Microalgae Applications in Wastewater Treatment

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Abstract Algal wastewater treatment is effective in the removal of nutrients (C, N and P), coliform bacteria, heavy metals and the reduction of chemical and biological oxygen demand, removal and/or degradation of xenobiotic compounds and other contaminants. Microalgae wastewater treatment technologies have long been in existence; however, uptake of the technology to date has been limited mainly due to considerations of land requirements and volumes of wastewater to be treated. This chapter gives an overview of algal applications in wastewater treatment with specific reference to nutrient removal, phycoremediation of heavy metals, high-rate algal ponds, symbiosis of algae with bacteria for wastewater treatment, and utilisation of wastewater-grown microalgae.

Keywords Microalgae · High-rate algal ponds · Wastewater treatment · Nutrient removal · Heavy metals

1 Introduction: Conventional WW Treatment Plants and Limitations

The conventional activated sludge biological nutrient removal (CAS-BNR) process is globally, one of the most applied biologically driven treatment methods for both industrial and domestic wastewaters. Although there are many variations of BNR configurations, most processes consist of steps such as raw sewage screening, primary treatment, secondary treatment, tertiary treatment, disinfection, and solids handling. In most cases, secondary treatment is achieved by manipulating three types of biochemical reactions (anaerobic, anoxic and aerobic) under which the microorganisms (mostly bacteria) can be favoured to perform the respective nutrient

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(COD, phosphorus and nitrogen) removal. Different aerobic technologies are used to supply oxygen to the microorganisms in conventional treatment processes which are usually quite energy intensive. Following the aeration step, microorganisms are separated from the liquid by sedimentation and the clarified liquid is the secondary effluent. A portion of the biological sludge (microorganisms) is recycled to the aeration basin and the remainder is removed from the process and is normally combined with primary sludge for sludge processing. The secondary effluent is subsequently directed to the disinfection tank(s).

Although conventional treatment technologies are simple and are important for wastewater treatment, they are expensive processes and require high energy input. Moreover, management of these sewage treatment plants in rural areas is also challenging due to the lack of local technical expertise. Most of these microorganisms are very sensitive to changes in operational and environmental conditions and therefore malfunctions occur readily under unfavourable conditions. Loss of nitrification (due to the decrease in activity of nitrifiers), bulking and foaming (due to the excess growth of filamentous bacteria) are some of the challenges that are often faced by the WWTP (Khin and Annachhatre 2004; de-Bashan and Bashan 2004). Moreover, the conventional treatment process generates large amounts of sludge, and handling and disposal of this waste sludge is one of the largest bottlenecks to the technology. The high operational and maintenance requirements of wastewater treatment plants including solid waste material handling make it economically unfeasible. Furthermore, tertiary treatment for total removal of organic ions via chemical treatment is prohibitively expensive and has the potential to generate additional pollution. Biological tertiary treatment of wastewater although effective, costs up to four times that of primary treatment (Abdel-Raouf et al. 2012). Algae-mediated wastewater treatment is a potential solution for treatment of wastewater in peri-urban and rural settings due to the low operational costs and lesser requirement for technical skill in operation of the system. This chapter provides a broad overview of the applicability of algae-mediated wastewater treatment for nutrient removal; algae-based wastewater treatment systems and remediation of heavy metal containing wastewaters.

2 Phycoremediation

Phycoremediation is broadly defined as the utilisation of algae for the removal of contaminants from water. Algal wastewater treatment is effective in the removal of nutrients (C, N and P), coliform bacteria, heavy metals and the reduction of chemical and biological oxygen demand, removal and/or degradation of xenobiotic compounds and other contaminants (Abdel-Raouf et al. 2012; Cai et al. 2013, Rawat et al. 2011; Olguín 2003). It is applicable to various types of wastewater including: human sewage, livestock wastes, agro-industrial wastes, industrial wastes, piggery effluent, food processing waste and other agricultural waste substrates (Abdel-Raouf et al. 2012; Cai et al. 2013). Wastewater treatment using algae offers several advantages over conventional techniques. These include lesser sludge formation, lower energy requirements, reduction in greenhouse gases, lower costs and concurrent production of energy-rich algal biomass which can be processed for a number of uses including biofuels, bio-fertilizers, biopolymers, bio-plastics, lubricants, paints, dyes and colourants (Batista et al. 2014; Cai et al. 2013).

The production of sludge, as compared to conventional activated sludge wastewater, is reduced as there is no need for the utilisation of flocculants for the removal of P. This indirectly reduces the sludge-handling costs and the requirement of land required for drying. Reduction in greenhouse gases is accomplished by the biological binding of carbon dioxide obtained from organic carbon (which would have been volatilised by bacterial respiration) or carbon dioxide from the atmosphere. Energy demand is lowered as there is no need to oxygenate the reactor (Rawat et al. 2011). Microalgae produce oxygen which is used by heterotrophic bacteria in the system which further reduce nutrient concentrations (Abdel-Raouf et al. 2012). Algal systems are easy to operate and require limited skilled labour (Aziz and Ng 1992). One of the major challenges to the technology is the substantially larger footprint as compared to CAS-BNR (Abdel-Raouf et al. 2012). This is mainly due to the requirement for shallow ponds. Algal treatment systems are favoured over conventional treatment processes for decentralised wastewater treatment due to the significantly lower cost of construction and operation and ease of operation without the requirement for skilled labour.

