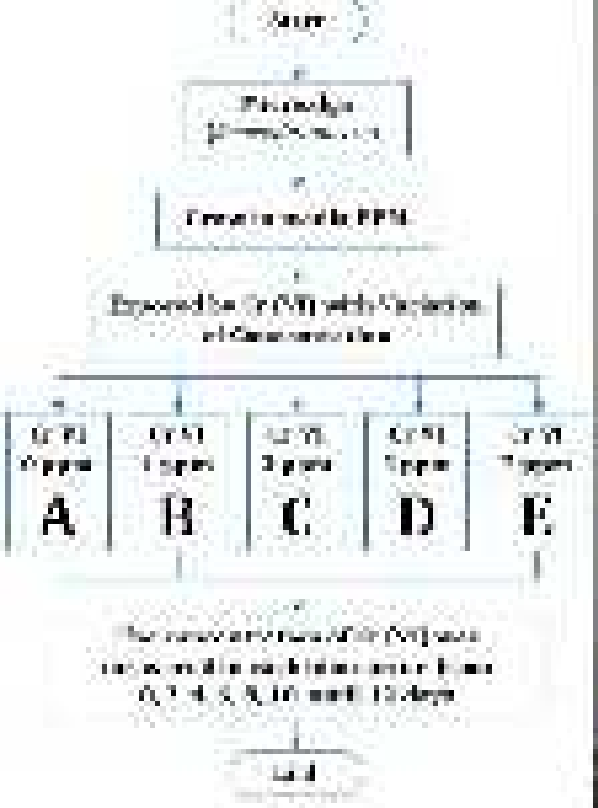
Scenedesmus sp. is a freshwater green alga that functions as an ionic biosorbent and can also be a bioindicator for water contaminated with hexavalent chromium Cr (VI) ion. This study aimed to observe the growth of *Scenedesmus sp.* exposed to Cr (VI) ion at various concentrations and analyze the remaining 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.

We believe that this manuscript is suitable for environmental chemistry and its sustainability for publication. We guarantee that the manuscripts submitted to the journal for review are original written directly by the authors mentioned and have not been published elsewhere. The manuscript is also not currently under consideration for publication by other journals and will not be submitted while it is being reviewed by this journal. This manuscript does not contain defamatory or other unlawful statements and does not contain any material that violates the personal rights or property rights of other people or entities.

Please address all correspondence concerning this manuscript to me at : rudi_biokimia@yahoo.com

Sincerely

Dr. Rudi Kartika


A
B
C
D
E


A
B
C
D
E
Cr (IV)
0.0 ppm
Cr (IV)
0.1 ppm
Cr (IV)
0.3 ppm
Cr (IV)
0.5 ppm
Cr (IV)
0.7 ppm
MICROALGAE GROWTH EXPOSED TO BIOREACTORS

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

Graphical Abstract

Abstract. *Scenedesmus sp* is a freshwater green alga that functions as a metal biosorbent and can also be a bioindicator for water contaminated with hexavalent chromium Cr (VI) metal. This study aimed to observe the growth of *Scenedesmus sp* exposed to Cr (VI) metal with various concentrations and analyze the remaining Cr (VI) metal that did not undergo biosorption by microalga. This research was conducted on *Scenedesmus sp* microalgae growth media using five bioreactors, each with a different Cr (VI) metal exposure concentration. The remaining metal 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 and 7.0 ppm indicating toxic conditions where microalgae growth was very poor.

Keywords: Hexavalent Chromium; Metal; *Scenedesmus sp*; Toxicity.

1. Introduction

Microalgae *Scenedesmus* sp is a biomass that has the most significant ability to bind metal ions such as carboxyl, amine, sulfate, and sulfonate. This microalga can be utilized to treat aquatic waste. The advantages of using this microalga are environmentally friendly, recyclable, and low maintenance costs (Wilan *et al.*, 2020). *Scenedesmus* sp is a cosmopolitan microalga that lives in brackish water and soil with a humid climate. *Scenedesmus* sp cells are cylindrical (8-20 m length and 3-9 m width) and usually live in colonies, with cells covered by three layers consisting of an inner layer (cellulose), a middle layer (membrane structure), and an outer layer net consisting of pectin and fine hairs (Prihantini *et al.*, 2007).

Scenedesmus sp is widely applied as a supplement and fish feed, as an additional pollutant removal agent for wastewater treatment, and as a source of biofuel production due to the fatty acid content of the culture. In addition, *Scenedesmus* sp also works well as a bio-indicator of water pollution using herbicides as a determinant (Fodorpataki *et al.*, 2009; Makareviciene *et al.*, 2011; Sudibandriyo and Putri, 2020).

The increasing industrial growth certainly impacts the increasing concentration of heavy metals, which results in environmental pollution because these heavy metals are not biodegradable and persistent. If it reaches a certain concentration, it can endanger aquatic ecosystems and human health (Suprapto *et al.*, 2020).

Several methods have separated heavy metals in waters, such as physical adsorption, chemical sedimentation, mechanical filtration, and ion exchange. However, the process has drawbacks, such as secondary pollution due to the chemicals used and the high cost. One alternative way that is environmentally friendly to overcome this is biomass or microorganisms, which is also known as biosorption. This method has high efficiency in wastewater detoxification, implementation is simple, and the cost is low. The adsorption of heavy metal ions by microorganisms is rapid and reversible. Biosorption occurs on live or dead biomass because it only occurs on the surface of the microalgae cell wall. However, dead biomass is more profitable because it does not require a supply of nutrients during its growth. Several factors affect biosorption: characteristics of biomass, temperature, pH, biosorbent concentration, contact time, and surface area of biomass. Biomass must be immobilized to avoid blockage of the reaction (Wilan *et al.*, 2020).

Many techniques have been applied to increase the ability of biosorbent, such as modification of the chemical composition to increase absorption by expanding the contact surface by increasing the porosity of the biosorbent and modification of the active groups on the surface of the cell wall of the biosorbent because it plays an important role in binding contaminants (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 processes, complex formation, micro-surface deposition, and ion exchange (Ekmekyapar *et al.*, 2012; Fomina and Gadd, 2014; Kusrini *et al.*, 2019). Microorganisms such as bacteria can absorb Pb metal by micrococcus sp and flavobacterium sp up to 100% at a concentration variation of 2.0 to 10 ppm with an exposure time of 3 to 30 days (Susanto *et al.*, 2019).

One of the most dangerous and toxic heavy metals in wastewater is chromium. 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 poorly absorption by soil and organic matter, making it difficult to mix with soil (Mnif *et al.*, 2017).

The Cr (VI) is released from various industries such as metallurgy, leather tanning, paint, textile, pulp, ore and petroleum refining, metal corrosion, and electroplating. Cr (VI) released into the environment is usually caused by leakage, poor storage, or improper disposal. The negative impact of chromium metal is toxic in the human body because it 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 (Khatoon and Rai, 2016; Khatoon *et al.*, 2013; Rahman and Singh, 2019).

This study aims to observe the growth of *Scenedesmus sp* exposed to Cr (VI) metal with various concentrations in the growth medium and then analyze the remaining Cr (VI) metal in the growth medium with an interval of 2 days that did not undergo biosorption by microalga. The absorption of Cr (VI) metal can be a bioindicator for the environment by providing information about the growth of microalgae *Scenedesmus sp*, which is disturbed at a certain concentration and is characterized by white growth media (not growing or dying). However, if the growth medium is green, the growth is normal (not disturbed by Cr (VI) metal.

2. Methods

2.1. Microalgae Cultivation Process and Exposed To Bioreactors

2.13 g of the growth media for algae or bold basal medium (BBM) was dissolved in 3 L of aquadest to achieve a BBM solution (Figure 1a). Five photobioreactors were prepared and then added with potassium dichromate ($K_2Cr_2O_7$) solution to achieve a Cr (VI) solution with a concentration of 0, 1, 3, 5, and 7 ppm. Next, the microalgae culture of *Scenedesmus sp* was inoculated into five photobioreactors that had been filled with BBM solution and aerated. The microalgae were given fluorescent lighting (1500 lux) with light conditions for 12 h and dark conditions for 12 h at 25 ˚C. The Cr (VI) with variation concentration (0, 1, 3, 5, and 7 ppm) was measured in each bioreactor starting from 0, 2, 4, 6, 8, 10, until 12 days (Figure 1b).

