Walisongo Journal of Chemistry
Walisongo Journal of Chemistry Vol. 7 Issue 1 (2024), 079-089
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v7i1.20749

Utilization Of Chlorella Pyrenoidosa As A Phytoremediator For Tannery Waste

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Abstract

This study aims to analyze the effect of phytoremediation on the bioremoval of COD, ammonia, and Cr (VI) from tannery wastewater and examine its effect on the growth of Chlorella populations. The research method consisted of two stages: first, preparation of liquid waste media. The second is culturing pure cultures followed by microalgae cultivation using leather tanning liquid waste media with a concentration variation of 0%, 10%, 20%, and 30% v/v. Filtrate samples after harvest were analyzed for COD, ammonia, and Cr (VI). The results obtained in this study show that Chlorella can grow in tanning waste media. The highest exponential phase occurs at a concentration of 20% with a growth rate of 0.557. Tannery liquid waste contains inorganic minerals utilized by Chlorella pyrenoidosa cells for growth. Cultivation of Chlorella pyrenoidosa can reduce leather tanning liquid waste parameters, namely COD, ammonia, and Cr (VI).

Keywords: Chlorella, Chromium, Microalgae, Phytoremediation, Tannery waste

Introduction

Tannery waste that is thrown directly into the environment can cause various diseases, including itching, asthma, cancer, and various other diseases (Wardhani, Dirgawati and Valyana, 2012). Solid and liquid waste has the potential to contain 6 valence chrome or Cr(VI) and three valence chrome or Cr(III). Cr(III) is less toxic, has low solubility, and is more challenging to penetrate plant and animal cell walls (Pradhan *et al.*, 2019). Conventional tannery waste was also found to have a COD content of 6606.2 mg/liter and BOD of 2277 mg/liter with maximum standard limits for COD and Cr (VI) according to the Regulation of the Minister of Environment of the Republic of Indonesia No. 5 of 2014 amounting to 110 mg/liter and 50 mg/liter (Nugroho and Anggriyani, 2018). If it is thrown into a river, it will disrupt the life of the biota that lives in the river. This is due to low oxygen levels as a result of being consumed by bacteria to oxidize organic substances (Hadiyanto *et al.*, 2019).

One of the preventive measures to reduce the concentration of leather tannery wastewater that can be applied is the phytoremediation technique. Phytoremediation is one way of dealing with waste and environmental pollution problems, derived from the word phyto, which means plant, while remediation (to remedy) means to repair (Ajayan *et al.*, 2015). Phytoremediation is an effort to clean pollutants using plants, including microalgae, macroalgae, and macrophytes. This method is easy to apply, efficient, inexpensive, and environmentally friendly (Gauje *et al.*, 2022).

Tannery liquid waste contains many nutrients and can be used to grow microalgae. One type of microalgae with a vast life span in the growth medium is *Chlorella pyrenoidosa* (Polontalo, Joelyna and Hadiyanto, 2021). The primary nutrient in the *Chlorella* microalgae culture media is Nitrogen (N). Nitrogen in wastewater is found in inorganic forms such as nitrite (NO_2^-) and nitrate (NO_3^+), but microalgae can generally use NO_3^+, NO_2^-, or ammonium (NH_4^+) as an N source with the same growth rate in both organic and inorganic forms (Nogueira *et al.*, 2018).

Factors influencing microalgae growth include abiotic factors (sunlight, temperature, nutrition, O_2, CO_2, pH, salinity), biotic factors (bacteria, fungi, viruses, and competition with other microalgae), and technical factors (harvesting method). The advantage of microalgae is that it can proliferate in the right climatic conditions (Ariyanti D and Handayani NA, 2010).

In the natural environment, algae play a very important role in controlling metal concentrations in lakes and seas. This relates to its ability to degrade or accumulate toxic heavy metals and organic pollutants such as phenolic, hydrocarbons, pesticides, and biphenyls from the environment and accumulate them so that the concentration in algae is higher than the concentration of pollutants in the environment (Al-Homaidan *et al.*, 2018). Several other types of microalgae that have been used as phytoremediators of leather tanning waste include Phormidium sp. (Das *et al.*, 2017) and Scenedesmus sp. (Da Fontoura *et al.*, 2017). Although research on phytoremediation of waste with microalgae has been carried out frequently, there has been no research related to the use of microalgae *Chlorella pyrenoidosa* as a phytoremediator of COD, ammonia, and Cr (VI) in leather tanning waste, and its effect on microalgae population growth. This study aims to examine the effect of the concentration of leather tanning waste on the growth of microalgae populations and to analyze the effect of microalgae phytoremediators on the bioremoval of COD, ammonia, and Cr (VI) from tannery wastewater.

Methodology

This research carried out stages such as media preparation, pure culture cultivation, and microalgae cultivation using liquid tannery waste media.

