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Review Article

Microalgal Remediation of Sewage Effluent

NIRBHAY KUMAR SINGH[1] and DOLLY WATTAL DHAR[2]
[1]*Department of Microbiology, C.P. College of Agriculture, S.D.A.U., S.K. Nagar, Gujarat 385 506, India*
[2]*Department of Microbiology, Indian Agricultural Research Institute (IARI), New Delhi 110 012, India*

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Microalgae (a term used for cyanobacteria and unicellular algae) are autotrophic microorganisms having extremely high photosynthetic efficiency and valued as rich sources of food items, feeds, high-value oils, chemicals, pharmaceuticals and pigments. Beside nutrients like nitrogen and phosphorous, the sewage water and sediments show terrible levels of organic matter, pathogens, fertilizers, pesticides and heavy metals. Cadmium, zinc, copper and nickel are probably the most toxic elements normally found in large amounts in sewage sludge. Once they are released into the environment, it becomes difficult to remove them by physical and/or chemical means. Use of microalgae for treatment of sewage waters have been in practice for a long period of time. They have been shown to be highly effective in uptake of nutrients like N and P and as accumulators and degraders of different kinds of environmental pollutants, including xenobiotic compounds. Several algae-based systems for wastewater treatment have been used for the studies regarding wastewater treatments. A great deal of interest has recently gained momentum in using microalgae for remediation of wastewaters because; in one hand they increase the O_2 content of waters *via* photosynthesis while on the other they remediate some heavy metals by biosorption, alkaline precipitation and production of metallothioneins, chelatins and polysaccharides. Moreover, microalgal communities play important roles in affecting the biochemical oxygen demand, chemical oxygen demand, turbidity, inorganic nutrients and pathogens (coliform bacteria); and at certain densities they keep the aerobic phase of the facultative ponds functioning.

Key Words: Heavy Metals; Microalgae; Nitrogen; Phosphorous; Sewage; Waste Water

1. Introduction

Microalgae are microscopic and photoautotrophic cell factories, have high surface area-to-volume ratio, capable of rapid uptake of nutrients and CO_2, possess much faster cell growth, have much higher photosynthetic efficiency and are considered to be the primary synthesizers of organic carbon in aquatic habitats. Since ancient time, microalgae have been used in human health food products [1], feeds for fish and livestock [2], high-value oils [3, 4, 5], chemicals, pharmaceutical products [6, 7] and pigments [7].

Sewage effluents often contain wastes from industries which contaminate the sludge with pollutants particularly, heavy metals, that are toxic if present in large concentrations. A large proportion of sewage effluent is dumped into the aquatic environment without proper treatment which deteriorates its aesthetic value. Besides heavy metals, sewage effluent is reported to contain high amounts of nutrients (particularly N and P), high levels of biodegradable organic matter, fertilizers, pesticides and bad odor [8, 9]. Untreated sewage not only causes eutrophication, but is also a major health hazard because of the likely presence of various kinds of pathogens [10]. The danger is serious when domestic and industrial wastes are mixed. In some cases the level of pollutants get amplified with time which ultimately lead to shift of bacterial and algal populations toward more resistant species such as the planktonic cyanobacteria that dominate the lake water, especially in the warm seasons. These species can be characterized by their greater ability to tolerate such high levels of pollutants and their efficiency for degrading highly persistent organic contaminants

**Author for Correspondence: E-mail : nirbhaysingh78@gmail.com*

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[9, 10] as well as accumulating heavy metals [11]. Therefore, they could efficiently be used in advanced technologies for bioremediation of the sewage effluent.

The wastewater treatment by microalgal cultures not generates additional pollution when the biomass is harvested. At the same time, it allows efficient recycling of nutrients [12]. The use of several microalgae including cyanobacteria in wastewater treatment is known for many years [13, 14, 15]. *Chlorella vulgaris, Scenedesmus dimorphus, Nostoc muscorum, Anabaena variabilis, Plectonema, Oscillatoria, Phormidium* and *Spirulina* are some of the microalgal strains that present a good option for biological treatment of agro-industrial wastewater [16, 17]. The selected environmental cyanobacterial species perform efficiently in suspension culture. Their application toward the removal of organic (BOD, COD, fats, oils and grease) and inorganic (Zn and Cu) as well as physical contaminants (solids; suspended and dissolved) from mixed domestic–industrial wastewater in a relatively short duration is well documented. Besides removing pollutants, the cyanobacteria have the ability to easily acclimatize to extreme environments and produce many valuable compounds [7].

