Phycoremediation: Algae as an Effective Agent for Sustainable Remediation and Waste Water Treatment

Olusola D. Ogundele[1*] , Adeniyi J. Adewumi[2] , David A. Oyegoke[1]

[1] Department of Chemical Sciences, Achievers University, Owo 341104, Ondo State, Nigeria
[2] Department of Geological Sciences, Achievers University, Owo 341104, Ondo State, Nigeria

Corresponding Author Email: ogundeleod@achievers.edu.ng

ABSTRACT

This article gives a brief review of the use of algae for remediation, including an introduction to its general principles, reported applicability, and utilization. Algae comprises of a broad fusion of photosynthesizing organisms. Based on size and morphology, algae are classified into macro- and microalgae. Algae were the first photosynthetic inhabitant on the earth surface as a result of their ability to utilize sunlight for synthesis of carbon dioxide, nutrients, water and the ability to increase atmospheric oxygen levels. Algae's growth within the environment is determined by the availability of nitrogen, carbon, phosphorous compounds, and other essential trace nutrients within the ecosystem. In line, algae enhance the air with oxygen (O_2) synthesized from the photosynthetic mechanism. Phycoremediation have always utilized algae species in the clean-up of various domestic, agricultural, municipal, and industrial wastewaters. Therefore, unlike conventional technologies that have the potential for secondary pollution, high operating costs, insufficient utilization of the natural resources, and a general public health burden brought on by the potential waterborne diseases, phycoremediation technology offers a sustainable, economical, and environmentally friendly method of remediating wastewater pollutants.

1. INTRODUCTION

Pollution and its adverse impacts on animals and people are major global concerns. Significant resources, including soil, air, and water, have been harmed by human activity. Water is essential to ecosystems, but it is now severely polluted due to industrialization and urbanization. Industries and home activities generate a lot of wastewater in many developing nations [1]. Without first undergoing pre-treatment, industries release their wastewater into natural water sources. As a result, heavy metals and other dangerous components are abundant in wastewater. In developing nations, wastewater is used for irrigation because there aren't enough water sources, and the water supply has dropped. Due to wastewater irrigation, trace metals and other toxins can build up in the animals, plants, and soil thereby negatively impacting people, animals, and the entire environment directly and indirectly [2]. Several approaches have been tried to lessen water pollution. Bioremediation is one of the successful, environmentally acceptable methods for removing hazardous pollutants from wastewater [3].

Given their capacity to photosynthesize and the overall effect of raising atmospheric oxygen levels, algae were the earliest photosynthetic microbes to populate the soil [4]. Because of this, the development of these micro-macroalgae within the environment depends on the presence of substances such as carbon, nitrogen, phosphorous, and other crucial trace nutrients in the surroundings. In a symbiotic manner of relationship, algae then enrich the surroundings with oxygen (O_2) produced by the photosynthesis process. As a result, algae can flourish in various habitats, biological zones, and ecosystems, including in soil, freshwater, saltwater, and wastewater from multiple origin [4]. Likewise, the concentration of specific organic wastes in an aquatic environment affects the algae that grow there [5]. In America, the photosynthetic treatment of home wastewater began in earnest in the 1950s with the study and multiplication of micro-macroalgae in wastewater remediation in a symbiotic association with bacteria. The photosynthetic algae were used in the holding and stabilization ponds to take up pollutants in the wastewater and to supply the needed oxygen by the aerobic bacteria for the breaking down of organic contaminants present in the wastewater. In addition, the algae used the carbon dioxide (CO_2) that the bacteria emitted during the photosynthesis process to fuel their growth, significantly enhancing the waste water's chemical composition and physical characteristics [6]. The high-rate algal ponds oxidation system, which has taken the role of the stabilization ponds, has an improved algae reactor and an increased oxygen supply [7].

However, the vigorous photosynthesis of algae is in charge of supplying a significant amount of oxygen (O_2) required to power the aerobic treatment mechanism and assimilate nutrients from wastewater into algal biomass [8]. The possibility for high scale production of algae through the phycoremediation process in the algae ponds and during other application initiatives has also been prompted by the rising usage of algae in wastewater treatment [9]. As indicated by the productivity, high growth rates and the sufficient algae nutrient removal effectiveness, which strongly depend on

series of abiotic parameters like light, pH, and temperature, wastewaters supply the necessary nutrients needed for the development of algae [10]. High pH may easily strip nutrients from ammonia by coagulation and precipitation technologies, whereas phosphorous is removed similarly. The majority of the agricultural businesses in Africa include those that produce tea, sugar, coffee, textiles, dairy products, paper, and other commodities that are known to contain significant levels of pollutants in their wastewater in enormous quantities [9, 10]. Although it can be difficult to treat industrial wastewater because of its particular physicochemical character caused by the presence of heavy metals, the goal is always to remove chemical toxins and harmful substances by phycoremediation rather than allowing algae biomass to build up [11].

Nevertheless, research has suggested that some industrial effluents may be phycoremediated to produce algal biomass. Therefore, phycoremediation is a different method for bioremediating excess nutrient contaminants that are found to be poisoning most agricultural wastewater [12]. Through phycoremediation, xenobiotics are biotransformed, and wastewater is transformed into something aquatic animals and humans can live in and utilize. It is recycled back into the production process at the same factories [13]. Because different species survive best in diverse environmental situations, phycoremediation research has traditionally used a variety of algae in the remediation of numerous agricultural, industrial, household, and municipal wastewaters. Therefore, unlike conventional technologies that have a high risk of secondary pollution, high operating costs, the inefficient use of natural resources, and a general health problem caused by the possibility of water related diseases, phycoremediation process promote a sustainable, economical, and environmentally friendly method of eliminating wastewater pollutants [8]. Studies by [14], successfully described large-scale remediation of domestic, agricultural, industrial, and municipal effluent by algae. Their research used different microalgae species that could quickly remove nutrients under laboratory conditions and achieved phycoremediation success of 75%, 88%, 90%, and 95%, respectively.

This article concentrated on using algae to remediate effluent water. Many different strains of algae are employed for wastewater bioremediation. Some algae are autotrophic organisms that needs more nitrogen and phosphorus for protein synthesis and metabolic activities. Different algae strains can take up many dangerous chemicals from wastewater, including heavy metals, pesticides, and other organic pollutants [15]. Physical adsorption refers to the method by which algae directly take up heavy metals through their cell wall. Chemisorption refers to the process by which contaminants enter the cytoplasm and are broken down by enzymes to produce nutrients [16]. Due to their ability to thrive in environments with high concentrations of heavy and trace metals including other harmful contaminants, algae are the microorganism most successfully utilized to remediate heavy metals from wastewater. Algae have a wide surface area, the capacity to develop autotrophically and heterotrophically, and the ability to absorb significant amounts of contaminants from wastewater. Additionally, they could be genetically altered.

