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Determination of Minimum Inhibitory Concentration of Chromium and Salinity on *Chlorella vulgaris*

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ABSTRACT

Heavy metal pollution, particularly chromium (VI) contamination, is a significant issue in Indonesian waters due to numerous chromium-producing industries. Research conducted in the downstream waters of Wonorejo found Cr(VI) levels ranging from 0.0025 to 0.018 mg/L, exceeding Indonesia's quality standard of 0.002 mg/L. Thus, it is crucial to treat industrial wastewater containing Cr(VI) before disposal into water bodies. One alternative for treating Cr(VI) waste is using biological agents like microalgae. *Chlorella sp*. was chosen for this study due to its abundance in Indonesian waters. The study aims to determine the minimum inhibitory concentration (MIC) of *Chlorella vulgaris* against Cr(VI) and salinity variations. The research involved propagating the microalgae to analyze growth rates and conducting MIC tests against salinity for 14 days with variations of 0, 20, 30, and 40 ppt. MIC tests against Cr(VI) were then performed using the optimal salinity (20 ppt) with variations of 0, 5, 10, 20, 30, and 40 mg/L. Results showed that *C. vulgaris* can thrive in salinities up to 40 ppt, with the optimal salinity being 20 ppt. The optimal Cr(VI) concentration for growth was 5 mg/L, resulting in a growth rate of 1.17 cells/mL/day. Based on statistical analysis only concentration of Cr(VI) that affected *C. vulgaris* cell density and not the salinity.

Keywords: *Chlorella vulgaris,* chromium (VI), microalgae, minimum inhibitory concentration, salinity.

INTRODUCTION

Madura Strait has experienced heavy metal pollution, including chromium, copper, mercury, and lead. Excessive levels of heavy metals can accumulate in organisms, leading to organ function disruptions. Some diseases caused by heavy metal pollution include neurological disorders and death (Romadhon et al., 2017; Sari et al., 2017; Tirta Wardana et al., 2023). Chromium (Cr) exists in two forms, one of which is Cr(VI), known for its toxic properties. Some industries that produce this element include tanning, paint manufacturing, metal coating, paper mills, and incineration (Hlihor et al., 2009; Wanta et al., 2022). The leather tanning industry in Indonesia has exceeded 60 factories as of 2013 and continues to grow each year (Waaly et al., 2018). A study indicated that the concentration of Cr (VI) in the downstream waters of Wonorejo ranges from 0.0025 to 0.018 mg/L (Romadhon et al., 2017). It exceeds the seawater quality standards of Indonesian regulation (KepMen LH No. 51/2004) with threshold level at 0.002 mg/L. This indicates the need for technology to remediate polluted brackish and seawater.

Remediation technologies vary based on the technique used, one of which is using microalgae. Microalgae are known for their ability to reduce CO_2 through photosynthesis. This process

produces oxygen, thereby increasing the dissolved oxygen (DO) levels in water (Aratboni et al., 2019). Other studies have shown that microalgae can reduce the levels of heavy metal lead (Pb) in water (Dyniari et al., 2019). One of the most commonly found microalgae in Indonesia is *Chlorella sp.,* including in the downstream waters of Wonorejo (Saputro et al., 2019). *Chlorella vulgaris* can remove various types of heavy metals such as arsenic, cadmium, and chromium. (Musah et al., 2022). Therefore, this study was conducted to assess the ability of *C. vulgaris* to survive in environments with high salinity and heavy metal contamination, specifically Cr (VI). This study will focus on MIC testing to determine the algae's tolerance limits to pollutants, environmental conditions, and pollutant concentrations. MIC tests were conducted on salinity and heavy metal Cr(VI) concentrations in microalgae *C. vulgaris.* Statistical analysis conducted in this study was Analysis of Variance (ANOVA) using Stat-Ease 360 Trial program to know the correlation between salinity level and concentration of Cr (VI) to *C. vulgaris*'s cells density.

METHODOLOGY

C. vulgaris preparation

Chlorella vulgaris inoculum were obtained from the Natural Feed Laboratory in Situbondo. Before being used in the MIC test, they were propagated for 14 days. The inoculum was added to reactors already filled with media. The volume added to each reactor was 150 mL. Nutrients in the form of walne, vitamins, and trace metals were added at a dose of 1 mL/L each week. The walne composition consisted of $NaNO_3$, H_3BO_3, Na_2 EDTA (anhydrous), $NaH_2PO_4 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$, $MnCl_2 \cdot 4H_2O$, and vitamins B1 and B12. The trace metal contained the metal elements needed for microalgae cell formation, namely $ZnCl_2$, $CoCl_2 \cdot 6H_2O$, $(NH_4)6Mo_7O_{24} \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$ (Andersen, 2005).

Reactor preparation

The equipment used in this study includes 50 mL beaker glass, 100 mL measuring cylinder, spray bottles, spatulas, and an analytical scale (Ohaus, United States). Other equipment needed to support microalgae growth includes a 1 L transparent plastic container as a reactor (Figure 1), a lamp, an aerator (Amara AA-350, China), and plastic tubing. The aerator flow rate of 2 L/min, connected to tubing leading to the 1 L plastic reactor. The lamp is a cool daylight 4W (Philips, China) placed 10 cm from the reactor.