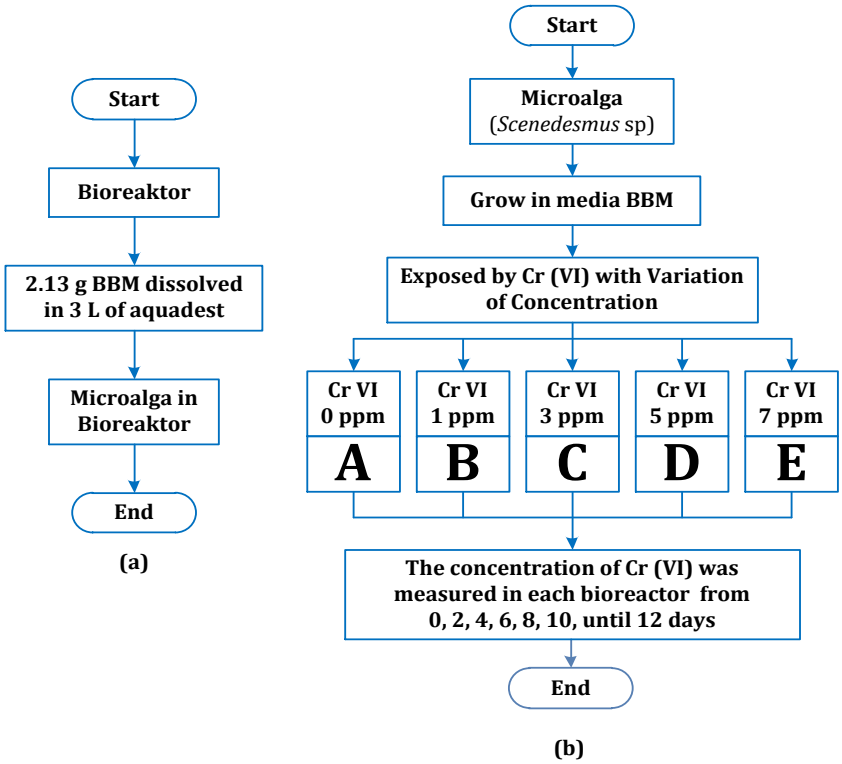

Figure 1 Scheme of (a) Microalga Cultivation, (b) Exposed To Bioreactors

2.3. Preparation of Cr (VI) Standard Solution

$K_2Cr_2O_7$ of 0.1414 g was dried in an oven, dissolved with 100 mL aquadest in a volumetric flask, and achieved Cr (VI) 500 ppm solution. After that, 10 mL of Cr (VI) 500 ppm solution was diluted with 100 mL aquadest in a volumetric flask and achieved Cr (VI) 50 ppm solution. Next, 10 mL of Cr (VI) 50 ppm solution was diluted with 100 mL aquadest in a volumetric flask and achieved a standard solution of Cr (VI) 5 ppm.

2.4. Curve Calibration

The Cr(VI) 5 ppm solution of 2; 4; 6; 8; 10; 12; 14; 16; 18; and 20 mL was put into a 100 mL volumetric flask, added five drops of H_3PO_4, adjusted the pH by adding 0.2 M H_2SO_4 until it reached pH 2. Next, diphenylcarbazide of 2 mL was added to each solution and filled aquadest up to the marked line. So it can be a standard solution for calibration curve with concentration of 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 and 1.0 ppm. Waited for 10 min and absorbance was measured at a wavelength of 540 nm.

2.5. Measurement of Chromium Concentration

The sample solution of 10 mL was filtered using a 0.45-micron folder membrane. Next, it was treated according to a calibration curve standard solution, and the concentration was measured at a wavelength of 540 nm.

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

In each treatment of 0; 2; 4; 6; 8; 10, and 12 days, the sample of 10 mL was taken and filtered with a vacuum filter using a millipore membrane (0.4 microns), then determined the concentration of Cr (VI). The Cr(VI) which undergoes biosorption is the concentration of Cr(VI) obtained (ppm) minus the concentration of Cr(VI) remaining in the medium.

3. Results and Discussion

Based on Table 1, it can be shown that the Cr (VI) metal concentration decreased with increasing contact time. The longer the exposure time, the longer the collision time, so the interactions between the biosorbent and metal ions also increase, which causes many active groups to bind metal ions and increase the amount of metal absorbed. Biosorption will increase significantly with increasing contact time until it reaches the equilibrium point or optimum conditions. The length of contact time affects the metal-binding process by the biosorbent surface before the surface reaches the saturation point. When the biosorbent has reached the optimum condition, the ability of the biosorbent to bind heavy metals will decrease because the surface capacity of the cell wall is saturated.

Table 1 Absorption of Cr (VI) with variations in concentration (ppm) againts time (days)

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	

Figure 2 displayed that the growth medium with exposure to Cr (VI) 1.0 ppm can be absorbed almost completely or 99.93%, which indicates that it can still be absorbed exposure concentrations of 1 ppm very well, and microalga can still grow as well. It means that the growth medium is not yet toxic to the growth of microalgae *Scenedesmus sp*. Based on the concentration of Cr (VI) exposed and remaining in the growth medium, the proportion of biosorption can be determined based on the following equation:

$$\%\ Biosorption\ of\ Cr\ (VI) = \frac{(Ce - Cr)ppm}{Ce\ ppm} \times\ 99{,}93\%$$

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

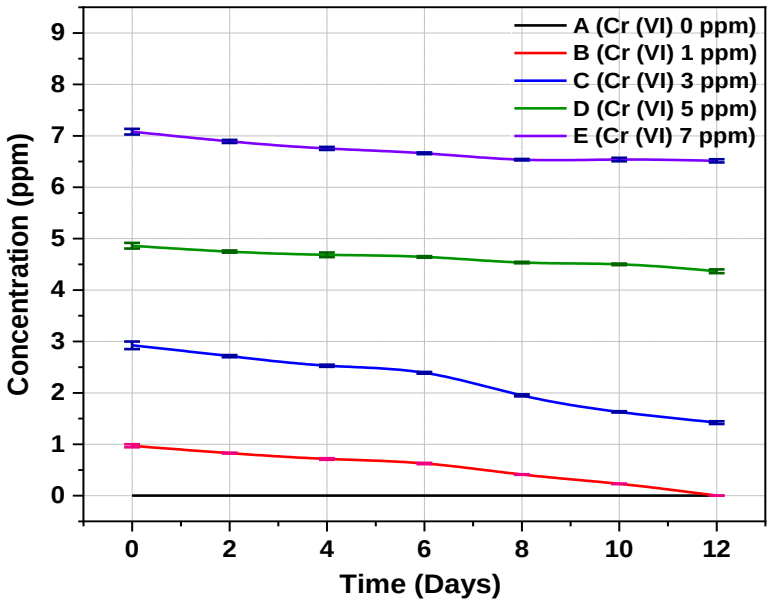

Figure 2 Absorption of Cr (VI) with variations in concentration againts time

At Cr (VI) 3.0 ppm, the exposure growth medium displayed that the metal absorption increased from the 2nd to the 12th day. However, the increase was smaller than the 1.0 ppm Cr (VI) metal exposure, which means that Cr (VI) metal was still absorbed but is toxic. Microalgae growth of *Scenedesmus sp* was disrupted compared to exposure to Cr (VI) metal 1.0 ppm because the metal ion cofactor required by the enzyme is non-competitively inhibited because the complex reagents exchange metal ions from the enzyme in greater amounts than their tolerance limit (Susanto *et al.*, 2019).

At Cr (VI) 5.0 ppm, the exposure growth medium showed a decrease in the absorption value due to Cr (VI) metal's increased toxicity on the growth of microalgae *Scenedesmus sp*. Likewise, the absorption of Cr (VI) 7.0 ppm on exposure to growth media, the absorption value becomes very small, and almost the microalgae *Scenedesmus sp* nothing grows. It indicates that the tolerance limit for growth with exposure to Cr (VI) is 7.0 ppm. The Cr (VI) 5.0 ppm and Cr (VI) 7.0 ppm have passed the tolerance limit, so both of them did not experience growth which was marked by a non-green color (white color). It is a bio-indication that the growth media already contains chromium metal with high concentrations (Susanto *et al.*, 2019).

The growth medium that was not exposed to Cr (VI) 0.0 ppm (reactor A) in Figure 3 did not detect the presence of Cr (VI), and the growth of microalgae was quite good. It could be shown from the greener color compared to other growth media. Meanwhile, in the growth media contaminated with Cr (VI) 1.0 ppm (reactor B), the absorption process had occurred from day 2nd to day 12th, and the concentration of remaining metals in the growth medium decreased to 0 ppm on day 12th (99.93% absorbed). It showed good absorption at exposure to a concentration of 1.0 ppm, and only the growth was slightly disturbed.

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

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

Notes: M = Metal, R = Protein radicals from microalga, 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 *et al.*, 2018).

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

In media exposed to Cr (VI) 3.0 ppm (reactor C) in Figure 3, it can be shown that there is a decrease in the Cr (VI) concentration of 50% in the growth medium until the 12th day. The growth media in reactor C has decreased by 50% compared to the growth medium in reactor B. The media exposed to Cr (VI) 5 ppm (reactor D) showed that the Cr (VI) concentration decreased only about 10.29% in the growth medium until the 12th day. Likewise, on growth media exposed to Cr (VI) 7 ppm (reactor E) showed that the Cr (VI) concentration decreased only about 8.05% in the growth medium, which shows a poor growth in reactor D and reactor E. Both of these reactors have the potential to be toxic to microalgae growth.

The results of this research can be implemented in a new way as a bioindicator for companies that produce Cr (VI) metal waste before being discharged into water bodies or the environment. The growth color, which shows a paler color (slowest growth), indicated high Cr (VI) waste. The wastewater treatment process containing Cr (VI) produced by the company can be carried out by first flowing it through a pond overgrown with Scenedesmus sp. microalga. If the growth of Scenedesmus sp microalga is well (green), then the waste treatment is good. Otherwise, if the growth of Scenedesmus sp microalga is not good (pale color), it means that the treatment of waste containing Cr (VI) is not good. The results of good waste treatment can be discharged into water bodies or the environment.