2. ALGAE

A diverse range of photosynthesizing organisms makes up algae. Algae are categorized into micro- and macro-algae based on their form and size as shown in Figure 1. Microalgae are a varied range of single-celled primary producers, whereas macroalgae are multicellular creatures that resemble plants. At some point in the year, microalgae can be found almost anywhere there is light and humidity. Microalgae can be found in deserts, hot springs, fresh and salt water, and snow and ice. The most considerable diversity of microalgae species is found in lakes and oceans. Microalgae are divided into groups according to distinct structural, chemical, and functional characteristics and their distinctive form and structure. Diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), blue-green algae, golden algae (*Chrysophyceae*), and (*Cyanophyceae*) are the most significant categories of microalgae in terms of abundance.
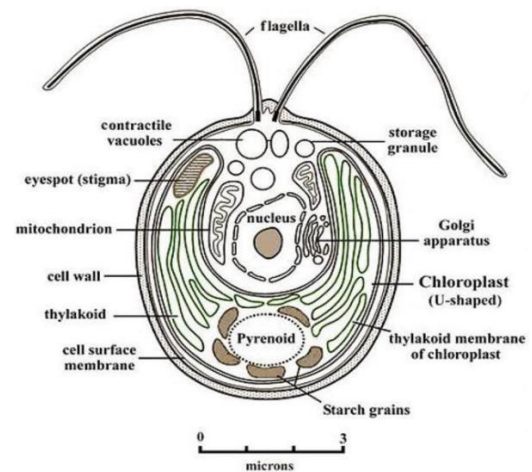

Figure 1. Microalgae

Between 200,000 and 800,000 different species of algae are thought to exist, of which 35,000 have been written about [17] Algae are responsible for using and producing more than half of the primary carbon dioxide on our planet, thus significantly impacting climate. The photosynthesizing blue-green algae are the source of the oxygen in our atmosphere (cyanobacteria). Algae also serve as a crucial foundation for numerous environmental nutrient chains. Algae consume nutrients essential for moving macronutrients like nitrogen and phosphorus [17]. Microalgae may use a wide variety of nutrients and can modify their metabolism and source of food in response to changes in their environmental conditions. Autotrophy is the most prevalent and significant trophy in microalgae. Autotrophic organisms produce energy by absorbing sunlight, oxidizing the substrate (often water), and decreasing CO_2. Contrarily, heterotrophic organisms get their power from organic substances made by other species. Photoautotrophic organisms produce chemical energy through photosynthesis using sunlight and atmospheric carbon dioxide.

Although most microalgae are photoautotrophs, they nevertheless require small amounts of organic substances, such as vitamins, to flourish. Using sunlight to generate energy, microalgae use the photosynthesis process to create organic molecules from water and carbon dioxide. Mixotrophic organisms obtain their energy through photosynthesis, which is carried out using both organic molecules and CO_2. Amphitrophy is a form of mixotrophy in which, depending on the presence of a carbon source and light, the organism can live either autotrophically or heterotrophically. To use organic substances, photoheterotrophic (also known as photoorganotroph) organisms need energy from sunlight. The

chemoautotrophs/chemoheterotrophs are a tiny category of algae that can oxidize inorganic materials to produce energy. For digestion, phagocytotic algae incorporate nutrient particles into food vesicles. It can be challenging to distinguish between these varied trophy tactics, and under most growth circumstances, switching between the numerous options is likely [18, 19]. Figure 2 below shows the structure of microalage.
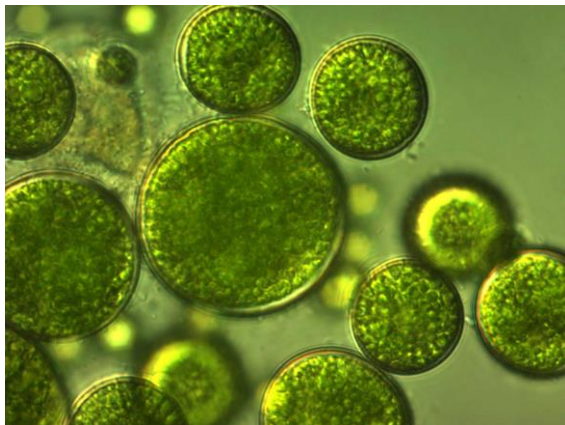

Figure 2. Microalgae structure

2.1 Algae's Environmental Requirements

Microalgae have a rapid growth rate and excellent photosynthetic efficiency; they can quadruple their mass in just 3 hours 30 minutes [18]. They frequently quadruple their biomass within 24 hours when the right circumstances are present. Lipid content in most algae species is substantial, typically between 20% and 50% of dry weight. Some algae strains have up to 70% lipid content [19]. Microalgae require the right amount of nutrients, the ability to exchange gases, and the transmission of radiation that is photosynthetically active for optimum growth. Even though microalgae can survive in the most challenging conditions, the ideal conditions for growing algae are important. Algae cultivation systems come in various forms, but they all aim to maximize the algae's production and growing environment [18, 19].

2.1.1 pH

Most types of algae can withstand pH changes of relatively significant magnitude. Most freshwater eukaryotic algae prefer settings with a pH of 5-7, while cyanobacteria prefer environments with a pH of 7-9 [20]. OH ions build up in the liquid during photosynthetic carbon fixation. pH steadily rises, and in dense algal cultures without adding CO_2, pH readings as high as 11 are not rare [19]. However, photosynthesis can be inhibited by pH values over 10 and 11. Algal auto-flocculation in the range of 10.2-12.0 is a possible effect of elevated pH [18, 19].

2.1.2 Temperature

Temperatures naturally vary throughout the day and the year. Temperature is another significant regulating factor for algae development after light. Most algae species thrive at temperatures between 20℃ and 30℃. There are species-specific requirements for temperature as well as other fundamentals. Algae develop more slowly at lower temperatures than ideal. The majority of algae species can survive temperatures that are up to 15°C below the perfect range. Only a few degrees can cause cell death in algae, making them more susceptible to temperatures higher than their optimum. As the rate of respiration rises, elevated temperatures reduce the net efficiency of photosynthesis. This effect is accelerated because CO_2 becomes less soluble than O_2 more quickly at higher temperatures [19]. Low temperatures at night may also be beneficial because they slow down respiration. According to the study [20], respiration can cause up to 25% of the biomass created through the day to be lost at night. The interaction between light and temperature can be problematic in outdoor cultures. Since the temperature of the cells are too low to process incoming photons in the early morning hours with intense light and a temperature below optimal, this can result in photoinhibition. According to literature, the optimal temperature for microalgae ranges between 20℃ and 30℃ [21, 22]. Too-high temperatures are a common problem for closed photobioreactors, which is why most need a heat exchange system [23].

2.1.3 Light

Light is the primary component that restricts the growth of algae. Algae require photosynthetically active radiation to make oxygen and organic matter and absorb carbon dioxide. The photosynthetic rate of the algae cells is negligible if the intensity of the light is too low. The efficiency of photosynthetic activity rises with light intensity until the cells reach a saturation point. After this, increasing light intensity has no further effect on photosynthesis. When the amount of light is too significant, the excess radiation damages the photosynthetic machinery, and the cells become photo-inhibited. As a result, the rate of photosynthetic activity declines as the light intensity rises. The saturation level is attained for most algae at around 1700 to 2000 mol m^{-2} s^{-1} [23]. Enough radiation into every algal cell poses a hurdle for the cultivation system. As the light is absorbed and shades the cells, the light intensity decreases as the depth of the culture increases. In a dense algal culture, light can only travel a few centimeters. The highest efficient photosynthesis occurs in algae with relatively low concentrations [23]. Too much direct sunlight can frequently limit photosynthesis at the surface. At the same time, because the radiation has been reflected or absorbed by cells closer to the surface, algae cells may experience photo deprivation further down.

2.1.4 Salinity

Different microalgae species have varying salinity optimums and tolerances. If salinity rises as a result of evaporation during hot weather, the range for the optimum can change. Through osmotic stress and changes in intercellular ionic ratios caused by the permeability of specific tissues, salinity impacts algae's growth and cell makeup. Salinity in cultures can be easily controlled by the addition of salt or fresh water [19].

