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Review

Potential Role of *Spirogyra* sp. and *Chlorella* sp. in Bioremediation of Mine Drainage: A Review

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Abstract: One of the biggest global challenges in the mining industry is managing the risks associated with contamination by potentially toxic elements (PTEs) resulting from their activity. The oxidation of sulfides is the main cause of polluted mine drainage through the leaching of PTEs from mine waste and mine galleries to the water systems. Mine drainage can be highly acidic and often has a high concentration of PTEs, particularly arsenic, one of the environment's most toxic elements. PTEs endanger the ecosystem's equilibrium and raise worries about human and animal health. Some species of algae which can be naturally present in mine drainage waters, such as *Spirogyra* sp. And *Chlorella* sp., have a high capacity for absorbing PTEs from wastewater and may thrive in harsh environments. As a result, algal-based systems in bioremediation were studied and carefully analyzed, since their capacity to remove heavy metals and hazardous contaminants from polluted mine water have already been shown in previous studies. Biofuels derived from microalgal biomasses are a viable alternative to fossil fuels that can lead to a circular bioeconomy. This study reviews and analyses Chlorophyta-based bioremediation systems with application to mine waters focusing on *Spirogyra* sp. and *Chlorella* sp., since they are naturally present in mine drainage and can serve as a study model to better understand their application in bioremediation.

Keywords: bioremediation; microalgae; mine drainage waters; potentially toxic elements (PTEs)

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1. Introduction

Mine drainage is a serious environmental problem because of its capacity to leak potentially toxic elements (PTEs) into numerous ecosystems. PTE bioaccumulation in organisms, with subsequent accumulation in the food chain, necessitates immediate and efficient solutions. Therefore, treatment of mine drainage to extract PTEs from mine drainage evades disastrous consequences for the environment and health, so that downstream contamination can be prevented and local contamination can be remediated.

Mine drainage is primarily caused by industrial mining operations and metallurgical extractions. Acid mine drainage (AMD) is quite common at inactive and abandoned mining sites due to the sulfides weathering (mostly iron sulfides, such as pyrite), caused by their exposure to water, atmospheric oxygen, and acidophilic iron-oxidizing bacteria. As a consequence, sulfuric acid and an acidic solution with high PTE concentrations are produced [1]. Furthermore, acid streams with high sulphate concentrations are typically produced by the weathering of sulfur-bearing minerals. The mineralogy of the ore resources extracted, as well as the surrounding geology and climate conditions, influence the composition of AMD. The high quantities of heavy metals in AMD endanger groundwater and surface water, and can have deadly consequences. [2]. The extent of these negative impacts includes the loss of biodiversity, human health poisoning threats, and the deterioration of aquatic ecosystems [3].

It is important to emphasize that to prevent damage to the environment and to strengthen ecological sustainability, bioremediation using algae has emerged, since their cultivation has low costs and is easy to carry out [4]. Additionally, algae are simple to handle, effective in removing excess nutrients from effluents in which they are inserted, and are not a source of secondary waste. When compared to other technologies, we can understand that they promote the circular economy and are an ecological alternative.

Current traditional wastewater treatment has been characterized by causing secondary pollution due to greater energy usage in moving and creating water sludge, as well as greenhouse gas emissions (GHGs). Many developed nations have lately passed environmental laws prompting mining companies to build effluent treatment plants. This causes significant issues for the operating firms, but they frequently become crucial when mine abandonment is planned. Mining companies in the most stringent nations face the conundrum of either adopting effective preventive measures or being compelled to pay for wastewater treatment for years after the mine is closed. Aside from general excellent water purity, the capacity to recover heavy metals is monetarily advantageous. Maintenance costs are high in current technologies, as seen in membrane technology and growth in the case of polymeric graphite. Other remediation processes pass to the inclusion of alkaline reagents, such as lime, limestone, sodium carbonate, or sodium hydroxide, to treat AMD contamination. This procedure attempts to neutralize acidic water and prevent heavy metal precipitation. However, upkeep needs are high, and significant quantities of sludge, primarily made of calcium sulfate and some metal hydroxides, are generated. In an oxidizing atmosphere, limestone becomes coated with reaction products, rendering it useless. Thus, the notion of resource recovery is founded on the freshly improved concept of circular bioeconomy, which is without a question the beginning of a new era, an era of sustainability [5–7].

Phyco-remediation using algae is one of the proposed cost-effective bioremediation methods that is generating great interest among researchers and companies due to its low operating costs, easy process design, easy adaptation of algae to extreme conditions, and the possibility of improved recovery of metals and sulphates [8].

Phyco-remediation employs macro or microalgae to remove or bio-transform contaminants from wastewater, including nutrients and xenobiotics, and offers certain benefits over conventional procedures, which are highly costly, consume a lot of energy, and produce a lot of sludge. [9]. Several studies on the use of various microalgal species for wastewater treatment have been undertaken during the last several decades. However, studies addressing *Spirogyra* (Charophyta) (Figure 1) are scarce but crucial, as it is found naturally in abandoned mining sites with high heavy metal contents. *Chlorella* sp. is also found in these types of environments, even though there are a lot of studies addressing *Chlorella* sp. as a bioremediation technology.

Spirogyra sp. and *Chlorella* sp. are two different species of microalgae that have unique characteristics. *Spirogyra* sp. is a filamentous alga and has helically shaped chloroplasts, whereas *Chlorella* sp. is a unicellular alga and has spherical chloroplasts. By evaluating two species with different characteristics, we are diversifying our study and understanding whether these differences have an impact on their use in bioremediation, since these species have been used together in studies on very few occasions, but with promising results for PTE removal [10].