4. Conclusions

Microalgae can absorb Cr (VI) well (99.93%) on exposure with a concentration of 1.0 ppm on the 12th day, with a concentration of 3.0 ppm is only able to absorb about 50% on the 12th day, but on with concentrations of 5.0 and 7.0 ppm tend not to absorb and even interfere with the growth of microalgae. The application of this technique can be used as an environmental bioindicator for companies that produce Cr (VI) metal waste before being discharged into water bodies or the environment based on the pale color it produces.

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List of Changes

Manuscript:

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

Response and Revision made by Author(s)

Reviewer #1:

No	Comments	Revision/Changes
1	**Introduction :** Remove figure 1	Thanks for the advice, I have removed Figure 1 "Microalgae *Scenedesmus sp"*
2	Introduction is too much and too long	Thanks for the advice, I have summarized the introduction
3	Revise the objective	Thanks for the advice, I have revised the objective
4	Improve the English.	Thanks for the advice I have improved the English and checked the grammar.
5	The last paragraph is not clear	Thanks for the advice I have improved the last paragraph by the revise the objective of the research
6	**Methodology :** Revise and arrange again for writing the experimental section	Thank for the advice, I have revised and rearranged the writing on experimental section.
7	Add the scheme for cultivation and scheme of bioreactor used	Thank for the advice, I have added the scheme of microalga cultivation (Figure 1a) and scheme of exposed to bioreactor (Figure 1b).
8	**Results and Discussion :** Add error bars for Figure 2.	Thanks for the advice. We have added error bars in Figure 2.
9	Remove Figure 3.	Thanks for the advice. We have removed Figure 3 "Metallothionein (MT) structure"
10	Add the scheme and or illustration, how microalga can eliminate the Cr (VI) ions	Thanks for the advice and question. We have explained illustration how the microalga can eliminate the Cr (VI) ions in the manuscript. The microalga in growth media can eliminate the CR (IV) ion was due to the presence of biosorption with bonds between metallothionein thiol groups and the non-competitive inhibitory effect of Cr (VI) ion form mercaptide salts with sulfhydryl groups of enzyme proteins.

No	Comments	Revision/Changes
11	Add mechanism reaction, what is the active component from microalgae can reduce/ adsorb the Cr (VI) ions?	Thanks for the advice and question. We have added a mechanism reaction in the manuscript with an explanation. $M + R - SH \rightleftharpoons MSR + H^+$ The active component from microalga can reduce the CR (IV) ion concentration was due to the presence of biosorption with bonds between metallothionein thiol groups, namely polypeptides containing about 30% of the amino acid cysteine and the non-competitive inhibitory effect of Cr (VI) ion form mercaptide salts with sulfhydryl groups of enzyme proteins.
12	Microalgae can absorb Cr (VI) well (99.93%) on exposure with a concentration of 1.0 ppm on the 12th day. This value is so high? Are you sure? Add formula to calculate this value in experimental section.	Thanks for the question. Yes, we are sure that the growth medium with Cr (VI) 1.0 ppm exposure can absorb almost completely or 99.93%, indicating that microalgae can still grow well. Based on the Cr (VI) concentration exposed and remaining in the growth medium, the proportion of biosorption can be determined with the following equation: $\%\ Biosorption\ of\ Cr\ (VI) = \frac{(Ce - Cr)ppm}{Ce\ ppm} \times 99{,}93\%$ Note: *Ce* = Concentration of Cr (VI) exposed in the growth medium (ppm). *Cr* = Concentration of Cr (VI) remaining in the growth medium (ppm).
13	This statement is confusing" with a concentration of 3.0 ppm is only able to absorb about 50% on the 12th day, but on with concentrations of 5.0 and 7.0 ppm tend not to absorb" how can be this happen?	Thanks for the question. Media exposed to Cr (VI) 3.0 ppm can decrease the Cr (VI) concentration by 50% until the 12th day. However, in media exposed to Cr (VI) 5 ppm and Cr (VI) 7 ppm showed that the Cr (VI) concentration decreased only about 10.29% and 8.05% in the growth medium. It happens because both of them have passed the tolerance limit, so they did not experience growth (a poor growth) and have the potential to be toxic for microalgae growth.
10	**References:** Ok.	Thank you
11	**Conclusion:** Improve and revise the conclusion. Add future work.	Thanks for the advice. We have improved and revised the conclusion. We also added about the future work that can be applied by industry.

No	Comments	Revision/Changes
12	**Other:** Authors have to revise and improve the abstract. Currently the abstract is too short and no objective and method.	Thanks for the advice. We have revised and improved the abstract by adding objectives and methods

Reviewer #2:

No	Comments	Revision/Changes
1	**Introduction :** I must request that further attention is paid to the use of English within the manuscript. It needs language editing.	Thanks for the advice. We have corrected the sentences in the introduction, improved the use of English in the manuscript, and re-checked the grammar of English by grammarly.com.
2	**Methodology:** As the authors did not discuss any significant limitations of the current work, it may be worthwhile to mention a few.	Thanks for the advice. The limitations of this study were concentration and exposure time. The concentrations analyzed were: 0.0; 1.0; 3.0, 5.0, and 7.0 ppm. Because the maximum concentration that can be absorbed is only 1.0 ppm above, it has decreased significantly. The exposure times are 2, 4, 6, 8, 10, and 12 days because the duration of 12 days has resulted in almost complete significant absorption at a concentration of Cr(VI) 1 ppm.
3	Abbreviations should probably be in the manuscript. Abbreviations that are not repeated must not be a part of the abstract. Perhaps, some abbreviations didn't define clearly in the text.	Thanks for the advice. We have corrected and defined some abbreviations that were previously unclear.
4	**Results and Discussion:** The section of "Results and discussion" is not well-presented. There is insufficient scientific discussion to the obtained results and documenting with the literature, also this section require additional support to strengthen the argument. Furthermore, the manuscript requires some proofreading.	Thanks for the advice. We have revised the results and discussion in more detail by presenting the calculation method and the reaction mechanism that can reduce metal with microalgae in the growth medium. We have also done proofreading of this section.
5	**References:** -	Thank you
6	**Other:** Overall, I believe that the presented paper contains adequate material to be published. I would suggest it to be published after major revision as above.	Thank you.

[IJTech] Manuscript Submission Notification for #R1-CE-5188

Dari: IJTech (noreply@ijtech.eng.ui.ac.id)

Kepada: rudi_biokimia@yahoo.com

Tanggal: Selasa, 25 Januari 2022 pukul 23.24 WIB

International Journal of Technology

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Manuscript Submission Confirmation

Dear Dr. Rudi Kartika,

Your revised manuscript entitled **"Biosorption of Hexavalent Chromium Cr (VI) Metal by Microalgae Scenedesmus sp as Environmental Bioindicator"** has been successfully submitted to International Journal of Technology (IJTech) Online System.

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[IJTech] Decision for manuscript #R1-CE-5188: Need to be Revised

Dari: IJTech (noreply@ijtech.eng.ui.ac.id)

Kepada: rudi_biokimia@yahoo.com; liliksulastri28@gmail.com; sittinurliana@gmail.com; ddy_iwn@yahoo.com; partomsimanjuntak@gmail.com; ahmad.hafizullah.r@gmail.com

Tanggal: Rabu, 2 Maret 2022 pukul 09.31 WIB

International Journal of Technology
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Decision Result : Revise

Dear **Dr. Rudi Kartika**

We have finished the review and made decision on your manuscript entitled **[Biosorption of Hexavalent Chromium Cr (VI) Metal by Microalgae Scenedesmus sp as Environmental Bioindicator]** which was submitted to International Journal of Technology.

We have decided that your manuscript **Need to be Revised**

We also send you the review result from the reviewers. Here is the detail review result:

Notes from Editor:
Please revise according to the reviewer's comment, and highlights in different colors that changed

Reviewer (1)

Introduction:
The manuscript has been well revised. Introduction is clear.

Methodology:
Acceptable

Results and Discussion:
Fig 2. Authors should give two figures; one figure is the plots of Cr(VI) concentration as a function of time, and another figure is the plots of the percentage of Cr(IV) removal as a function of time. Authors also need to present the plots of the percentage of Cr(IV) removal as a function of initial Cr concentration. This latter figure could tell the ideal ratio of Cr and microalgae for the optimum Cr removal.

References:
Acceptable

Other:
1. Data analysis needs to be improved.
2. Some sentences are really difficult to be understood. English of this manuscript needs to be improved.

Originality	3 *(average)*
Technical	3 *(average)*
Methodology	3 *(average)*
Readability	3 *(average)*
Practicability	4 *(above average)*

Organization	3 *(average)*
Importance	3 *(average)*

Attachment from reviewer:
-

Reviewer (2)
Introduction:
ok
Methodology:
1. Improve Fig. 1(a). Bioreaktor should be bioreactor.
Results and Discussion:
1. See pages 4. the number is still in bahasa
2. Why authors use value 99.3%?. it is not common fomula for calculation of efficiency?
3. authors have to concern for some items such as subscript and or superscript for nd, th and so on. please check it all.
4. add 1 table for comparison several types of adsorbent and or method to adsorp the Cr ions.
References:
Authors have to add more references related with adsorption of Cr ions and type of adsorbent used.
Other:
1. the english should be improved all manucript
2. no need copy paste graphical abstract in manuscript.
3 graphical abstract is not interesting. This figure is similar with Fig. 1a and Fig. 3
3. Revise the title. currently the title is not interesting.