2.2 Microalgae's Nutrient Requirements

The most important nutrients are phosphorous, nitrogen, and carbon. Algae also require trace levels of micronutrients. Algae formation in wastewater reduces production costs because adding nutrients to the water might be expensive. Algae can efficiently take up and eliminate nutrients from sewage and produce biomass. Wastewater treatment facilities could potentially cut costs if they combined algae production with treatment [24]. In the growing media, such as wastewater,

nutrients are available to the algae cells besides light and carbon. Any nutrient deficiency or deficit could disrupt metabolism, reducing productivity and growth [23].

2.2.1 Nitrogen

Nitrogen is one of the most crucial nutrient for microalgae growth. Depending on its availability, the nitrogen content of the algae ranges from 2% to 10%. Species and groups have different needs for nitrogen. Algae culture browning and an accumulation of organic compounds are common signs of nitrogen constraint. A decrease in chlorophyll and a rise in carotenoids are the causes of the coloring. For example, polysaccharides and polyunsaturated fatty acids are two examples of accumulated carbon molecules [18]. Algae can use numerous nitrogen molecules. Similar growth rates have been seen when nitrate (NO_3), ammonium (NH_4^+), and urea supplies are present.

2.2.2 Phosphorous

Although phosphorous makes up less than 1% of algal biomass, it is nevertheless a crucial ingredient for growth and cellular functions. The preferred form of phosphorous delivery is orthophosphate. Due to its ease of binding to ions like carbonate and iron, phosphorus is a significant growth limiting factor. Because of the precipitation, the phosphorus is unavailable for the algae to absorb. Algae can store extra phosphorus in polyphosphate bodies (luxury storage), allowing for internal and external phosphorous supply. The amount of phosphorus available affects the biomass generated in terms of lipid and carbohydrate composition. Being a constituent of phospholipids, nucleic acids, and phosphorylated sugars which is also crucial for cellular energy conversion and regulating protein activity, phosphorus often makes up less than 1% of the dry weight of cells in microalgae [25]. Another crucial factor is the nitrogen to phosphorus ratio. Maintaining the proper N/P ratio in the culture for the desired species of algae is one strategy to keep that species' dominance [18]. When phosphorous is scarce, algae are known to excrete alkaline phosphatases. By doing this, the algae have access to organic phosphorous for reabsorption. The algae may use organic waste products as a source of energy. At night, mixotrophic algae use this energy. Extracellular organic substance production changes daily, lagging behind the development curve by six hours and declining during the night [18].

2.2.3 Carbon

40% to 50% of the dry weight of an algal cell is made up of carbon [19]. Algae produce oxygen by photosynthesis and use atmospheric carbon dioxide for this purpose [20]. The supply of carbon dioxide can be challenging to manage while seeking to maximize production. The addition of carbon dioxide accelerates the growth of biomass and the lipid content of algal cells. Mass transfer can be a challenge while cultivating algae in open ponds. Thus, closed reactors require the addition of carbon dioxide [24].

2.2.4 Micronutrients

Microalgae also require tiny amounts of nitrogen and phosphorus in addition to carbon. selenium (Se), manganese (Mn), Sulfur (S), sodium (Na), potassium (K), iron (Fe), calcium (Ca), magnesium (Mg), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), vanadium (V), and cobalt (Co), are all significant micronutrients. In addition, the production of several molecules and enzyme processes depend on trace elements [18].

2.3 Cultivation Systems

Microalgae can be grown using a wide range of various growth methods. The systems range from open to closed, with cultures in suspension and immobility. To attain optimal production, all systems must work to meet the ideal growth circumstances outlined in the preceding chapter. Low production and maintenance costs, as well as efficient use of land, are other goals.

2.3.1 Open Systems

In industrial processes, commercial production, and wastewater treatment, algae are most frequently grown in open systems. A natural body of water, a pond, or a cascade system are examples of open systems. Open systems have very inexpensive construction and running costs but are susceptible to environmental impacts. Simple water tanks or big mud-brick ponds with externally provided nutrients are the usual for open systems. Photosynthesis depends on sunlight, and CO_2 is generated from the atmosphere. The tank or pond is typically built in the shape of a track with a paddlewheel serving as the mixer and circulation for the algae nutrients and cells. In order to keep the ground from absorbing the liquid, the tank is often constructed from concrete slabs or are simply excavated into the ground and insulated with polyethylene. The major problems associated with open system are low productivity and contamination. Monocultures of one species cannot be sustained, and there is a significant chance of contamination. Only species that survive conditions other species cannot, such as high pH or salinity, are successfully grown in large-scale commercial monocultures in open systems. In addition, the preservation of steady irradiance and temperature is challenging since the growing conditions of this system are vulnerable to weather conditions and climatic factors [23].

2.3.2 Closed Systems

There have been many different closed reactor designs created. The closed system, which uses closed cylindrical reactors, is particularly from durable technology and lowers the possibility of contamination. This method achieves a better productivity in the usage and fixing of the CO_2 injected when compared to open systems. This system enables proper maintenance of microalgae culture while ensuring conditions that are conducive to the growth of a particular strain and preventing the invasion of contaminating microorganisms. Although the building, maintenance, and operation expenses of the closed systems are significantly greater than those of the open systems, the closed photobioreactors can retain increased cell density than the open systems giving better results. A closed reactor, commonly referred to as a photobioreactor (PBR), can offer better cell densities, biomass yields, and lower harvesting costs. More delicate strains of algae can be grown, and contamination and environmental factors are easier to manage. Compared to an open system, the capital and operating expenditures are higher [23]. About 30 to 50 g m^{-2} day^{-1} production in closed reactors has been reported [24]. Most photobioreactors aim to increase light output by maximizing the surface to volume ratio and maintaining a manageable running cost, culture volume, mixing, and cleaning. Temperature management and ideal light penetration depth are

trade-offs. To prevent sedimentation, photobioreactors need a rather vigorous mixing process.

Additionally, sterile environments are necessary for the production of delicate species. Chemical sterilization is required, but it is expensive. The most likely method of scaling up is to increase the number of units rather than the reactor size [23]. Flat-plate or tubular reactors are the most frequently used closed reactors. A division for illumination, gas exchange facility, and harvest must be circulated in most closed reactor systems. Reactors made of tubing might have a horizontal, plane, vertical, or spiral shape. Vertical tube-shaped reactors are also known as bubble column or airlift reactors because the gas transfer and mixing is accomplished by bubbling air or air that has been enhanced with CO_2 from the reactor's bottom. Optimizing light collection is a benefit of vertical tubular reactors, but the buildup of oxygen constrains the length of the tubes. Although the amount of land needed can be decreased with the helical tubular reactor, light provision is still limited [23]. The formation of hazardous oxygen in tubular reactors is the biggest challenge.

2.4 Harvesting Techniques

The most challenging aspect of producing algae is harvesting or getting the algae out of the water. According to estimates, the recovery accounts for 20% to 30% of overall production expenses. In addition, the separation is difficult due to the microalgae's small size. Typically, cyanobacteria are as small as 0.2 mm to 2.0 mm, and unicellular eukaryotic algae range in size from 3 mm to 30 mm. In addition to the algae's microscopic size, their concentrations are typically somewhat diluted, necessitating a lot of water usage. Algae concentrations of 200 to 600 mg per liter are typical for cultures.

The difficulty lies in reducing harvesting costs and developing techniques that enable algal biomass utilization for bioproduct manufacture. There are four categories of harvesting techniques: biological, chemical, electrical, and mechanical. Before being further processed into biofuel, the algae is typically dried, requiring one or more dewatering phases. In open ponds, the microalgae slurry typically contains 0.05% of the dry weight. The dry weight concentration may increase to 2% through flocculation or sedimentation. The quantity of the dry weight may approach 30% after centrifugation and automated dehydration. The wet slurry can either be dried further to 85% dry weight or turned directly into biofuel [24].