MIC test against salinity

The media used in this study was artificial brackish water made from NaCl (Merck, Germany) with concentrations of 0, 20, 30, and 40 ppt. Distilled water was used, and it was supplemented with

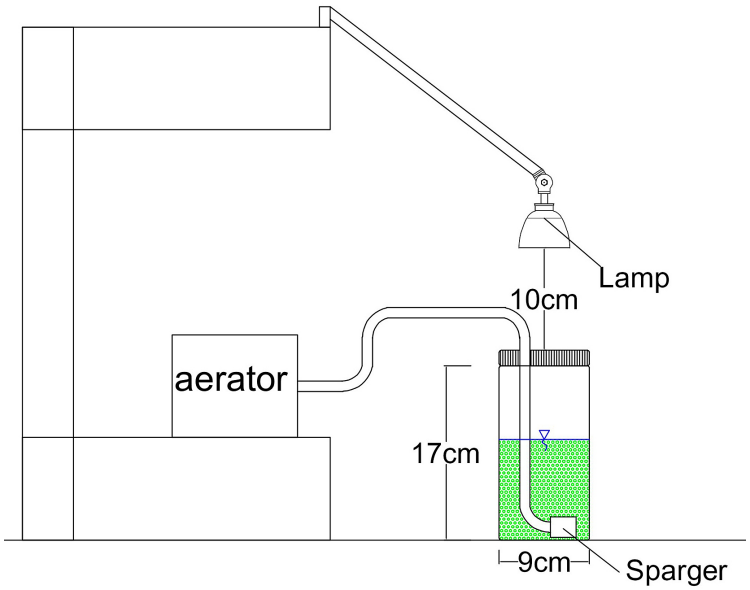

Figure 1. MIC test reactor design

walne, vitamin, and trace metal fertilizers as the nutrient source for *C. vulgaris*. After adding NaCl to the reactor as needed, distilled water was added up to 350 mL. Walne, vitamin, and trace metal fertilizers were added at 0.5 mL each. These three nutrients were added once a week. The test was conducted under a light intensity of 6,000 lux with planned lighting duration of 12 hours/day and an aerator with a flow rate of 2 L/min running 24 hours/day for a 14-day observation period.

MIC test against Cr(VI)

The media used in this study is the same as the media used for the MIC test on salinity. At this stage, the salinity used is the result of the MIC test on salinity. The source of Cr(VI) ions used is Potassium Dichromate or $K_2Cr_2O_7$ (Smart lab, Indonesia). The variations of Cr(VI) concentrations used are 5, 10, 20, 30, 40 mg/L based on the chromium concentrations in the Madura Strait (Romadhon et al., 2017) then increased to the limits researched in other studies by (Lee et al., 2017). The reactor, nutrients, and distilled water added are the same as for the MIC test on salinity. The MIC test was conducted under a light intensity of 6,000 lux (cool daylight 4 W) with a planned lighting duration of 12 hours/day and an aerator (Amara AA-350, China) with a flow rate of 2 L/min running 24 hours/day for 14 days observation period.

Data analysis

This study was conducted in batch form and in duplicate. The parameters tested were temperature, pH, and microalgae cell density. Observations were made daily for 14 days. Temperature and pH were measured using an alcohol thermometer (GEA, China) and a pH meter (Trans Instruments, Singapore). Microalgae cell density was measured using a microscope (Yazumi XSZ-107 BN, China) and a hemocytometer (Marienfeld, Germany) based on the number of cells in one of the squares on the hemocytometer (BTI, 2015). Measurement using a hemocytometer was done by selecting 5 squares according to the size of the microalgae. In this study, the squares used were 0.2×0.2 mm with a thickness of 0.1 mm, resulting in a reading volume of 4×10^6 mL. The formula used to calculate cell density is the average number of cells from the 5 selected squares divided by the volume of the square. Thus, the resulting unit is cells/mL. In this study, the cell density was further divided by the number of days (14 days) to determine the average growth rate each day.

$$\rho\, cell = \frac{\bar{x} \times fp}{V} \quad (1)$$

where: $\rho\, cell$ – cell density (cells/mL), $\bar{x}$ – average number of cells per square (cells), fp – dilution factor (if needed), V – volume of the square, mL.

ANOVA analysis

Statistical analysis in this research was used ANOVA method using Stat-Ease 360 Trial program. The use of ANOVA will show the data set we input significant or not based on the model they run. Statistical significance can be utilized to address scientific inquiries (Brereton, 2019). In this study the questions or inquiries are whether the salinity level variation used affect cells density and whether the variation of Cr (VI) affect the cells density for 14 days of observations. Data used for ANOVA can be seen in Table 1 below.

RESULTS AND DISCUSSION

MIC test against salinity

Physical observations of the salinity test can be seen in Figure 2. In the control (0 ppt), the color intensity continued to increase until the day – 12, but on the day – 13 and 14, the color returned to a lighter shade. Meanwhile, at a concentration of 20 ppt, the most noticeable difference in color intensity was between the day – 7 and day – 10. When observed physically, *C. vulgaris* can grow in salinities ranging from 0 to 40 ppt, with the reactor at a concentration of 20 ppt showing the best results as its color intensity is darker compared to the others. The optimum temperature range for *C. vulgaris* is 20–42 °C (Rusdiani et al., 2016). In this salinity MIC test, it can be seen that the media temperature is still within the optimum temperature range. The temperature observations showed sufficient stability, with a range of ± 1.5 °C in all reactors during the 14-day observation period (Figure 3). The temperature at 0 ppt salinity is more stable compared to 20 ppt, 30 ppt, and 40 ppt. This temperature range is considered optimal as it does not disrupt nutrient absorption by the microalgae (Gatamaneni et al., 2018; Juneja et al., 2013). According to Henry's law, temperature and pressure differences can affect gas solubility (Elperin et al., 2007). Aside from temperature, pH