Some waste contaminants (including heavy metals and salts) cannot be converted to harmless materials and will remain in the environment permanently, but many other contaminants (such as organic chemicals and pathogens) can be degraded to harmless components at varying rates [18]. Increasing human population, upcoming industries and townships and release of untreated sewage in water bodies may lead to increase of eutrophication in the aquatic ecosystems [19]. Removal of ions by traditional methods is highly expensive and is an energy consuming effort. However, use of biological systems serves as a cheap and efficient way of nutrient and heavy metals removal from wastewaters.

Microalgae have received more attention in recent years, especially in the tropical and subtropical regions as an alternative bio-system for wastewater treatment [12, 20, 21]. Algal systems have traditionally been employed as a tertiary treatment process [15, 22] and have recently been proposed as a potential secondary treatment system [23]. One of the major problems in using microalgae for wastewater treatment is their recovery from the treated effluent [12, 24]. Immobilization technology, which entraps the microalgal cells into a matrix, solves the harvest problems to a considerable limit [25]. This technology offers a greater degree of operational flexibility and easy separation [26]. Many authors have reported higher nutrient removal efficiency in the immobilized algal biomass than the freely suspended cells of the same species [27, 28].

Selection of new biological wastewater treatment systems depend largely on the necessities of the human communities and cost competitiveness of the systems. All the microalgal strains are not equally effective for wastewater treatment. Their usefulness in wastewater treatment depends mainly on two major parameters: (i) the capacity of the microalgal strains to grow under the prevailing environmental conditions and (ii) its efficiency to remove the pollutants. The operation and efficiency of the wastewater treatment plants located in the areas with extreme environmental conditions (very cold/very hot and low insolation/high insolation), depends upon the choice of the microorganisms that can proliferate under the specific harsh environmental conditions [29, 30]. Therefore, microalgal strains destined for remediation must pass the scrutiny of adaptability to the local wastewater scheme [31]. However, adaptation of microalgae to various growth regimes often requires varying acclimatisation periods [32, 33, 34].

2. Advantages over Other Microorganisms

Recently, there has been increasing interest in utilising microalgae as pollution control agents than other microorganisms isolated from the soil. It also has an edge over the conventional systems of wastewaters treatment. The photoautotrophic nature and ability of some species to fix atmospheric nitrogen enable microalgae to be producers, as opposed to consumers, and make their growth and maintenance inexpensive [35]. Microalgae are also known to inhabit heavily polluted environments where, they are widely distributed and dominate the micro floral populations [36, 37, 38]. They acquire natural resistance and selectivity against environmental pollutants due to their presence in the polluted systems. However, their viability and metabolic activities are not affected by decrease in the levels of biodegradable pollutants that they may break down. They have been regarded as highly

effective accumulators and degraders of different kinds of environmental pollutants including pesticides [9, 10], crude oil [39], naphthalene [40], phenanthrene [41], phenol and catechol [42, 43] and xenobiotics [44].

They have been efficiently used as a low-cost method for remediating dairy wastewater [45], dissolved inorganic nutrients from fish farms [46] and nutrients (N and P) from sewage effluents [30, 47, 48, 49, 50]. Moreover, they have been proved to be a more cost effective way to remove biochemical oxygen demand, pathogens, phosphorus and nitrogen than activated sludge [17, 51, 52]. Traditional wastewater treatment processes involve the high energy costs of mechanical aeration to provide oxygen to aerobic bacteria to consume the organic compounds in the wastewater whereas, microalgae provide an efficient way to consume nutrients and provide the aerobic bacteria with the needed oxygen through photosynthesis [53]. Roughly one kg of BOD removed in an activated sludge process requires one kWh of electricity for aeration, which produces one kg of fossil CO_2 from power generation. By contrast, one kg of BOD removed by photosynthetic oxygenation requires no energy inputs and produces enough algal biomass to generate methane that can produce one kWh of electric power [54]. They are also reported as efficient agents for the assimilation of organic matter from contaminated media [18, 55, 56] as well as transformation and removal of heavy metals [57, 58]. Moreover, some hydrogen producing cyanobacteria are being considered as an alternative energy source in the United States of America as well as in Sweden. Recently many cyanobacterial members has been fully sequenced and characterized in order to optimize their beneficial use for reclamation of wastewaters [59, 60, 61, 62, 63].