Materials

The material used in this research was *Chlorella pyrenoidosa* culture, previously isolated from cultivation ponds in the Sukoharjo area, Central Java, Indonesia. In addition, the leather tanning liquid waste used as a cultivation medium comes from the Post Tanning and Finishing Workshop, Politeknik ATK Yogyakarta. Other chemicals used as growth nutrients for *Chlorella* are $NaHCO_3$ pro analyze (Merck), Urea, and TSP, as well as NaOH pro analyze (Merck), used when harvesting biomass. The tools used in this study were: Analytical balance, pH meter, container, erlenmeyer, measuring cup, pipette, aerator and hose, filter cloth, and TL lamp.

Media Preparation and Pure Culture Cultivation

The medium used for microalgae cultivation is leather tanning liquid waste taken using jerry cans. The leather tanning waste used is freshly produced waste. Liquid waste is precipitated and filtered to separate solids that are not dissolved in water (Yang *et al.*, 2017). The characteristics of the liquid waste used in this study are shown in Table 1.

Table 1. Characteristics of tannery liquid waste

Parameters	Parameter Values	Quality Standards*
pH	6.0	6.0-9.0
COD (mg/L)	1250	110
Ammonia Total (NH_3-N) (mg/L)	0.8	0.5

 | ISSN 2621-5985 (online) | ISSN 2549-385X (print)
Volume 7, Nomor 1, 2024

Cr (Total) (mg/L)	0.38	0.6

*Minister of Environment Regulation Number 5 of 2014 concerning Waste Water Quality Standards

Cultivation media preparation was carried out by diluting one liter of *Chlorella* pyrenoidosa into two liters, then adding the nutrients: 0.64 grams of NaHCO3, 0.1 grams of urea, and 0.1 grams of TSP. During cultivation, oxygen is provided through an aerator. Absorbance (Optical Density) was checked daily with a spectrophotometer at a wavelength of 680 nm. If the absorbance is close to 0.6, the microalgae are ready for use (Polontalo, Joelyna,.

Cultivation of Chlorella pyrenoidosa Using Tannery Liquid Waste Media

Cultivation was carried out using 500 ml glass bottles, light lighting for 24 hours, and aeration during cultivation. Variable changes were made with 0%, 10%, 20%, and 30% v/v tannery waste mixed in 200 ml *Chlorella*. Cultivation was carried out for 10 days with optical density measurements daily using a spectrophotometric wavelength of 680 nm. Additional nutrients are given every 2 days with the amount of 25 mg/L TSP, 25 mg/L Urea, and 200 mg/L $NaHCO_3$ (Hadiyanto *et al.*, 2019).

Cultivation of *Chlorella* as a control was carried out with a 1:1 ratio between distilled water and *Chlorella* with additional nutrients in the form of 50 mg/L TSP, 50 mg/L Urea, and 400 mg/L $NaHCO_3$. After 10 days of cultivation, microalgae were harvested to produce wet and dry biomass. Harvesting was done by flocculation using NaOH (1 L microalgae: 0.2 mL NaOH). After deposition, a filtration process is carried out using two layers of satin cloth for several hours. After collecting everything in the satin cloth, the harvest is dried to remove some remaining water content. Next, the yield is weighed using an analytical balance (Kawaroe *et al.*, 2012).

Testing and Data Analysis

The tests carried out in this research were COD, total ammonia, and Cr (VI) levels. COD is tested based on the Indonesian National Standard (SNI) 6989.2-2019, while total ammonia testing is carried out based on SNI 06-6989.30-2005. Cr (VI) levels were tested based on SNI 6989.71:2009.

Microalgae growth was analyzed by measuring the Optical Density (OD) using a UV-Vis spectrophotometer. Optical density (OD) is an indirect method widely used for biomass measurement because it can directly correlate with the number of cells in the media and is easily adaptable to automatic measurement systems, making this OD method a valuable tool in growth monitoring and control. and microalgae biomass (Lúcia *et al.*, 2011). The specific growth rate (μ) is calculated based on the following equation (1) (Asuthkar *et al.*, 2016).

$$\mu = \frac{\ln(X_2) - \ln(X_1)}{\Delta_t} \quad \ldots(1)$$

where $\mathbf{X_2}$ is the number of cells in the exponential phase (cells/mL), $\mathbf{X_1}$ is the initial number of cells (cells/mL) and Δ_t is the time required to increase the concentration from $\mathbf{X_1}$ to $\mathbf{X_2}$ (days).

The percentage of waste parameter removal can be calculated using the equation (2) (Bellén *et al.*, 2016).

$$R = \frac{(C_o - C_f)}{C_o} x\ 100 \quad \ldots(2)$$

where R is the percentage of removal (%), C_0 is the initial concentration of the parameter (mg/L) and C_f is the final concentration of the parameter (mg/L).

