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Review Article

Advances in Synthesis and Applications of Microalgal Nanoparticles for Wastewater Treatment

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Rapid industrialization, economic development, and population overgrowth are the major reasons responsible for the release of organic and inorganic substances into the environment, further leading to environmental pollution and contamination of water. Nowadays, it is truism that wastewater treatment has raised concern worldwide and is the need of the hour. Therefore, it is necessary to conserve sustainable energy and adopt advanced wastewater treatment technologies. Microalgae culture is gaining tremendous attention as it provides a combined benefit of treating wastewater as a growth medium and algae biomass production which can be used for several livestock purposes. Microalgae are ubiquitous and extremely diverse microorganisms which can accumulate toxic contaminants and heavy metals from wastewater, making them superior contender to become a powerful nanofactory. Furthermore, they are versatile, relatively convenient, and easy to handle, along with various other advantages such as synthesis can be performed at low temperature with greater energy efficiency, less toxicity, and low risk to the environment. Comparing with other organisms such as fungi, yeast, and bacteria, microalgae are equally important organisms in the synthesis of nanoparticles; therefore, the study of algae-mediated biosynthesis of nanometals can be taken towards a newer branch and it has been termed as phytonanotechnology. Here, an overview of recent advances in wastewater treatment processes through an amalgamation of nanoparticles and microalgae is provided.

1. Introduction

Water pollution is rising at an alarming rate mainly caused by waste generated through manmade activities including domestic, industrial, and agricultural wastes which are being discharged directly into the water bodies. Uncontrolled discharge of nutrient-rich waste poses serious eutrophication threat. Eutrophication occurs due to excessive presence of nutrients in the water body, further, which is responsible for the dense growth of plants and algae. Due to spread of huge range of contaminants, water resources are facing severe threat despite recognition of sustainable water management strategies all over the world. Therefore, it is imperative to explore alternative advanced technologies for wastewater treatment and ensure that appropriate treatment standards are being followed to meet the local conditions. In wastewater treatment system, the removal of principle pollutants such as suspended solids, biochemical oxygen demand (BOD), nutrients (organic and inorganic), toxicity, and coliform bacteria is the main goal to get purified wastewater [1–6]. A conventional wastewater system includes the removal of dissolved organic matter and suspended solids by sedimentation process. The preliminary treatment of sewage removes 60 percent of large solid materials through a well-designed sedimentation tank and approximately 35 percent of BOD delivered by sewers responsible for obstructing the flow through the plant or damage equipment. Materials such as heavier grit particles, rags, fecal materials, and woods can be removed by passing the sewage through screen bars. The secondary treatment process aims to reduce 85 percent of suspended solids and BOD exerted by reducing organic matter [2]. This can be primarily achieved by a mixed population of heterotrophic bacteria that have the capability of utilizing the organic constituent for energy and growth. Fixed film and suspended growth reactors are some of the operation system for secondary wastewater treatment. Tertiary treatment processes aim to remove 95 percent of the organic ions. It can be accomplished either biologically or chemically, but it is a quite expensive process. Advanced treatment based on technologically complex techniques includes chemical precipitation, reverse osmosis, carbon adsorption, or ozonation. These techniques remove nutrients, such as phosphorus or nitrogen, which can stimulate eutrophication in surface water. For small scale operations, systems such as land application, filtration, and lagoon storage are used to remove fine particles. Several primary and secondary treatment plants have been introduced in number of places to eliminate settled materials and to oxidize the organic material from wastewater. Also, even after tertiary treatment, 100% removal of incoming waste load cannot be expected and as a result, many organisms remain in the water bodies [3].