3. Algae-based Systems for Wastewater Treatment

Wastewater treatment ponds that have been used widely for studying the treatment processes and can be categorised into four main groups-facultative ponds, high-rate algal ponds, algae seedling ponds and maturation ponds. A method involving integration of different ponds in a system called 'advanced integrated wastewater ponding system (AIWPS)' has also been advocated for microalgal wastewater treatment [64, 65]. However, of the major types of algal ponds, high rate algal pond is regarded as one of the most efficient systems for wastewater treatment. Moreover, the role of high rate algal pond (HRAP) in the secondary treatment of sewage effluent has been widely studied [66].

HRAP systems were first designed for wastewater treatment by Oswald [15, 67], and thereafter were used in other parts of the world. High rate algal pond is a low-cost microalga based wastewater treatment system and is designed to achieve two goals: the secondary treatment of wastewater and the production of algal biomass. It is a coherent combination of intensified oxidation pond and algal reactor. Microalgae supply the oxygen needed for the bacterial degradation of organic matter, while bacteria supply mineral compounds to the algae for their nutrition. The HRAP is characterized by shallow depths, mechanical mixing and short residence times, implying smaller surface areas than those of conventional stabilization ponds [20].

4. Nutrients Removal from Wastewater

Cyanobacteria can assimilate a wide array of nitrogenous compounds like ammonium, nitrate, nitrite, urea, and amino acids. Diazotrophic cyanobacteria can also assimilate atmospheric nitrogen (N_2) [68]. However, the reduction of dinitrogen gas to ammonia by nitrogenases is a highly endergonic reaction requiring metabolic energy in the form of ATP. The capability of nutrient removal in the absence of organic carbon has often been used for wastewater treatment in algal pond systems [69, 70]. The cyanobacterial genera *Synechococcus*, *Merismopedia*, *Leptolyngbya*, *Limnothrix* and *Nostoc* were isolated and identified by morphological and molecular analyses from the waste stabilization pond system from Sao Paulo, Brazil. Many studies have been performed at different scales confirming the high ability for cyanobacterial species for nutrient uptake (N and P) from contaminated media and system. This was shown by *Phormidium bohneri* [71], the thermophilic cyanobacterium *P. laminosum* [72], *P. tenue* and *Oscillatoria* O-210 [47, 73] and other species [30, 47, 48, 49, 50]. In addition to the high efficiency and low cost, the removal of nutrients using cyanobacteria offers a very simple technology compared to other complicated one where, integration between chemical (coagulation) and biological method (activated sludge) is performed to achieve higher efficiencies [74].

The application of oxygenic phototrophs in the treatment of waste streams that are relatively rich in nutrients and low in organic carbon has many advantages. In a heterotrophic biofilm O_2 transfer by diffusion is limited to approximately 20 nmol $cm^{-2}min^{-1}$. However, the areal net oxygen production in an active phototrophic biofilm at a light intensity of 1,000 µmol photons $m^{-2}s^{-1}$ is approximately a hundred times higher [75]. Hence, the oxygen that is produced by phototrophs can cover a great part of the demand of bacterial nitrification and the heterotrophic consumption of organic carbon. In addition, oxygenic phototrophs assimilate nutrients for building biomass with carbon dioxide as carbon source. In contrast to wastewater treatment by bacterial nitrification and denitrification, where a large part of the nitrogen escapes as N_2 gas to the atmosphere, the nitrogenous compounds in case of phototrophic biofilm are retained in algal biomass. However, the right operational conditions for process control and system optimization parameters must be defined as it determine the efficiency of nutrient removal in an experimental phototrophic biofilm system [76].

4.1. Ammonical-N Removal

Several feasible mechanisms and pathways by which nitrogen in its various forms can be transformed and removed from wastewaters have been proposed and supported by research carried out in many parts of the world. However, there are still much debate regarding the relative importance of the various mechanisms and pathways through which, nitrogen is removed from wastewaters. A microbial candidate for wastewater treatment has to show a significant capacity to remove pollutants under the intended environmental conditions. After organic nitrogen gets hydrolyzed to ammonium; ammonia volatilization has generally been assumed to be the main nitrogen removal mechanism in wastewater stabilization pond. Moreover, ammonium is generally used as a model common pollutant of domestic wastewater [30]. This hypothesis is based on the fact that in pond high pH values (9, even 10) and temperatures can reach, thereby, improving the mass transfer rate of NH_3 to the atmosphere [77, 78, 79]. But, microalgal uptake is also considered to be an important mechanism for ammonium removal during warm summer months when pH and temperatures reach their annual maxima. However, alternate mechanism for permanent nitrogen removal by microalgal uptake of ammonium and subsequent sedimentation followed by partial retention in the pond sludge has also been proposed [80, 81]. Moreover, ammonia volatilization has been suggested to dominate nitrogen removal during summer while nitrogen removal *via* biological uptake and further sedimentation could be the dominant removal mechanism in winter [82].