Table 1. Data set for ANOVA

No.	Salinity (ppt)	Cell *d*ensity *l*og (cells/mL)	Cr (VI) (mg/L)	Cell *d*ensity *l*og (cells/mL)
1	0	6.67	0	6.26
2	0	6.89	0	6.56
3	0	6.96	0	6.71
4	0	6.99	0	6.87
5	0	6.92	0	6.92
6	0	6.84	0	6.97
7	0	7.12	0	7.05
8	0	7.22	0	7.09
9	0	7.26	0	7.1
10	0	7.17	0	7.12
11	0	7.2	0	7.2
12	20	6.59	5	6.49
13	20	6.92	5	6.66
14	20	7.04	5	6.68
15	20	7.14	5	6.79
16	20	7.15	5	6.76
17	20	7.16	5	6.74
18	20	7.19	5	6.82
19	20	7.21	5	6.75
20	20	7.26	5	6.79
21	20	7.29	5	6.82
22	20	7.29	5	6.81
23	30	6.75	10	6.48
24	30	6.97	10	6.6
25	30	7	10	6.67
26	30	7.15	10	6.71
27	30	7.17	10	6.74
28	30	7.2	10	6.69
29	30	7.15	10	6.74
30	30	7.25	10	6.73
31	30	7.22	10	6.74
32	30	7.26	10	6.72
33	30	7.17	10	6.71
34	40	6.6	20	6.43
35	40	6.86	20	6.62
36	40	6.82	20	6.62
37	40	7	20	6.66
38	40	7.04	20	6.69
39	40	7.13	20	6.7
40	40	7.01	20	6.76
41	40	7.11	20	6.68
42	40	7.11	20	6.71
43	40	7.15	20	6.74
44	40	7.13	20	6.7
45			30	6.46
46			30	6.61

No.	Salinity (ppt)	Cell density log (cells/mL)	Cr (VI) (mg/L)	Cell density log (cells/mL)
47			30	6.64
48			30	6.66
49			30	6.68
50			30	6.68
51			30	6.69
52			30	6.7
53			30	6.65
54			30	6.74
55			30	6.75
56			40	6.37
57			40	6.6
58			40	6.53
59			40	6.58
60			40	6.6
61			40	6.61
62			40	6.61
63			40	6.6
64			40	6.61
65			40	6.63
66			40	6.65

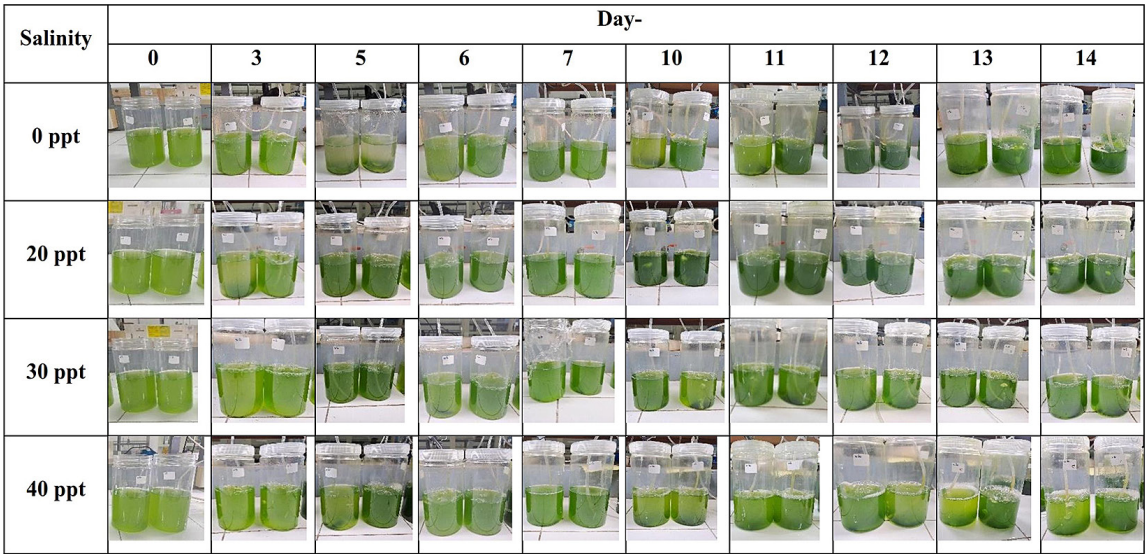

Figure 2. Physical observation results of *C. vulgaris* against salinity concentration

is also a determinant of the optimal or suboptimal growth environment for microalgae. *C. vulgaris* grows optimally at a neutral pH, with a pH range between 6.3–7.3 (Dyniari et al., 2019; Rusdiani et al., 2016). The pH observations in the salinity MIC test can be seen in Figure 4.

