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REMOVAL OF HEAVY METALS USING *CHLORELLA VULGARIS*: A REVIEW

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ABSTRACT

In this review, the researchers describe a natural process that can remove heavy metals from the environment. The use of Chlorella vulgaris has more potential than other bioremediation processes. Chlorella vulgaris has been recognized as a biomaterial capable of removal and could be a potential alternative method for the physicochemical absorption of heavy metals. The removal of heavy metals using Chlorella vulgaris on living cells can occur rapidly, independent of absorption of metabolism to the cell surface and intracellular absorption. Non-living cells have also successfully removed heavy metals from liquid waste. As one of the innovative removal technologies, it depends on algae's biosorption and bioaccumulation capabilities, which dominate the bioremediation process. This study shows that the composition of Chlorella vulgaris constituents, such as proteins, lipids, carbohydrates, pigments, vitamins, and minerals, which have various amino acid, hydroxyl, carboxyl, and sulfate functional groups, are the main factors in the absorption process of heavy metals in liquid waste, so they have the potential sustainability in its use as a bio adsorbent in the removal of heavy metals in liquid waste in the future.

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INTRODUCTION

Heavy metals that are toxic to the environment can accumulate in the food chain, and some of them can be considered mutagenic and carcinogenic at high concentrations. Therefore, heavy metals threaten the balance of the environment and human health (Barquilha *et al.*, 2019; Li *et al.*, 2019; Morosanu *et al.*, 2019; Almomani and Bohsale, 2020).

In the environment, the most common heavy metals found are lead (Pb), chromium (Cr), arsenic (As), zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), and nickel (Ni). Whose existence is abundant and used in various industries (Yen *et al.*, 2017; Rangabhashiyam *et al.*, 2019; Putra *et al.*, 2024). Studies link exposure to high concentrations of heavy metals with changes in cellular and nervous system activity, as well as to gastrointestinal irritation, depression, and lung cancer (Cheng *et al.*, 2017; Lalhmunsiama *et al.*, 2017; Barquilha *et al.*, 2019; Morosanu *et al.*, 2019; Fathony *et al.*, 2023; Putra *et al.*, 2023).

Waste treatment processes often used in industry are mainly carried out conventionally, such as separation using membranes, chemical precipitation, and ion exchange, which are successfully used to remove pollutants in liquid waste (Efome *et al.*, 2019; Ibrahim *et al.*, 2019; Garba *et al.*, 2020; Wulandari *et al.*, 2023). However, this process is less efficient and not economically appropriate because it requires money in the management process (Giwa *et al.*, 2019; Putra *et al.*, 2022). However, this process is less efficient and not economically appropriate because it requires much money in management (Jokar *et al.*, 2019; Almomani and Bohsale, 2020; Apiratikul, 2020).

The biosorption method used to adsorb heavy metals here utilizes microalgae, where the microalgae can selectively absorb metals from liquid waste and accumulate them in their cells. One type of microalgae used for heavy metal absorption is *Chlorella vulgaris*. The absorption of heavy metals using *Chlorella vulgaris* is due to the presence of polysaccharide components, proteins, and lipids as components of the cell wall, which contain amino, hydroxyl, carboxyl, and sulfate functional groups that act as binding sites for heavy metals. (C. and J., 2012; El-Sheekh *et al.*, 2019; Putra and Fitri, 2021). *Chlorella vulgaris* can grow in a polluted environment because it has polyamine groups that can adapt to polluted water ecosystems. Polyamine can act as a molecule that can protect microalgae against the risk of stress from the growing environment (C. and J., 2012; Fitri *et al.*, 2021). In this review, the researcher wants to describe research related to the use of *Chlorella vulgaris* both in the form of living and dead cells and its application in removing heavy metals from liquid waste.

METHODOLOGY

This article reviews several research articles that have been reported. In the process of searching for articles, the author used "Google Scholar" and "Science Direct" as the retrieval database, using the keywords "*Chlorella vulgaris*" and "Heavy Metals." from 2015 to 2020. Based on the analysis, this article describes the growth of *Chlorella vulgaris* and its application to study the absorption of heavy metals in liquid waste. The review stages of this article include: 1) Describing the characteristics and growth of *Chlorella vulgaris* and the methods of biosorbent preparation 2) The biosorption mechanism of *Chlorella vulgaris*. 3)

Application of *Chlorella vulgaris* to adsorb heavy metals in wastewater treatment, 4) To realize the use of *Chlorella vulgaris* sustainably, economic and environmental impacts are analyzed for further study.