Absorption of ammonium within the cells, which was later removed together with the beads where they were immobilized, has also been demonstrated [83]. At the lowest temperatures, removal of ammonium was reported to be less efficient. However, when the microalga was immobilized in alginate beads with *Azospirillum brasilense*, a technology under development for wastewater treatment [30, 84], growth of *Chlorella sorokiniana* was stimulated by ammonium and removal of ammonium increased. Microalgae in nature or in culture are frequently associated with bacteria [85]. These associations may exert positive or negative influence on growth of microalgae. Under certain conditions, the growth of microalga and removal of ammonium by *C. sorokiniana* UTEX 2805, when immobilized, improved positive interaction patterns, similar to other *Chlorella* species used to treat wastewater [30, 86].

The capacity to remove pollutants quickly and efficiently from wastewater dictates the nature of the microbial agent to be used [47]. Good biological agents should remove pollutants in short retention times of 1–4 days [30, 83]. It is also known that culturing conditions, including the level of nitrogen, affects the removal of ammonium from wastewater by *C. vulgaris* [87]. This study showed that removal of ammonium by *C. sorokiniana* UTEX 2805 is more efficient when concentrations of ammonium are higher, conditions of temperature and light intensity are extreme, and the helper immobilization matrix are present. When the intensity of light increases photosynthetic pigments in microalgae (*Scenedesmus quadricauda* and *C. sorokiniana*) also increase. This happens similar to the xanthophyll cycle pigments that participate in dissipating the excess of absorbed light in higher plants [88]. It is also known that inoculation with *Azospirillum brasilense* enhances the production of auxiliary photosynthetic pigments in wheat plants [89]. Similarly, the level of some photosynthetic pigments increased after exposure of

strains of *Chlorella vulgaris* and *Chlorella sorokiniana* cultivated under regular conditions with the added influence of *A. brasiliense* [90].

4.2. Nitrate-N Removal

Nitrate removal from wastewater may be accomplished by bacterially mediated denitrification, or chemically and physically [91]. These treatment processes, however, require input of external energy sources and/or chemical additives and generate concentrated waste streams that must be disposed. Therefore, they are often problematic and expensive [12, 92]. Since nitrate may be taken up effectively by photosynthetic microorganisms, such as cyanobacteria, which require mostly fixed nitrogen, inorganic carbon, and light for growth, the use of photosynthetic organisms would minimize the need of chemicals and fossil fuels for nitrate removal, thus leading to an efficient resource recovery and recycling.

Heterocystous cyanobacteria (*e.g. Nostoc, Anabaena, Cylindrospermum, Aulosira,* and *Scytonema*) that can fix nitrogen from the air are reported to be generally inefficient in uptake of nitrate when nitrate is present in the medium at relatively low concentrations. Moreover, many nitrogen-fixing cyanobacteria are commonly known to excrete ammonium into the medium and therefore increase the alkalinity [93]. However, the non-nitrogen-fixing cyanobacteria have capacity to efficiently remove nitrate from wastewater even though nitrate is present in the medium at relatively low concentrations. They require minimal amount of chemical additions and supply of naturally available solar energy rather than that of fossil fuel energy or organic carbon. However, few issues like (i) optimization of nitrate uptake under outdoor conditions, (ii) possible toxicity of cyanobacterial products that may be excreted, and (iii) scale-up potential for continuous-flow reactor systems; needs to be further investigated before their practical applications are considered [48].

The nitrate assimilation process is ultimately driven by light energy and photosynthesis, which provides ATP and reducing power for nitrate uptake and reduction. However, high light intensity may introduce photo-oxidative cell damage in cyanobacteria due to absorption of excess light that cannot be used productively for photosynthesis [94]. At low irradiance, photosynthesis and respiration occur at roughly the same rate, and a lack of significant nitrate uptake is not unexpected [48]. The nitrate uptake rate as a function of light intensity follows a similar pattern as the growth curve, consistent with the requirement of photosynthesis for efficient nitrate uptake [95]. Efficient and long-term nitrate uptake may readily be achieved through manipulation of both the specific growth rate and cell density. This is however different from the phenomenon observed at a higher rate of nitrate uptake on a volumetric basis associated with a higher initial cell density [96]. The conditions for the most efficient long term nitrate uptake clearly include a rapidly growing, healthy, phosphate-sufficient cyanobacterial culture [48]. Nitrate uptake can also be affected by spectral quality and light intensity [97], temperature [98], and cell density and nitrate level [22, 96], as well as physiological acclimation of the culture [22].