Originality	2 *(fair)*
Technical	1 *(poor)*
Methodology	1 *(poor)*
Readability	1 *(poor)*
Practicability	1 *(poor)*
Organization	1 *(poor)*
Importance	2 *(fair)*

Attachment from reviewer:
-

Please login into application https://ijtech.eng.ui.ac.id/login for more detail.

You must respond to this revise and resubmit request before **09 Mar 2022**, after which point we will presume that you have withdrawn your submission from International Journal of Technology (IJTech) Online System.

Yours sincerely,

Dr. Eny Kusrini
Managing Editor
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COVER LETTER

04th of March 2022
Dear Dr. Nyoman Suwartha

We wish to submit revision-1 an original research article entitled "Biosorption of Hexavalent Chromium Cr (VI) using Microalgae *Scenedesmus sp* as Environmental Bioindicator" for consideration by the IJTech. We confirm that this work is original and has not been published elsewhere.

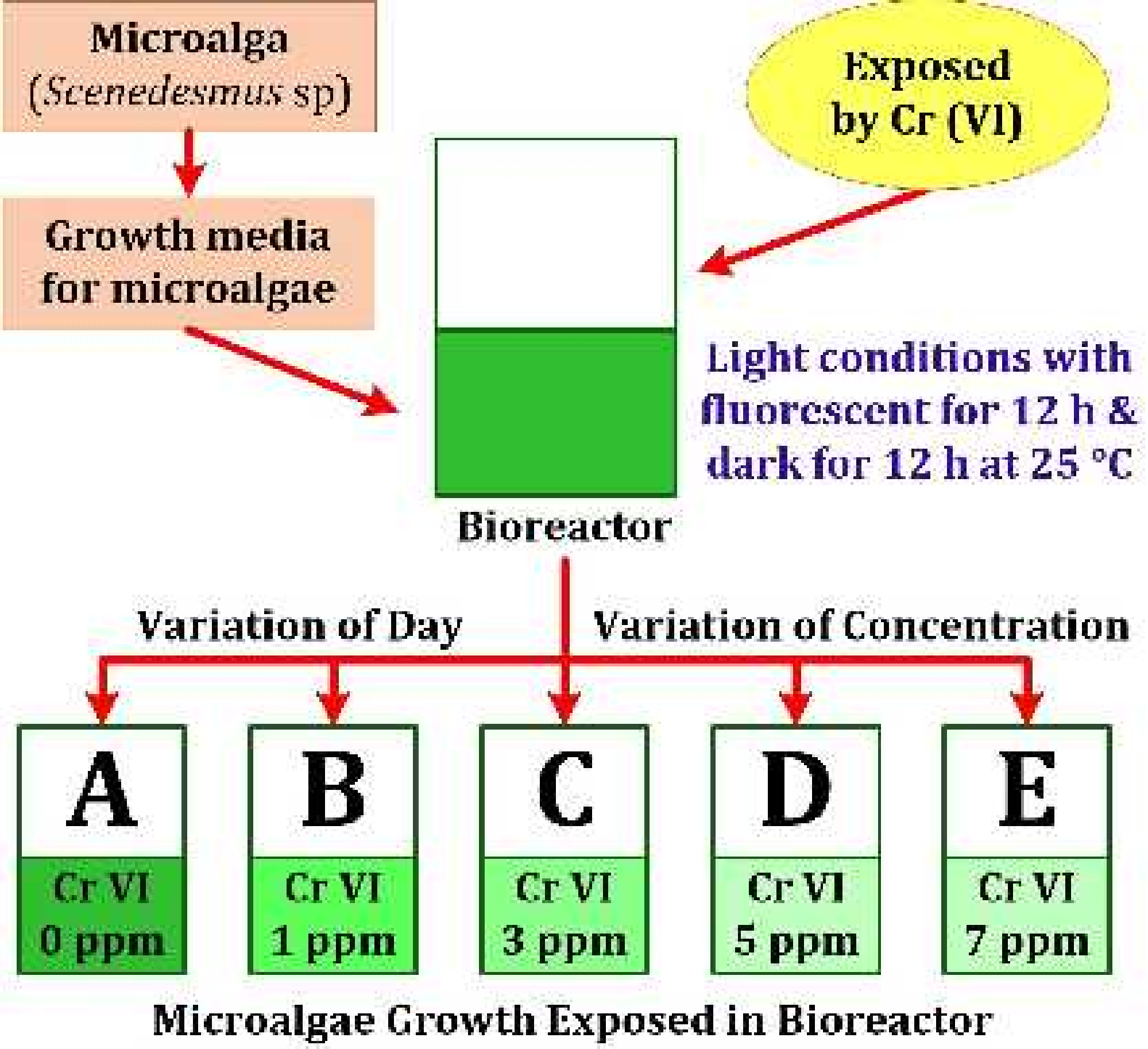
Scenedesmus sp. is a freshwater green alga that functions as an ionic biosorbent and can also be a bioindicator for water contaminated with hexavalent chromium Cr (VI) ion. This study aimed to observe the growth of *Scenedesmus sp.* exposed to Cr (VI) ion at various concentrations and analyze the remaining 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.

We believe that this manuscript is suitable for environmental chemistry and its sustainability for publication. We guarantee that the manuscripts submitted to the journal for review are original written directly by the authors mentioned and have not been published elsewhere. The manuscript is also not currently under consideration for publication by other journals and will not be submitted while it is being reviewed by this journal. This manuscript does not contain defamatory or other unlawful statements and does not contain any material that violates the personal rights or property rights of other people or entities.

Please address all correspondence concerning this manuscript to me at : rudi_biokimia@yahoo.com

Sincerely

Dr. Rudi Kartika



Microalgae Growth Exposed in Bioreactor

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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: Hexavalent Chromium; Biosorption; *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 *et al.*, 2007).

Scenedesmus sp. is widely utilized as a supplement and fish feed, as an additional pollutant removal agent for wastewater treatment, and as a source of biofuel due to the fatty acid content of the culture. In addition, *Scenedesmus* sp. also functions well as a bio-indicator of water pollution using herbicides as a determinant (Fodorpataki *et al.*, 2009; Makareviciene *et al.*, 2011; Sudibandriyo and Putri, 2020).

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doi: 10.14716/ijtech.v0i0.0000

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. The adsorption of heavy metal ions by microorganisms 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 surface area of biomass. 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 (Ekmekyapar *et al.*, 2012; Fomina and Gadd, 2014; Kusrini *et al.*, 2019). 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 time of 3 to 30 days (Susanto *et al.*, 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 (Khatoon and Rai, 2016; Khatoon *et al.*, 2013; Rahman and Singh, 2019).

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).

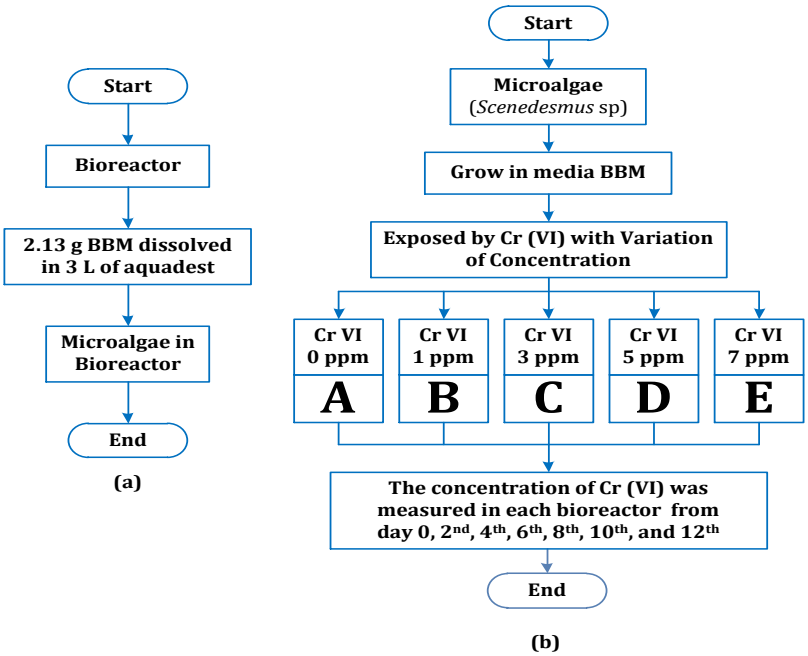

Figure 1 Scheme of (a) Microalgae Cultivation, (b) Exposed To Bioreactors

2.3. 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 solution of Cr (VI) 5 ppm.

2.4. 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_2SO_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.

2.5. 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.

2.6. 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 number of 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 more heavy metals because the surface of the cell wall is saturated.

Table 1 Absorption of Cr (VI) with variations 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 *et al.*, 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 (IV) 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 concentration of chromium. The growth medium is not yet toxic to the growth of microalgae *Scenedesmus sp*.