RESULTS AND DISCUSSION

3.1 Chlorella vulgaris

Algae are two different types: micro and macroalgae. Microalgae are photosynthetic unicellular microorganisms that live in seawater and fresh water. They consist of four groups: diatoms, green algae, golden algae, and blue-green algae (Anastopoulos and Kyzas, 2015). *Chlorella vulgaris* is a unicellular microalga belonging to the green microalgae of the Chlorophyta family (Ahmad *et al.*, 2020). For more details, the taxonomic classification of *Chlorella vulgaris* is as follows: Domain: Eukaryotic, Kingdom: Protista, Division: Chlorophyta, Class: Trebouxiophyceae, Order: Chlorellales, Family: Chlorellaceae, Genus: *Chlorella*, Species: *Chlorella vulgaris*, where the word "chloro" is defined as green while "ella" is defined as small size (Safi *et al.*, 2014). *Chlorella vulgaris* is a spherical or ellipsoidal cell where the diameter of the cells ranges from 2 to 10 min (Ahmad *et al.*, 2020).

3.2 Growth Chlorella vulgaris

The growth of *Chlorella vulgaris* is influenced by several factors, including nutrition, pH, salinity, temperature, and light. Among these factors, light greatly influences the photosynthetic mechanism and is important in determining the optimal conditions for culture. Phytoplankton photosynthesis is influenced by natural factors, such as temperature and radiation (Edwards *et al.*, 2015, 2016). Natural bioactive compounds found in *Chlorella vulgaris*, such as carotenoids, phenolic compounds, sulfate polysaccharides, and vitamins, have functions that can affect cell regulation, immune response, and as antioxidants (Silva *et al.*, 2019).

Characteristics of light sources with wavelength and intensity also affect the growth of *Chlorella vulgaris* and microalgae in general (Blanken *et al.*, 2013). Another factor that can affect the life of microalgae is the type or source of light with a wavelength ranging from 400-700 nm. The use of appropriate wavelengths of light in the photosynthesis process can increase biomass growth and the quality and content of nutrients, pigments, and microalgae bioactive compounds (Khalili *et al.*, 2015). According to research reported by Balubi (2019), the growth of *Chlorella vulgaris* was significantly affected by salinity, with better growth at high salinity (40 and 45 ppt) than at low salinity (30 and 35). The growth and nutrient composition of *Chlorella vulgaris* may vary depending on the nutrient composition in the growing medium and environmental conditions such as light intensity, temperature, and salinity (Hay, 2016; Iba *et al.*, 2018).

3.3 Composition Chlorella vulgaris

The main composition of *Chlorella vulgaris* consists of protein, fat, carbohydrates, pigments, minerals, and vitamins. The table below shows the abovementioned components (Safi *et al.*, 2014).

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Tabel 1. *Chlorella vulgaris* amino acid profile per 100 g of protein.

Amino Acid	**Concentrations in 100 g**
Aspartic acid	10.94
Threonine	6.09
Serine	7.77
Glutamic acid	9.08
Glycine	8.60
Alanine	10.90
Cysteine	0.19
Valine	3.09
Methionine	0.65
Isoleucine	0.09
Leucine	7.49
Tyrosine	8.44
Phenylalanine	5.81
Histidine	1.25
Lysine	6.83
Arginine	7.38
Tryptophan	2.21
Ornithine	0.13
Proline	2.97

The composition of lipids contained in *Chlorella vulgaris* consists of three main fractions: phospholipids (PL), glycolipids (GL), and neutral lipids (NL).