Nitrate is taken up by cyanobacteria, involving a common high affinity transport system consisting of NrtABCD permease (an ABC-type transporter) and to a lesser extent enters the cells by diffusion [98, 99, 100]. Inside the cell, nitrate is reduced to nitrite by nitrate reductase and nitrite to ammonium by nitrite reductase [101]. Ammonium is then incorporated into carbon skeletons mainly through the operation of the glutamine synthetase-glutamate synthase cycle [99]. Fixed nitrogen storage components, such as C-phycocyanin and/or cyanophycin, may be formed and accumulated in the cells under certain physiological conditions [102, 103]. Regulation of nitrate assimilation is controlled by several regulatory products that affect the expression of the *nrtABCD, narB* (encoding nitrate reductase), and *nirA* (encoding nitrite reductase) genes that together form the *nirA-nrtABCD-narB* operon in several cyanobacteria [101, 104]. Nitrate uptake can also be influenced by the availability of phosphate ion (PO_4^{3-}), which has an important role in cellular energetics as part of ATP and influences the activity of many enzymes required for cell metabolism, including the nitrate reduction process [101, 105].

4.3. Phosphate Removal

Wastewater generally contains a significant amount of phosphate which at many times exceeds the permissible limit [106, 107]. Extremely low phosphate level apparently limits the cyanobacterial growth in water, influencing not only the overall

growth rate but also the final cell density. The first few cell divisions after transfer to distil water probably depend mostly on the intracellular phosphate reserves that have been carried over from the artificial growth medium (such as BG-11). However, long-term growth and survival of the cells in the media require addition of phosphate. Gonzalez *et al*. [16] studied phosphorus removal efficiencies obtained by the two microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus* in a bioreactor. The removal ranged from 20% after 144 h to 55% after 216 h. However, their findings reflect lower efficiencies of phosphorus removal by *C. vulgaris* and *S. dimorphus* than those reported by Lavoie and de la Noue [22] for *S. obliquus* (75%) and de la Noue and Proulx [27] for *Phormidium* (71-90%). The removal efficiencies of phosphorus by *C. vulgaris* were compared in cylindrical and triangular bioreactors. It was observed that cylindrical bioreactor was more efficient in removing PO_4^{3-} than the triangular bioreactor [16]. PO_4^{3-} concentration was reported to reduce from 15 mg/L (influent) to approximately 3 mg/L (effluent) after 28 days of treatment resulting in sustained P removal efficiencies of 80% for approximately three weeks [108].

There are two major ways to remove phosphorus from wastewater: (i) direct cellular absorption under aerobic conditions, and (ii) sedimentation to anoxic conditions. Phosphorus removal under aerated conditions can be explained by its interaction with the nitrogen in the water keeping nitrogen as the limiting nutrient factor in the medium [16]. Exhaustion of ammonia in the medium leads to saturation in the phosphorus cellular absorption mechanisms [25]. Moreover, the high oxygenation rate leads to the absence of a sedimentation zone in the bioreactors which prevent phosphorus chelate precipitation [16].

An interesting feature of some cyanobacteria is that they can accumulate inorganic phosphorus and store it internally as polyphosphates [109]. During anoxic or aerobic conditions, the internally stored PHB is oxidized and used for growth, phosphate uptake, glycogen formation and maintenance [110, 111]. It was also postulated that, in the aerobic phase, phosphate-accumulating organisms oxidize the stored PHB to generate adenosine triphosphate, which is used for cell growth. Phosphate is also transported into the cell and stored as poly-P, resulting in phosphate removal from solution. If there is a low level of PHB in the cell, phosphate uptake decreases [112]. However, Lo *et al*. [113] indicated that the addition of an anaerobic period in the activated sludge treatment system remarkably increased the phosphorus removal, reaching 99%. The photosynthetic activity in phototrophic biofilms results in an increasing pH due to the change of the carbon dioxide equilibrium in water. This increase in pH causes precipitation of dissolved phosphates, in addition to phosphorus removal by assimilation. This photosynthesis induced pH increase has also shown potential for the reduction of faecal coliform bacteria in wastewater streams [114].