2. Microalgal Wastewater Treatment System

Several investigations are in process to provide effective, productive, and viable solutions for the development of reduction in pollutants, maintenance of natural resources, and processing of waste products. The polluted seas, rivers, and lakes are aesthetically displeasing by manmade activities which importantly are a public health hazard since they harbored toxic contaminants and human pathogens and increased the risk of spreading excreta-related diseases through the waterborne route [3]. Carbon dioxide emission is the main pollutant responsible for global warming. Several carbon dioxide sequestration techniques have been developed globally. Therefore, serious interests in natural means for wastewater treatment have reemerged. Over the past few years, the use of aquaculture systems as engineered systems in wastewater either domestic or industrial treatment and recycling has increased immensely. Aquatic treatment systems consist of shallow ponds (one or more) in which one or more species of water-tolerant vascular plants such as duckweed or water hyacinth are grown. Water hyacinth systems are capable of removing nitrogen, high levels of BOD, refractory trace organic matter, and suspended solids (SSs). Marine algae are the most diversified species on the earth's surface, and still many of their species are unexplored. They are responsible for producing more than half of the oxygen present on the earth. The term microalgae belongs to various algal divisions such as *Charophyta, Bacillariophyta*, and *Chlorophyta* and Kingdoms, including Plantae, Protozoa, and Chromista [4–6]. They are too small to be seen properly without microscope and are mostly eukaryotes except for prokaryotic cyanobacteria. The photosynthetic rate of microalgae is much faster in comparison with that of other terrestrial plants (Figure 1). Microalgae offer a cost-effective approach for removing inorganic nutrients and contaminants from wastewater while producing valuable biomass (phospholipids and carotenoids).

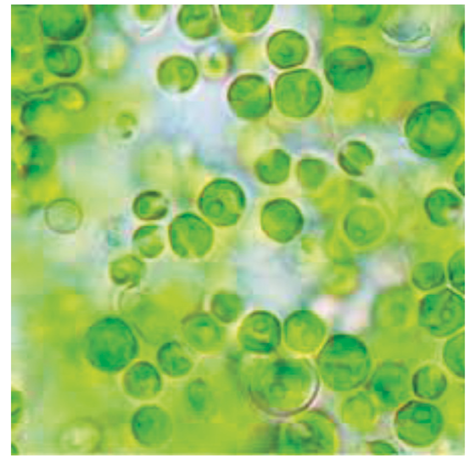

Figure 1: Microalgae (*Cholera vulgaris*).

Biotreatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses of high calorific values and incorporating nutrients such as nitrogen and phosphorus responsible for eutrophication. This process is known as phycoremediation. The algal biomass can be used for composting, animal feed, methane production, and production of liquid fuels and fine chemicals. In 2016, the MEGA-BIO program was launched by United States Department of Energy to investigate algae as a biofuel platform. The algal systems can treat human sewage, livestock wastes, agro-industrial wastes, and industrial wastes. Also, microalgal systems for the treatment of other wastes such as piggery effluent, the effluent from food processing factories, and other agricultural wastes have been studied [6, 7]. Certain factors responsible for successful microalgal wastewater treatment include abiotic factors such as temperature, light, pH, oxygen, carbon dioxide, salinity; biotic factors such as competition among pathogens; operational factors such as dilution rate, mixing, depth, and harvesting frequency; and inhibitory substances such as ammonia [6].

2.1. Light. Microalgae require light as an energy source for its growth and maintenance. They synthesize food by converting light energy into chemical energy. 90% of the light energy is dissipated as heat. In certain parts of the world, artificial light is used for microalgal growth as it is hard to rely on sunlight in those regions. Furthermore, depth of cultivation tank and dense microalgal culture also affect light availability.

2.2. Temperature. Optimum temperature should be maintained for algal growth. Larger variation in temperature can have a negative impact on microalgae growth cultures.

2.3. Aeration and Mixing. They are important factors for wastewater treatment, resulting in aerobic degradation of organic matter. Aeration serves following purposes including respiration of microorganisms and acts as a source of carbon dioxide and mixing. Mixing provides good distribution of the nutrients, improves the mass transfer, limits the formation of the anaerobic zone, and prevents the light limitation.

2.4. pH. pH has a greater influence on metabolism of microalgae. It plays a vital role in determining biochemical composition of the wastewater. pH affects solubility of mineral salts, CO_2, and O_2 in the wastewater. A high pH level causes reduction in BOD, disinfection, ammonia stripping, and precipitation of phosphate and other metals.

2.5. Inhibitory Substances. In wastewater, there are numerous microorganisms competing for their survival. Some of them produce inhibitory substances which can affect the growth of other microorganisms. Microalgae are highly sensitive and can be infected by different organisms like protozoa and viruses; therefore, they can be used as sensitive indicators in toxicity tests. Infection of microalgae can be controlled by acidification of the wastewater responsible for killing protozoa and rotifers, exposure of wastewater to high concentration of ammonia, and using a microalgal culture system having a short duration of anaerobic stages.