2.4.1 Biological Harvesting Techniques

Algal autoflocculation and bioflocculation are two different types of spontaneous flocculation. High pH levels lead to autoflocculation. Calcium phosphate residue, which is positively charged, balances out the negatively charged algal cells, causing flocculation. Dissolved carbon dioxide raises pH, further promoting the saturation of phosphate and calcium ions. The term "bioflocculation" refers to flocculation brought on by secreted polymers. The use of flocculating microorganisms has similarly shown excellent recovery outcomes. Algal recovery with both techniques has reached over 90% [24].

2.4.2 Chemical Harvesting Techniques

Chemicals can be used to increase the particle size in the algal suspension. Usually, this chemical flocculation is done before using any other harvesting technique. Cells of microalgae have a negative charge. The addition of electrolytes neutralizes the charge on the algal cells, and the addition of synthetic polymers flocculates the cells. Finding flocculants that don't prevent sludge and algae utilization in the subsequent process is complicated. Typically, ferric chloride ($FeCl_3$) and aluminum sulfate ($Al_2(SO)_3$) are used to neutralize the cells charges. Sulfate and aluminum use has been demonstrated to prevent the growth of bacteria in wastewater sludge, and the disposal and land application of treated aluminum sludge are equally troublesome. Natural polymers do not negatively impact biomass in the same way as synthetic polymers, although their application as flocculants has received less research. At the small scale, cationic starch and polysaccharide chitosan have produced successful outcomes [24].

2.4.3 Electrical Harvesting Techniques

By using electrophoresis, efforts have been made to remove algae from water bodies. The electric field forces charged algae out of the fluid. Hydrogen produced by water electrolysis propels microalgae to the surface. The benefit of this approach is that no additional chemicals are required [26]. However, high power and cost requirements make using this approach on a big scale challenging [25, 26]. In addition, functional magnetic particles can entrap algae cells in an external magnetic field. It has been done using Fe_3O_4 nanoparticles in a somewhat acidic environment. This novel approach has a high recovery efficiency and is reasonably quick and easy. However, the approach is constrained by the challenge of creating functional magnetic particles.

2.4.4 Mechanical Harvesting Techniques

The mechanical processes of centrifugation, filtration, sedimentation, and dissolved air flotation are used to recover suspended algae. When utilizing biofilm, algae can be mechanically gathered by scraping off the surface. Centrifugation is a quick and effective method of extracting algae from the water. All types of algae can be processed using this technique. However, the high operating and investment costs present a hurdle. Centrifugation is not considered viable for usage on a big scale due to the high expenses. At a relatively low cost, bigger species of algae and filamentous algae strains can be harvested using various filtration techniques. However, due to membrane fouling and replacement costs, the cost and energy requirements of filtration for suspended microalgae are significant. The most efficient filtration method is tangential flow [26]. Algal sedimentation is a low-cost, relatively slow process that results in solids concentrations of 1.5%. This technique can be combined with chemicals to hasten sedimentation. Sludge removal in wastewater treatment is accomplished via dissolved air flotation. This approach is thought to be more effective than sedimentation for recovering algae. Additionally, this approach is used with chemical flocculation therapy [24].

2.5 Decontamination Mechanism of Heavy Metals by Algae

Peptides found in algae bond to heavy metals as shown in Figure 3. Heavy metals bond to peptide chains in algae to produce organometallic complexes, which enter vacuoles to regulate the cytoplasmic concentration of heavy metals. Algal cells perform this examination to see if heavy metals are

harmful. Metallothioneins and phytochelatins are the names of the peptide chains. The PCs are peptides created by enzymes, whereas the MTs are polypeptides encoded by genes. Metallothioneins of class III are another name for phytochelatins [27]. Class-II and class-III metallothioneins are present in algae. There are no class-I metallothioneins in algae. Certain heavy metals, including Cd^{2+}, Ag^+, Zn^{2+}, Hg^{2+}, Au^{2+}, Pb^{2+}, and Bi^{3+}, can trigger the synthesis of Mt III. Mt III peptide molecules have a crucial role in algae because their presence enables the organisms to endure high concentrations of heavy metals. The sulfide group of the cysteines found in MT III peptides play a significant role in the proteins' involvement in metal binding. Cysteine's sulfide groups have a partial negative charge that metal cations are chemically attracted to. This leads to the creation of HM-MT, which can absorb heavy metals and reduce their harmful effects on cells [27]. Therefore, the degree of pollution directly relates to the biosynthesis of Mt III [28]. The mechanism for the removal of heavy metals is shown in Figure 4.
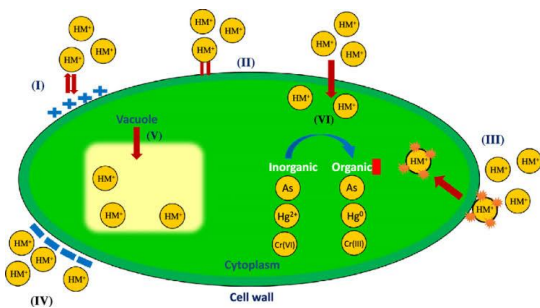

Figure 3. Decontamination mechanism of heavy metals by algae [29]

3. WASTEWATER TREATMENT

Since every site and circumstance is different, there is no one universal method for cleaning wastewater. Pollution causes are many, and the wastewater's composition is complex. Industrial and domestic effluent from rural and urban areas can be the source, and it may occasionally contain unpredictable concentration of precipitation and snowmelt. Wastewater can come from industrial or manufacturing facilities, as well as from agricultural land and waste treatment facilities [30]. Depending on how the water is collected, wastewater is typically a mixture of water from many sources [31]. Characterizing and identifying the chemical components is crucial for efficiently treating wastewater. The concentrations of the chemicals are used to gauge how well the wastewater is being treated [31]. Depending on where it comes from, the wastewater has different compositions. It comprises a variety of organic, inorganic, and synthetic substances [31]. The main chemical elements in municipal waste include urea, proteins, lipids and carbohydrates. Urine produces urea, which contributes significantly to the nitrogenous materials in the wastewater system [30]. Ammonia, lipids, oil, soaps, and some other artificial compounds made of carbon, hydrogen, sulfur, oxygen, iron and phosphorus are numerous biodegradable substances in wastewater. Total dissolved solids, pH, temperature, color, and odor are wastewater indicators, among others [31]. Heavy metals and poisonous substances can be found in industrial effluent, while petroleum compounds, silt, and pesticides can be found in runoff from melting snow and rain. Wastewater is a haven for various microorganisms,

mainly bacteria, viruses, and protozoa. Most microorganisms are benign, but some are pathogenic. Preventing eutrophication and contamination of natural water bodies is the primary objective of wastewater treatment [30].