Microalgae are versatile in converting contaminants into disposable or even recyclable non-hazardous products, allowing the treated water to also be reused or safely disposed of, and since *Spirogyra* sp. and *Chlorella* sp. are found in mine drainage, the study of their physico-chemical characteristics can lead to the development or evolution of bioremediation techniques in a more sustainable way [11].

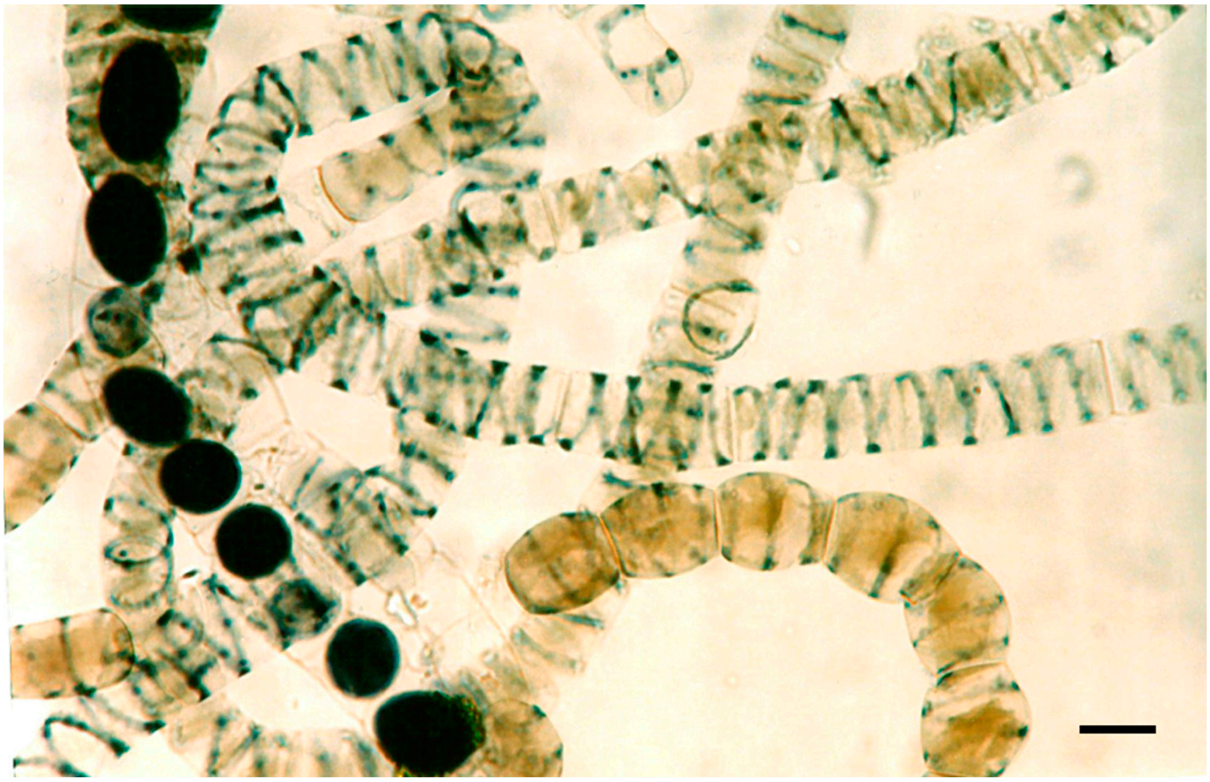

Figure 1. *Spirogyra* under microscopic view (Scale bar, 30 µm).

Some studies have already been carried out, where *Spirogyra* sp. and *Chlorella* sp. stood out from other microalgae in the adsorption of PTEs [12,13].

This review report aims to analyze studies using algae, in particular *Spirogyra* sp. and *Chlorella* sp., as a bioremediation technology for mine drainage treatment, and to understand the state of development of this technology in the eyes of the scientific community.

2. Potentially Toxic Elements

Environmental pollution by PTEs is widely acknowledged to be one of the most serious risks to human health. PTEs can form naturally in the environment because of parent rock weathering. Nonetheless, due to a variety of human activities, such as mining, industrial work, and use of fertilizers, PTE concentrations have significantly increased, attracting widespread attention due to their persistence in the environment, proclivity to bioaccumulate in the food chain and toxicity to humans (Table 1) and other organisms [14]. The environmental impact of a PTE is always determined by the geochemical associations that it has in the soil, and these associations are determined by the metal's origin, as well as its reactivity in the environmental conditions [15].

It Is critical to stress that anthropic sources of PTEs, such as mining, result in increased PTE persistence in soils for many years, even after the pollution sources have been abandoned or deactivated. PTEs have complex geochemical behavior in the aquatic environment, which can make these elements accessible [16].

PTEs have varying mobility, and the potential impact of pollution might be accelerated or delayed by various associations and geochemical circumstances. One of the most commonly utilized methods is operational (chemical) availability, which offers signs of potential biological availability, as well as an overall environmental risk [15].

The predicted availability/mobility decreases as the extraction circumstances get more severe, along with the phases becoming more solid and stable. This is extremely reliant on the type of soil components, meteorological conditions, and any anthropogenic disturbance, thus soils make it very changeable in space and time.

Chemical precipitation, ion exchange, coagulation, and activated carbon are among the mitigation options used for PTEs, as are biological processes such as bioremediation, phyco-remediation, and so on. Biological approaches are widely utilized because they are cost-effective, efficient, and environmentally benign. Mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) have been identified as the major metals/metalloids produced by metal mining and smelting activities [17]. The World Health Organization has identified the same metals/metalloids as a major public health problem.