Significant concentrations of heavy metals and toxic organic compounds have been measured in municipal wastewater. Subsequently, the ability of wastewater treatment systems to tolerate and remove toxicity is of considerable importance. Microalgae are efficient absorbers of heavy metals, uptake carbon dioxide, provide oxygen, and can boost biogas generation from the produced biomass [8–10]. Bioaccumulation of metals by algae may create a feasible method for remediating wastewater contaminated with metals [11, 12]. Some of the alternate algae culture and wastewater treatment systems include the following.

2.5.1. Hyperconcentrated Culture System. Hyperconcentrated cultures consist of algal biomass >1.5 g/l. The algae cultures are either concentrated by flocculation or can be settled by using a flocculent (chitosan). It has been demonstrated that they can accelerate the removal of inorganic nutrients compared to normal algal cultures. Advantages associated with hyperconcentrated cultures are reduced residence time and requirement of a small land area.

2.5.2. Immobilized Cell System. Immobilization of microalgae can overcome the problem of harvesting and recovery of algal biomass from the treated effluent. In comparison with suspended cells, immobilized living cells on a suitable support can simplify the treatment process as it contributes to increased cell retention time in the reactor. A hollow fiber-immobilized cyanobacterial system has been reported for high rates of hydrogen production and for the removal of inorganic nutrients from wastewater.

2.5.3. Tubular Photobioreactors. These reactors consist of closed, clear tubes inside which algae are grown. These tubes can be placed either flat on the ground or can be arranged as long vertical rows (Figure 2). Circulation of algae is achieved by means of a pump (centrifugal, peristaltic, diaphragm, or lobe), and these photobioreactors consists of a gas exchange unit, through which carbon dioxide can be added and oxygen produced can be stripped from the system. Various parameters are taken under consideration such as control of oxygen and carbon dioxide, circulation speed, temperature control, and growth of algae in the inner surface of tubes [13].

2.5.4. Waste Stabilization Ponds (WSPs). WSPs consist of anaerobic, maturation, and facultative ponds arranged in single or several parallel series. They comprise large and shallow basins in which treatment of raw sewage is achieved by means of natural resources (mainly sunlight). They proved to be the most cost-effective, easily operated, and reliable method to treat wastewater [5]. Hopefully in the coming future, WSPs can lead to a more ecologically sustainable wastewater treatment system. Biochemical oxygen demand (BOD) removal is performed in anaerobic and facultative ponds by the sedimentation process and through aerobic bacteria, respectively. Maturation ponds are used to remove fecal coliform and excreted pathogens due to the presence of diverse algal population (Figure 3).

3. Diatoms

Diatoms are ubiquitous, widespread, single-celled eukaryotic microalgae, thus making them an ideal organism for huge range of applications including environmental indication, oil exploration, and forensic examination [14]. According to the ongoing research, diatoms have a wide range of applications in both nanotechnology and biotechnology fields such as chemo- and biosensing, analyzing ecological problems, nanofabrication techniques, immunodiagnostics, particle sorting, drug delivery, and eutrophication. They are responsible for consuming anthropogenic carbon emissions. Diatoms due to their physiological features and biological aspects can be used for waste degradation. Being sensitive to environmental stress, they can easily detect the degree of water contaminants [15, 16]. They are the dominant group of phytoplankton responsible for the removal of nutrients and dissolved oxygen matter present in the water bodies. Diatoms algae have been proved to be cost-effective for wastewater treatment which are the best food for Zooplankton. The major obstacle is to accelerate diatom growth, for which there is requirement of silica. Nualgi invented by T Sampath Kumar in 2004 is an innovative product which uses nanosilica as a micronutrient along with iron and nine trace metals (Mg, Co, B, Ca, Mn, Zn, Mo, etc.) to trigger the growth of diatoms [17–19]. Only 1 Kg of nualgi is needed to remove 400–1400 Kg of carbon from water and replacing it with oxygen achieved by the growth of diatoms. Furthermore, they are consumed by small fishes, thus restoring food chain in the water. Due to its nanosize, nualgi has the capability to remain suspended in water, further increasing the bioavailability of nutrients to microalgae, and can invade extremely small spaces in the subsurface. In comparison with

Figure 2: Photobioreactor [13].