To safeguard the environment and the public's health, the wastewater treatment process must adhere to rules and restrictions. The main goal of treating wastewater is typically to enable the disposal of effluents without endangering public health or causing intolerable harm to the environment. Ordinarily, untreated wastewater must undergo some kind of treatment before being discharged. The wastewater treatment method that results in effluent that satisfies the recommended chemical and microbiological quality requirements in various countries is the one that should be used. Reducing and eliminating suspended particles, biodegradable organic matter, pathogens, and hazardous substances is the primary goal of wastewater treatment [30, 31]. Physical, chemical, and biological treatment methods are all available for wastewater. Physical treatment techniques such as filtration or sedimentation remove suspended materials. Chemical treatment techniques, such as flocculation, sedimentation, disinfection, or precipitation, try to destroy or transform contaminants by chemical reactions. Biological treatment techniques use microbes to convert or eliminate pollutants and decrease nutrients and biodegradable organic materials [31]. There are five levels of sewage treatment technology: preliminary, primary, secondary, tertiary, and quaternary. The coarse materials are eliminated during the initial treatment. Bigger entities are eliminated from the sewage when it goes through the 20–60 mm spacing bar. The silt and grit are stabilized by slowing the flow while allowing biological materials to proceed to the next stage. The majority (up to 75%) of the leftover solids settle by gravitational force in sedimentation chambers during the process' primary treatment stage. Chemical coagulants are employed occasionally. Solids and organic materials are processed during secondary treatment.
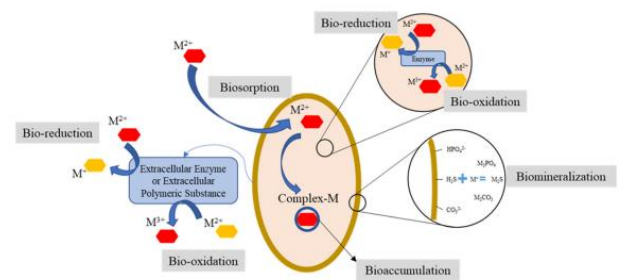

Figure 4. Heavy metals removal mechanisms [32]

A diverse community of bacteria uses the residual organic matter for energy and growth. Several strategies fulfill the biological oxygen need (BOD) aerobically [30]. The microbial population may be suspended in activated carbon reactors or adhered to on a biofilm surface. Biological oxidation systems can effectively eliminate pathogenic microorganisms. It is occasionally necessary to use in chemical and biological remediation [30]. The tertiary and quaternary practices are included in the advanced remediation steps. Some carbon-based ions are eliminated during the tertiary treatment process, either chemically or biologically. The biological approach is less expensive and does not result in secondary contamination compared to the chemical method. Heavy metals, residual organic molecules, and soluble minerals are all eliminated

during the quaternary process. Complex techniques are employed in advanced treatment processes to focus on specific nutrients like phosphorous or nitrogen. The cost of the cleaning process increases with the number of processes. The tertiary procedure is roughly four times more expensive than the primary treatment step, while the quaternary phase is eight to sixteen times costly [29].

As shown in Table 1, the remediation potential of some alage species have been studied. *Scenedesmus obliquus* was examined by the study [41] for its dual use in effluent bioremediation and biochemical constituent buildup in algal cells. Micro-element absorption and removal efficiency for the microalgae cultured in wastewater were 71.2% COD, 81.9% NH_4^+, 100% NO_3, and 94% PO_4^{3-}. *Scenedesmus obliquus*'s growth profile showed a definite growth rate of 0.42 $1.d^{-1}$ and a carrying capacity of 0.88 g L^{-1}. The dry weight yields for lipids, proteins, and carbohydrates were 26.5%, 28.5%, and 27.5%, respectively. Following biochemical extraction on the de-oiled biomass, yields of protein and carbohydrates were 25.3% and 21.4%, respectively. Multiple functional groups, including NeH, CH_3, CH_2, CeN, and SieO, were visible using Fourier transform infrared spectroscopy on the algae surface, supporting the growth of biological components in algae. The sequential steps of dehydration (60–200°C), devolatilization (200–500°C), and solid residue breakdown (500–600°C) were shown by the thermal analysis of microalgal biomass. A cost-value analysis of algae grown in effluent water was created, considering amortization, operating expenses, energy savings, and ecofriendly advantages. The payback period for phycoremediation was 14.8 years, with a net profit of 16885 US dollars per year (i.e., shorter than the project lifetime). The proposed phycoremediation method was, therefore, financially feasible.

Table 1. Phycoremediation potential of some algae species

Source	Location	Algae Specie	Condition	Identification	Reference
Water	China	*Desmodesmus sp*	25°C, 60 µmol $m^{-2}s^{-1}$	Optical microscope analysis	[33]
Soil	China	*Scenedesmus sp*	25°C, 60 µmol $m^{-2}s^{-1}$	Optical microscope analysis	[33]
Domestic sewage	India	*Tetraselmis indica*	27°C, 135 µmol $m^{-2}s^{-1}$	18S rRNA gene sequence analysis	[34]
Swine waste water	Taiwan	*Chlorella sorokiniana*	27°C, 150 µmol $m^{-2}s^{-1}$	23S rRNA gene sequence analysis.	[35]
clinical waste	Malaysia	*Aspergillus spp*	26°C, 140 µmol $m^{-2}s^{-1}$	scanning electron microscope (SEM)	[36]
---	Canada	*Haematococcus pluvialis*	23°C	18S rRNA gene sequence analysis.	[37]
---	Portugal	*Tetraselmis sp*	---	---	[38]
Fresh water	----	*Chlorella sp*	25°C, 70 µmol $m^{-2}s^{-1}$	---	[9]
Seasalter Shellfish Limited	United Kingdom	*Tetraselmis suecica*		---	[39]
Waste water	Vietnam	*Scenedesmus sp*	27°C, 50 µmol $m^{-2}s^{-1}$	---	[40]

Polybags, photobioreactors, and raceway ponds were used to cultivate an indigenous microalga, *Scenedesmus obliquus*, in rice mill paddy-soaked effluent in the study [42] study. With ammoniacal nitrogen (NH_3-N) removal of 96.12% and a phosphate (PO_4-P) removal of 97.58%, photobioreactors was found to have the highest biomass productivity (BP) of 340 mg/L/d. The PBR culture system produced the highest amounts of lipids (12% of biomass), protein (40%), and carbohydrate (20%), which raceway ponds and polybags then followed.

Chlamydomonas reinhardtii, Chlorella sp., Parachlorella kessleri-I, and *Nannochloropsis gaditana* were investigated by the study [43] for their effectiveness in phycoremediation of municipal waste and prospective usage in biodiesel generation. *P. kessleri-I* outperformed the other three studied strains in terms of growth rate and biomass output in 100% municipal waste. After ten days of growth in 100% municipal waste taken from Delhi, it effectively removed all key nutrients with a rate of up to 98% phosphate. Compared to growth in control media, *P. kessleri-I* growth in municipal waste led to a 50% increase in biomass and a 115% increase in lipid output. International criteria were reached by the fatty acid methyl ester (FAME) and fuel characteristics of lipids extracted from cells cultured in municipal waste.

Five *Scenedesmus* and three *Desmodesmus* species of microalgae were cultured from soil and water in China and identified by 18S rRNA gene sequence analysis by [33]. *Scenedesmus sp. HXY2* had the best nutrient removal efficiency (> 95%) and thrived in environments with high levels of total organic carbon and ammonia. *Scenedesmus sp. HXY2* had 7.2 106 cells mL^{-1} of biomass on day 12. This species has a lipid content of 15.56% and a productivity of 5.67 mg L^{-1} day^{-1}. *Scenedesmus sp. HXY2'*s lipids were acceptable for biodiesel synthesis, as evidenced by the amount of unsaturated fatty acids (60.07%). *Scenedesmus sp. HXY2* can grow in sewage containing a lot of ammonia and organic matter while also purifying the sewage and producing lipids.