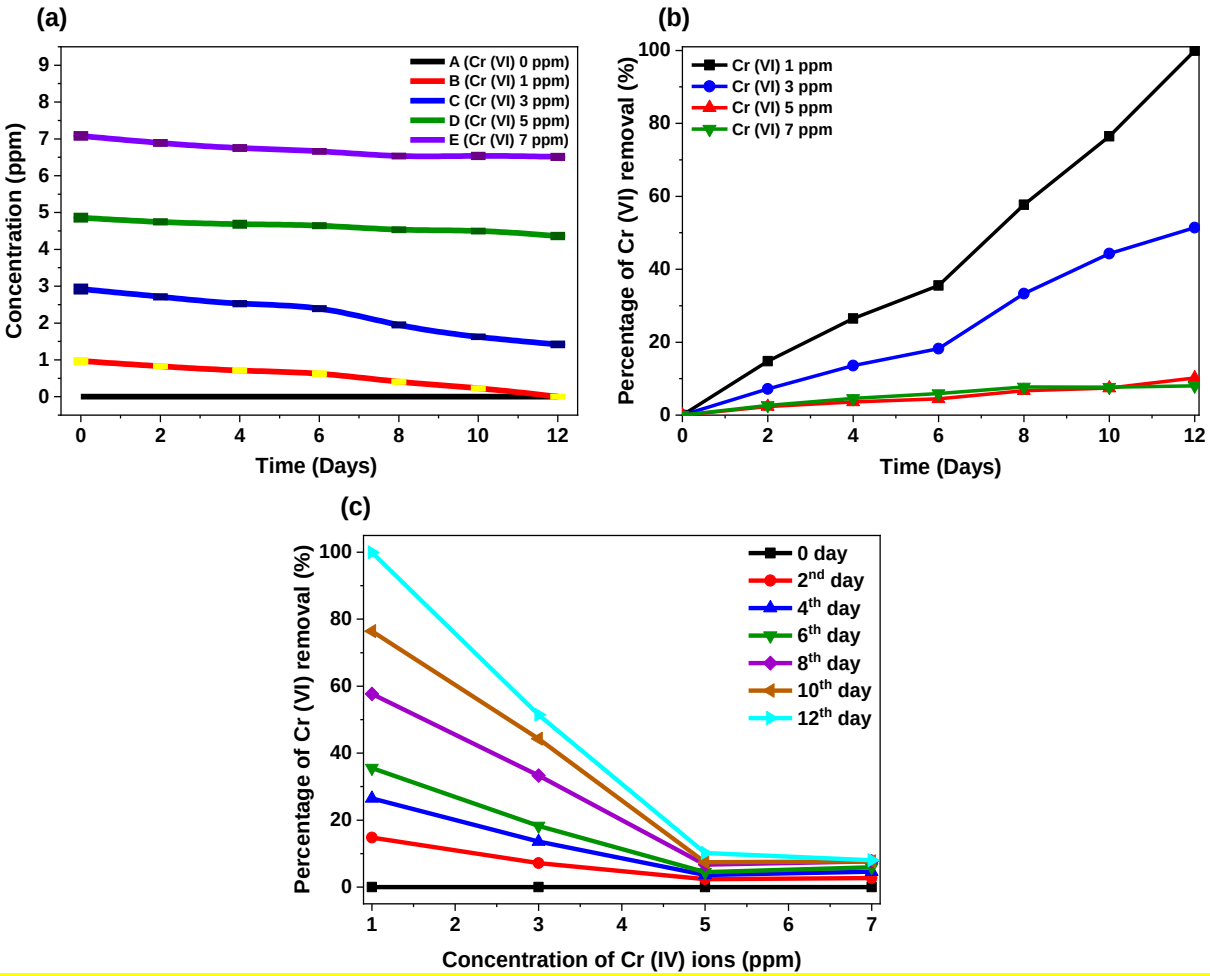

Figure 2 (a) Plots of Cr (VI) concentration as a function of time, (b) plots of the percentage of Cr (VI) removal as a function of time, and (c) the plots of the percentage of Cr (VI) removal as a function of Cr (VI) concentration

At Cr (VI) 3.0 ppm, the 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 *et al.*, 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 *et al.*, 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 *et al.*, 2018), and (2) the non-competitive inhibitory effect of Cr (VI) ion to form mercaptide salts with sulfhydryl groups of enzyme proteins :

$$M + R - SH \rightleftharpoons MSR + H^{+}$$

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 *et al.*, 2018).

Figure 3 Microalgae growth exposed to bioreactors Cr (VI) 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 had 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 that have been used as bio-sorbents and the mechanism that occurs in the absorption of Cr (VI) ions which is 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 : (Fernández-López *et al.*, 2014; Jobby *et al.*, 2018)

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) ion. 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, while the mediums with 5.0 ppm and 7.0 ppm chromium were toxic to the microalga, and very little chromium was 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.

References

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List of Changes

Manuscript:

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

Response and Revision made by Author(s)

Reviewer #1:

No	Comments	Revision/Changes
1	**Introduction :** The manuscript has been well revised. Introduction is clear.	Thank you.
2	**Methodology :** Acceptable	Thank you.
3	**Results and Discussion :** Fig 2. Authors should give two figures; one figure is the plots of Cr (VI) concentration as a function of time, and another figure is the plots of the percentage of Cr (VI) removal as a function of time.	Thanks for the advice. We have added a graph plot of the percentage of Cr (IV) removal as a function of time and have given two graphs; (1) the plots of Cr (VI) concentration as a function of time, and (2) the plots of the percentage of Cr (VI) removal as a function of time.
4	Authors also need to present the plots of the percentage of Cr (VI) removal as a function of initial Cr concentration. This latter figure could tell the ideal ratio of Cr and microalgae for the optimum Cr removal.	Thanks for the advice. We have added the graph plots the percentage of Cr(IV) removal as a function of time.
5	**References:** Acceptable	Thank you
6	**Conclusion:** -	Thank you
7	**Other:** Data analysis needs to be improved.	Thank you for the advice. We have improved by adding a Table 2 about data percentage of (Cr (VI) ions removal with variations in concentration and time.
	Some sentences are really difficult to be understood. English of this manuscript needs to be improved.	Thanks for the advice. We have improved it via **https://proofreadingmalaysia.com/**

Reviewer #2:

No	Comments	Revision/Changes
1	**Introduction :** Ok	 Thank you
2	**Methodology:** Improve Fig. 1(a). Bioreaktor should be bioreactor.	 Thanks for the advice. We have fixed it.
3	**Results and Discussion:** See pages 4. the number is still in bahasa.	 Thanks for the advice. We have fixed it.
4	Why authors use value 99.3%?. it is not common formula for calculation of efficiency?	Thanks for the advice. We have corrected and changed it to 100%.
5	Authors have to concern for some items such as subscript and or superscript for nd, th and so on. please check it all.	Thanks for the advice. We have fixed it.
6	Add 1 table for comparison several types of adsorbent and or method to adsorp the Cr ions.	Thanks for the advice. We have added it.
7	**References:** Authors have to add more references related with adsorption of Cr ions and type of adsorbent used.	Thanks for the advice. We have added four references related to the adsorption of Cr ions and the type of adsorbent used.
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9	No need copy paste graphical abstract in manuscript.	Thanks for the advice. We have removed and changed it.
10	Graphical abstract is not interesting. This figure is similar with Fig. 1a and Fig. 3.	Thanks for the advice. We have removed and changed it.
11	Revise the title. currently the title is not interesting.	Thanks for the advice. We have removed the word “Metal” and changed the word “by” with “using”

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Author(s) : Rudi Kartika, Ahmad Hafizullah Ritonga, Lilik Sulastri, Sitti Nurliana, Deddy Irawan, Partomuan Simanjuntak

Dear **Dr. Rudi Kartika** ,

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

Rudi Kartika[1*], Ahmad Hafizullah Ritonga[2], Lilik Sulastri[3], Siti Nurnila[4], Dedy Irawan[5], Partomuan Simanjuntak[6]

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[3]*Sekolah Tinggi Teknologi Industri dan Farmasi (STTIF), Bogor-16151, Indonesia*
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[6]*Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Serpong-15314, Indonesia*

Abstract. *Scenedesmus sp.* is a freshwater green alga that functions as an ionic biosorbent~~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; ~~Biosorption;~~ *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 *et al.*, 2007).

Scenedesmus sp. is widely utilized as a supplement and fish feed, as an additional pollutant removal agent for wastewater treatment, and as a source of biofuel due to the fatty acid content of the culture. In addition, *Scenedesmus* sp. also functions well as a bio-indicator of water pollution using herbicides as a determinant (Fodorpataki *et al.*, 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'~~The~~ adsorption of heavy metal ions ~~by microorganisms~~ 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~~biosorbent~~ concentration, contact time, and biomass surface area~~surface area of biomass~~. 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~~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 (Ekmekyapar *et al.*, 2012; Fomina and Gadd, 2014; Kusrini *et al.*, 2019). 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 ~~time~~ of 3 to 30 days (Susanto *et al.*, 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 (Khatoon *et al.*, 2013; Khatoon and Rai, 2016; ~~Khatoon *et al.*, 2013;~~ Rahman and Singh, 2019).

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

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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).