To further understand the real problem of PTEs, it is essential to look at some of the chronic health conditions associated with PTE exposure. Cd is a carcinogen that can harm the lungs, heart, and reproductive system. Mercury has a negative effect on both the central and peripheral nervous systems. Lead is a metal that may accumulate in the blood and bones and has been shown to damage reproductive, hepatic, and immune system functions [18]. Arsenic can lead to acute, subacute, or chronic poisoning symptoms, such as skin lesions and cardiovascular difficulties [19]. These effects highlight the alarming need to address the PTEs as soon and as efficiently as possible.

Table 1. Heavy metal risks for human health.

Heavy Metal	Associated Risk	References
Cadmium	Renal damage Infertility Organ dysfunction, damage, and cell death Bone diseases Lung injuries Cancer	[20]
Arsenic	Cancer in the lungs, kidneys, and bladder Cardiovascular dysfunction Skin and hair changes Liver damage	[21]
Lead	Lung dysfunction Cardiovascular dysfunction Reduced pulmonary function	[22]
Mercury	Brain, lung, and kidney damage Nervous and immune system diseases Cardiovascular system diseases	[23]

Consequently, these are the four most widely studied metals/metalloids in the literature, mainly because of their high toxicity to humans. Among them, mercury, cadmium, and arsenic have a significant presence in mining waste and leachate discharge sites.

3. Review of Acid Mine Drainage

The primary concerns related to mining pollution are acid production and metal dissolution, and there is no universally efficient treatment strategy, since geochemical and physical features vary widely from site to site. When pyrite (FeS_2) is exposed to the environmental conditions, it oxidizes, releasing hydrogen ions which cause acidity, sulphate ions, and soluble metal cations. This oxidation process occurs naturally in rocks, but at a slow rate. Water, and eventually carbonates, can act as a buffer with the acid generated; however, mining increases the exposed surface area of sulfides, allowing for the generation of excessive acid that exceeds the natural buffering capacity of the water.

$$2FeS_2\ (s) + 7O_2\ (aq) + 2H_2O \rightarrow 2Fe^{+2} + 4SO_4{}^{-2} + 4H^+ \tag{1}$$

$$2Fe^{+2} + \frac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{+3} + H_2O \tag{2}$$

$$2Fe^{+3} + 6H_2O\langle - \rangle 2Fe(OH)_3\ (s) + 6H^+ \tag{3}$$

$$14Fe^{+3} + FeS_2\ (s) + 8H_2O \rightarrow 2SO_4{}^{-2} + 15Fe^{+2} + 16H^{+} \quad (4)$$

The pyrite is converted to dissolved iron ions (Fe^{2+}) and dissolved H^+ and $SO_4{}^{-2}$ ions through the oxidative process (1). When exposed to dissolved or atmospheric oxygen, the ferric ions ($2Fe^{3+}$) react immediately with pyrite to raise the acid content of the water (4) and stimulate the creation of colonies of acidophilic bacteria, which further enhances the acidification process. [24].

Acidic drainage effluent is formed because of the movement of water through surface-exposed rocks and mine shafts caused by mining activity. The interaction of oxygen, water, sulfide, and non-sulfide minerals produces acidic sulphate-rich wastewater, known as AMD.

The use of algae to remove heavy metals assists in the monitoring of heavy metal levels in a specific environment. However, due to the toxicity of accumulated heavy metals, algal turnover makes continuous systems considerably more appealing in terms of efficiency and simplicity of maintenance.

There has been research on the properties of heavy metal absorption kinetics by microalgae, and these properties would be crucial in developing successful and long-lasting phytoremediation systems. The methods of heavy metal detoxification by microalgae have already been studied by analyzing the biomolecular way in which algae separate and collect the environmental chemicals to which they are exposed, as well as examining the production of metallothionein peptides in algae [25].

4. Algae and Their Bioremediation Capacity

Because of its low cost and great efficacy in metal and sulphate removal, bioremediation with algal strains is a new and appealing biological way of AMD therapy. Microalgae can operate as sorbents, and the metabolism of an algal biomass creates high alkalinity, which helps counteract the acidic character of the drainage stream and hence aids in metal precipitation. However, variations in the pH, oxygen content, and temperature of the acid stream have a significant impact on the treatment efficiency of this approach [2]. However, in this fascinating world of microalgae, there are always several possibilities, and in this situation, non-living algae may also be employed, and there are noticeable differences in metal ion accumulation when compared to living algal cells [26].

Non-living biomass biosorption benefits include heavy metal biosorption that is multiple times higher in non-living microalgae than in living microalgae [27]. Metal ions associated to the algal cell wall, for example, can be eliminated by washing the biomass with different desorption agents [28]. Living microalgae, on the other hand, have little mechanical and chemical resistance to physical and chemical recycling treatments. A non-living algal biomass also eliminates the hazards of exposure to highly hazardous settings and does not need intense maintenance or the addition of further growth nutrients, being a cost reduction when considering a scale-up process [29].

To obtain the best removal effectiveness, the interaction between algal strains, dead or living cells, and contaminants should be adjusted, since several factors that we will discuss afterwards influence non-living and living algal heavy metal ion biosorption in different ways [30].

5. Factors That Affect Microalgal Bioremediation Capacity

5.1. pH

pH is one of the most critical factors influencing metal adsorption by microalgal biomass. Metal intake pH dependency is intimately connected to metal chemistry in solution, as well as the acid-base characteristics of different functional groups on the microalgal cell surface. When related to acid mine drainage, pH is one of the most important variables, since the acid mining drainage is often extremely acidic ($pH < 3.0$), making metal ions available in solution, which can lead to competition between hydrogen ions and metal ions for the same adsorption site on the algae [31]. However, $pH > 7.0$ is not good either, since it leads to metal ion precipitation into hydroxides, making them unavailable to be adsorbed, which can make adsorption rates drop. As a result, there is already an optimal pH value range for each metal's sorption helping to promote adsorption, which is typically in the range of 4.0–6.0 [32]. The best pH range for microalgae, especially the *Chlorella* sp., has been researched, and it has been shown that pH 6 appears to be most favorable for growth and lipid accumulation, which is relatively similar to the optimal pH value range for metal sorption [33].

