Immobilized Algae Bioremediation Technology

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A green technology that immobilizes algae through a carrier to improve biosorbent's stability and adsorption performance is immobilization technology. An environmentally friendly technology is bioremediation, which uses the metabolic potential of microorganisms to remove heavy metals through a series of physicochemical interactions which occur between the functional groups of microorganisms and the heavy metals.

Keywords: water remediation ; immobilized algae ; biosorption ; heavy metals

1. Mechanisms of Heavy Metal Removal by Algae

Heavy metals are metallic elements with densities greater than 5 g/cm^3 and are toxic at lower concentrations [1]. Heavy metals are classified into three groups [2][3], (i) transition elements, which contain certain minor amphoteric oxides (titanium (Ti), zirconium (Zr), hafnium (Hf), rutherfordium (Rf), vanadium (V), Niobium (Nb), tantalum (Ta), chromium (Cr), molybdenum (Mo), tungsten (W), manganese (Mn), technetium (Tc), rhenium (Re), ferrum (Fe), ruthenium (Ru), osmium (Os), and zinc (Zn)); (ii) rare earth elements, including lanthanides (with lanthanum (La)) and actinides (with actinium (Ac)); and (iii) elements of the p-group dominated by gallium (Ga), indium (In), thallium (Tl), stannum (Sn), plumbum (Pb), antimony (Sb), bismuth (Bi), and polonium (Po) as the main elements of the p-group. The p-group elements are the elements of the third main group to the seventh main group plus the zero group.

Without changing their own activity, algae are capable of forming cellular protein-heavy metal complexes [4]. Organometallic complexes are further divided inside the vesicles to control the amount of heavy metal ions in the cytoplasm and lessen their hazardous effects [5]. A three-stage mechanism, involving the extracellular precipitation/accumulation of heavy metals by living cells, complexation or cellular adsorption in living and dead cells, and intracellular internalization requiring microbial activity or metabolic processes, allows algae to remove heavy metals from the environment [6][7].

Biosorption activities known as rapid extracellular passive processes can be carried out by both living and non-living biomass. The primary mechanism of heavy metal adsorption by active or passive algal biomass is biosorption, which has been demonstrated to be a practical method for removing heavy metals from industrial effluent [8]. Within a few seconds, heavy metal ions are passively absorbed after interacting with negatively charged functional groups found on the algae cell surface. Heavy metals bind to cell walls that include sulfate, carboxyl, amino, and hydroxyl groups, and the attachment of heavy metal ions occurs via chelation/complexation, adsorption, electrostatic interactions, surface precipitation, and ion exchange to functional groups on the cell surface [9]. Positively or negatively charged ions will bind to the surface of the biosorbent that has been negatively charged in the ion exchange process and has grown to be the predominant mechanism [10]. Electrostatic repulsion between positively charged surfaces and metal cations may be influenced by the protonation of the functional groups of algal biomass particles and the amino and hydroxyl groups of carriers [11]. Cd(II) is transferred from aqueous solutions to algal cell surfaces by membrane flow or boundary layer diffusion, and the immobilized algal cells have more carboxylate groups, resulting in the faster transfer of Cd(II) [12]. Additionally, biosorption can create complexes with functional groups found on the surface of cells [13]. A diverse variety of biopolymers, such as humic compounds, lipids, nucleic acids, polysaccharides, proteins, and glyoxylates, are also found in cyanobacterial extracellular polymer components in algae [14]. Cyanobacterial extracellular polymers play a crucial function in the biosorption of heavy metals and serve as a barrier against hostile external conditions [15]. Polysaccharides enable heavy metals to readily bind to algae surfaces, lipids, and proteins. Moreover, heavy metals have a tendency to precipitate and accumulate on the cell surface when the pH of the solution changes rapidly during biosorption or when the concentration of the metals rises to saturation. This process is another way that algae bind to heavy metals. The heavy metals adsorbed on the surface erode the algal cell surface, while the immobilization process results in a smoother algal cell surface, and some carriers preferentially bind to metal ions, reducing the solution metal concentration, thus making it possible to protect algal cells from adsorption [16]. Algae will produce more extracellular polymeric substances (EPS) rich in negatively charged groups in response to heavy metal ions [17]. These EPS appear to be able to generate an extracellular protective barrier on the surface of the cell wall to prevent the harmful effects of heavy metals in the intracellular environment because they feature a lot of charged hydrophobic groups that are suited for the active binding of heavy metals [18][19].