Table 2. Composition of Carbohydrate *Chlorella vulgaris*

Neutral sugars	**Percentage (%)**
Rhamnose	45–54
Arabinose	2–9
Xylose	7–19
Mannose	2–7
Galactose	14–26
Glucose	1–4

Tabel 3. Composition of Pigmen Chlorella vulgaris

Pigments	**μgg^{-1} (dw)**
β-Carotene	7–12,000
Astaxanthin	550,000
Canthaxanthin	362,000

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Lutein	52–3830
Chlorophyll-a	250–9630
Chlorophyll-b	72–5770
Pheophytin-a	2310–5640
Pheophytin-b	N/A
Violoxanthin	10–37

Tabel 4 Composition of Mineral Chlorella vulgaris

Minerals	Mineral content (g 100 g^{-1})		
	Maruyama et al (2018)	Tokusoglu and Unal (2020)	Panahi et al (2019)
Microelements			
Na	N/A	1.35	N/A
K	1.13	0.05	2.15
Ca	0.16	0.59	0.27
Mg	0.36	0.34	0.44
P	N/A	1.76	0.96
Macroelement			
Cr	N/A	tr	tr
Cu	N/A	tr	0.19
Zn	N/A	tr	0.55
Mn	N/A	tr	0.40
Se	N/A	tr	N/A
I	N/A	N/A	0.13
Fe	0.20	0.26	0.68

tr: traces; N/A: not available

Tabel 5. Composition of Vitamin *Chlorella vulgaris*

Vitamins	Content (mg 100 g^{-1})		
	Maruyama et al (2019)	Yeh et al. (2020)	Panahi et al (2019)
B1 (Thiamine)	2.4	N/A	1.5
B2 (Riboflavin)	6.0	N/A	4.8
B3 (Niacin)	N/A	N/A	23.8
B5 (Pantothenic acid)	N/A	N/A	1.3
B6 (Pyridoxine)	1.0	N/A	1.7
B7 (Biotin)	N/A	N/A	191.6
B9 (Folic acid)	N/A	N/A	26.9

B12 (Cobalamin)	tr	N/A	125.9
C (Ascrorbic acid)	100.0	39.0	15.6
E (Tocopherol)	20.0	2787.0	N/A
A (Retinol)	N/A	13.2	N/A

tr: traces; N/A: not available

3.4 Biosorption and Bioaccumulation Chlorella vulgaris

Biosorption is the removal of heavy metals by passive binding to inanimate biomass from aqueous solutions. This implies that the disposal mechanism is not metabolically controlled. While bioaccumulation describes an active process, metal removal requires the metabolic activity of living organisms. In recent years, research on biosorption mechanisms has increased since biomass can absorb heavy metals from industrial waste, such as the mining industry, or electroplating to remove heavy metals from liquid waste (Davis *et al.*, 2003; Putra and Fitri, 2021).

Biological technology has three main advantages to removing these pollutants: 1) biological processes can be carried out in situ at the contaminated site; 2) bioprocess technology is usually environmentally friendly, with no secondary pollution; and 3) cost-effective. From different biological methods, bioaccumulation and biosorption have been shown to have good potential to replace conventional methods of removing heavy metals (Abdi and Kazemi, 2015)**.**

3.5 Mechanisms Biosorption

Usually, the biosorption process is quite complex, and the entire metal uptake mechanism results from a combination of different elementary mechanisms, such as electrostatic interactions, ion exchange, complexation, chelation adsorption, micro-deposition, etc., which occur simultaneously or sequentially (Volesky, 1987; Kim, 2015)**.**

In the biosorption system, two types of biosorption processes can be carried out by *Chlorellla vulgaris*: passive mode, dead *Chlorella vulgaris* cells, and active mode, living *Chlorella vulgaris* cells. The passive model does not depend on energy because of chemical interactions with functional groups of biomaterials, mainly consisting of cells and cell walls. In general, the biosorption process in dead microalgae follows a chemical mechanism. In contrast, the main important factors that determine the properties of elementary processes are 1) the types of functional groups present on the surface of the microalgae, 2) the nature of the heavy metal species from the aqueous solution, and 3) characteristics of the solution (pH, ionic strength, presence of competing ions, etc.) (Bulgariu and Gavrilescu, 2015). Meanwhile, the active model depends on metabolism, transport systems, and metal deposition. So passive metal uptake can occur when cells are metabolically active (Javanbakht *et al.*, 2014; Febria *et al.*, 2023).