Result and Discussion

Effect of waste concentration on the growth rate of microalgae

The growth rate describes the growth rate of microalgae cells per unit of time, which can be used as a benchmark to determine the carrying capacity of the medium or nutrients for the growth and division of microalgae cells. The growth of *Chlorella pyrenoidosa* cells in liquid waste media was observed for 10 days with varying concentrations of 0%, 10%, 20% and 30% tannery wastewater to determine the effect of tannery wastewater concentration on microalgae growth. The growth of microalgae occurs in various phases, namely (i) the lag phase, which is the phase in which bacteria adapt to their environment, (2) the log/exponential phase, which is a phase of fast bacterial growth, characterized by active cells that can be observed, (3) the stationary phase, which is the phase in which the rate of growth and death is balanced so that the total number of living bacteria remains, (4) the phase of decreased growth and ends in (5) the phase of cell death (Istirokhatun, Aulia and Utomo, 2017). Graphs of optical density and growth rate in this study are shown in Figure 1 and Figure 2.

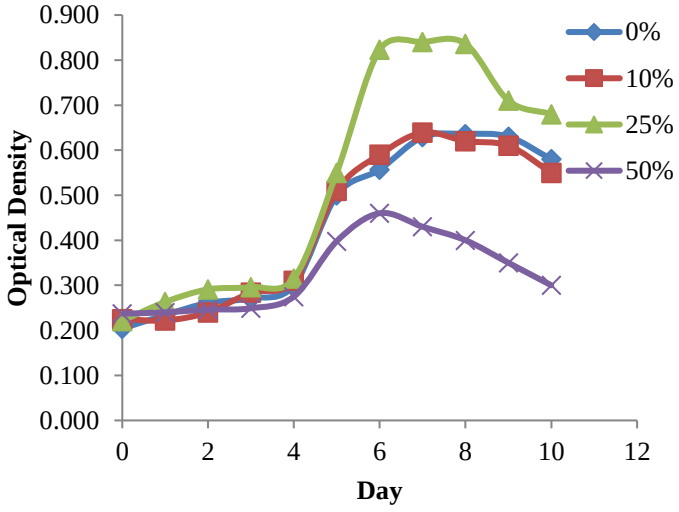

Figure 1. Optical density/cell density of *Chlorella pyrenoidosa*

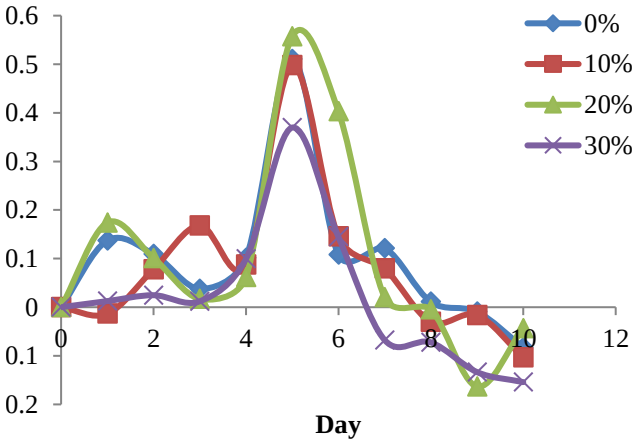

Figure 2. Growth rate of *Chlorella pyrenoidosa* at various concentrations of leather tannery wastewater

The lag phase in this study occurred in the first four days. This can be seen from the increased optical density value of Chlorella. In this lag phase, *Chlorella* can adapt well to the environment where cell division occurs. The ability of microalgae to adapt is influenced by organic and inorganic compounds or materials in the media, which are sources of nutrition and can also be limiting nutrients for growth. If one of the nutrients is not available in the waste or the amount is too large, the process of reducing organic compounds and the growth of microalgae will be hampered (Istirokhatun, Aulia and Utomo, 2017).

The exponential phase (log phase) begins with cell division and is characterized by an increase in growth rate so that population density increases (Kawaroe *et al.*, 2012). In this study, the time and cell density of the exponential phase of various concentrations were varied. Based on Figure 2, the lowest exponential phase occurs at a concentration of 30% with a growth rate value of 0.370. A waste concentration of 30% has a high enough nutrient content to be used as a source of nutrition for microalgae growth. However, if the levels of available nutrients are too high and exceed the tolerance limit for *Chlorella* cells to be able to utilize them, then these nutrients can be toxic,

causing *Chlorella* growth to be hampered. The temperature, light, aeration, and pH factors provided during the culture process also support microalgae growth.

The highest exponential phase occurs at a concentration of 20% with a growth rate of 0.557. This is because the leather tanning liquid waste contains inorganic minerals in the form of ions, which are more easily absorbed and utilized by *Chlorella pyrenoidosa* cells for growth. In addition, the amount of nutrients in the leather tanning wastewater is proportional to the amount of microalgae that utilize these nutrients.

The stationary phase in this study could not be observed closely because the growth calculation of microalgae cell density was carried out once every 24 hours. The distance between the decreasing phase and the stationary phase is generally relatively short, so according to researchers' needs, the intensity must be calculated more than once every 24 hours.

The next phase is decreased growth, characterized by decreased cell density. Decrease in *Chlorella pyrenoidosa* cell density at each different concentration. At concentrations of 0%, 10%, 20%, and 30%, the decline in growth began to occur on the 6th day. The cell density begins to decrease because the nutrients in the waste have begun to decrease over time, and the death rate is higher than the growth rate (death phase). The next phase is the cell death phase. The microalgae cell death phase was observed on the 8th day, which indicated more microalgae cells that died than cells that were still alive. This can be seen from the growth rate curve, which shows a negative value or is below the X-axis.