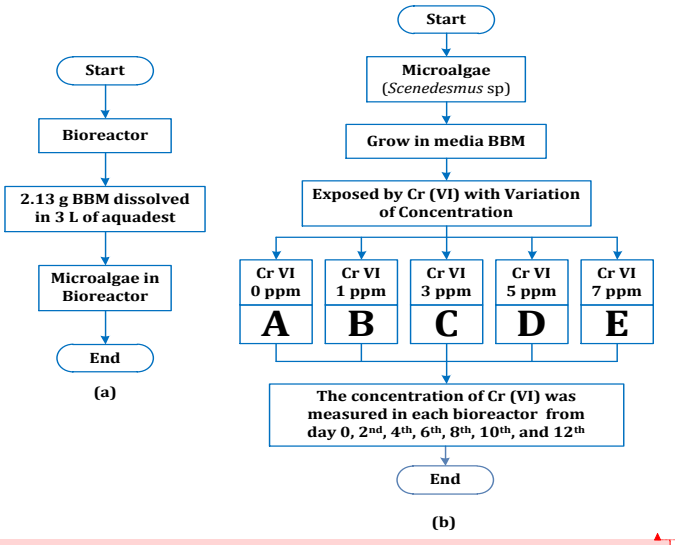

Figure 1~~1~~ Scheme of (a) Microalgae Cultivation, (b) Exposed To Bioreactors

2.3. 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 ~~solution of~~ Cr (VI) 5 ppm solution.

2.4. 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_2SO_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

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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.

2.5. 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.

2.6. 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 ~~number of~~ possible interactions between the biosorbent~~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~~biosorbent~~ surface before the surface reached the saturation point. When the biosorbent~~biosorbent~~ has reached the equilibrium point, the biosorbent~~biosorbent~~ will not bind any ~~more heavy~~heavier metals because the surface of the cell wall is saturated.

Table 1~~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 *et al.*, 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 22 Percentage of Cr (VI) ion removal with variations in concentration and time

	Cr (IV) 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~~concentration of chromium~~. The growth medium is not yet toxic to the growth of microalgae *Scenedesmus sp*.

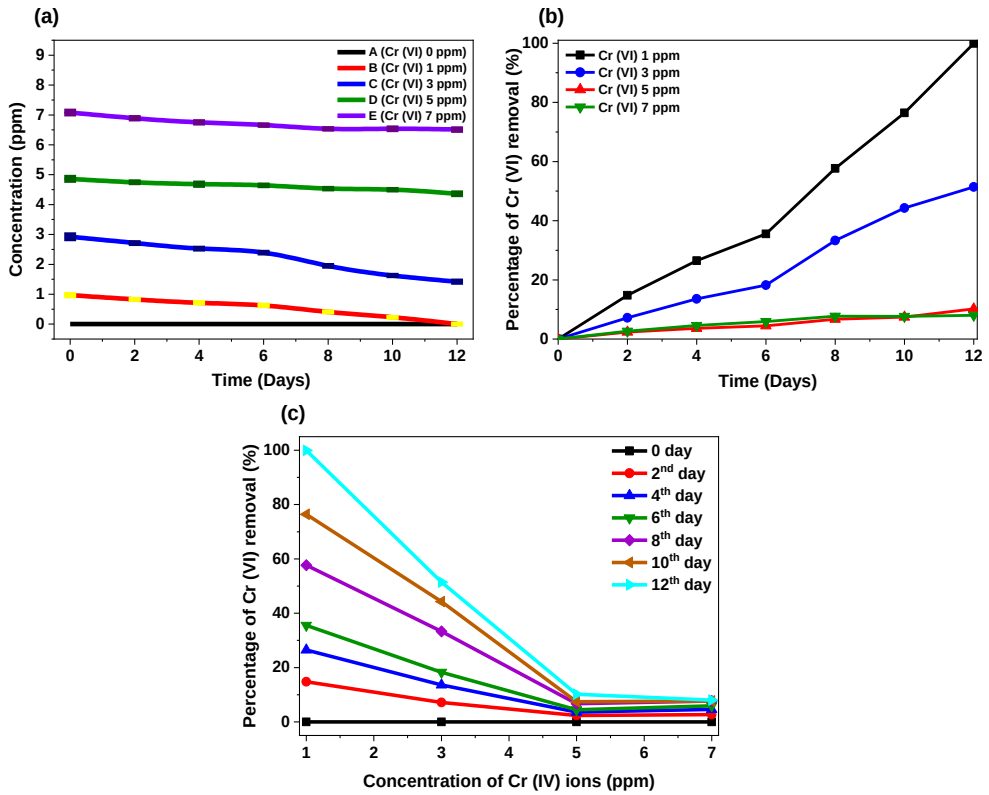

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Figure 22 (a) Plots of Cr (VI) concentration as a function of time, (b) plots of the percentage of Cr (VI) removal as a function of time, and (c) the plots of the percentage of Cr (VI) removal as a function of Cr (VI) concentration

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 *et al.*, 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 *et al.*, 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 *et al.*, 2018), and (2) the non-competitive inhibitory effect of Cr (VI) ion to form mercaptide salts with sulfhydryl groups of enzyme proteins :

$$M + R - SH \rightleftharpoons MSR + H^{+}$$

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 *et al.*, 2018).

Figure 33 Microalgae growth exposed to bioreactors Cr (VI) 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 had occurred from day second to twelfth, and

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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 ~~that have been~~ used as bio-sorbents and the mechanism that occurs in the absorption of Cr (VI) ions ~~which is~~ stated in Table 3.

Table 3~~3~~ Several types of biosorbents~~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 : (Fernández-López *et al.*, 2014; Jobby *et al.*, 2018)

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~~ion~~. 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~~, while the~~ mediums with 5.0 ppm and 7.0 ppm chromium were toxic to the microalga, with~~and~~ very little chromium ~~was~~

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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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 *et al.*, 2007).

Scenedesmus sp. is widely utilized as a supplement and fish feed, as an additional pollutant removal agent for wastewater treatment, and as a source of biofuel due to the fatty acid content of the culture. In addition, *Scenedesmus* sp. also functions well as a bio-indicator of water pollution using herbicides as a determinant (Fodorpataki *et al.*, 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 (Ekmekyapar *et al.*, 2012; Fomina and Gadd, 2014; Kusrini *et al.*, 2019). 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 *et al.*, 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 (Khatoon *et al.*, 2013; Khatoon and Rai, 2016; Rahman and Singh, 2019).

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).

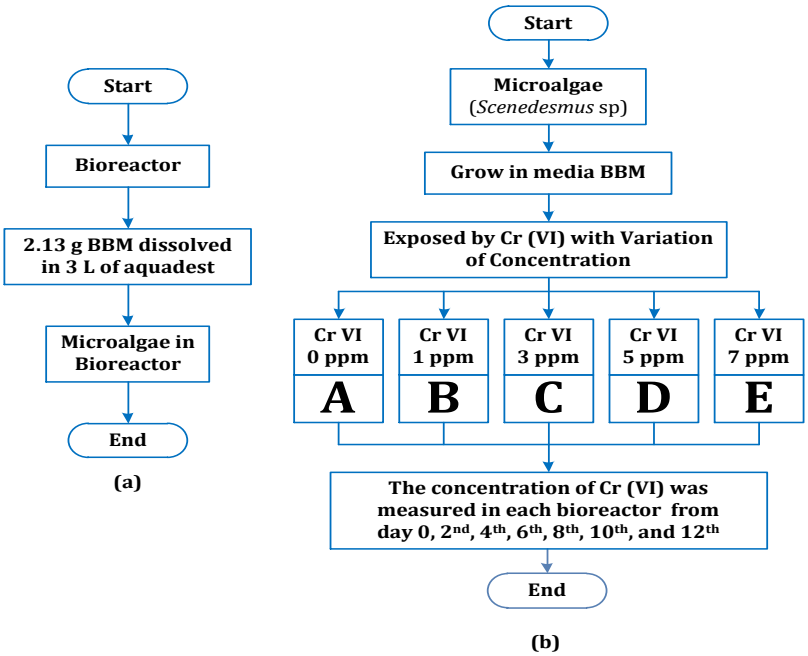

Figure 1 Scheme of (a) Microalgae Cultivation, (b) Exposed To Bioreactors

2.3. 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.

2.4. 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_2SO_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.

2.5. 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.

2.6. 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 *et al.*, 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 (IV) 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*.