Active bioaccumulation is the transport of heavy metals across the cell membrane to the cytoplasm or other organelles and requires energy to accumulate intracellular heavy metals; however, the process is a slow intracellular active accumulation of compartmentalization [20]. Depending on the kind of biomass, chemicals are absorbed, and nutrients are taken up through the surface of the biomass, which either accumulates or metabolizes substances. Ion-selective transport proteins found in the cell membrane are necessary for the whole process, which, from the absorption of metal ions to the movement of these ions throughout the cell or any organelle, takes a long time [21].

Algae must safeguard cells against non-essential metals and maintain intracellular ion concentrations at appropriate levels. As a result of structural/binding proteins, such as metallothioneins, binding to the adsorbed ions, the host cell is spared the inhibitory effects of a high concentrations of metal ions [22]. The sulfhydryl groups in phytochelatin peptides synthesized by microalgae through enzymatic synthesis are responsible for metal binding as organometallic complexes stored in the organelles of microalgal cells [23]. Additionally, acidic calcifiers and polyps promote the accumulation and storage of heavy metals [24].

Biotransformation in algae is mainly applied to the enzymatic and biochemical transformation of heavy metals but has also been used for detoxification pathways in algae. Enzymatic biotransformation is due to the non-degradable nature of heavy metals, converting them into less harmful inorganic complex forms [25]. In contrast, biotransformation is the use of electron transfer to reduce highly valued heavy metals and which will then be converted into organic heavy metal compounds [26].

Furthermore, the mechanisms of algal adsorption can differ due to the different properties of heavy metal ions [27]. The primary mechanism of adsorption of cadmium cations by algal biomass is apparently chelation, and the adsorption of nickel ions is mainly ion exchange [28]. The binding processes of lead cations, in contrast, combine ion exchange, chelation, and reduction events with the precipitation of metallic lead on algal biomass. Lead cations have a greater affinity for algal biomass [29]. Sarojini et al. [27] verified that algae adsorb Cr ions mainly through electrostatic interactions and ion exchange. To combat arsenic toxicity, microalgae oxidize As(III) to As(V), which then undergo methylation, volatilization, and extracellular excretion as they are transformed into less toxic forms [30]. Higher Cd concentrations have a considerable impact on cellular processes linked to energy consumption, DNA replication, cell cycle, and signal transduction [31]. The process by which algal cells remove heavy metal ions is shown in **Figure 1**.

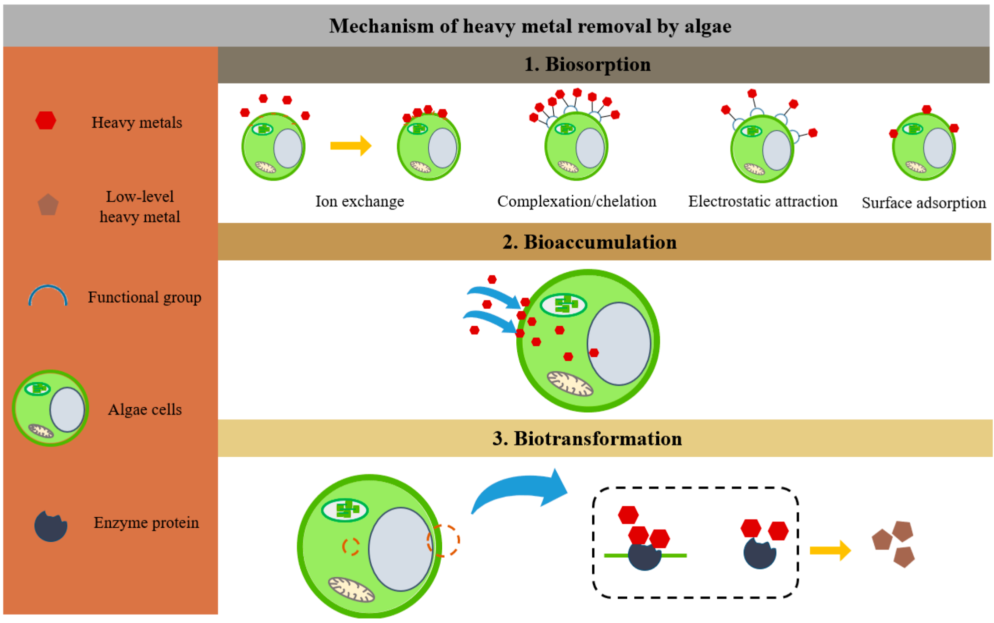

Figure 1. Removal of heavy metals by algae through biosorption, bioaccumulation, and biotransformation mechanisms.