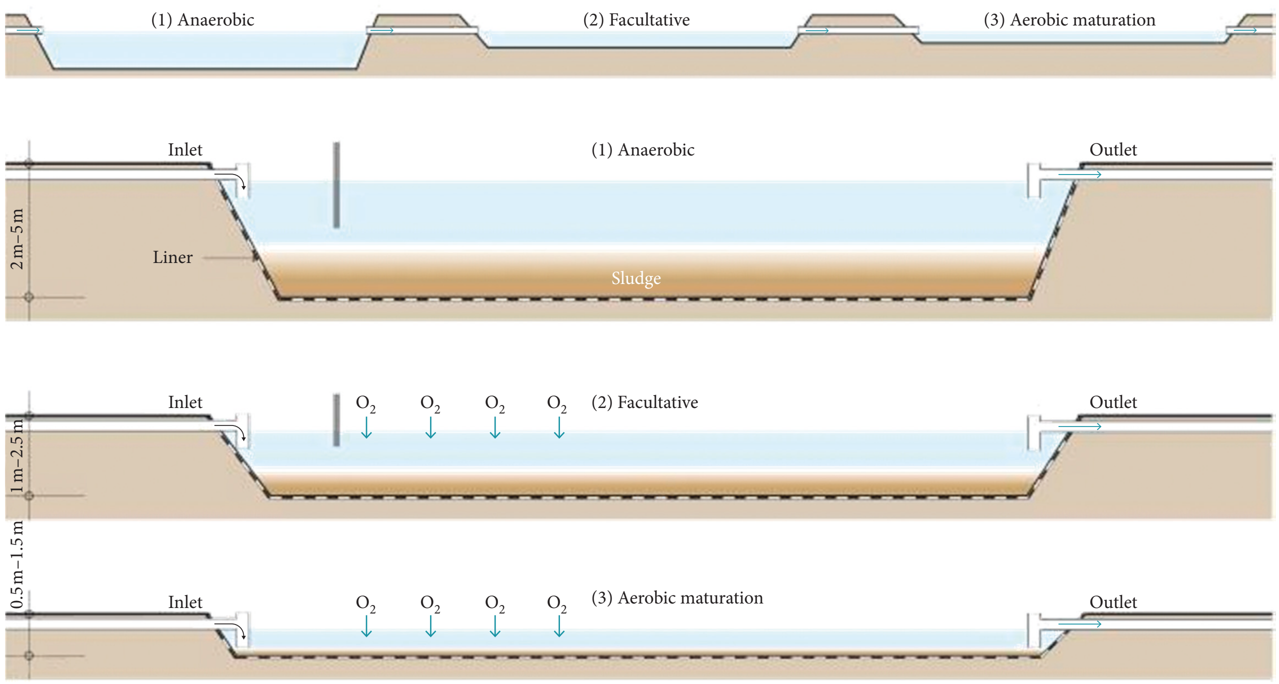

Figure 3: Waste stabilization ponds.

other methods, nualgi is harmless and do not require electricity to treat wastewater [20].

4. Microalgal Nanoparticles

Some of the major limitations associated with the microalgal culture system are highly time-consuming, tedious task, harvesting of microalgae, and extensive use of arable land. To avert these consequences, microalgal nanoparticles tend to be an innovative research area for enhanced removal efficiency. Nanotechnology refers to an enabling technology that studies nanometer sized objects to develop, use, and understand material structures, systems, and devices with fundamentally novel properties and roles resulting from their small structure. The efficient physiochemical and crystallographic properties of nanoparticles make nanotechnology an outstanding area to focus. This has driven research towards the use of nanoparticles as they have some unusual thermal, electronic, biological, optical, chemical, and physical properties compared to their bulk scale counterparts [20, 21]. Nanoparticles can be used for diverse purposes such as for medical treatment, in solar and fuel cells for efficient energy production, and in water and air filters to reduce pollution, and they can also be used as catalysts in current manufacturing processes to eliminate the use of toxic materials. Conventionally and most commonly used methods to synthesize nanoparticles are wet methods (physical and chemical). These methods are categorized under two approaches—top down and bottom up approaches (Figure 4). Chemically, nanoparticles are synthesized by growing in a liquid medium containing reducing agents and stabilizing agents such as potassium bitartrate, sodium