5.2. Temperature

The data available in the literature on the influence of temperature on the adsorption of PTEs by microalgae is not entirely consistent. As an example, increasing temperature lead to an increase in Ni2 adsorption by the dry biomass of *C. vulgaris* [34]. Nevertheless, the same author claimed that increasing the temperature (from 20 to 50 °C) affected the biosorption capacity of cadmium(II) (from 85.3 to 51.2 mg/g) in a prior research paper [35], although other publications suggest that temperature has no influence on metal sorption [26], remaining unanimous and requiring further study.

5.3. Biomass Concentration

The quantity of metals taken from solution by microalgae is plainly impacted by the biomass concentration: it increases with the latter, which might be attributable to a greater number of metal-binding sites accessible. However, increasing the biomass level is only possible to a limited extent, because after a certain concentration, the values may lead to a decrease in metal binding [36]. This may be explained by a possible overlap of biomass leading to a reduced surface area available for sorption, as well as a decrease in the average distance between the adsorption locations that are available [37].

5.4. PTE Interactions

Mine drainage often contains significant amounts of PTEs, resulting in a complex combination of heavy metals. Metal cation interactions may be studied using multiple metal solutions, which are more reflective of actual environmental issues than research on a single metal [26]. The presence of other metals/co-ions in solution has a substantial effect (generally inhibits) on the sorption of PTEs into the microalgal biomass, owing to the competitive interactions between them and the adsorption binding sites on the cell surface, or precipitation [38].

5.5. Metals Speciation

PTE chemical speciation is a major component that impacts heavy metal toxicity. Their potential mobility, bioavailability, and environmental behavior are highly reliant on their precise chemical forms and current states, which are mostly influenced by pH [39]. Metals in mine drainage can take many different chemical forms, including free ions, complexes with inorganic/organic ligands, and adsorbates on particulate phases. Nevertheless, free metal ions in solution are the most harmful to living creatures and bind the most to microalgae [27].

Because this procedure is so reliant on so many variables, algal-based bioremediation is frequently employed together with other treatment techniques, and is thus classified as a secondary or tertiary treatment.

As previously stated, the bioremediation of heavy metals and sulphates by algae is extremely variable, since it relies on the PTE interaction, biomass concentration, metal speciation, pH, temperature, and the season during which the removal process is carried out [40]. The climate conditions can strongly influence the removal of contaminants, because algae are sensitive to parameters such as light, temperature, and water availability.

Depending on the degree of saturation and aeration of a region, several strategies are used. In situ procedures are those that are used on soil and groundwater on-site, with little disruption. Ex situ procedures are those that are used on soil and groundwater that have been removed from the site by excavation (soil) or pumping (water) [41]. Different bioremediation strategies, and their application benefits and limitations are summarized in Table 2.

Table 2. Bioremediation methods, benefits, and limitations [41–43].

Techniques	Examples	Advantages	Constraints	Factors to Consider
In situ	In situ bioremediation- Biosparging Bioventing Bioaugmentation Bioslurping Permeable reactive barrier (PRB)	Noninvasive and least expensive Relatively inactive Natural attenuation mechanisms Treatment for soil and water	Environmental restrictions Treatment period lengthened Difficulties with monitoring Heavy metal and organic compound concentration inhibit the activity of some indigenous microorganisms Acclimatization of microorganisms is frequently required for in situ bioremediation	The depth of pollution, the kind of pollutant, the degree of pollution, the cost of remediation, and the geographical location of the contaminated site are all factors to consider
Ex situ	Landfarming Composting Biopiles Windrows Biofilter	Cutting costs Low price Can be completed on-site	Heavy metal pollution and chlorinated hydrocarbons, such as trichloroethylene, are not covered Non-permeable soil needs further treatment Before placing the pollutant in the bioreactor, it can be removed from the soil by soil washing or physical extraction	See above
Bioreactors	Slurry reactors Aqueous reactors	Kinetics of rapid deterioration Environmental characteristics have been optimized Improves mass transferInoculants and surfactants are used effectively	Excavation is required for soil. Capital at a somewhat high cost Operating costs are rather expensive	Process requires bioaugmentation Amendment toxicity Contaminant concentrations that are toxic

Algal bioremediation treatment can be carried out in situ, with the algae ideally growing in the contaminated effluent, followed by the collection of algal biomasses, drying to recover the content of adsorbed metal, and finally the conversion of heavy metals into recoverable oxides or other salts. Alternatively, an ex situ treatment would entail the algal biomass being grown in the laboratory and the adsorption capacity of the algae in the laboratory verified through the collection of samples of the effluent under study [4]. It should also be noted that after the heavy metal extraction, the algal biomass can be reused to increase the potential for biofuel production.

6. Uptake Mechanism of Heavy Metals in Microalgae

Algae employ absorption and adsorption methods to remove nutrients, heavy metals (depending on the species), and other components from wastewater. While these components are removed from the wastewater, simultaneous algae develop, because they need some of the nutritional elements to proliferate. Because of the occurrence of various algal species with thick cell walls, algae have a high accumulation capacity, making them an inexpensive supply of heavy metal adsorbents [44].

Metal biosorption by microalgae is a two-stage process (Figure 2). The first stage typically involves an initial fast, reversible, and passive adsorption onto the surface of the cell wall, where metal ions adsorb functional groups, via electrostatic interactions, followed by a second stage that is a much slower, irreversible, active process that includes the transport of metal cations across the cell membrane into the cytoplasm, with posterior binding to intracellular compounds [26].