2. Immobilized Algae Technology

Immobilization is carried out by attaching microalgae to the external surface of a supporting biological carrier. Cell immobilization techniques, metabolic processes with reduced susceptibility to senescence and significant stability over time, have been inspired by the attachment of living microorganisms to one another and to solid surfaces [32]. The target cells will be encapsulated by a porous polymer layer, thus allowing the process to diffuse the substrate into the cells [33]. The small particle size of the free particle biosorbent, the strong densification, and the uneven distribution on the reaction bed make the process less efficient and more difficult to separate [34]. The combined synergistic impact of immobilized systems can improve resistance to cell growth disruption, prevent photoinhibition and minimize cytotoxicity, and considerably aid microalgal cells in tolerating and adapting to environmental stress or toxicity [35][36]. Immobilized algae boost volumetric output, increase substrate usage, and increase resistance to harmful elements (e.g., extreme pH, temperature, and toxic compounds) [37]. Immobilized *Sargassum* contrasts with free *Sargassum* for Cu(II) ions, and immobilized adsorbents have high metal uptake, improving biosorption of nickel ions by 49% and copper ions by 36% [38].

Furthermore, during immobilization, the mobility of algal cells is affected by the limited intracapsular space, which can lead to high shear stresses from chemical forces and interactions between the support matrix and microalgal cell walls [39]. Immobilization processes prevent biomass loss from the process and improve operational flexibility, and the immobilization or sequestration of cells in small confined spaces may trigger interactions that enhance nutrient uptake [40]. Therefore, cell immobilization technology will accelerate the rate of nutrient uptake by microalgae, thus increasing the efficiency of wastewater treatment systems, which can further increase productivity and thus reduce production costs [41][42]. Additionally, compared to suspended systems, the substrate may restrict or lessen the degree of photon accessibility of algal cells, which will result in less biomass formation. However, the morphological and physicochemical characteristics of immobilized algae can be altered by homogenizing the intracapsular and extracapsular phases as well as by enhancing the substrate's characteristics in order to improve intracapsular mobility and achieve effective mass transfer performance. The creation of improved reactor designs, as well as the provision of infrastructure and logistics, are necessary for the scale-up of immobilized algae technology to create algal beads on a commercial scale [43]. Algal immobilization techniques include adsorption, encapsulation, entrapment, and self-immobilization [44]. **Figure 2** summarizes the advantages and disadvantages of immobilization techniques.

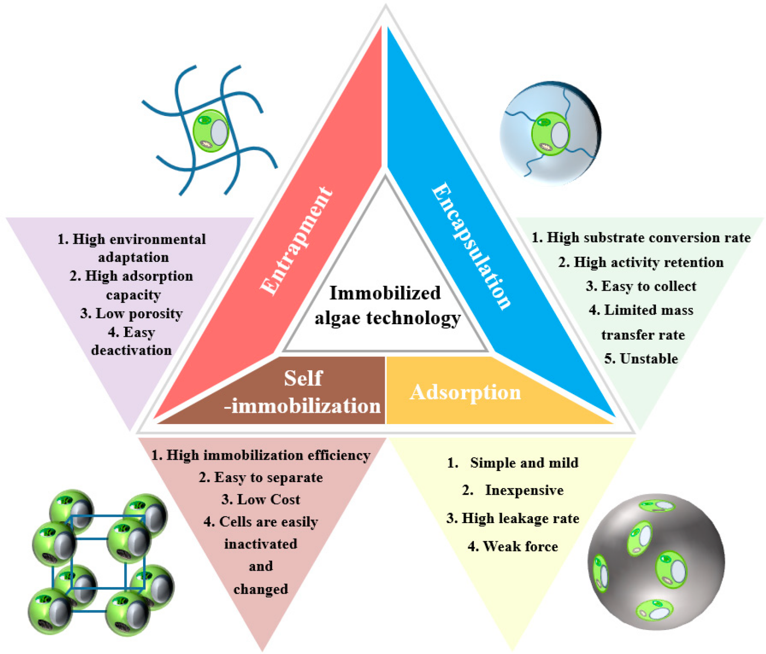

Figure 2. Advantages, disadvantages, and characterization of methods for the immobilization of algae by entrapment, encapsulation, adsorption, and self-immobilization.