The cadmium and copper adsorption capacities of *Scenedesmus abundans'* living and nonliving biomass were compared [44]. The findings demonstrated that *S. abundans* at lower concentrations effectively removed copper and cadmium concentrations greater than 4.00 mg L^{-1}. Additionally, it was revealed that biomass outperformed non-living biomass and that lower algal concentrations were more efficient than higher ones at eliminating heavy metals from effluent or polluted water. The adsorption of Cr^{6+} by two algae species, *Chlorella Vulgaris* and *Zoogloea ramigera*, was studied [45]. According to the study, *C. Vulgaris* and *Z. ramigera's* adsorption capacity rose when the concentration of

metal ions was increased to 75 and 200 mg L^{-1}, respectively. Temperatures between 25 and 50 °C and a pH of 2.0 resulted in the greatest metal adsorption.

Studies [46] investigated the algal biomass of *Sargassum*. Fe (III) concentration ranged from 15 to 40 mg L^{-1} in this experiment as suspended particles, while the concentration of Cu^{2+} ion was 25 mg L^{-1}. The removal of metal from a solution comprising iron (Fe) and copper (Cu) ions was accomplished using a flow-through sorption column. They showed that algae can take up to 2.3 meq g^{-1} of metal ions from solution and that its ability to bind those ions declined in the order: Cu>Ca>Fe. Deep filtering was used to remove Fe (III) ions, and a biosorption approach was used to remove Cu^{2+}. Using two species of *Scenedesmus* algae.

Travieso et al. [47] looked at how zinc, cadmium, and chromium were affected by two algal species, *Scenedesmus acutus* and *Chlorella Vulgaris*. In the research, 96 hours was the absolute minimum time required for algal inoculation. *Scenedesmus acutus* and *Chlorella Vulgaris* both had growing rates of 0.020 h^{-1} and 0.0150 h^{-1} in the culture conditions, respectively. The generation time were 37 hours 53 minutes and 45 hours 35 minutes. Maximum concentrations of 45 mg L^{-1} for Cr, 2 mg L^{-1} for Cadmium, and 600 mg L^{-1} for Zinc are not toxic to *chlorella vulgaris*. *Scenedesmus acutus* displayed a maximum resistance of 100 mg L^{-1} for Zinc, 2 mg L^{-1} for Cadmium and 15 mg L^{-1} for Chromium. Harris and Ramelow [48] investigated the binding ability of algae species (*Chlorella Vulgaris* and *Scenedesmus quadricauda)*, for the metals such as zinc, copper, silver and cadmium. The findings demonstrated that the uptake of metal by algae was strongly dependent on pH and that both *Chlorella Vulgaris* and *Scenedesmus quadricauda* showed extremely similar binding capabilities for zinc, copper, silver and cadmium. Silver was the most heavily adsorbed metal over the whole pH spectrum. Algae absorbed the majority of the metals from water solutions within a minute. Silver, Copper, Cadmium, and Zinc are in decreasing order of metal binding capacity.

Three algae species (*Chlamydomonas reinhardtii, Chlorella vulgaris* and *Chlorella pyrenoidosa*) were used to remove cadmium (Cd) from contaminated water. Dried weight algae were used in this experiment to evaluate adsorption. These algae species' results revealed a strong initial uptake of metals after which a gradual uptake of metal. Was observed. In that order, *chlorella vulgaris, Chlorella pyrenoidosa, and Chlamydomonas reinhardtii* demonstrated about 96, 79, and 57% adsorption saturation, respectively [49]. Kaewsarn and Yu [50] examined the Cd^{2+} adsorption capacity of pre-treated aquatic algae biomass by employing column and batch experiments. The results showed the pre-treated biomass of *Padina sp.* was most effective at removing wastewater's 0.52 mmol g^{-1} content of Cd^{2+} at pH 5.0. 90% of this experiment's Cd^{2+} was adsorbed within 36 minutes.

Aksu and Kutsal [51] explored the removal of Pb^{2+} from effluent by observing the adsorption of metals from industrial effluent utilizing *Chlorella Vulgaris*. Metal absorption was investigated in a sole batch mechanism. The results show that the *Chlorella Vulgaris* mechanism is a promising alternative method for treating effluent water. The residual or adsorbed metal concentration was determined using the Freundlich adsorption isotherm technique. Green algae *Cladophora crispata* was used [52] to absorb Pb^{2+} and Cr^{4+}. Lead adsorption at 5.0 pH and 25°C was optimal, whereas chromium adsorption at 1.0 pH and 25°C was shown to be optimal. *Zoogloea ramigera* and *Rhizopus arrhizus*, two algae

strains, had their adsorption potential tested. Findings show that increasing the concentration of metal ions to 200–300 and 150-200 mg L^{-1} respectively, enhanced the amount of Pb^{2+} that *Rhizopus arrhizus* and *Zoogloea ramigera* could adsorb. For both microorganisms, the ideal pH range was 4.5 to 5.5, and the perfect temperature range was 25-45°C.

El-Enany and Issa [53] examined two algal strains. One strain, *Nostoc linckia*, was metal-tolerant, but *Nostoc rivularis* was sensitive to metals. *Nostoc linckia* and *Nostoc rivularis* were raised in sewage water at 25, 50, and 75% levels. *Nostoc linckia* had 50% growth rate while *Nostoc rivularis* had 25% growth rate. Both strains increased chlorophyll, protein, oxygen, and respiration levels in 25% of the sewage water. In the lower level sewage water, *Nostoc linckia* demonstrated 30 times metal uptake greater for zinc and ten times greater for cadmium than in moderate level, and *N. rivularis* showed metal uptake ten times greater for zinc and two times greater for cadmium. The adsorption of Cu^{2+} by two algae species, *Zoogloea ramigera* and *Chlorella Vulgaris*, was examined by authors [54]. The results of this experiment demonstrated that *C. Vulgaris* and *Z. ramigera* both had higher adsorption capacities when metal ions concentrations were increased to 100–125 and 150–200 mg L^{-1}, respectively, at a pH range of 4.0 to 4.5 and an optimal temperature of 25°C.

Durvillaea potatorum, a marine alga, was used by authors [55] in a binary adsorption system to remove Cu^{2+} and Cd^{2+} from solution. While the biosorption capacity for both Cu^{2+} and Cd^{2+} was comparable to that of a single biosorption mechanism, it was discovered that the adsorption capacity of algae for both Cu^{2+} and Cd^{2+} in a dual mechanism was lesser than that of a single biosorption system. The ideal pH for this experiment was 5.0. Metal ions, light, temperature, and biosorption rate are all unaffected. The algae absorbed 90% of the copper and cadmium in this experiment within 10 and 30 minutes, respectively, and equilibrium was noticed after 60 minutes. Spirulina maxima were employed by Kosaric et al. [56] to treat municipal garbage from London. According to the study, spirulina maxima effectively removes phosphate and nitrogen from wastewater[57-62].

4. CONCLUSION

Research articles from a wide range of disciplines have shown that the use of phycoremediation is highly effective and has potential for further use. The most advantageous method is phycoremediation because it is economical, less stressful, simple to use and does not generate potentially dangerous byproducts, and the residual waste can be used to generate biofuel. This review also revealed that phycoremediation has received very little attention. Therefore, there is need for more intense research to be carried out on this environmentally friendly method to remediate water resources, eliminate toxins and heavy metals pollution.