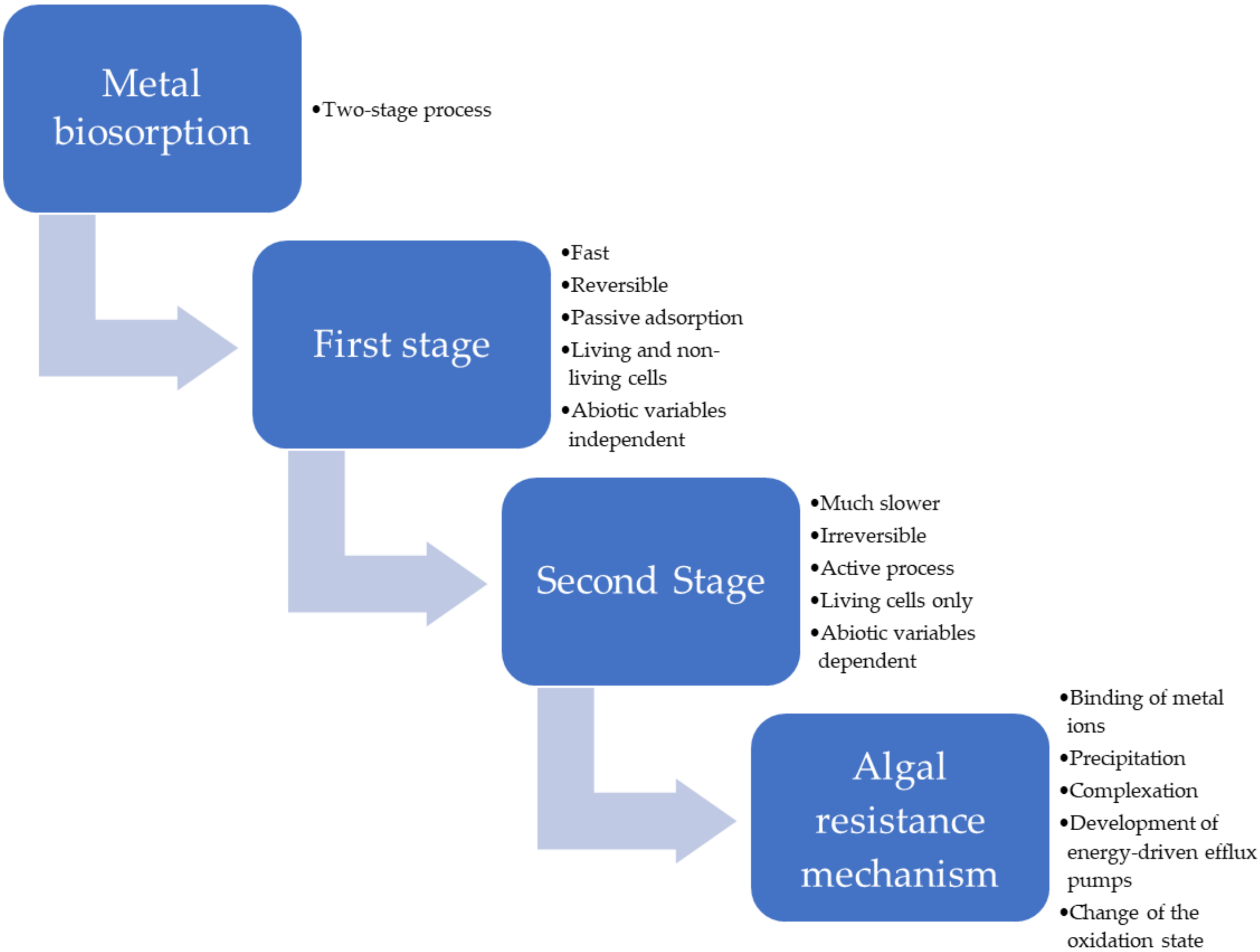

Figure 2. Metal biosorption by microalgae.

The first stage of metal biosorption happens in both living and non-living cells, while the second stage happens exclusively in living cells [45]. The first quick stage of biosorption is uncompromised by metabolism, light, temperature, or the presence of metabolic inhibitors, whereas the second slower stage of absorption is dependent on metabolism and other abiotic variables [46]. The ability of biomaterials to adsorb metals is determined by the composition of their cell surface, and is enhanced by the presence of negatively charged functional groups in conjunction with the chemical composition of the outside solution being treated. This is especially true for the competing anionic groups and pH, both of which alter protolysis and, as a result, induce such changes [26].

The algal resistance mechanism consists of the following stages: binding of metal ions at cell surfaces; precipitation of insoluble metal complexes thereon; complexation of metal ions with excreted metabolites that may extracellularly mask a toxic metal; development of energy-driven efflux pumps that keep toxic element levels low in the cell interior; and the change of the oxidation state, allowing the toxic form of a metal to be enzymatically (and intracellularly) transformed to a less hazardous one [47].

7. Previous Studies on the Application of *Spirogyra* and *Chlorella* to Remove Heavy Metals and Toxic Contaminants

Microalgal advantages include rapid metal uptake capability, time and energy savings, eco-friendliness and user-friendliness, year-round occurrence, ease of handling, recyclables and reusables, low cost, a faster growth rate (when compared to higher plants), high efficiency, a large surface-to-volume ratio, the ability to bind up to 10% of their biomass, high selectivity (which improves their performance), no toxic waste generation, no synthesis required, usefulness in both the batch and continuous systems, and relevance to waterways with significant metal concentrations or minimal contaminants. However, for the scale-up to be efficient, there is a need to select microalgae that are adapted to contaminated environments and are native to the targeted area, due to reducing ecological problems and impacts, and having an easy solution to cultivate and control the microalgal biomass [48]. Thus, *Chlorella* sp. is well documented for its presence in contaminated waters, being one of the most abundant microalgae in the world. *Chlorella* sp. is one of the most studied microalgae for removing heavy metals in bioremediation processes [49]. The *Spirogyra* sp. is also found in these types of environments, but is different due to being a filamentous microalga, unlike *Chlorella* sp. [50,51]. This characteristic of *Spirogyra* sp. makes the harvest of the microalgae from the cultivation system easier and low-cost [49].