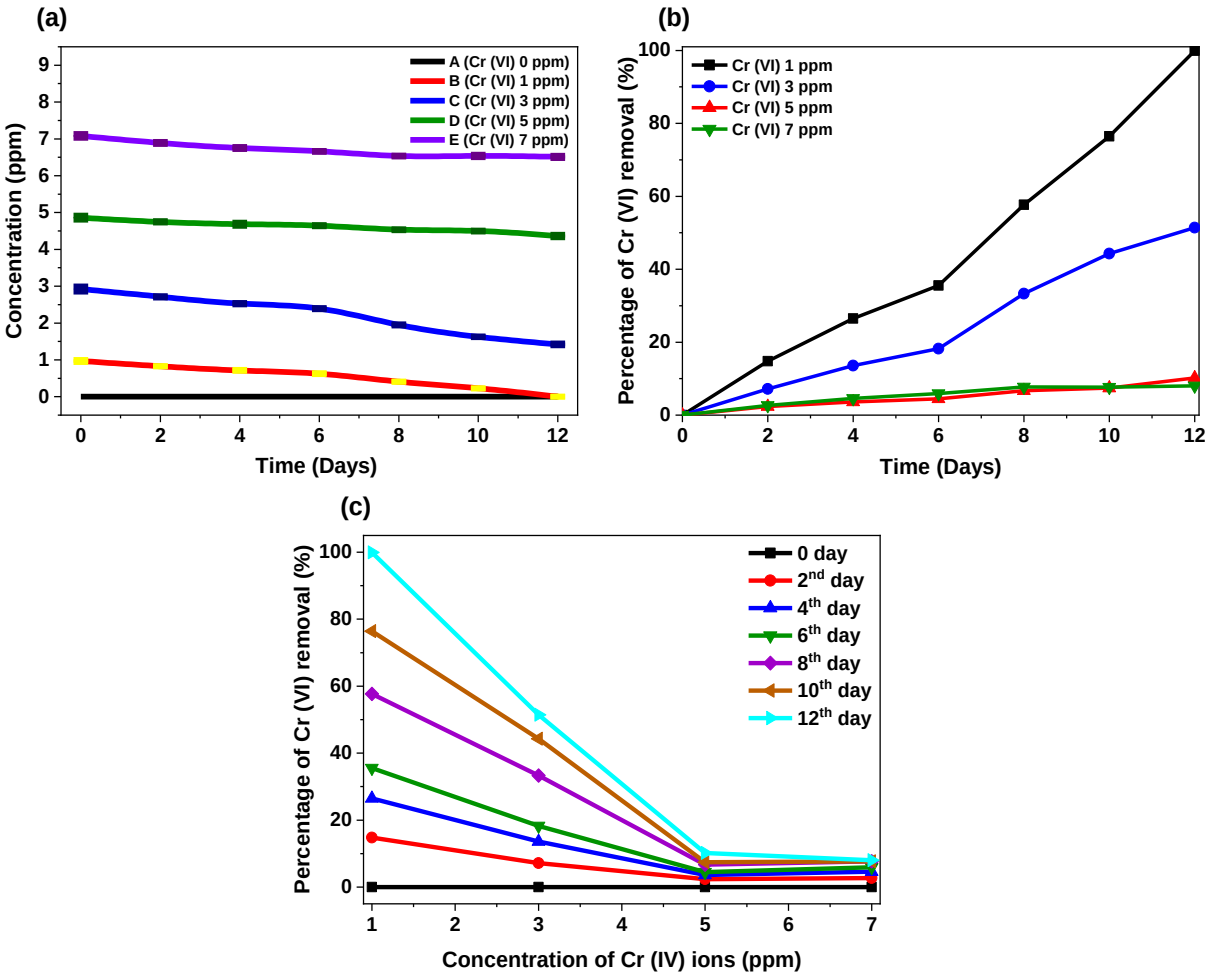

Figure 2 (a) Plots of Cr (VI) concentration as a function of time, (b) plots of the percentage of Cr (VI) removal as a function of time, and (c) the plots of the percentage of Cr (VI) removal as a function of Cr (VI) concentration

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 *et al.*, 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 *et al.*, 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 *et al.*, 2018), and (2) the non-competitive inhibitory effect of Cr (VI) ion to form mercaptide salts with sulfhydryl groups of enzyme proteins :

$$M + R - SH \rightleftharpoons MSR + H^{+}$$

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 *et al.*, 2018).

Figure 3 Microalgae growth exposed to bioreactors Cr (VI) 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 : (Fernández-López *et al.*, 2014; Jobby *et al.*, 2018)

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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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).

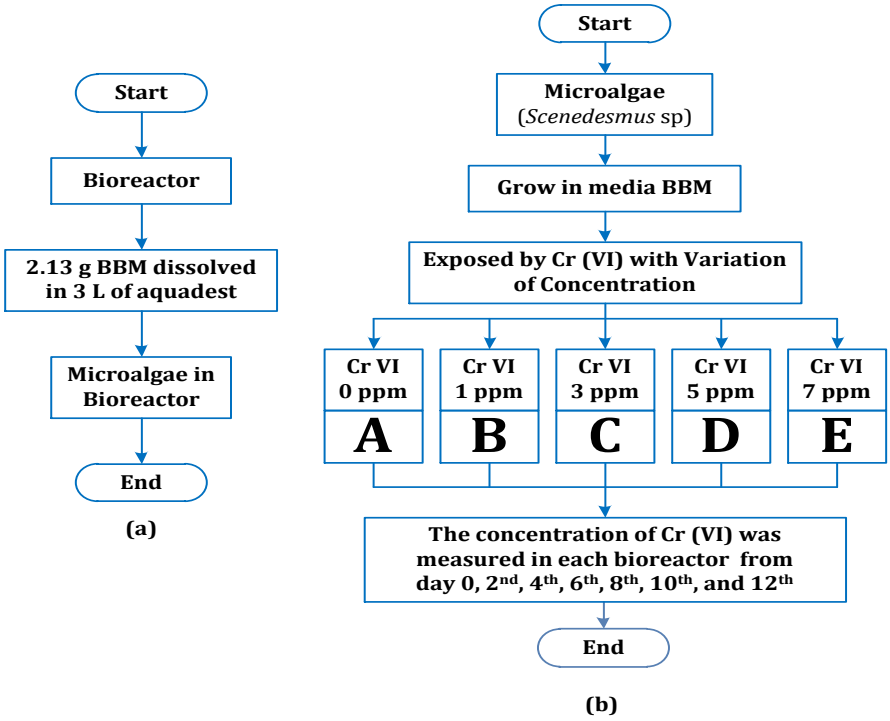

Figure 1 Scheme of (a) Microalgae Cultivation, (b) Exposed to Bioreactors

2.3. 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.

2.4. 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_2SO_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.

2.5. 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.

2.6. 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*.

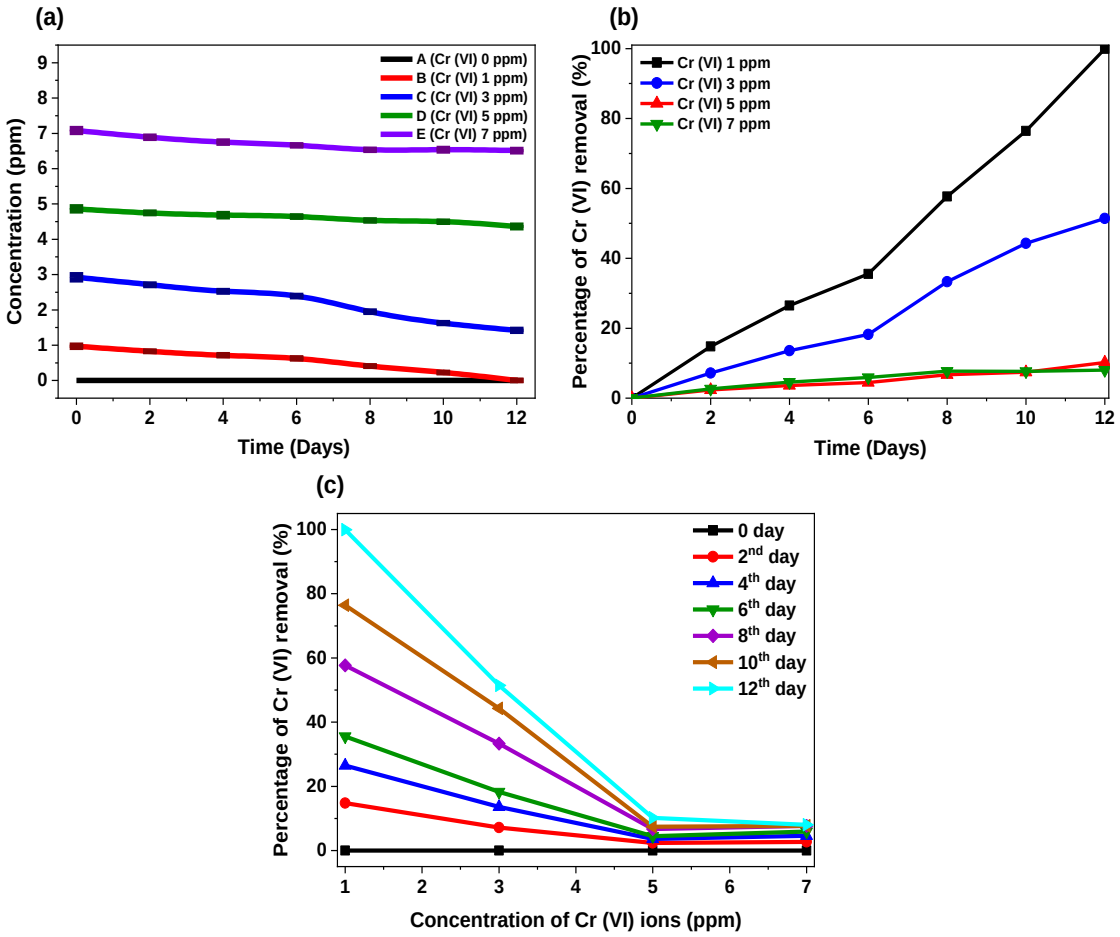

Figure 2 (a) Plots of Cr(VI) concentration as a function of time, (b) plots of the percentage of Cr(VI) removal as a function of time, and (c) the plots of the percentage of Cr(VI) removal as a function of Cr(VI) concentration

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 :