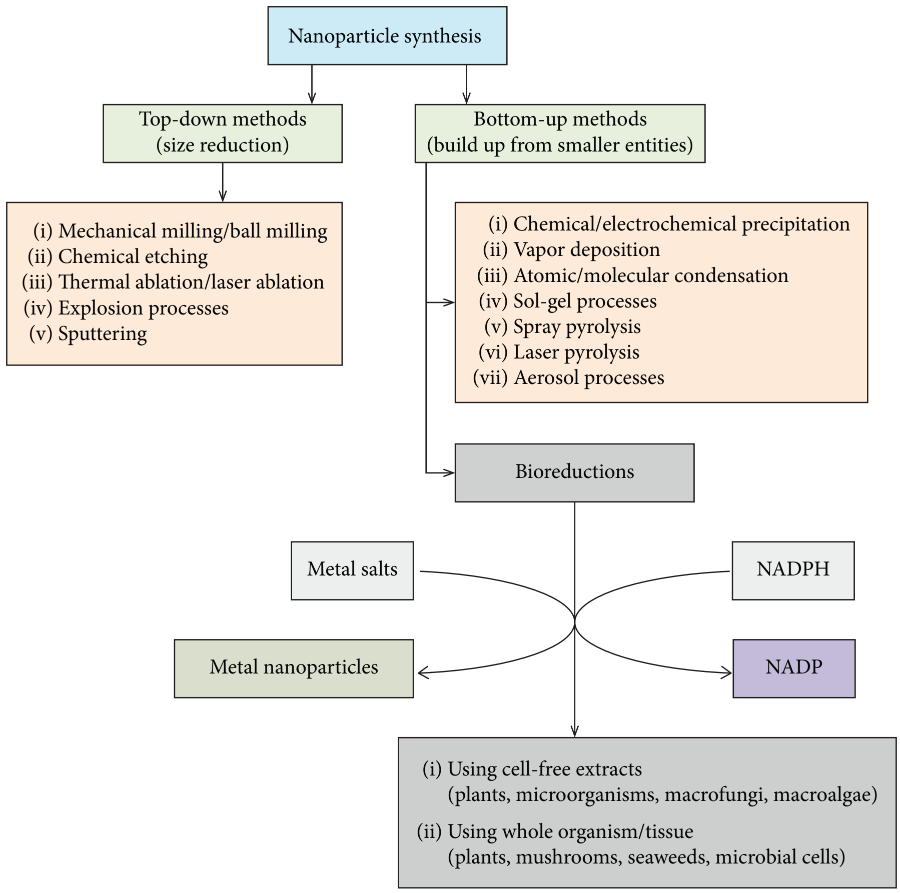

Figure 4: Production of nanoparticles [24].

dodecyl benzyl sulfate, methoxypolyethylene glycol, polyvinyl pyrrolidone, or sodium borohydride. Furthermore, physical methods consist of pyrolysis and attrition. Unfortunately, physical and chemical methods used in both approaches have some consequences due to their negative environmental impact, laborious production technique, and unaffordable cost. Research has shown that due to high surface energies, nanoparticles attract atoms and molecules, further changing their surface properties [22, 23]. Thus, they are unable to exist in the environmental surroundings in their naked form. Because of these environmental interactions, nanoparticles are not suitable for therapeutic use. This mechanism of action has led to awareness spreading with respect to developing nontoxic and ecofriendly procedures for the assembly and synthesis of nanoparticles.

Nanoparticle synthesis through a biosynthetic route (via microorganisms or plant extracts) is a much simpler technique in comparison with physical and chemical methods and has attracted considerable interest all over the world [23–25]. Microorganisms such as fungi, bacteria, and yeast play vital role in remediation of metal ions. Among all biological systems, microalgae have gained lot of interest since they can bioremediate toxic metals, further converting them to more amenable forms. Microalgae possess photosynthetic capabilities, converting solar energy and carbon dioxide into useful biomasses and accumulating nutrients such as nitrogen and phosphorus responsible for eutrophication of water bodies [26]. Numerous methodologies have been developed to synthesize metallic nanoparticles using microalgae from their corresponding aqueous salt solutions [27]. This could modify the size and shape of nanocrystals with superior quality (Figure 5). There are four ways to synthesize microalgae nanoparticles:

(i) Direct exploitation of extracted biomolecules from disrupted cells of microalgae
(ii) Exploitation of cell-free supernatants made of microalgae culture media
(iii) Biosynthesis of nanoparticles of different natures from whole cells of microalgae
(iv) Using living cells of microalgae

Several microalgal species have been exploited for the biosynthesis of nanomaterials by using their extracted biomolecules [29, 30]. To obtain gold nanoplates, the microalgal biomass is first lyophilized and then it is subjected to RP-HPLC, i.e., reverse-phase high performance liquid chromatography, until the gold shape-directing protein (GSP), which is responsible for directing the shape of nanoparticles, is isolated. Furthermore, this protein is brought into the contact of HAuCl4 aqueous solution, thus producing gold nanoparticles of different shapes. In case of silver nanoparticles, PLW (proteins of low molecular weight) and PHW (proteins of high molecular weight)

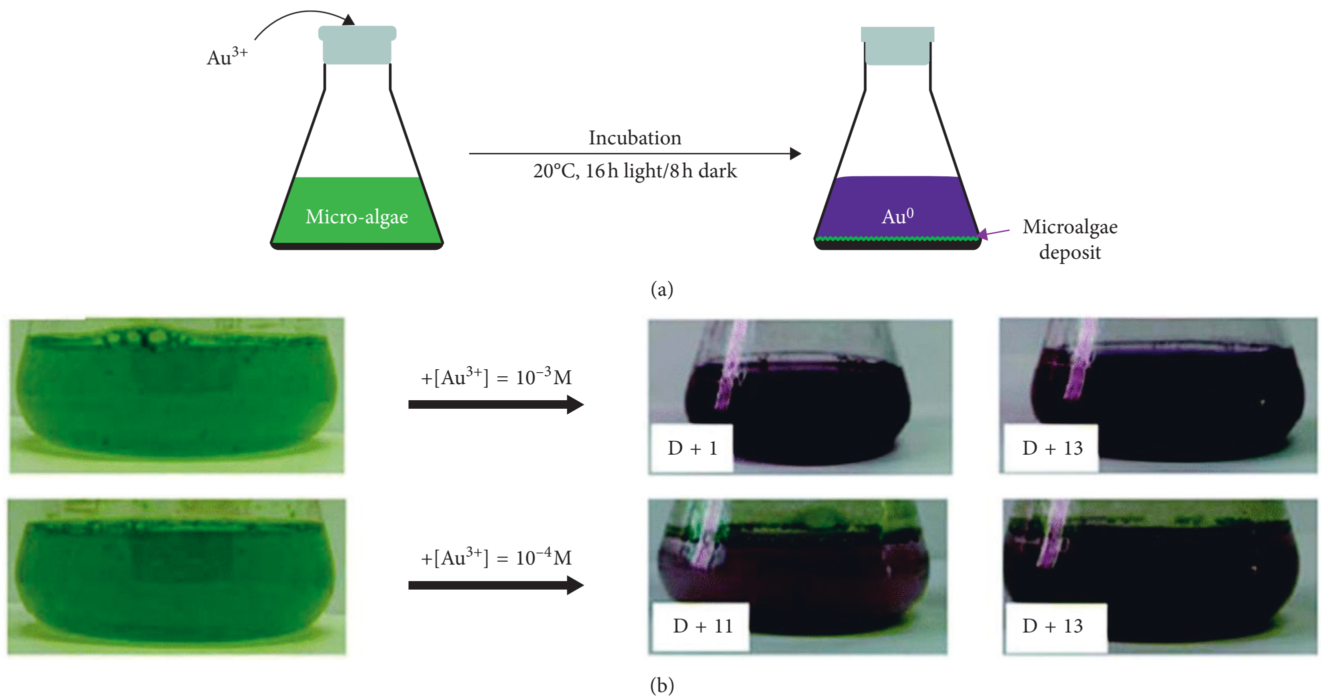

Figure 5: Algae-mediated biosynthesis of nanoparticles [28].

contained in the biomass of microalgae are responsible for reducing silver ions into their metallic counterpart. Tang et al. used a fine powder of *Spirogyra insignis* (Charophyta) for the biosynthesis of silver and gold nanoparticles [31]. Patel et al. synthesized silver nanoparticles by exploiting cell-free supernatants of cyanobacteria and chlorophyta species [30]. Harvesting whole cells of microalgae is a much easier method for the biosynthesis of metallic nanoparticles. For sustainable and huge amount of production, microalgae are one of the best biological resources for designing photobioreactors.