Although studies considering *Spirogyra* application are scarce, they are of extreme importance, particularly because these microalgae are naturally present in mining runoff from deactivated and/or abandoned mines, where the effluents usually have significant heavy metal contents, which are harmful to human health and the environment [52].

The bioremediation potential of *Spirogyra* sp. and *Oscillatoria* sp. was studied and compared the accumulation capability between *Spirogyra* sp. (Charophyta) and *Oscillatoria* sp. (Cyanobacteria) for Cd. The results showed that the highest accumulation by *Spirogyra* was 7.6354 mg/L at 30 ppm concentration, while *Oscillatoria*'s highest accumulation was 2.9404 mg/L at 30 ppm Cd concentration [12]. This only corroborates what has been said and tested, showing that despite the low number of studies, *Spirogyra* has a huge potential as an alternative for ecological bioremediation. The substantial ability of *Spirogyra maxima* has been shown to remove lead and manganese from wastewater [53]. The adsorption capacity of Cu^{2+} and Ni^{2+} by live *Spirogyra* sp. is 29 and 521 mg g^{-1}, respectively, showing the good potential of *Spirogyra* for wastewater treatment [54]. *Spirogyra* also has a great absorption capacity for other heavy metals confirmed, corroborated by studies indicating that the *Spirogyra* species can also carry out an effective removal of Cu (II) [55] and As, even though there is a more efficient removal of As (III) than As (V) [56].

The maximal As (V) sorption capacity of living and dead *Spirogyra* sp. is 315 and 207 mg/g, respectively, which is relatively high in comparison to other arsenic sorbents described [57].

With its high metal biosorption and desorption capacities, the *Spirogyra* biomass has the potential to be used as an effective and cost-effective biosorbent material for the removal and recovery of heavy metals from wastewater streams, as well as a competing technology in the existing bioremediation market. According to [58], *Spirogyra* was successfully used as a bioindicator of Pb, Al, Ca, Na, atrazine, and 2,4-D, through the changes of OD, at the wavelengths of 663 nm and 450 nm, showing that is indeed a good candidate as a bioindicator for the presence of pollutants.

The removal of contaminants in wastewater by *Spirogyra* sp. has also been studied, whereby removal efficiencies were 3.1, 46.65, and 30.70% after 24 h for chemical oxygen demand, nitrate, and phosphate, respectively [59].

Green filamentous algae, *Spirogyra*, can reduce CH_4 emissions from a eutrophic river, and their bloom has a high optical density concentration, which promotes CH_4 consumption by increasing sediment CH_4 oxidation [60].

The arsenic phyco-remediation has been studied, using *Chlorella* sp., whereby after 168 h of treatment with an initial arsenic content of 50 mg/L at pH 9.0 and inoculum size of

10% (*v*/*v*), the microalgae *Chlorella* sp. demonstrated a removal percentage of 85.217% and 88.1534% of As(III) and As(V), respectively, in synthetic wastewater [61].

Chorella sp. can eliminate over 35% of Cu and accumulate huge levels of Cd (11,232 mg kg^{-1}) present in the culture medium at high concentrations of Cu and Cd (both 500 M) [62].

Microalgae, particularly, the genus *Chlorella* sp., has a high ability to remove heavy metals, such as Cu, U, and Cd [25].

At an optimal pH for copper (pH = 5), and cadmium and lead (pH = 6), an equilibrium duration 60 min, and adsorbent dose 5 g L^{-1}, the removal percent of cadmium, lead, and copper, using *Chlorella vulgaris* as an adsorbent, was 87.52, 90.09, and 84.75%, respectively [63].

There was already a study where the *Chlorella* sp. dry biomass was used to show that it can remove 131.36 mg/g of lead and 43.41 mg/g of zinc at pH 5 [32].

As shown above, the results of the various studies are not comparable due to the different methods of analysis and cultivation. Furthermore, the results are presented in the form of different SIs, therefore, they are not comparable with each other.

There are two main options for using algae in bioremediation, either dried or live. Some studies show that dried algae have higher sorption values than live algae, which is quite interesting, as it opens a wide range of different applications. The fact that they sorb more when dry can be explained by the increased surface area caused by the disintegration of cell membranes during the fabrication of a dry biomass sample [56].

The occurrence of diverse algae species opens a variety of possibilities for the treatment of AMD. The utilization of certain algae species in wastewater treatment and biofuel generation is determined by their growth rate, lipid content and productivity, resistance to potential contaminants, and strong growth features with an enhanced tolerance for varying environmental circumstances [64].

8. Future Perspective

One of the most difficult obstacles today is the lack of a single, dependable, and effective strategy for treating AMD. This challenge is what arouses the interest and curiosity of researchers around the world in the search for effective and efficient techniques for the control and management of AMD.

In addition to environmental considerations, there are other biological and operational issues that might negatively impact AMD treatment, such as contamination, autoinhibition, and harvesting during microalgal development. This is why it is critical to monitor algal development, culture protection, sterilization, and ultra-filtration of the culture media on a regular basis. More studies are also required so that the contamination and self-inhibition concerns can be understood, regulated, or reduced. Another critical factor is the selection of the algal species, as not all species can be effective in the treatment of the AMD [4].

Most studies have shown that algae can successfully remove heavy metals and other pollutants, but only in the laboratory. As a result, there is a need to scale up technology to handle the issue of AMD management and treatment, which has not been solved efficiently until now.