5. Removal of Heavy Metals

Knowingly and/or unknowingly, heavy metals have been released into the environment over long periods of time through activities of human beings. Cadmium, zinc, copper and nickel are probably the most toxic elements normally found in large amounts in sewage sludge. After release of heavy metals into the environment, it becomes difficult to remove them by physical and/or chemical means and most of them exhibit toxic effects on a wide array of organisms. Heavy metals in sewage become bound to the organic matter which settles out in sedimentation tanks. Although the initial concentration of metals may be very small, the removal of water concentrates them in the sludge [115]. The conventional processes used for sewage effluent treatment are precipitation as hydroxides/sulphides, oxidation/reduction and ion exchange. These chemical treatments for recovery of metals from effluents before they leave the factory are expensive and not eco-friendly [116]. Therefore, a great deal of interest has recently been generated in using microbes, particularly microalgae as biosorbents for metal removal. Microalgae represent the best biological treatment for wastewater because they increase O_2 content of waters *via* photosynthesis and sorption of some heavy metals from contaminated waters. Cyanobacteria are photosynthetic prokaryotes; many of which have remarkable affinity for heavy metals [117, 118]. However, several other mechanisms have been devised for removal of heavy metals from wastewater.

5.1. Biosorption of Heavy Metals

Biosorption of heavy metals by microalgal biomass has been an important component in the integrated

approach to the treatment of aqueous effluents [116, 119]. The selective uptake and/or degradation capabilities of the selected species are attributed to the changes in the composition of the cell wall on which metal ions binding takes place as well as variation in their growth under different conditions [120]. Biosorption consists of several mechanisms like ion exchange, chelation, adsorption and diffusion through cell walls. These passive mechanisms can occur at cellular level or microbial community level.

In contrast, the active mode of metal uptake and concentration is called bioaccumulation which is dependent on the cellular metabolism. On prolonged contact with the metal bearing solution, the living biomass also become capable to sequester metal intracellularly by an active process involving expenditure of energy. Biosorption is possible by both living and nonliving biomass; however, bioaccumulation is mediated only by living biomass [121]. Bioaccumulation is regarded as a growth dependent process and is difficult to define in a variety of effluents in contrast to biosorption which is growth independent. Therefore, microbial biomass can be used and exploited more effectively in biosorption rather than bioaccumulation [122].

Major advantages of metal removal by biosorption include, low cost, and high efficiency of heavy metal removal from diluted solutions. However, in order not to simply displace heavy metal pollution, methods will have to be developed to extract heavy metals easily from biomass [123]. Sorption of heavy metals on phytoplankton cell surfaces is dependent on a number of factors ranging from the concentration of inorganic ions, dissolved organic matter, pH and the nature of particulates [124, 125]. Under comparable experimental conditions, however, species-specific differences in the ability to accumulate metals have been observed which is supposed to be under influence of various molecular mechanisms [126, 127]. The selective uptake and/or degradation capabilities of the selected species are attributed to the changes in the composition of the cell wall on which metal ions binding takes place as well as variation in their growth under different conditions [120].

5.2. Metallothioneins

Many algae are known to detoxify metals *via* evolutionarily strongly conserved metal-binding proteins of low molecular weight and high cysteine and metal contents [128, 129, 130]. Metallothioneins are known for detoxification of metal ions in animals and fungi [127, 131, 132, 133]. Ma Clean *et al*. [134] first reported the presence of Cd-binding material in fresh blue-green algae (*Anacystis nidulans*). Mallich and Rai [135] found that cyanobacterium (*Anabaena dolilolum*) synthesized low molecular weight Cd-binding protein (3.3 kDa) in response to Cd and they concluded that, this protein may play a role in metal tolerance. Also, Bierkens *et al*. [136] concluded the induced production of a shock protein (70 kDa) in grown algae in response to heavy metals. Moreover, an important study by Torres *et al*. [137] concluded that marine algae in response to Cd synthesized metallothioneins that sequester the metal in harmless form. Occurance of these metal-binding proteins in organisms growing in a mining refuse area also supports the postulate that they are involved in detoxification [138, 139]. Additional function of metallothioneins include control of intracellular redox potential, cellular repair processes, growth and differentiation, where they are likely to serve as the source of Zn for newly synthesized apoenzymes, as well as regular molecules in gene expression [140].