Based on the observations, the pH in reactors with 20, 30, and 40 ppt salinity was lower compared to the control reactor (0 ppt). Although the pH appeared lower, the pH values remained neutral throughout the two weeks. This is because the decomposed NaCl does not produce ions, either cations or anions. Salinity affects the biomass of microalgae and fatty acid production. Fatty acids are a source used as an alternative for biodiesel (Aratboni et al., 2019). Furthermore, among the observed parameters in this study, microalgae cell density is an important result. This is because the

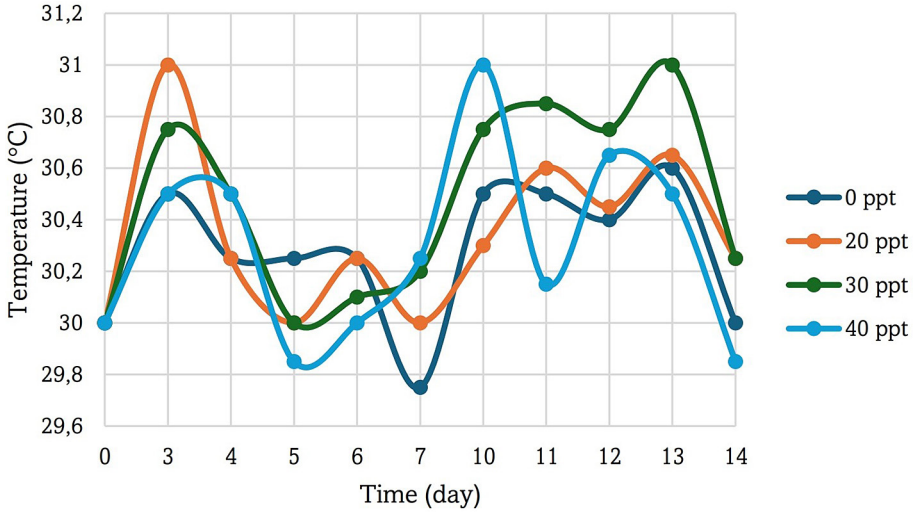

Figure 3. Temperature observation results of MIC test against salinity concentration

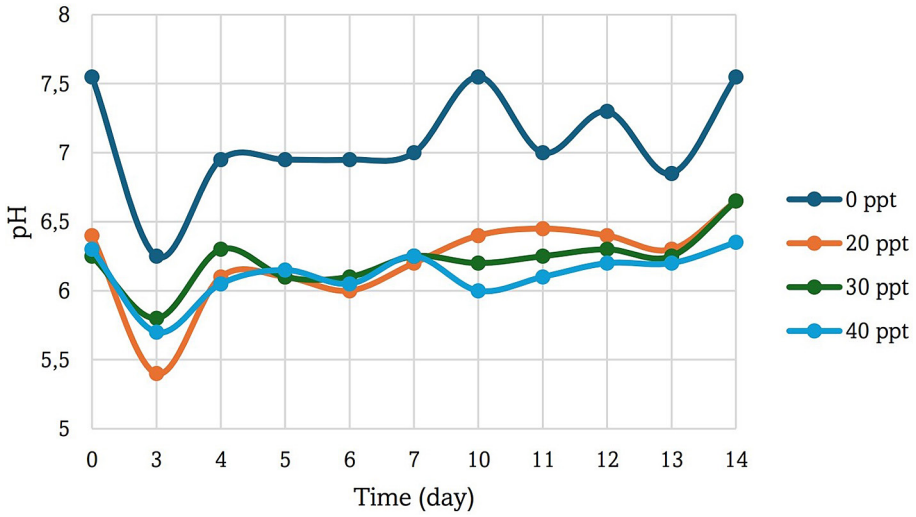

Figure 4. pH observation results of MIC test against salinity concentration

main purpose of the MIC is to determine the maximum limits of salinity and Cr (VI) concentration at which *C. vulgaris* can still survive. The observations of microalgae density can be seen in Figure 5.

Based on the observations in Figure 5, there was considerable growth at concentrations of 20 ppt and 30 ppt. Both concentrations showed growth phases until the 5th day, followed by a small or stationary growth. The 40 ppt concentration did not show identifiable phases, as it continued to fluctuate within a similar range. Similar to other studies, higher salinity concentrations tend to result in fewer microalgae (Almutairi et al., 2021; Hanifa, 2019). The growth rates of microalgae at salinities of 0, 20, 30, and 40 ppt were 1.16, 1.18, 1.14, and 1.15 cells/mL/day, respectively. Based on the MIC results, the optimal salinity for *C. vulgaris* growth is 20 ppt. This indicates that Walne, known as the best fertilizer for *C. vulgaris* (Satriaji et al., 2016), can be used in conjunction with salinity. The presence of salinity can stimulate microalgae growth up to the optimum concentration (20 ppt). In aquatic environments, 20 ppt falls within the range of brackish water salinity, which is 0.5–33 ppt (Gray et al., 2011), making it suitable for testing in the downstream waters of Wonorejo, which are affected by Cr (VI) accumulation (Nursanti et al., 2021; Romadhon et al., 2017; Suharjo, Ernawati, 2022).

MIC test against Cr (VI)

The MIC test for Cr (VI) was conducted for 14 days under consistent salinity conditions of 20 ppt

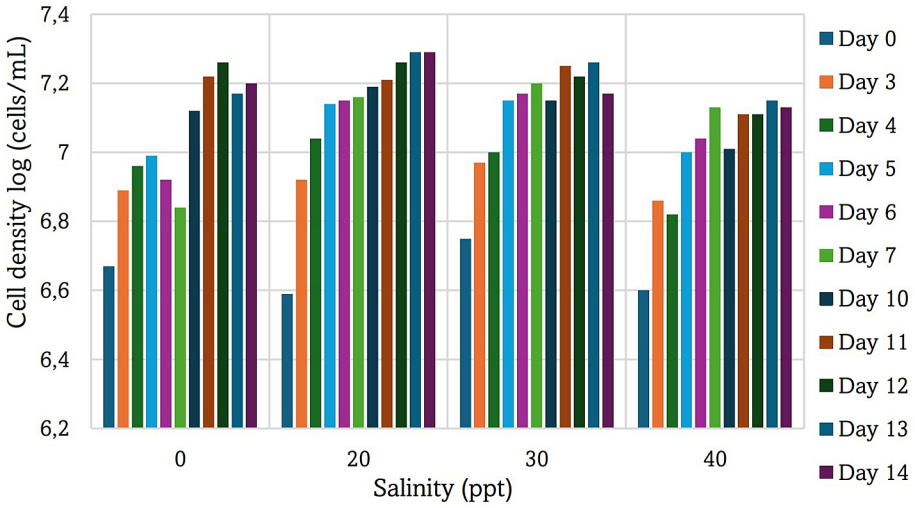

Figure 5. Cell density observation results of *C. vulgaris* against salinity concentration