However, the research has not been applied at a large or industrial scale; it is mainly carried out in the laboratory and at small indoor and outdoor scales, due to the complexity of creating an efficient and economic feasible plan for viability to implement in AMD sites. Thus, the studies and research are in progress, so that in the near future it will be possible to implement this type of in situ bioremediation with bioreactors.

8.1. Circular Bioeconomy Approach

The environmental friendliness and cost efficacy of microalgae make them an interesting option for industrial-scale mining wastewater treatment on a global scale. As a result, microalgae have huge potential for broad usage in large-scale applications for heavy-metal-containing industrial wastewater, to increase the feasible recycling of wastewater.

To promote a circular economy, the steps for bioremediation should be designed in such a way that any waste is avoided.

Therefore, Figure 3, schematically shows which relationships should exist between microalgal cultivation and wastewater bioremediation to facilitate this circular process. It is also very important to note that the applicability to AMD wastewater is very similar.

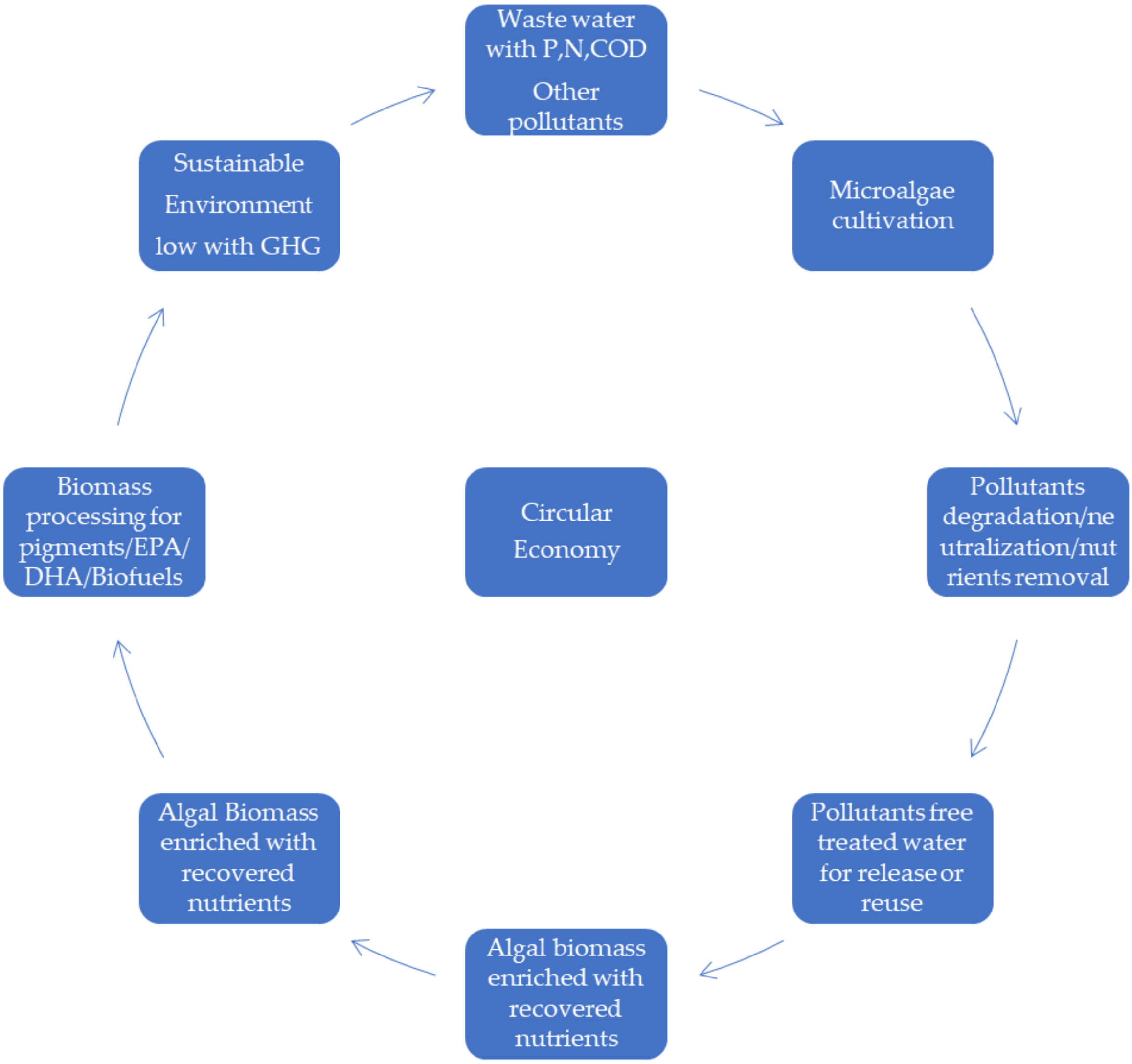

Figure 3. Integration of wastewater treatment with microalgal cultivation and resource recovery in a circular bioeconomy perspective.

Alternative flocculation harvesting methods must be investigated in order to advance in the economic potential and complete the functioning of microalgae in the treatment process.

Auto-flocculation should be investigated for extracting microalgae from growing media. The flocculation technique is a different method of extracting the algal biomass. The investigation of the organic compounds that can be added to the medium, to aid microalgal flocculation and the understanding of the mechanisms, should be prioritized. Focusing on robust microalgal species that are resistant to infection by other microorganisms, aggregation formation, and a tendency to auto-flocculate would also be a wise decision, since it would shield microalgae from potential natural predators [65].

More study is needed to reduce protozoa and other rotifer infection in microalgal farms [66]. It has been observed that rotifers and protozoa can feed on 200 microalgal cells per minute individually, under favorable conditions, implying that these organisms might kill all microalgae in the culture system and cause the culture to collapse [67].