Microalgal live cells are used to synthesize metallic nanoparticles through a one-step procedure comprising of an aqueous solution of metallic salts which is to be added directly to the cells maintained under their culturing conditions. After the nanoparticles are synthesized, they are released into the culture medium wrapped within the matrix which is further responsible for forming colloids. The latter settles down in the photobioreactor due to its heavy weight. If desired, repetitive cycles for nanoparticle biosynthesis can be performed by adding a fresh culture medium. Moreover, microalgae when entrapped within organic vesicles maintained their nanoparticle biosynthetic capabilities. Merin et al., Mohseniazar et al., Dahoumane et al., Dahoumane et al. and Rosken et al. reported the biosynthesis of silver nanoparticles from various microalgal species such as *Chlorophyta, Haptophyta*, and *Ochrophyta* [25, 32–34]. Variety of analytical techniques are used to characterize the morphology of metal nanoparticles such as atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), UV–Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), powder X-ray diffractometry (XRD), and X-ray photoelectron spectroscopy (XPS). Mahdieha et al. synthesized silver nanoparticles by using live biomass of *Spirulina platensis* which was further confirmed through the X-ray diffraction spectrum [35]. Luangpipat et al. [36] described an efficient biological route for intracellular production of gold nanoparticles in *Cholera vulgaris* which were recognized by transmission electron microscopy (TEM). They incubate *Cholera vulgaris* with a solution of gold chloride which were harvested by centrifugation. Synchrotron based X-ray absorption spectroscopy and X-ray powder diffraction further confirmed these nanoparticles to be metallic gold [37].

Synthesis of nanoparticles is related to algal strain and is dose dependent. Several biomolecules such as polysaccharides, pigments, and peptides are responsible for the reduction of metal ions. Proteins (amino group or cysteine residues) and sulfated polysaccharides are used to stabilize and cap the metal nanoparticles in aqueous solutions. In comparison with other biosynthesizing methods, synthesis of algal nanoparticles takes comparatively shorter time. Microalgae can be considered as a powerful nanofactory, capable of producing nanoparticles not only of silver ions but also of other metal ions such as gold, cadmium, and platinum [20]. Metallic nanoparticles can be synthesized by both live and died dried biomasses. Algae such as *Chlorella vulgaris, Spirulina platensis*, and *Lyngbya majuscule* have been reported for the efficient biosynthesis of silver nanoparticles [29].

5. Hybrid of Nanobiomaterials

Nanofibers such as porous nanofibers, 2-dimensional nanofibers/nets, bioinspired nanofibers, and cable-like nanofibers can be fabricated by special electrospinning methods [35]. Due to their unique physical, biological, chemical, or optical properties, they exhibit great potential for various diverse applications ranging from superhydrophobic materials, quartz crystal microbalance-based sensors, filtering materials, layer-by-layer template materials, colorimetric sensors, dye-sensitised solar cells, and so on [38–40]. Their large porosity, adjustable functionality, and high surface-to-volume ratio have proved to be effective in liquid filtration and particulate separation when compared to conventional polymeric and nonwoven membranes. Electrospinning has the unique ability for generating ultrathin fibers of different materials including synthetic and natural polymers, ceramics, polymers loaded with nanoparticles, and polymer alloys [41]. Nonwoven electrospun nanofiber meshes are a promising approach for membrane preparation, especially for environmental engineering and biotechnology applications. Biomacromolecules or ligand molecules can be hybridized with nanofiber membrane and thereby can be used for numerous applications including protein purification, enzymatic catalysis or synthesis, chemical analysis and diagnostics, and wastewater treatment [42–44]. At a pilot scale, electrospun nanofiber membranes have shown to remove particulates in wastewater. They have been tested as affinity membranes, prepared by immobilizing a specific capturing agent for a specific target molecule on its membrane surface. Pan et al. (2017) investigated the synergistic effect of algae and TiO2/Ag nanomaterial for the photoremoval of Cr(IV) under UV irradiation [45]. Keskin et al. developed polysulfone nanofibrous web by an electrospinning technique on which microalgae *Chlamydomona reinhardtii* were immobilized for the removal of reactive dyes from water [46]. Eroglu et al. demonstrated nitrate removal from liquid effluents by immobilizing microalgal cells on electrospun nanofiber mats. In the future, they can play a crucial role in environmental industry for removing organic waste and heavy metals from wastewater [47].