REFERENCES

[1] Qadir, M., Wichelns, D., Raschid-Sally, L., McCornick, P.G., Drechsel, P., Bahri, A., Minhas, P.S. (2010). The challenges of wastewater irrigation in developing countries. Agricultural water management, 97(4): 561-568. https://doi.org/10.1016/j.agwat.2008.11.004

[2] Mapanda, F., Mangwayana, E.N., Nyamangara, J., Giller, K.E. (2005). The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agriculture, Ecosystems & Environment, 107(2-3): 151-165. http://dx.doi.org/10.1016/j.agee.2004.11.005

[3] Kumar, R., Goyal, D. (2010). Waste water treatment and metal (Pb^{2+}, Zn^{2+}) removal by microalgal based stabilization pond system. Indian journal of microbiology, 50(1): 34-40.

[4] Olguín, E.J. (2003). Phycoremediation: key issues for cost-effective nutrient removal processes. Biotechnology Advances, 22(1-2): 81-91. https://doi.org/10.1016/S0734-9750(03)00130-7

[5] Pacheco, M.M., Hoeltz, M., Moraes, M.S., Schneider, R.C. (2015). Microalgae: cultivation techniques and wastewater phycoremediation. Journal of Environmental Science and Health, Part A, 50(6): 585-601.

[6] Oswald, W.J., Gotaas, H.B., Golueke, C.G., Kellen, W.R., Gloyna, E.F., Hermann, E.R. (1957). Algae in waste treatment [with discussion]. Sewage and industrial wastes, 29(4): 437-457.

[7] Huppe, H.C., Turpin, D.H. (1994). Integration of carbon and nitrogen metabolism in plant and algal cells. Annual review of plant biology, 45(1): 577-607.

[8] Lim, S.L., Chu, W.L., Phang, S.M. (2010). Use of Chlorella vulgaris for bioremediation of textile wastewater. Bioresource technology, 101(19): 7314-7322. https://doi.org/10.1016/j.biortech.2010.04.092

[9] Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J., Ruan, R.R. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresource technology, 101(8): 2623-2628.
https://doi.org/10.1016/j.biortech.2009.10.062

[10] Li, Y., Chen, Y.F., Chen, P., Min, M., Zhou, W., Martinez, B., Ruan, R. (2011). Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresource technology, 102(8): 5138-5144.
https://doi.org/10.1016/j.biortech.2011.01.091

[11] Hodaifa, G., Martínez, M.E., Sánchez, S. (2008). Use of industrial wastewater from olive-oil extraction for biomass production of Scenedesmus obliquus. Bioresource technology, 99(5): 1111-1117. https://doi.org/10.1016/j.biortech.2007.02.020

[12] Chinnasamy, S., Bhatnagar, A., Claxton, R., & Das, K. C. (2010). Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresource technology, 101(17): 6751-6760. https://doi.org/10.1016/j.biortech.2010.03.094

[13] Olguín, E.J. (2012). Dual purpose microalgae–bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery. Biotechnology Advances, 30(5): 1031-1046. https://doi.org/10.1016/j.biotechadv.2012.05.001

[14] El-Sheekh, M.M., El-Naggar, A.H., Osman, M.E., Haieder, A. (2000). Comparative studies on the green algae Chlorella homosphaera and Chlorella vulgaris with respect to oil pollution in the river Nile. Water, air, and soil pollution, 124(1): 187-204.

[15] Oswald, W.J. (2003). My sixty years in applied algology. Journal of Applied Phycology, 15(2): 99-106.

[16] Dwivedi, S. (2012). Bioremediation of heavy metal by algae: current and future perspective. Journal of advanced laboratory research in biology, 3(3): 195-199.

[17] Rajkumar, R., Yaakob, Z., Takriff, M.S. (2014). Potential of micro and macro algae for biofuel production: a brief review. Bioresources, 9(1): 1606-1633. http://dx.doi.org/10.15376/biores.9.1.1606-1633

[18] Grobbelaar, J.U. (2013). Inorganic algal nutrition. Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 123-133. https://doi.org/10.1002/9781118567166.ch8

[19] Mata, T.M., Martins, A.A., Caetano, N.S. (2010). Microalgae for biodiesel production and other applications: a review. Renewable and Sustainable Energy Reviews, 14(1): 217-232. https://doi.org/10.1016/j.rser.2009.07.020

[20] Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25(3): 294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

[21] Bernard, O., Rémond, B. (2012). Validation of a simple model accounting for light and temperature effect on microalgal growth. Bioresource Technology, 123: 520-527. https://doi.org/10.1016/j.biortech.2012.07.022

[22] Ras, M., Steyer, J.P., Bernard, O. (2013). Temperature effect on microalgae: a crucial factor for outdoor production. Reviews in Environmental Science and Bio/Technology, 12(2): 153-164.

[23] Griffiths, M. (2013). Microalgal Cultivation Reactor Systems. In Biotechnological Applications of Microalgae: Biodiesel and Value-Added Products, 51-75.

[24] Christenson, L., Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnology advances, 29(6): 686-702. https://doi.org/10.1016/j.biotechadv.2011.05.015

[25] Mayers, J.J., Flynn, K.J., Shields, R.J. (2014). Influence of the N: P supply ratio on biomass productivity and time-resolved changes in elemental and bulk biochemical composition of Nannochloropsis sp. Bioresource technology, 169: 588-595. https://doi.org/10.1016/j.biortech.2014.07.048

[26] Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresource technology, 102(1): 71-81. https://doi.org/10.1016/j.biortech.2010.06.159

[27] Satoh, M., Karaki, E., Kakehashi, M., Okazaki, E., Gotoh, T., Oyama, Y. (1999). Heavy-metal induced changes in nonproteinaceous thiol levels and heavy-metal binding peptide in Tetraselmis tetrathele (Prasinophyceae). Journal of phycology, 35(5): 989-994. https://doi.org/10.1046/j.1529-8817.1999.3550989.x

[28] Howe, G., Merchant, S. (1992). Heavy metal-activated synthesis of peptides in Chlamydomonas reinhardtii. Plant Physiology, 98(1): 127-136. https://doi.org/10.1104/pp.98.1.127

[29] Koul, B., Sharma, K., Shah, M.P. (2022). Phycoremediation: A sustainable alternative in wastewater treatment (WWT) regime. Environmental Technology & Innovation, 25: 102040. https://doi.org/10.1016/j.eti.2021.102040

[30] Abdel-Raouf, N., Al-Homaidan, A.A., Ibraheem, I. (2012). Microalgae and wastewater treatment. Saudi Journal of Biological Sciences, 19(3): 257-275. https://doi.org/10.1016/j.sjbs.2012.04.005

[31] Rawat, I., Kumar, R., Bux, F. (2013). Phycoremediation by high-rate algal ponds (HRAPs). Biotechnological Applications of Microalgae: Biodiesel and Value-Added Products. CRC Press, Boca Raton, 179-199.

[32] Kurniawan, S.B., Ramli, N.N., Said, N.S.M., Alias, J., Imron, M.F., Abdullah, S.R.S., Othman, A.R., Purwanti, I.F., Hasan, H.A. (2022). Practical limitations of bioaugmentation in treating heavy metal contaminated soil and role of plant growth promoting bacteria in phytoremediation as a promising alternative approach. Heliyon, 8(4): e08995. https://doi.org/10.1016/j.heliyon.2022.e08995

[33] Ye, S., Gao, L., Zhao, J., An, M., Wu, H., Li, M. (2020). Simultaneous wastewater treatment and lipid production by Scenedesmus sp. HXY2. Bioresource technology, 302, 122903. https://doi.org/10.1016/j.biortech.2020.122903

[34] Chandra, R., Ghosh, U.K., Nayak, J.K. (2017). Phycoremediation potential of marine microalga Tetraselmis indica on secondary treated domestic sewage for nutrient removal and biodiesel production. Environmental Science and Pollution Research, 24(26): 20868-20875.