Effect of pH on microalgae growth

The pH value of the culture media is a controlling factor that determines the biological ability of microalgae to utilize nutrients (Megawati, Yusuf and Maslukah, 2014). The appropriate pH for the growth of *Chlorella* ranges from 4.5 - 9.3. The increase in media pH can be seen in Figure 3. The lowest cell concentration occurs in the treatment media with a value of around pH 6, according to (Purnamasari, 2020)This is due to the acidic cultivation conditions in the media, which cause the cells' ability to absorb nutrients to be less than optimal, thereby affecting the subsequent growth process. The initial acidic pH disrupts cell metabolism.

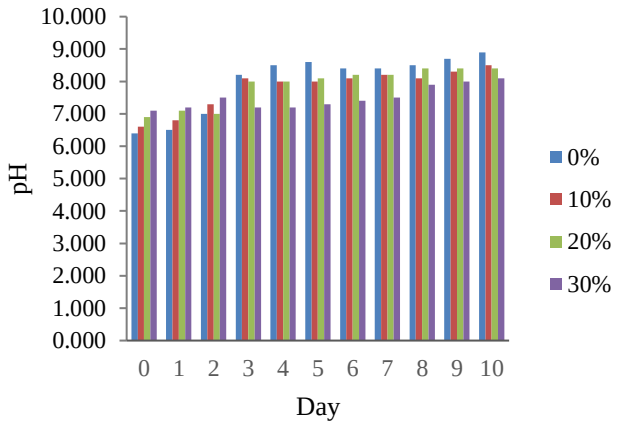

Figure 3. pH of *Chlorella pyrenoidosa* culture media

Based on the results of the pH test, it is known that the lowest pH is (6.4) and the highest pH is (8.99). This pH value increases pH from low to high on the 1st to 10th observations. According to Purnamasari (2020), there was a change in the pH value, which was different from the initial pH treatment due to the photosynthetic activity of the microalgae. During photosynthesis, free CO_2 is the primary type of inorganic carbon used by microalgae in the form of carbonate ions (CO_3^{2-}) and bicarbonate ions (HCO_3^-). Absorption of free CO_2 and bicarbonate by microalgae causes a decrease in dissolved CO_2 concentration and increases the pH value. This is in line with the research (Nurhayati, Hamzah and Pambayun, 2014), that the photosynthetic activity of microalgae takes dissolved carbon and results in an increase in pH.

Another factor that causes an increase in pH in the waste media is the decomposition of proteins and other nitrogen compounds. Ammonium (NH_4^+), nitrate (NO_3^-), and nitrite (NO_2^-) are forms of organic nitrogen compounds that have undergone

decomposition (Farahdiba, Budiantoro and Yulianto, 2019). In general, nitrogen compounds used in microalgae cell metabolism are in the form of ammonium. Ammonium is produced through the dissociation process of ammonium hydroxide. Ammonium hydroxide is ammonia dissolved in water. According to (Prihantini, Putri and Yuniati, 2005), the increase in pH is caused by an increase in the concentration of ammonium in the media, this also applies to a pH above 9. If the pH of the water is >9, then the enzyme whose role is to form ammonium when the metabolism of biota cells is disrupted so that living creatures in aquatic biota will die (Nurhayati, Hamzah and Pambayun, 2014).

The degree of acidity (pH) of the media determines the solubility and availability of mineral ions, affecting cells' nutrient absorption. Changes in the pH value can drastically affect the work of enzymes and inhibit the process of photosynthesis and the growth of some microalgae. *Chlorella* culture with bean sprout extract medium is the most optimal for population growth with an initial pH of 7, with a population peak on day 10 (Prihantini, Putri and Yuniati, 2005).

Table 2. Removal of COD in leather tannery wastewater

Waste Concentration (% v/v)	Max. growth rate	COD		COD Removal (%)
		Start (mg/L)	End (mg/L)	
10	0.50	1270	544	57.16
20	0.56	1298	469	63.87
30	0.37	1342	747	44.33

Based on Table 2, the calculation results show that the most effective removal of COD content from leather tannery wastewater is carried out by microalgae at a concentration of 20%, namely 63.87%. This is because the composition between microalgae and nutrients is ideal at this concentration. The COD removal efficiency decreases when the wastewater concentration is increased to 30%. This shows that a concentration of 20% is optimal for COD removal in liquid waste by *Chlorella pyrenoidosa*. For COD content, the higher the nitrogen content in the liquid waste, the more significant the reduction in COD value. This increase in nitrogen levels will make microalgae grow better, as seen from the more rapid growth of *Chlorella* cells. In addition, when CO_2 levels increase, the reduction in COD value gets smaller. This is because the addition of CO_2 will inhibit growth, and the ability to reduce COD also decreases (Istirokhatun, Aulia and Utomo, 2017).