Another challenge in microalgal cultivation in wastewater is the failure to provide a sterile environment for the microalgae.

Research should be addressed to the co-culture of natural communities of microalgae, in order to develop stable consortia that perform better while covering all ecological niches, reducing the chance of culture collapse and contamination, and increasing treatment efficiency [68]. The growth method should be chosen with the characteristics of large-scale microalgae growing in wastewater systems in mind. The use of enhanced species, in conjunction with gene editing technology, might be a viable hypothesis for solving many of the present difficulties.

It is crucial to focus on the extraction techniques of heavy metals from an algal biomass when we consider a circular bioeconomy, since the desorption of heavy metals from used algal biomasses into biosorption systems that make biomasses recyclable in several cycles, is a focus of research [69].

Adsorbed metal ions can be removed from biomasses using alkaline solutions, such as NaOH or $CaCl_2$, or mineral acids, such as HCl, and HNO_3, as eluants of adsorbed metals [70]. Metal ion reaction centers were reduced in the second and third cycles of three sequential adsorption/desorption cycles; however, almost all adsorbed metal ions could be recovered [71].

Other application to promote a circular bioeconomy is using microalgae biomass, after the desorption of contaminants and a series of toxicological tests, as a potential source for food industry, nutraceutical, pharmaceutical products, and biofuel production.

The presence of favorable nutritional content in the algae *Spirogyra porticalis* (Charophyta) has been shown through a nutraceutical profile, which might be extremely beneficial in the treatment of a variety of oxidative-stress-related issues [72].

8.2. Biorefinery as a Solution

Building integrated biorefineries is a popular topic these days, to analyze microalgal biomasses more efficiently. The integrated microalgal biorefinery hypothesis is a macro-cascade of sequential extractions, aimed at maximizing the monetization of all biomass constituents. Consequently, a manufacturing platform for transforming polluted microalgae into safe and value-added commodities, such as fatty acids, pigments, and polymers, has been developed.

The final microalgal waste might be directed toward bio-fermentation (to make biomethane or bioethanol), with leftover fractions of the processes used to produce soil biofertilizer and heavy metal recovery. Spectroscopic and chromatographic methods can be used to test biomasses to ensure their safety and quality [73].

Biodiesel from lipids, bioethanol from starch, photosynthetically generated biohydrogen, and anaerobic fermentation of an algal biomass to produce biogas are all third-generation biofuels that may be produced from microalgal biomasses (main methane). However, the most researched and viable approach is biodiesel generation from neutral-storage lipids, primarily triacylglycerol (TAGs) [74].

Green energy is generated by algal-based biofuel, which leads to the manufacture of a cost-effective product. Biofuels derived from photosynthetic, organism-based feedstocks, such as aquatic microalgae, provide significant potential for meeting global energy demands, while providing carbon-neutral solutions and permitting CO_2 sequestration from the atmosphere [75]. Is also important to note that *Spirogyra* has the ability to be a raw material for bioethanol production, since it can be converted to ethanol via the hydrolysis and fermentation processes [76].

Although ADM can be a low-cost method for marginally increasing lipid content, there are additional dangers related to the safety of the culture. To make use of this low-cost medium, however, numerous physico-chemical and biotic factors (such as metal concentration, other contaminants, and microbial contaminations) must be controlled during microalgal culture. Metal or microbiological contaminations are the most serious issues in microalgal culture systems, having a significant influence on microalgal producers in general, and not just in biofuel production [77]. As a result, there is a need to fully characterize the microalgal culture conditions, such as temperature and aeration, which the chosen organisms can endure, in order to acquire the optimum lipid production and biomass amount. Additional research in this culture medium allows us to understand the tolerance of the targeted organisms to changes in cultivation parameters that increase lipid synthesis, while having no detrimental effects on biomass production or contaminations [48].

Because biofuel can be combined with gasoline, it is becoming increasingly popular for its potential as a low-cost product. The EU standard EN 228 allows for a 5% mix, while still maintaining the petrol fuel quality criteria. As a result, heavy metals must be kept within the limits set by fuel control agencies across the world [73]. Thus, if all the aspects of ADM cultivation meet the criteria for developing new biofuels, it can be an excellence source, although it needs to be analyzed in the targeted contaminated area and in the context of the microalgae chosen for cultivation [48].

Another option for microalgal biomasses can be the production of bioplastics. Even though it is still a juvenile process, this can be important as a sustainable and environmentally friendly future product. Bioplastics from algae could add value at an economic level when allied with biofuels. Ophthalmic lenses made of polylactic acid (PLA) are another option. Following microalgal bioremediation, lipid maximization, and extraction, it is possible to obtain feedstock for the production of PLA, and the lipids may be used as a feedstock for the production of other chemicals, providing yet another opportunity to succeed in the transition to a more circular economy [78].

9. Conclusions

Spirogyra sp. and *Chlorella* sp. are native to AMD sites, which makes them suitable and therefore promising for AMD remediation, as evidenced by the good removal values of heavy metals presented in this review. However, the toxicity limits should still be performed to understand what is the maximum concentration that they can adsorb before they start to die. As demonstrated above, the results from various studies cannot be comparable due to the methods of analysis and cultivation being very different. Moreover, the results are in different Sis, thus not comparable between each other.

These two species can be reused to promote the circular bioeconomy through different applications notably addressing the biorefinery area, thus promoting a greener bioremediation strategy.

More work already exists and must continue to be done showing techniques capable of desorbing metal ions from the microalgae used in bioremediation.

This review suggests future studies regarding the toxicity of metals for these species, and other potential reuses of these algae, namely the recovery of heavy metals adsorbed during the bioremediation processes.

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