The following is the heavy metal biosorption mechanism, shown in Figure 1, and can be explained according to the place where the heavy metal absorbs from the solution:

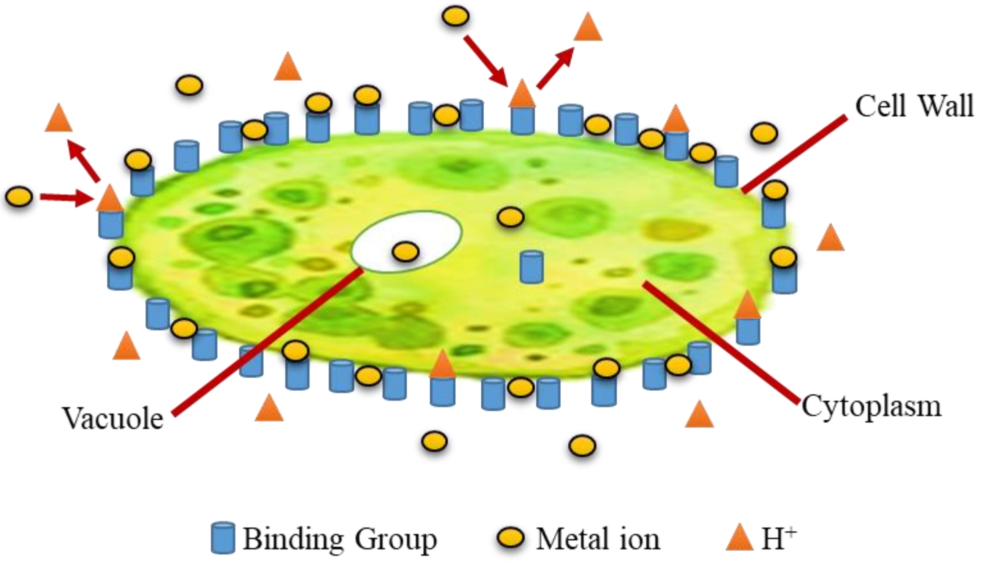

Figure 1. Biosorption of heavy metals by algal cells (Bilal *et al.*, 2018)

3.5.1. *Extracellular accumulation/precipitation*

Some prokaryotic (bacteria, Archaea) and eukaryotic microorganisms (algae, fungi) possess and produce extracellular polymeric substances (EPS), such as polysaccharides, glycoproteins, lipopolysaccharides, dissolved peptides, etc. This component has a negatively charged functional group that absorbs metal ions (Flemming and Wingender, 2001a, 2001b).

3.5.2. *Absorption/precipitation on the surface of the cell*

The cell wall tends to be the first cellular structure to come into contact with metal ions, excluding any possible extracellular layers primarily associated with bacterial cells. Two primary mechanisms of metal uptake by cell walls are as follows: stoichiometric interactions between functional groups of cell wall compositions, including phosphates, carboxyls, amines, and phosphodiesters, and physicochemical inorganic deposition by adsorption or inorganic precipitation. Currently, complexation, ion exchange, adsorption (by electrostatic interactions or van der Waals forces), inorganic microprecipitation, oxidation, and reduction have been proposed to explain metal uptake by organisms (Liu, Tang, and Lo, 2002).

3.5.3. *Intracellular accumulation/precipitation*

When the extracellular metal ion concentration is higher than intracellular, metal ions can penetrate the cell across the cell walls and biomass membranes by unrestricted diffusion. Metal ions can also enter the cell if the cell wall is disturbed by natural forces (e.g., autolysis) or artificial forces (mechanical or alkaline forces, etc.). The above process does not depend on metabolism. However, the intracellular accumulation/precipitation processes discussed here are primarily concerned with the living cell biomass and are energy-driven processes dependent on active metabolism. Metal ions are transported across the cell membrane, converted into other species, or deposited in the cell by active cells, including transport (Wang and Chen, 2006).

3.6. *Application Of Chlorella vulgaris In Removal of Heavy Metal*

To commercialize the biosorption using *Chlorella vulgaris* in wastewater treatment, it is necessary to continue to explore various aspects relevant to various applications in various studies. In Table 6 below, several studies related to using *Chlorella vulgaris* from 2015 to 2020 have been reported.