Based on the study's results, the final COD value in the 20% waste concentration was 469 mg/L with the highest removal efficiency of 63.87%, which still did not meet the quality standard of 110 mg/L. Furthermore, to reduce the COD value, which is still high, namely by re-cultivating the harvested microalgae into the liquid waste medium (Megawati, Yusuf and Maslukah, 2014)Because harvesting was taken during the middle of the cultivation period, namely the exponential phase, and the COD had fallen ± 50% from the initial COD, further cultivation only took half the cultivation period, which is around 5-7 days. Using a technique like this will provide benefits in COD removal and the amount of biomass that can be harvested.

The research results show that the cell growth phase seen from cell density or maximum growth rate is inversely proportional to the COD content in tofu liquid waste. The greater or the greater the number of microalgae that grow by dividing, the more the COD content decreases. This is because microalgae cells grow by utilizing organic substances as nutrients.

Ammonia removal

Ammonia is the primary source of nitrogen besides nitrate, which can be used by microalgae for their metabolic processes, while their toxicity limits the use of nitrites. If nitrate and ammonia-N are present together, nitrate will not be absorbed until all the ammonia-N has been absorbed. Almost all microalgae have the urease enzyme, as do higher plants. Urea is a source of N in the

growth of various microalgae, even by microalgae that do not have urease (Nurhayati, Hamzah and Pambayun, 2014).

This indicates that in the treatment with a 20% microalgae concentration, the pH was adjusted to pH 8, and the 10th day of observation showed the lowest ammonia levels. At a pH of 8, microalgae will carry out their metabolism optimally so that a large number of cells will utilize ammonia from leather tanning waste in large quantities, reducing the amount of ammonia as a waste pollutant.

The decomposition of proteins and other nitrogen compounds will increase the pH value. Ammonium (NH_4^+), nitrate (NO_3^-), and Nitrite (NO_2^-) are forms of organic nitrogen compounds that have undergone decomposition (Farahdiba, Budiantoro and Yulianto, 2019). In general, nitrogen compounds used in microalgae cell metabolism are in the form of ammonium. The reaction for the formation of ammonium is as follows (Nurhayati, Hamzah and Pambayun, 2014):

$$NH_3 + H_2O \leftrightarrow NH_4OH \leftrightarrow NH_4^+ + OH^- \quad (3)$$

If reaction (3) moves to the right, the ammonium concentration in the medium will increase, and the pH will become alkaline. This data is supported by cell growth, where initially, microalgae will experience a lag phase on day one. On days 2 to 4, microalgae experience a logarithmic phase, and on days 4 to 5, microalgae experience an exponential phase. There is a stationary phase on days 6 to 7, and on days 8 to 10, there is death. The allowance for ammonia in this study can be seen in Table 3.

Table 3. Removal of ammonia in leather tannery wastewater

Waste Concentration (% v/v)	Max. growth rate	Ammonia		Ammonia Removal (%)
		Start (mg/L)	End (mg/L)	
10	0.50	0.8	0.5	37.5
20	0.56	0.85	0.28	67.06
30	0.37	0.92	0.41	55.43

Cr (VI) Removal

The commonly used leather tanning agent is trivalent chrome mineral. Therefore, chrome is the metal focused on in this research. Other heavy metals were also detected in tannery waste, such as Cd, Pb, Zn, Cu, Fe, and Ni, but the metal whose levels most exceeded the safe limit was Cr. The presence of other metals besides Cr may influence the saturation response of phytochelatin compounds when recognizing the presence of non-essential compounds such as metals, thereby influencing the phytoremediation process (Sharma and Mehra, 2023).

Chromium is commonly found in waters as trivalent and hexavalent chromium. Trivalent chromium is an essential element for plants and animals, but hexavalent chromium is toxic (Kawaroe *et al.*, 2012). Provision for metal Cr (VI) from tannery wastewater in this study can be seen in Table 4. The highest removal of Cr (VI) occurred in media with 30% waste concentration. The higher the metal concentration, the greater the metal absorbed by the sample. This is because, in cells, there are active phytochelatin compounds that will bond longer with the amount of metal concentration, so as long as the phytochelatin compounds formed are not at saturation point, metal binding (Auliyah, 2021).

Table 4. Removal of Cr (VI) in leather tanning wastewater

Waste Conc. (% v/v)	Max. growth rate	Cr (VI)		Cr (VI) Removal (%)
		Start (µg/L)	End (µg/L)	
10	0.50	11.5	< 6	>47.83
20	0.56	13.2	< 6	>54.54
30	0.37	19.2	< 6	>68.91

There are 2 types of absorption process of *Chlorella pyrenoidosa* as an accumulator, namely passive uptake and active uptake. Passive uptake means heavy metals bind to cell walls employing ion

exchange and complex sequences between metal ions and function groups. In general, the interaction between metals and functional groups through ion exchange interactions or complex formation is called the adsorption process (Adi and Nana, 2010). Adsorption occurs due to the sharing of electrons by the adsorbent, which is a covalent bond. Cell wall components in the cytoplasm that can bind ions include carboxyl, hydroxyl, sulfate and sulfonate groups, phosphates, amines, and sulfhydryl groups (Salam, 2019).