2.1. Adsorption

Adsorption is a process that forms a physical bond between the surface of the water-insoluble carrier and immobilized algae through weak molecular forces like van der Waals interactions and ionic and hydrogen bonding, which are relatively gentle and quick. As a result, during use, there is a significant amount of cell leakage from the carrier due to the adsorption process [45]. Shen et al. [46] uncovered that effective adsorption of Fe_2O_3 on microalgal surfaces resulted in nanoscale spherical iron oxide covering the microalgae, opening the door to the potential of immobilizing microalgae using metal oxides. Through adsorption on the surface of the substance and passage through the algal cells, surface-immobilized algae lowered the heavy metal burden in the effluent. The growth of algal cells adsorbed onto the biofilm surface reduces the recovery cost because the method is easier to perform [47]. Adsorption-type immobilized algae have a lower cell concentration than encapsulated cells, and cells leak from the surface of the carrier during algal growth [48].

2.2. Encapsulation

Encapsulation is a permanent kind of immobilization in which cells are confined in a capsule space created by membrane walls. The cells can float freely in the inner space of the capsule despite being physically constrained [49]. Whitton et al. [50] investigated how light affected calcium alginate beads, encapsulating immobilized microalgae for nutrient remediation. The use of the enclosed carriers increased the substrate conversion and simplicity of collection by shielding the microbes from environmental stress/shock loading and hazardous byproducts. Alginate bead encapsulation techniques have drawbacks, such as poor swelling and mechanical qualities, which can cause damage or mass loss during adsorption [51]. Additionally, the encapsulation method limits the mass transfer rate, is unstable at a specific pH, and easily dissolves in buffers. Qin et al. [52] developed novel algae-encapsulated macro-capsules combined with membrane separation, where dual encapsulation created a restricted microaerobic environment with higher biomass harvesting and activity, the improved stability of live cells, and reduced cell leakage rates.

2.3. Entrapment

The method of the entrapment of cells in a polymer matrix and self-adhesion of the cells to the surface of solid support is entrapment and is the most commonly used immobilization method [53]. This method captures algal cells into a supporting matrix, namely a fiber or natural gel polymer. Maswanna et al. [54] entrapped green alga *Tetraspora* sp. CU2551 in alginate substrates with 10–50 times higher hydrogen production than cyanobacteria, which was considered a promising biological system. It has a bigger specific surface area, can adsorb a higher density of bacteria and algae, can sustain a greater pollution load, and is more adaptive to environmental conditions than adsorption immobilization and self-immobilization on the carrier surface [55]. Kube et al. [56] discovered that immobilizing algal cells by enclosing them in alginate beads assisted in the beginning and sustained larger densities of algae in the reactor, which enabled the quick removal of heavy metals. Saxena et al. [57] used freshwater diatom *Nitzschia palea* entrapped in calcium alginate hydrogel beads by gelation method without swelling behavior and in a more stable form to consume the nitrate, phosphate, and ammonia load in the water column. The gelation reaction was also shown to be reversible. Entrapment immobilization suffers from the high inactivation of algal cells [58]. The low porosity of immobilized algal cells via natural polymers can lead to restricted nutrient diffusion and thus affect the bio-removal efficiency of immobilized cells [48].

2.4. Self-Immobilization

The filamentous fungus can serve as immobilization carriers for mycorrhizal self-immobilization since they can spontaneously cluster into spheres and immobilize various mycorrhizal species [59]. Applying multifunctional reagents and crosslinking immobilization encourages the creation of channels between functional groups on the outer cell membrane [60]. The successful use of crosslinked polyethyleneimine polymer on immobilize *C. vulgaris* cells was achieved [61]. Electrostatic interaction between the negatively charged microalgae surface and the positively charged adsorbent amine results in a significant improvement in immobilization efficiency [62]. Carrier-free engagement can reduce the cost of materials and replace time-consuming and expensive technologies [63][64]. Artificially induced conditions of leading to the formation of algal cell aggregates have fewer mass transfer limitations and can better enhance cell growth, resulting in higher cell density. However, the cellular makeup of microalgae can be unintentionally altered when algal cells are exposed to chemicals and severe environments that could harm the cell surface and decrease their metabolic activity [65].

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