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

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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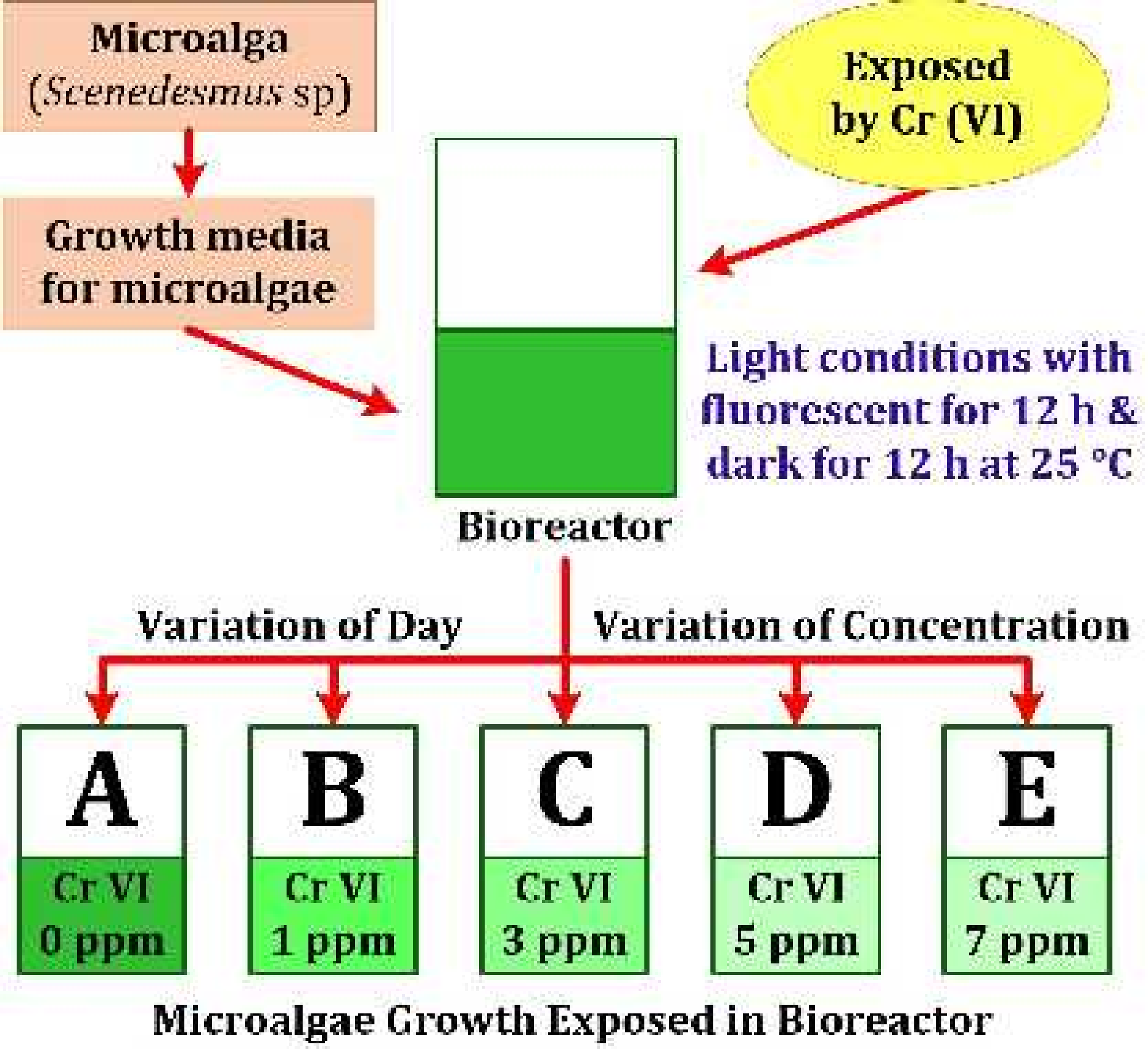

Microalgae Growth Exposed in Bioreactor

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Thank you for your contribution to IJTech and we look forward to a good collaboration in the next future.

Yours sincerely,

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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).

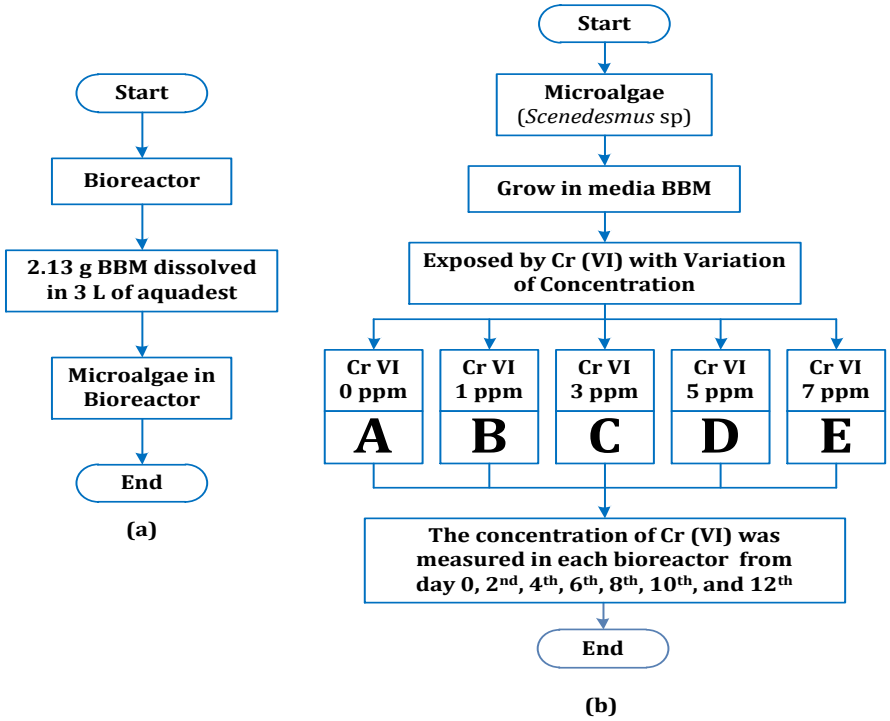

Figure 1 Scheme of (a) Microalgae Cultivation, (b) Exposed to Bioreactors

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_2SO_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*.

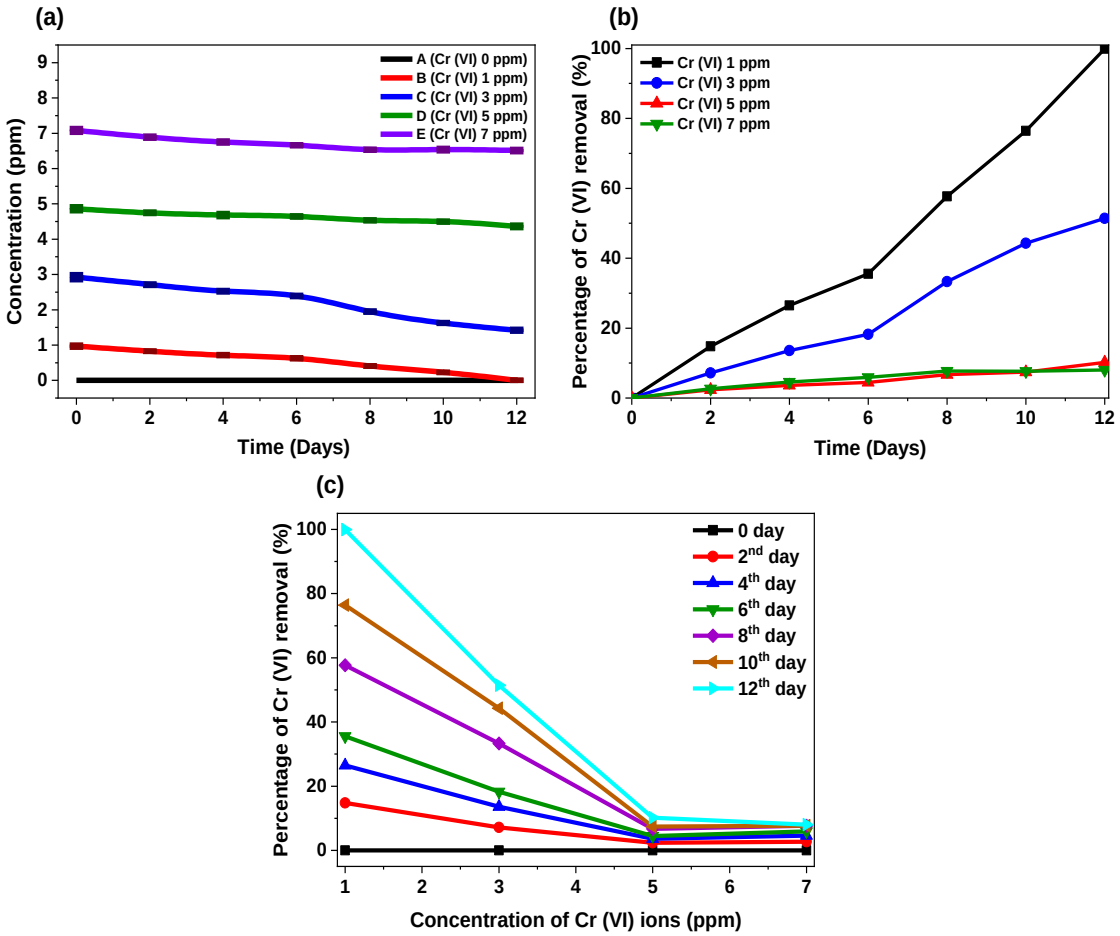

Figure 2 (a) Plots of Cr(VI) concentration as a function of time, (b) plots of the percentage of Cr(VI) removal as a function of time, and (c) the plots of the percentage of Cr(VI) removal as a function of Cr(VI) concentration

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 :

$$M + R - SH \rightleftharpoons MSR + H^{+}$$

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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