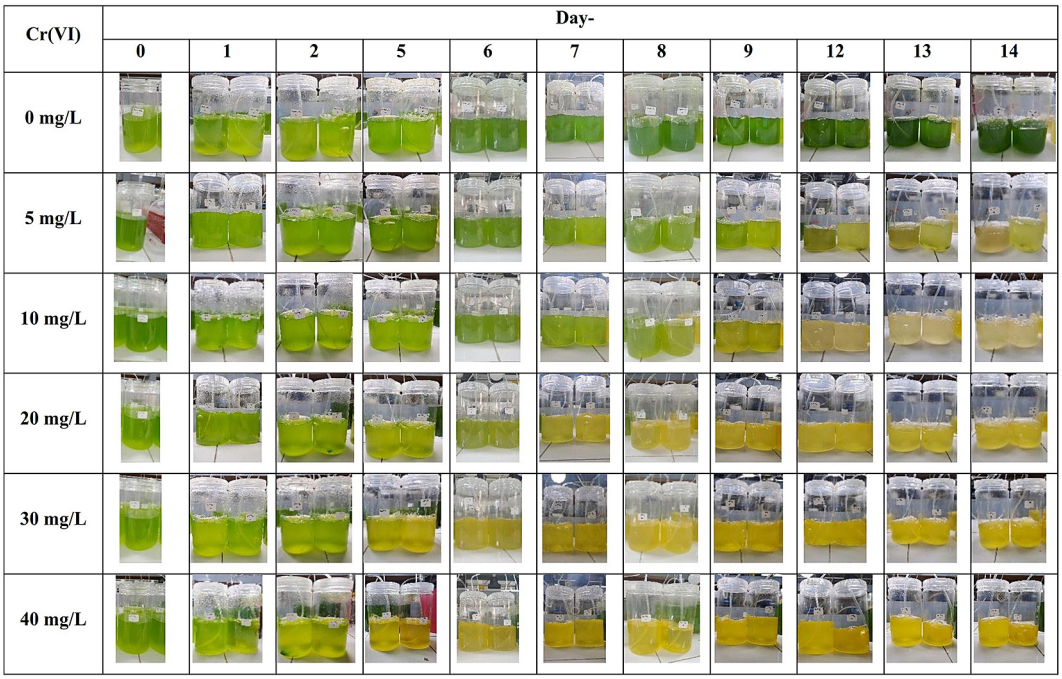

Figure 6. Physical observation results of *C. vulgaris* against Cr (VI) concentration

across all reactors. The physical observations of *C. vulgaris* can be seen in Figure 6. Based on the physical observations, *C. vulgaris* can grow at concentrations of 5 mg/L and 10 mg/L as there is still an increase in green color intensity. Additionally, there is a preliminary suspicion that Cr(VI) is adsorbed by *C. vulgaris* and affects its chlorophyll color. The accumulation of heavy metals at high levels by microalgae can lead to the formation of reactive oxygen species (ROS). ROS can inhibit chlorophyll formation and disrupt cell proliferation.

The observations in the Cr (VI) MIC test showed that the temperature did not significantly change and remained within the optimum temperature range for *C. vulgaris*, which is 20–42 °C (Figure 7). The stable temperature is crucial for the algae's metabolism. Fluctuations can disrupt the carbon source, slowing down carbon binding and electron transfer processes. Additionally, it can affect the amount of carotenoids in chlorophyll, thereby impacting photosynthesis (Juneja et al., 2013).

The pH tends to become more acidic as the concentration of Cr(VI) increases. This observation can be seen in Figure 8. This phenomenon occurs because dichromate ($Cr_2O_7^{2-}$) is easily soluble and reacts with water. This reaction binds hydrogen

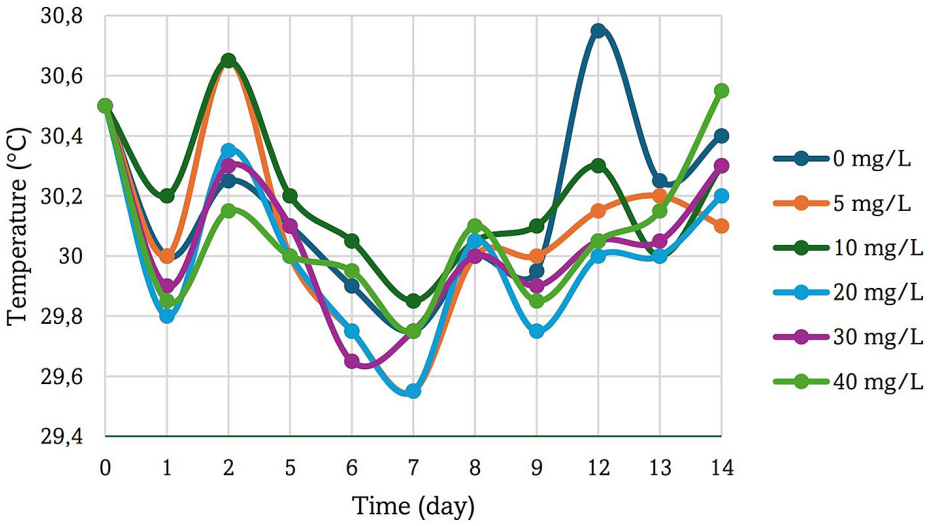

Figure 7. Temperature observation results of MIC test against Cr(VI) concentration