5.3. Chelatins and Polysaccharides

Many oxygenic phototrophic microorganisms have the capability to absorb or accumulate metals in one way or another, and there is considerable potential for the application of algal biofilms in the detoxification of wastewaters polluted with heavy metals [141, 142]. Removal of heavy metals using microorganisms could be achieved through the production of extra-cellular components, such as chelatins and polysaccharides, which are capable of complexing metal ions [143]. In the last fifty years, more than 100 cyanobacterial strains, belonging to 20 different genera, have been investigated with regard to the production of exocellular polysaccharides into the culture medium which could be an effective cation-chelating material [144]. Extracellular polysaccharides that are negatively charged at elevated pH levels generated by oxygenic photosynthesis may account for the metal-binding properties of phototrophic biofilms [141, 145, 146, 147]. Parker *et al*. [148] showed that mucilage sheaths isolated from the cyanobacteria *Microcystis aeruginosa* and *Aphanothece halophytica* exhibited strong affinity for heavy metal ions such as copper,

lead, and zinc. In addition to biosorption and bioaccumulation, the elevated pH inside the photosynthetically active biofilms may favour removal of metals by precipitation [113]. These facts suggest the possibility to manipulate exocellular polysaccharides-producing cyanobacteria, a multiproduct strategy to produce a wide range of biopolymers suited to various industrial applications, in addition to the residual biomass effective in the recovery of heavy metals from polluted waters [144].

5.4. Alkaline Precipitation of Heavy Metals

Alkaline precipitation of heavy metals from acidic water streams is a popular and long standing treatment process as well as another application where cyanobacteria are involved in heavy metals removal. The ability of certain algal species to increase the alkalinity of the surrounding medium as a by-product of their inorganic carbon accumulating mechanism has been documented but the potential use of this alkalinity in the precipitation of metals has not been widely reported [149]. The cyanobacterium *Spirulina* sp. was utilized in a continuous treatment system of tannery effluent to precipitate heavy metals (Fe and Zn) using the alkalinity generated by this alga. *Spirulina* sp. reduce the treatment running costs which is considered as additional benefit of treating either a tannery or sewage effluent using cyanobacteria [143, 150]. Moreover, the increased efficiency, coupled with the reduced operating costs involved in the algal treatment system for saline wastes produced from tannery suggests that the system has considerable potential for the treatment of waste waters containing metals. Also, microalgal biomass has been effectively and directly used to treat acid mine drainage (AMD) over a short period of time [151]. To overcome the problem of heavy metal toxicity on *Spirulina*, a system combining the principles of passive treatment with some simple, low-cost reactors associated with active treatment methods has been developed. In this system, the growing culture separated from the untreated effluent resulted in a system with the potential to be as effective as active treatment processes, but with the cost and sustainability advantages of passive treatment systems [152].

6. Physico-Chemical Properties of Sewage Effluent

Very little is known about the survival and influence of blue-green and green algae on the physico-chemical properties of sewage water. Microalgal communities play significant roles in the wastewater treatment systems by providing conditions to reduce the biochemical oxygen demand (BOD), chemical oxygen demand (COD), inorganic nutrients like N and P and pathogens (coliform bacteria) [153, 154] and at certain densities, they keep the aerobic phase of facultative ponds functioning [155]. Cyanophyceae have been reported to have a great competitive advantage under condition of high turbidity [156].

Microalgal abundance is affected by pH to a great extent. In one study when the pH was lowered from 6.6 to 5.0, algal abundance increased [157]. Because an increase in algal abundance was observed when lowering the pH, it can be expected that algal abundance should *decrease* when the pH is raised. Reduced growth was observed in a group of pH-tolerant algae when the pH exceeded 9.5. The most pH-sensitive species in the experiment decreased in abundance when the pH was raised to 8.8, and did not grow at all when the pH exceeded 9.0 [158]. Earlier studies have also shown that wastewater with high pH has negative effects on algal abundance. Only carbonate and bicarbonate ions are available to submerged photosynthetic plants in water with high pH [159]. If the ability of algae to photosynthesize is impaired as the pH is increased, then algal abundance will decrease in water with high alkalinity [158].