The active uptake process occurs slowly, namely the process of absorption, detoxification and accumulation occurs in living *Chlorella* sp cells. Metal absorption by absorption also occurs in cell walls where the absorbed metal is bound by the sulfur (S) group of the amino acid cysteine. After that, the receptor protein will recognize the presence of incoming metals so that the gene will code for the formation of Metallothionin Protein (MTP). MTP is a metal binding protein. The high cysteine content causes the protein to have a strong affinity for metals (Tripathi *et al.*, 2019). Two sulfur compounds bound to the amino acid cysteine will react with the protein metal so that the MTP protein forms a structure like Figure 4.

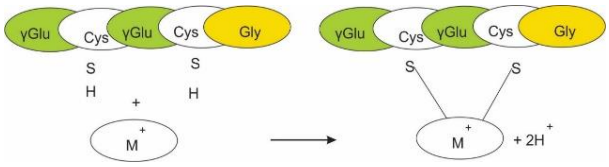

Figure 4. Phytochelatin forms a complex with the heavy metal M^+

Chlorella pyrenoidosa is a group of microalgae that is reported to have phytochelatin compounds that can bind metals, where the phytochelatin ligand is composed of several amino acid groups, namely glutamine, cysteine , and glycine. Phytochelatin is formed from the reduction of glutathione and is an essential molecule in fighting heavy metals bound to different thiol groups on the amino acid cysteine. The reaction of metals with thiol groups, especially glutathione (GSH), reduces the abundance of metals in the cellular environment (Auliyah, 2021). The metal will be bound with the active sulfhydryl group (-SH), a soft base belonging to the amino acid cysteine. The sulfhydryl group easily binds heavy metals and enters cell organelles. Furthermore, the metal will accumulate in the vacuole through an enzymatic process (Gratão, Alves and Lima, 2019). Figure 5 shows the bioaccumulation of heavy metal ions in microalgae.

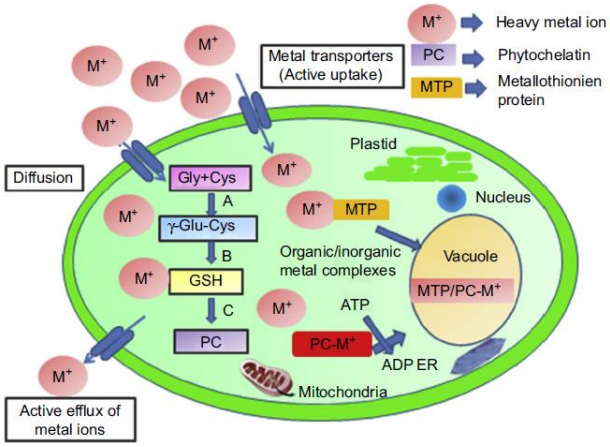

Figure 5. Bioaccumulation of heavy metal ions in microalgae (Gratão, Alves and Lima, 2019)

Metal absorption by phytochelatin ligands can occur when traces of environmental conditions expose metal. The formation of phytochelatin is a form of adaptation to the environment, detoxifying the peptide-thiol compounds contained therein. Phytochelatin binds heavy metals, whereas heavy metal ions will bind to phytochelatin (PC) to form PC-heavy metal bonds (Auliyah, 2021).

Based on Figure 4, the mechanism of absorption of heavy metals is the response of phytochelatin compounds when recognizing the presence of non-essential compounds such as Cr (VI), the sulfhydryl (-SH) group bound to two different cysteine compounds will deprotonate the H^+ atom and be replaced by Cr metal (Daneshvar *et al.*, 2019). PC/MTP that binds to heavy metals is transported to the vacuole, which stores ions and metabolites. Inside the cell, it will form MTP

continuously as long as heavy metal ions are still in solution bound to the S group of the cell wall protein until, at a certain point, the cell will experience saturation and be in the death phase (Adi and Nana, 2010).

The high absorption of metals by *Chlorella pyrenoidosa* is also influenced by the pH in a solution; namely, changes in pH affect the arrangement of functional groups. When the pH increases, the absorption carried out by *Chlorella pyrenoidosa* also increases. Changes in pH in the environment are caused by Chlorella sp's growth and photosynthesis activities, which require free CO_2. The absorption of free CO_2 causes the concentration of dissolved CO_2 to decrease and causes an increase in the pH value. At high pH, the surface of *Chlorella* sp will be negatively charged, and deprotonation of the sulfhydryl groups occurs so that the absorption capacity of metal ions increases (Auliyah, 2021).

Figure 6 shows the tannery liquid waste removal parameters after using it as a microalgae cultivation medium. The parameters measured are COD, ammonia, and Cr(VI).