Table 6. Several heavy metal biosorption studies used *Chlorella vulgaris*

No	Biosorption Applications	References
1	Biosorption of toxic metals from industrial wastewater by algae strains Spirulina platensis and Chlorella vulgaris: Application of isotherm, kinetic models and process optimization.	(Almomani and Bohsale, 2020)
2	Potential Microalga *Chlorella vulgaris* For Bioremediation Of Heavy Metal Pb(II)	(Halima *et al.*, 2019)
3	Optimization of heavy metal biosorption onto freshwater algae (Chlorella colonies) using response surface methodology (RSM)	(Jaafari and Yaghmaeian, 2019)
4	Application of modified Spirulina platensis and *Chlorella vulgaris* powder on the adsorption of heavy metals from aqueous solutions	(Sayadi *et al.*, 2019)
5	Adsorption of inorganic mercury from aqueous solutions onto dry biomass of Chlorella vulgaris: kinetic and isotherm study	(Solisio *et al.*, 2019)
6	Biosorption of Cadmium from Aqueous Solution by Free and Immobilized Dry Biomass of Chlorella vulgaris	(El-Sheekh *et al.*, 2019)
7	Study of sorption and desorption of Cd (II) from aqueous solution using isolated green algae Chlorella vulgaris	(Kumar *et al.*, 2018)
8	Potential of Microalgae *Chlorella vulgaris* As Bioremediation Agents of Heavy Metal Pb (II) On Culture Media	(Dewi and Nuravivah, 2018)
9	The use of autotrophic *Chlorella vulgaris* in chromium (VI) reduction under different reduction conditions	(Yen *et al.*, 2017)
10	Insight into the mechanism of Cd(II) and Pb(II) removal by a sustainable magnetic biosorbent precursor to Chlorella	(Lalhmunsiama *et al.*, 2017)

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	vulgaris	
11	Biosorption capacity and kinetics of cadmium(II) on live and dead Chlorella vulgaris	(Cheng *et al.*, 2017)
12	Biosorption of some toxic metals from aqueous solution using non-living algal cells of Chlorella vulgaris	(Goher *et al.*, 2016)
13	The isotherm and kinetic studies of the biosorption of heavy metals by non-living cells of chlorella vulgaris	(Ali *et al.*, 2016)
14	Enhanced removal of Zn(II) or Cd(II) by the flocculating *Chlorella vulgaris* JSC-7	(Alam *et al.*, 2015)

3.7 Sustainable of Chlorella vulgaris

The content of liquid waste varies, including heavy metal content from various industrial processes (Masindi and Muedi, 2018); using conventional methods such as membrane methods, precipitation, ion exchange, and electrochemistry requires a large amount of money on an industrial scale (Tran *et al.*, 2017; Efome *et al.*, 2019; Ibrahim *et al.*, 2019; Garba *et al.*, 2020). The existence of non-conventional methods of using macro and microalgae (Leong and Chang, 2020) for the removal of heavy metals have the advantage and potential of using algal biomass sustainably because it is effective and efficient in the use of costs on an industrial scale (Wang *et al.*, 2019).

Liquid waste with different heavy metal contents can be used as a medium for cultivating adequate amounts of highly productive algae with a high absorption capacity to remove heavy metals. However, some heavy metals in the liquid waste can interfere with algae growth, although the effect can be minimized by diluting or adding organic compounds into the liquid waste (Abinandan and Shanthakumar, 2015).

The potential of algae biosorption in reducing the toxic effects of heavy metal ions can be seen in cellular structure, pretreatment, modification, and potential applications of genetic engineering in biosorption performance. Evaluation of pretreatment, immobilization, and factors affecting biosorption capacities, such as initial metal ion concentration, biomass concentration, initial pH, time, temperature, and multi-metal ion interference and development of algae production engineered to increase heavy metal absorption capacity and selectivity. These parameters can lead to low-cost micro and macroalgae cultivation with bioremediation potential for utilization and sustainability of macro and microalgae utilization. (Keyvan *et al.*, 2016)

CONCLUSIONS

In this review, heavy metals are toxic compounds that must be removed from the environment. Conventional processing methods require a large amount of money. This review shows that biosorption is the most economical and environmentally friendly method for removing heavy metals from wastewater. This method is expected to be an alternative for removing toxic heavy metals from industrial waste. In this biosorption process, *Chlorella vulgaris* acts as a natural chemical substrate where the functional groups present from the biomass are the binding sites for heavy metals from liquid waste. The advantages offered by this method are that it is simple, cost-effective, and highly efficient, minimizes secondary chemical or biological waste, and regenerates biosorbents for removing heavy metals. Cultured *Chlorella vulgaris* can be regenerated relatively easily and reused. The metal removal capacity with *Chlorella vulgaris* is better than that of other conventional adsorbents using the technology used today. So, from some of the above assessments, *Chlorella vulgaris* can be used as a basis for futuristic applications and a strong candidate for future wastewater treatment.

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