Dissolved oxygen (DO) is present in some amount in almost all water. It is required for many organisms that live in the river, but extremes in either direction can be harmful for aquatic organisms. The level of DO in the water depends on temperature, water velocity and turbulence, and the existing organisms in the water. An increase in the redox potential is expected as temperature increase due to increase in O_2 production by vegetation and algae [160]. The growth of the marine alga *Dunaliella tertiolecta* in the effluents of an aluminium plating plant and to the waste water from a pharmaceutical plant was found to be inhibited or stimulated at low concentration and inhibited at high concentration [161]. The biomass of microalgal groups displayed significant negative correlations with biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspend solids (TSS). Conversely, a significant positive correlation was found with dissolved oxygen, pH and nitrates [155]. High microalgal densities have been reported from

wastewater treatment systems, since these are able to withstand the broad variations in physicochemical parameters that occur in the wastewater treatment system [153, 162].

7. Pathogens Removal in Algae-based Systems

Untreated sewage not only causes eutrophication, but is also a major health hazard because of the likely presence of various kinds of pathogens. Helminths and protozoan parasites are the predominant ones responsible for disease transmission associated with raw wastewater, with only limited cases of bacterial and viral disease [163]. Their cosmopolitan distribution, very low doses required to produce infection and long persistence in the environment make these organisms the main problem in contamination with wastewater, mainly in rural areas [164, 165]. Moreover, presence of tough walls makes these parasites extremely resistant to disinfection with chlorine and monochloramines [166, 167].

Cryptosporidium parvum, a protozoan parasite causing diarrhoeal disease in a wide range of vertebrates including humans is considered to be one of the most important waterborne pathogen [168]. International guidelines strongly recommend the use of low-cost, highly efficient pathogen removal systems for wastewater treatment and stabilization ponds systems are the most efficient for their removal [163, 169]. High rate algal ponds and wastewater stabilization ponds follow basically the same removal mechanisms and are highly efficient according to faecal bacteria indicators [170]. However, experiments revealed that inactivation of oocysts by HRAP were higher than in conventional wastewater treatment systems. The HRAP physico-chemical conditions were responsible for more than 97 % of the reduction of infection cases because of the fact that oocysts lose their infectivity shortly after contact with the water environment. With regard to the helminths, a 90 % reduction in viability was achieved after 10 days exposure to conditions in the HRAP. The effect of ions and general osmotic conditions on the viability of nematode eggs in HRAP was responsible for 50-60 % of egg mortality. This implies that mortality due to the ionic environment in the HRAP could be more important than physical retention and other removal factors [66].

8. Conclusions

Microalgal treatment of wastewater has been investigated for over five decades as an environmentally sound alternative to remove nutrients and heavy metals from wastewaters and thus improve their quality. Several cyanobacterial species have been employed and identified as a working model for such studies because of their rapid ammonium and nitrate uptake, phosphate removal, heavy metals removal, influence on physico-chemical properties of sewage effluent and high growth rate. Several regulations have been put into operation which may lead to more stringent effluent standards for sewage treatment facilities located in ecologically sensitive areas. Nutrient removal efficiency could be increased depending upon many factors, including algal species, immobilization matrix, cell and bead concentration, aeration, and retention time. However, metal uptake, in addition to cation concentrations, is reported to be affected by light intensity, pH, biofilm density, presence of metal binding humic substances, and the tolerance of individual algal species to specific heavy metals. Water hardness is a crucial factor that influences metal uptake efficiency because cations such as Ca^{2+} and Mg^{2+} compete with trace metals for binding sites on cell membranes and extracellular polysaccharides [171]. Their ability for rapid genetic manipulation is an added advantage which, may lead to performance improvements of cyanobacteria for sewage water remediation in future.

Microalgae have been identified as efficient agents for the assimilation of organic matter from contaminated water and therefore, reduction of the BOD and COD. Their capabilities for the multi-contaminants removal can be greatly enhanced using efficient strains in fixed applications as biofilm or immobilized cells. They can also be used as a consortium instead of the individual application which would significantly reduce the treatment time required for best removal [18, 56]. Microalgae have been shown to be a more cost effective way to remove biochemical oxygen demand, pathogens, phosphorus and nitrogen than activated sludge. Economic feasibility of microalgal wastewater treatment can be enhanced further by linking microalgal biomass production with wastewater treatment and thereby using efficient methods of biomass harvesting and drying, metabolic and genetic engineering, systems

biology approaches, selection of efficient strains and high-value co-product strategy. These technologies allow microalgae to be used as economic and low-maintenance technology for contaminated systems [172]. However, the beneficial application of microalgae in remediation of contaminated waters in natural or manmade aquatic environment is still not optimally manipulated. Application of bioremediation using indigenous microalgal strains for decontamination of the domestic and industrial effluents polluted with organic contaminants and heavy metals provides a viable and sustainable approach for managing the environmental resources.

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