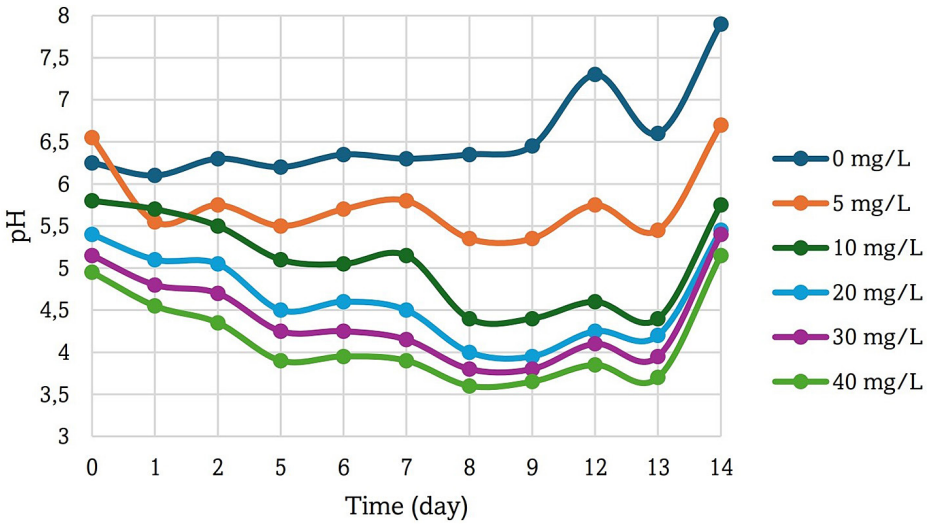

Figure 8. pH observation results of MIC test against Cr(VI) concentration

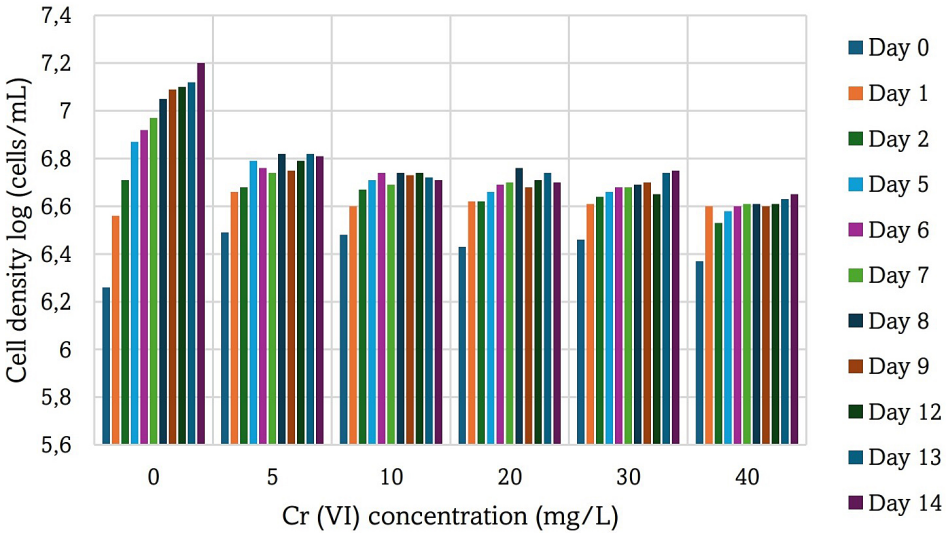

Figure 9. Cell density observation results of *C. vulgaris* against Cr(VI) concentration

atoms, thus lowering the pH of the solution (Unceta et al., 2010). An acidic environment can inhibit the growth of microalgae, leading to reduced survival rates. The microalgae may die and transform into adsorbents (Aththanayake et al., 2022). In the observation of microalgae cell density, the slow growth of microalgae is due to the acidic pH environment. The increase in the number of microalgae cells can be seen in Figure 9.

Based on the cell density results, it can be observed that all reactors, except the control, experienced prolonged growth, resulting in a graph resembling a stationary phase. The most noticeable increase occurred on the first day. Reactors with concentrations of 5, 10, and 20 mg/L continued to increase until the eighth day. On the other hand, reactors with concentrations of 30 and 40 mg/L showed minimal growth after the first day. The growth rates at concentrations of 0, 5, 10, 20, 30, and 40 mg/L were 1.17, 1.07, 1.04, 1.05, 1.06, and 1.04 cells/mL/day, respectively. Thus, based on the observations, *C. vulgaris* thrived best at a concentration of 5 mg/L in a salinity of 20 ppt. A concentration of Cr(VI) at 5 mg/L is relatively high, considering that concentrations in mining areas and rivers range between 0.3 and 1.1 mg/L. The efficiency of Cr(VI) removal from various studies using aqueous media and nutrients ranges from 12.93% to 34.78%. Further research can investigate Cr(VI) degradation in environments with brackish or seawater salinity.