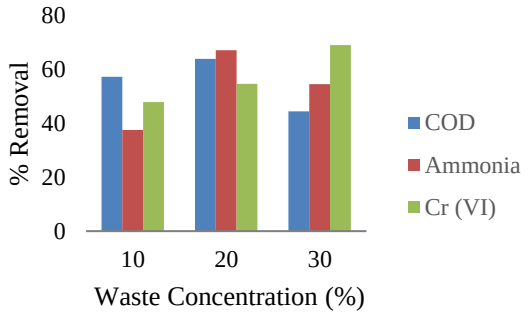

Figure 6. Percentage of removal of leather tannery wastewater parameters

Conclusion

Based on the research that has been carried out, it can be concluded that *Chlorella pyrenoidosa* can grow in liquid tannery waste media. The highest exponential phase occurs at a concentration of 20% with a growth rate of 0.557. This is because tannery liquid waste contains inorganic minerals utilized by *Chlorella pyrenoidosa* cells for their growth. Cultivation of *Chlorella pyrenoidosa* can reduce levels of leather tannery wastewater parameters, namely (1) COD with a maximum removal efficiency of 63.87% at 20%v/v liquid waste; (2) Ammonia with a maximum removal efficiency of 67.06% at 20%v/v of liquid waste; (3) Cr (VI) with a maximum removal efficiency of 68.91% at 30%v/v of liquid waste.

References

Adi, E. and Nana, D. (2010) 'Reducing ion Pb concentration in electroplating waste water with biosorption process and stirring', *Jurnal Teknik Kimia*, 5(1), pp. 373–379.

Ajayan, K. V. *et al.* (2015) 'Phycoremediation of Tannery Wastewater Using Microalgae Scenedesmus Species', *International Journal of Phytoremediation*, 17(10), pp. 907–916. doi: 10.1080/15226514.2014.989313.

Al-Homaidan, A. A. *et al.* (2018) 'Potential use of green algae as a biosorbent for hexavalent chromium removal from aqueous solutions', *Saudi Journal of Biological Sciences*. King Saud University, 25(8), pp. 1733–1738. doi: 10.1016/j.sjbs.2018.07.011.

Ariyanti D and Handayani NA (2010) 'Mikroalga Sebagai Sumber Biomasa Terbarukan: Teknik Kultivasi Dan Pemanenan', *Metana*, 6(2), pp. 35–40.

Asuthkar, M. *et al.* (2016) 'Effect of Different Wavelengths of Light on the Growth of *Chlorella Pyrenoidosa*', *International Journal of Pharmaceutical Sciences and Research*, 7(2), pp. 847–851. doi: 10.13040/IJPSR.0975-8232.7(2).847-51.

Auliyah, Y. A. (2021) *Fitoremidiasi Logam Tembaga (Cu) oleh Mikroalga Chlorella sp Hasil Kultivasi Media*

Ekstrak Tauge Berdasarkan Variasi Konsentrasi. Universitas Islam Negeri Maulana Malik Ibrahim.

Bellén, M. *et al.* (2016) 'Using Scenedesmus sp. for the Phycoremediation of Tannery Wastewater', *Tecciencia*, 12(21), pp. 69–75.

Daneshvar, E. *et al.* (2019) 'Hexavalent chromium removal from water by microalgal-based materials: Adsorption, desorption and recovery studies', *Bioresource Technology*. Elsevier, 293(August), p. 122064. doi: 10.1016/j.biortech.2019.122064.

Das, C. *et al.* (2017) 'Bioremediation of tannery wastewater by a salt-tolerant strain of *Chlorella vulgaris*', *Journal of Applied Phycology*. Journal of Applied Phycology, 29(1), pp. 235–243. doi: 10.1007/s10811-016-0910-8.

Farahdiba, A. U., Budiantoro, W. and Yulianto, A. (2019) 'Ammonia removal from Yogyakarta Domestic Wastewater (WWTP-SEWON) by microalgae reactor with CO2 addition', *IOP Conference Series: Earth and Environmental Science*, 245(1), pp. 2–10. doi: 10.1088/1755-1315/245/1/012019.

Da Fontoura, J. T. *et al.* (2017) 'Influence of light intensity and tannery wastewater concentration on biomass production and nutrient removal by microalgae Scenedesmus sp.', *Process Safety and Environmental Protection*. Institution of Chemical Engineers, 111, pp. 355–362. doi: 10.1016/j.psep.2017.07.024.

Gauje, B. *et al.* (2022) 'Simultaneous phytoremediation of tannery effluent and production of fatty acids rich biomass by *Chlorella sorokiniana*', *Journal of Applied Phycology*. Springer Netherlands, 34(2), pp. 929–940. doi: 10.1007/s10811-022-02683-5.

Gratão, P. L., Alves, L. R. and Lima, L. W. (2019) 'Heavy Metal Toxicity and Plant Productivity: Role of Metal Scavengers', *Plant-Metal Interactions*, pp. 49–60. doi: 10.1007/978-3-030-20732-8.

Hadiyanto, H. *et al.* (2019) 'Pretreatment of Herbal, Tofu and Fertilizer Waste Using UV/Ozon Technique and Its Utilization for Spirulina sp Cultivation', *IOP Conference Series: Earth and Environmental Science*, 248(1). doi: 10.1088/1755-1315/248/1/012071.