[35] Chen, C.Y., Kuo, E.W., Nagarajan, D., Ho, S.H., Dong, C.D., Lee, D.J., Chang, J.S. (2020). Cultivating Chlorella sorokiniana AK-1 with swine wastewater for simultaneous wastewater treatment and algal biomass production. Bioresource technology, 302: 122814. https://doi.org/10.1016/j.biortech.2020.122814

[36] Efaq, A.N., Nagao, H., Al-Gheethi, A.A. (2017). Inactivation of Aspergillus spores in clinical wastes by supercritical carbon dioxide. Arabian Journal for Science and Engineering, 42(1): 39-51.

[37] Haque, F., Dutta, A., Thimmanagari, M., Chiang, Y.W. (2017). Integrated Haematococcus pluvialis biomass production and nutrient removal using bioethanol plant waste effluent. Process Safety and Environmental Protection, 111: 128-137. https://doi.org/10.1016/j.psep.2017.06.013

[38] Schulze, P.S., Carvalho, C.F., Pereira, H., Gangadhar, K.N., Schüler, L.M., Santos, T.F., Barreira, L. (2017). Urban wastewater treatment by Tetraselmis sp. CTP4 (Chlorophyta). Bioresource technology, 223: 175-183. https://doi.org/10.1016/j.biortech.2016.10.027

[39] Michels, M.H., Vaskoska, M., Vermuë, M.H., Wijffels, R.H. (2014). Growth of Tetraselmis suecica in a tubular photobioreactor on wastewater from a fish farm. Water research, 65: 290-296. https://doi.org/10.1016/j.watres.2014.07.017

[40] Pham, T.L., Bui, M.H. (2020). Removal of nutrients from fertilizer plant wastewater using Scenedesmus sp.: formation of bioflocculation and enhancement of removal efficiency. Journal of Chemistry, 2020: 8094272. https://doi.org/10.1155/2020/8094272

[41] Ansari, F.A., Ravindran, B., Gupta, S.K., Nasr, M., Rawat, I., Bux, F. (2019). Techno-economic estimation of wastewater phycoremediation and environmental benefits using Scenedesmus obliquus microalgae. Journal of environmental management, 240: 293-302. https://doi.org/10.1016/j.jenvman.2019.03.123

[42] Umamaheswari, J., Shanthakumar, S. (2019). Phycoremediation of paddy-soaked wastewater by indigenous microalgae in open and closed culture system. Journal of environmental management, 243: 435-443. https://doi.org/10.1016/j.jenvman.2019.05.023

[43] Singh, A.K., Sharma, N., Farooqi, H., Abdin, M.Z., Mock, T., Kumar, S. (2017). Phycoremediation of municipal wastewater by microalgae to produce biofuel. International Journal of Phytoremediation, 19(9): 805-812. https://doi.org/10.1080/15226514.2017.1284758

[44] Terry, P.A., Stone, W. (2002). Biosorption of cadmium and copper contaminated water by Scenedesmus abundans. Chemosphere, 47(3): 249-255. https://doi.org/10.1016/S0045-6535(01)00303-4

[45] Aksu, Z., Sag, Y., Kutsal, T. (1990). A comparative study of the adsorption of chromium (VI) ions to C. vulgaris and Z. ramigera. Environmental Technology, 11(1): 33-40. https://doi.org/10.1080/09593339009384836

[46] Kratochvil, D., Volesky, B. (1998). Biosorption of Cu from ferruginous wastewater by algal biomass. Water Research, 32(9): 2760-2768. https://doi.org/10.1016/S0043-1354(98)00015-3

[47] Travieso, L., Canizares, R.O., Borja, R., Benitez, F., Dominguez, A.R., Valiente, V. (1999). Heavy metal removal by microalgae. Bulletin of environmental contamination and toxicology, 62(2): 144-151.

[48] Harris, P.O., Ramelow, G.J. (1990). Binding of metal ions by particulate biomass derived from Chlorella vulgaris and Scenedesmus quadricauda. Environmental science & technology, 24(2): 220-228. https://doi.org/10.1021/ES00072A011

[49] Matheickal, J.T., Yu, Q. (1997). Biosorption of heavy metals from wastewater using Australian biomass. Developments in Chemical Engineering and Mineral Processing, 5(1-2): 101-114. https://doi.org/10.1002/apj.5500050210

[50] Kaewsarn, P., Yu, Q. (2001). Cadmium (II) removal from aqueous solutions by pre-treated biomass of marine alga Padina sp. Environmental pollution, 112(2): 209-213. https://doi.org/10.1016/S0269-7491(00)00114-7

[51] Aksu, Z., Kutsal, T. (1991). A bioseparation process for removing lead (II) ions from waste water by using C. vulgaris. Journal of Chemical Technology & Biotechnology, 2(1): 109-118. https://doi.org/10.1002/jctb.280520108

[52] Özer, D., Aksu, Z., Kutsal, T., Çaglar, A. (1994). Adsorption isotherms of lead (II) and chromium (VI) on Cladophora crispata. Environmental technology, 15(5): 439-448. https://doi.org/10.1080/09593339409385448

[53] El-Enany, A.E., Issa, A.A. (2000). Cyanobacteria as a biosorbent of heavy metals in sewage water. Environmental toxicology and pharmacology, 8(2): 95-101. https://doi.org/10.1016/S1382-6689(99)00037-X

[54] Aksu, Z., Sag, Y., Kutsal, T. (1992). The biosorpnon of copperod by C. vulgaris and Z. ramigera. Environmental Technology, 13(6): 579-586. https://doi.org/10.1080/09593339209385186

[55] Yu, Q., Kaewsarn, P. (1999). Binary adsorption of copper (II) and cadmium (II) from aqueous solutions by biomass of marine alga Durvillaea potatorum. Separation Science and Technology, 34(8): 1595-1605. https://doi.org/10.1080/01496399909353759

[56] Kosaric, N., Nguyen, H.T., Bergougnou, M.A. (1974). Growth of Spirulina maxima algae in effluents from

secondary wastewater treatment plants. Biotechnology and Bioengineering, 16(7): 881-896. https://doi.org/10.1002/bit.260160703

[57] Benemann, J.R., Tillett, D.M., Weissman, J.C. (1987). Microalgae biotechnology. Trends in Biotechnology, 5(2): 47-53.

[58] Benemann, J.R., Van Olst, J.C., Massingill, M.J., Carlberg, J.A., Weissman, J.C., Brune, D.E. (2003). The controlled eutrophication process: using microalgae for CO2 utilization and agricultural fertilizer recycling. Greenhouse gas control technologies-6th international conference, 2(2003): 1433-1438. https://doi.org/10.1016/B978-008044276-1/50227-0

[59] Chu, W.L. (2012). Biotechnological applications of microalgae. IeJSME, 6(1): S24-S37.

[60] Dominic, V.J., Murali, S., Nisha, M.C. (2009). Phycoremediation efficiency of three micro algae Chlorella vulgaris, Synechocystis salina and Gloeocapsa gelatinosa. SB Acad Rev, 16(1): 138-146.

[61] Gerbens-Leenes, P.W., Xu, L., De Vries, G.J., Hoekstra, A.Y. (2014). The blue water footprint and land use of biofuels from algae. Water resources research, 50(11): 8549-8563.

[62] Grima, E.M., Acién Fernández, F.G., Robles Medina, A. (2013). Downstream processing of cell mass and products. Handbook of microalgal culture: applied phycology and biotechnology, 267-309.