ANOVA analysis

In this research we used the set data to know the correlation between salinity or concentration of Cr(VI) to *C. vulgaris* cell density. The result of salinity data against cell density yielded a p-value of 0.1686, indicating insignificance (> 0.05). The results from the model were not deemed significant, as shown in Table 2

Table 2. ANOVA result of salinity correlation with *C. vulgaris* cell density

Source	Sum of squares	df	Mean square	F-value	p-value	Signification
Model	0.1184	2	0.0593	1.86	0.1686	not significant
A-Salinity	0.0013	1	0.0013	0.0405	0.8415	
A^2	0.1184	1	0.1184	3.72	0.0607	
Residual	1.30	41	0.0318			
Lack of fit	0.0087	1	0.0087	0.2677	0.6077	not significant
Pure error	1.30	40	0.0324			
Cor total	1.42	43				

Std. dev.	0.1784	R^2	0.0832
Mean	7.06	Adjusted R^2	0.0385
C.V. %	2.53	Predicted R^2	-0.0607
		Adeq precision	2.6704

Table 3. ANOVA result of Cr(VI) concentration correlation with *C. vulgaris* cell density

Source	Sum of squares	df	Mean square	F-value	p-value	Signification
Model	0.6194	3	0.2065	10.98	<0.0001	significant
A-Cr(VI)	0.0051	1	0.0051	0.2734	0.6029	
A^2	0.0597	1	0.0597	3.18	0.0797	
A^3	0.1038	1	0.1038	5.52	0.0220	
Residual	1.17	62	0.0188			
Lack of Fit	0.0052	2	0.0026	0.1356	0.8735	not significant
Pure Error	1.16	60	0.0193			
Cor Total	1.79	65				

Std. Dev.	0.1371	R^2	0.3469
Mean	6.70	Adjusted R^2	0.3153
C.V. %	2.05	Predicted R^2	0.2401
		Adeq Precision	9.1766

Adequate precision evaluates the signal-to-noise ratio. A ratio of 2.6704 suggests an insufficient signal, indicating that this model should not be relied upon to explore the design space.

Meanwhile, the result of concentration of Cr(VI) data against cell density yielded a p-value of < 0.0001, indicating significance (< 0.05). The results from the model were deemed significant, as shown in Table 3. Adequate Precision evaluates the signal-to-noise ratio. A ratio above 4 is considered favorable. With ratio of 9.177, the signal is deemed adequate, indicating that this model can be effectively used to explore the design space.

From statistical analysis we can see that salinity did not affect or does not have a significant correlation for *C. vulgaris* cell density (in this case was for salinity 0, 20, 30, and 40 ppt). Meanwhile using 20 ppt as base for salinity and Cr(VI) concentration as variables showed that it has significant correlation. Which means, Cr(VI) concentration affecting *C. vulgaris* cell density.

CONCLUSIONS

Based on this research, the conclusion is that the microalgae species *C. vulgaris* can survive in salinities up to 40 ppt, but it grows best at a salinity of 20 ppt during the 14 days MIC test. This is indicated by a growth rate of 1.18 cells/mL/day. In the MIC test against the heavy metal Cr(VI), *C. vulgaris* can tolerate Cr(VI) concentrations up to 40 mg/L, but the best cell density with good growth rate is seen at a Cr(VI) concentration of 5 mg/L. This is indicated by a growth rate of 1.17 cells/mL/day over the 14-day observation period. Other factors such as pH and temperature also have a significant effect. pH and temperature must always be at their optimum levels to avoid damaging the metabolism of the microalgae. In this study, a neutral pH at room temperature between 29–31 °C was found to be the optimum condition. Based on statistical analysis only concentration of Cr(VI) that affected *C. vulgaris* cell density and not the salinity.

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REFERENCES

1. Aratboni, H.A., Rafiei, N., García-Granados, R., Alemzadeh, A., Morones-Ramirez, J. 2019. Biomass and lipid induction strategies in microalgae for biofuel production and other applications. Microbial Cell Factories, 18. https://doi.org/10.1186/s12934-019-1228-4
2. Almutairi, A.W., El-Sayed, A.E.-K.B., Reda, M.M. 2021. Evaluation of high salinity adaptation for lipid bio-accumulation in the green microalga Chlorella vulgaris. Saudi Journal of Biological Sciences, 28(7), 3981–3988. https://doi.org/10.1016/j.sjbs.2021.04.007
3. Andersen, R.A. (Ed.). 2005. Algal culturing techniques. Elsevier/Academic Press.
4. Aththanayake, A.M.K.C.B., Rathnayake, I.V.N., Deeyamulla, M.P., Megharaj, M. 2022. Potential use of Chlorella vulgaris KCBAL01 from a freshwater stream receiving treated textile effluent in hexavalent chromium [Cr(VI)] removal in extremely acidic conditions. Journal of Environmental Science and Health, 57(9), 780–788. https://doi.org/10.1080/10934529.2022.2113281
5. Brereton, R.G. 2019. ANOVA tables and statistical significance of models. Journal of Chemometrics, 33(3), e3019. https://doi.org/10.1002/cem.3019
6. BTI. 2015. Algae to Energy – Using and Re-using a Hemocytometer to Count Algae Cells. BTI Science, 12, 1–3.
7. Dyniari, Y.I.P., Farikhah, F., Rahim, A.R. 2019. Dinamika populasi *C. vulgaris* dalam paparan logam berat timbal (PB) dengan konsentrasi yang berbeda skala laboratorium. Jurnal Perikanan Pantura, 2(1), 42–50. https://doi.org/10.30587/jpp.v2i1.810
8. Satriaji, D.E., Zainuri, M., Widowati, I. 2016. Study of growth and n, p content of microalgae Chlorella vulgaris cultivated in different culture media and light intensity. Jurnal Teknologi, 78, 2–4. https://doi.org/10.11113/jt.v78.8148
9. Elperin, T., Fominykh, A., Krasovitov, B. 2007. Evaporation and condensation of large droplets in the presence of inert admixtures containing soluble gas. Journal of the Atmospheric Sciences, 64, 983–995. https://doi.org/10.1175/JAS3878.1
10. Gatamaneni, B.L., Orsat, V., Lefsrud, M. 2018. Factors affecting growth of various microalgal species. Environmental Engineering Science, 35(10), 1037–1048. https://doi.org/10.1089/ees.2017.0521
11. Gray, S., Semiat, R., Duke, M., Rahardianto, A., Cohen, Y. 2011. Seawater Use and Desalination Technology. Treatise on Water Science, 4(04), 73–109. https://doi.org/10.1016/B978-0-444-53199-5.00077-4
12. Hanifa, F. 2019. Pengaruh perbedaan salinitas dan dosis pupuk walne terhadap pertumbuhan populasi Chlorella sp pada skala laboratorium. Jurnal Online Mahasiswa