Istirokhatun, T., Aulia, M. and Utomo, S. (2017) 'Potensi *Chlorella Sp*. untuk Menyisihkan COD dan Nitrat dalam Limbah Cair Tahu', *Jurnal Presipitasi : Media Komunikasi dan Pengembangan Teknik Lingkungan*, 14(2), pp. 88–96. doi: 10.14710/presipitasi.v14i2.88-96.

Kawaroe, M. *et al.* (2012) 'Laju Pertumbuhan Spesifik dan Kandungan Asam Lemak pada Mikroalga Spirulina platensis, Isochrysis sp. dan Porphyridium cruentum', *Ilmu Kelautan*, 17(3), pp. 125–131.

Lúcia, H. R. R. *et al.* (2011) 'Algal density assessed by spectrophotometry: A calibration curve for the unicellular algae Pseudokirchneriella subcapitata', *Journal of Environmental Chemistry and Ecotoxicology*, 3(8), pp. 225–228. doi: 10.5897/jece2011.025.

Megawati, C., Yusuf, M. and Maslukah, L. (2014) 'Sebaran Kualitas Perairan Ditinjau dari Zat Hara, Oksigen Terlarut dan pH Di Perairan Selat Bali Bagian Selatan', *Jurnal Oseanografi*, 3(2), pp. 142–150.

Nogueira, S. M. S. *et al.* (2018) 'Use of Spirulina platensis in treatment of fish farming wastewater', *Revista Ciencia Agronomica*, 49(4), pp. 599–606. doi: 10.5935/1806-6690.20180068.

Nugroho, A. R. and Anggriyani, E. (2018) 'the

Reduction of Bod, Cod and Chrome in the Tanning Wastewater', *MATTER: International Journal of Science and Technology*, 4(3), pp. 25–33. doi: 10.20319/mijst.2018.43.2533.

Nurhayati, C., Hamzah, B. and Pambayun, R. (2014) 'Pengaruh pH, konsentrasi isolasi chlorella vulgaris dan waktu pengamatan terhadap tingkat cemaran limbah cair crumb rubber', *Jurnal Dinamika Penelitian Industri*, 25(2), pp. 97–106.

Polontalo, N. F., Joelyna, F. A. and Hadiyanto, H. (2021) 'Production of Bioelectricity from Microalgae Microbial Fuel Cell (MMFC) Using Chlorella pyrenoidosa and Batik Wastewater', *IOP Conference Series: Materials Science and Engineering*, 1053(1), p. 012096. doi: 10.1088/1757-899x/1053/1/012096.

Pradhan, D. *et al.* (2019) 'Biosorption for removal of hexavalent chromium using microalgae Scenedesmus sp.', *Journal of Cleaner Production*. Elsevier B.V., 209, pp. 617–629. doi: 10.1016/j.jclepro.2018.10.288.

Prihantini, N. B., Putri, B. and Yuniati, R. (2005) 'The Growth of *Chlorella sp*. in Tauge Extract Medium (TEM) with Various Initial pH', *MAKARA of Science Series*, 9(1). doi: 10.7454/mss.v9i1.457.

Purnamasari, D. (2020) *Isolasi Dan Kultivasi Mikroalga Untuk Pengolahan Limbah Dari Sungai Cisadane Tangerang*, *Repository.Uinjkt.Ac.Id*. Available at: http://repository.uinjkt.ac.id/dspace/handle/123456789/55035%0Ahttp://repository.uinjkt.ac.id/dspace/bitstream/123456789/55035/1/DIAN PURNAMASARI-FST.pdf.

Salam, K. A. (2019) 'Towards sustainable development of microalgal biosorption for treating effluents containing heavy metals', *Biofuel Research Journal*, 6(2), pp. 948–961. doi: 10.18331/BRJ2019.6.2.2.

Sharma, A. and Mehra, R. (2023) 'Analysis of heavy metals and toxicity level in the tannery effluent and the environs', *Environmental Monitoring and Assessment*, 195(5). doi: 10.1007/s10661-023-11154-4.

Tripathi, S. *et al.* (2019) *Microalgae: An emerging source for mitigation of heavy metals and their potential implications for biodiesel production*, *Advanced Biofuels: Applications, Technologies and Environmental Sustainability*. Elsevier Ltd. doi: 10.1016/B978-0-08-102791-2.00004-0.

Wardhani, E., Dirgawati, M. and Valyana, K. P. (2012) 'Application of electrocoagulation method for leather tanning industrial waste water treatment', *Seminar Ilmiah Nasional, Masalah Lingkungan di Indonesia 8, Kampus Universitas Gadjah Mada*, 12, pp. 1–16.

Yang, Y.-C. *et al.* (2017) 'Biomass and Lipid Production of *Chlorella sp*. Using Municipal Wastewater under Semi-continuous Cultivation', *International Proceedings of Chemical, Biological and Environmental Engineering*, 101(3), pp. 18–25. doi: 10.7763/IPCBEE.