Nanomaterials such as magnetic nanoparticles offer various advantages including controlled size and an enhancement of contrast in magnetic resonance imaging, and they can be manipulated externally. Because of their potential benefits in biology and medicine field such as drug delivery, medical imaging, and protein purification, researchers have designed multifunctional magnetic nanoparticles. Liu et al. synthesized magnetic chitosan nanocomposites to remove heavy metal ions from wastewater. Due to their exceptional properties, they can be easily removed from the water with the help of an external magnet [48]. Fakhrullin et al. reported a single-step technique to functionalize live unicellular *Chlorella pyrenoidosa* algal cells with biocompatible polyelectrolyte (polyallylamine hydrochloride) stabilized with supermagnetic nanoparticles for the development of bioelectronic devices and biosensors [49]. For algal synthesis of nanomaterials on large scale, there are certain parameters that should be taken under consideration such as the effect of environmental conditions on the final form of nanoparticles, kinetics, and mechanisms for nanoparticle formation (pH, strain selection, mean light intensity, ionic strength, etc.) and biological transport of metal cations.

6. Applications of Microalgal Nanoparticles

According to some recent studies, fresh water algae like *Scenedesmus quadricauda, Chlorella vulgaris, Selenastrum capricornutum*, and *Scenedesmus platydiscus* are capable of accumulating and degrading polycyclic aromatic hydrocarbons. Algal systems have conventionally been employed as a tertiary wastewater treatment process. They have also been proposed as a potential secondary treatment system. Algae can be used in treatment processes for a range of purposes, some of which are used for the removal of coliform bacteria, reduction in both chemical and biochemical oxygen demand, removal of N and/or P, and the removal of heavy metals [9]. Biomedical applications of algal nanoparticles comprise wound healing and antifungal, anticancer, and antibacterial activities. It has been reported that metallic nanoparticles (gold, silver, and copper oxide) synthesized using *Bifurcaria bifurcate, Galaxaura elongate, Sargassum plagiophyllum, Caulerpa racemose, Microcoleus* sp., and *Chlorococcum humicola* have antibacterial activity against Gram-positive and Gram-negative bacteria. Diatoms absorb global carbon dioxide and release oxygen, thereby increasing the dissolved oxygen (DO) level in the aquatic bodies. This can lead to the improvement in biodiversity, aquatic life, and water quality. In the future, algal nanoparticles can be used as antibiofilm agents against multidrug-resistant bacteria as they are capable of penetrating extrapolysaccharide (EPS) and cell membranes. Moreover, they can be further explored in nanocomposites and biosensing applications.

7. Conclusion

The integration of nanotechnology into biotechnology has created a new science nanobiotechnology. Era of nanobiotechnology is a prominent gift for the development of science worldwide. This new technology corresponds to the current scientific urge for improving the existing strategies for nanoparticle biosynthesis and inventing new ones. Biogenic synthesis of nanoparticles can reduce environmental contaminations and decrease human health hazards resulting from currently used conventional manufacturing processes. Algae can be used in wastewater treatment for a range of purposes, including reduction of BOD, removal of N and/or P, inhibition of coliforms, and removal of heavy metals. Hence, microalgal treatment of wastewater, through biological and physicochemical mechanisms, could represent an attractive addition to existing biological treatment used to purify wastewater. It has already been demonstrated that microalgae have the capability to accumulate intracellular concentrations lipids and carotenoids. Currently, research is going on to produce recombinant proteins from algae. More research is needed not only to identify the compounds responsible but also to better understand the mechanism of nanoparticle formation by microalgae. As the field is quite new, there are various aspects about the biotechnological potential of green-synthesized nanoparticles which are yet to be clarified. It would be desirable to develop a technology in which the specific size and shape of the particles could be obtained using a specific strain of algae and cyanobacteria.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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