Bidang Perikanan dan Ilmu Kelautan, 6, 1–14.

13. Hlihor, R., Apostol, L., Pavel, L., Betianu, C., Sluser, B., Căliman, F., Gavrilescu, M. 2009. Overview on chromium occurrence in the environment and its remediation. Bulletin of the Polytechnic Institute of Iasi, Section Chemistry and Chemical Engineering, LV, 19–34.
14. Juneja, A., Ceballos, R.M., Murthy, G.S. 2013. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A Review. Energies, 6(9), Article 9. https://doi.org/10.3390/en6094607
15. Lee, L., Hsu, C.-Y., Yen, H.-W. 2017. The effects of hydraulic retention time (HRT) on chromium (VI) reduction using autotrophic cultivation of *Chlorella vulgaris*. Bioprocess and Biosystems Engineering, 40(12), 1725–1731. https://doi.org/10.1007/s00449-017-1827-6
16. Musah, B.I., Wan, P., Xu, Y., Liang, C., Peng, L. 2022. Contrastive analysis of nickel (II), iron (II), copper (II), and chromium (VI) removal using modified Chlorella vulgaris and Spirulina platensis: Characterization and recovery studies. Journal of Environmental Chemical Engineering, 10(5), 108422. https://doi.org/10.1016/j.jece.2022.108422
17. Nursanti, V., Hidayaturrahman, H., Hadiko, G. 2021. Studi pelepasan dan penanganan kromium dari air limpasan tambang PT. Vale Indonesia Tbk. Jurnal Rekayasa Pertambangan. 1(1).
18. Romadhon, R.P., Mahmiah, Rahyono. 2017. Akumulasi logam berat Cr6+ pada air di Perairan Wonorejo Surabaya. Seminar Nasional Kelautan XII, 424, B86–B93.
19. Rusdiani, R.R., Boedisantoso, R., Hanif, M. 2016. Optimalisasi teknologi fotobioreaktor mikroalga sebagai dasar perencanaan strategi mitigasi gas CO_2. Jurnal Teknik ITS, 5(2), F188–F192. https://doi.org/10.12962/j23373539.v5i2.16942
20. Saputro, T.B., Purwani, K.I., Ermavitalini, D., Saifullah, A.F. 2019. Isolation of high lipids content microalgae from Wonorejo rivers, Surabaya, Indonesia and its identification using rbcL marker gene. Biodiversitas Journal of Biological Diversity, 20(5), 1380–1388. https://doi.org/10.13057/biodiv/d200530
21. Sari, S.H.J., Kirana, J.F.A., Guntur, G. 2017. Analisis kandungan logam berat Hg dan Cu terlarut di Perairan Pesisir Wonorejo, Pantai Timur Surabaya. Jurnal Pendidikan Geografi, 22(1), 1–9. https://doi.org/10.17977/um017v22i12017p001
22. Suharjo, M.H., Ernawati, R. 2022. Analisis Pencemaran Logam Kromium Heksavalen di Daerah Sungai pada Pertambangan Nikel. 6(2), 11978–11984.
23. Unceta, N., Séby, F., Malherbe, J., Donard, O. 2010. Chromium speciation in solid matrices and regulation: A review. Analytical and Bioanalytical Chemistry, 397, 1097–1111. https://doi.org/10.1007/s00216-009-3417-1
24. Waaly, A., Ridwan, A., Akbar, M. 2018. Development of sustainable procurement monitoring system performance based on Supply Chain Reference Operation (SCOR) and Analytical Hierarchy Process (AHP) on leather tanning industry. MATEC Web of Conferences, 204, 01008. https://doi.org/10.1051/matecconf/201820401008
25. Wanta, K.C., Saptoaji, D., Miryanti, Y.I.P.A., Kristijarti, A.P. 2022. The effect of pH, initial concentration, and salinity on the biosorption process of chromium (VI) ions using microalgae Chlorella sp. IOP Conference Series: Earth and Environmental Science, 963(1), 012039. https://doi.org/10.1088/1755-1315/963/1/012039
26. Wardana, M.T., Kuntjoro, S. 2023. Analisis kadar logam berat timbal (Pb) di Perairan Pelabuhan Teluk Lamong dan korelasinya terhadap kadar Pb kerang darah (*Tegillarca granosa*). Lentera Bio, 12(1), 41–49.