2.1 Nutrient Removal

Uncontrolled growth of algae in aquatic bodies receiving nutrient rich wastewater streams results in eutrophication and its associated complexities (Conley et al. 2009), which include very low oxygen levels with increased depth in water bodies and the formation of dead zones (Diaz and Rosenberg 2008). Since conventional wastewater treatment technologies are largely designed for organic carbon removal; they are not always efficient in removing other nutrients, thus giving the need for tertiary treatment (Arceivala and Asolekar 2007). The controlled growth of algae in semi-engineered systems such as; oxidation ponds, facultative ponds, or lagoons etc., are effective tertiary treatment strategies for removal of residual nutrients from wastewater and have been practiced globally with varying degrees of success (Brockett 1977; Saqqar and Pescod 1996; Mahapatra et al. 2013). Renewed interest in the production of biofuels from microalgae has resulted in search for cheaper and more readily available cultivation media for growing algae with high productivities. Cultivation of algae on nutrient-rich wastewater presents a unique opportunity to achieve both objectives of nutrient removal and production of algal biomass for producing biofuels or other high-value products (Han et al. 2014; Rawat et al. 2011).

Microalgae are able to take up nutrients from surrounding environments in excess to their immediate requirements and store them within their cell for future utilisation in cell synthesis (Droop 1974). This ability allows microalgae to remove nutrients at high rates during their exponential growth phase. Droop observed this property of microalgae while working with vitamin B12 and introduced the concept of cell quota to explain such storage of essential nutrients (Droop 1968). Subsequently many researchers have found evidence of luxury uptake and storage of nitrogen and phosphorus by microalgae (Nambiar and Bokil 1981; Powell et al. 2009). It could be postulated that microalgae have developed the ability of storing nutrients in the cell to deal with the variable levels of nutrients in the natural environment (Leadbeater 2006). Earlier research postulated that luxury uptake of nitrogen and phosphorus was a mechanism to maintain the average stoichiometry in the cell as close to Redfield ratio (16N: 1P) as possible (Redfield 1958). Recent literature provides evidence of deviations from this ratio and establishes vast variations in the uptake rates of nitrogen and phosphorus in accordance to their levels in the surrounding environments (Klausmeier et al. 2004). This flexibility in nutrient uptake allows microalgae to effectively tolerate the variability in different wastewaters to a certain level without having detrimental effects on the culture. For example, *Chlorella vulgaris* has been reported to treat wastewater having N/P molar ratios from 1.80 (Travieso et al. 2006) to 2017 (Ryu et al. 2014) effectively. The potential to take up nutrients varies between different algal species (Boelee et al. 2011; Zhang et al. 2008) and also depends on environmental factors such as temperature and light (Richmond 1992). Species belonging to various divisions such as chlorophytes, cyanobacteria, and diatoms have been widely utilised for nutrient removal effectively (Cai et al. 2013). Removal of algae from the system is vital to avoid recycling of nutrients back to the receiving waters.

2.1.1 Nitrogen Removal

The presence of nitrogen in wastewater is due to various anthropogenic activities. Nitrogen is primarily in the form of ammonia, but can also be present in other forms such as nitrate, nitrite, or organic nitrogen (Metcalf & Eddy et al. 1998). High levels of unionised ammonia or nitrate/nitrite are toxic to aquatic life and humans (Bryan et al. 2012; Braissant 2010). In addition, their presence has great potential to lead to eutrophication. Hence, nitrogen removal from wastewater is essential before it can be safely discharged.

Microalgae show a great potential to effectively remove nitrogen as it is an essential macronutrient for their growth. Nitrogen is required for synthesis of peptides, proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), etc. (Conley et al. 2009; Cai et al. 2013). Microalgae assimilate the inorganic nitrogen present in wastewater, such as ammonium, nitrate and nitrite; and convert them to various organic nitrogen species required for cell synthesis. Microalgae prefer ammonium over other inorganic nitrogen species due to the fact that it can easily be converted to the amino acid glutamine without any redox reaction and thus utilises less cellular energy (Cai et al. 2013; Flynn et al. 1997). Nitrate and nitrite are also assimilated by microalgae depending on their availability in the wastewater, and are reduced to ammonium inside the cell. Such reduction is mediated by various enzymes and involves many intermediate products during such reduction pathways (Dortch et al. 1984). Pathways usually involve reduction of nitrate to nitrite mediated by an enzyme nitrate reductase, and then nitrite to ammonium by nitrite reductase (Flynn et al. 1997; Cai et al. 2013). In addition to assimilation of nitrogen in the cell, indirect removal in the form of ammonia stripping also occurs due to increased pH with algal cultivation (García et al. 2000).

2.1.2 Phosphorus Removal

The presence of phosphorus in wastewater streams is also predominantly due to human activities, particularly the application of phosphorus fertilizers in agriculture. Phosphorus is mainly present as phosphates, such as orthophosphate, polyphosphate, or organic phosphate. The bio-availability of phosphorus varies with the chemical speciation (Dueñas et al. 2003). The presence of phosphorus in water has potential to lead to eutrophication (Abdel-Raouf et al. 2012). Hence phosphorus removal from wastewater is essential.

Microalgae have the ability to take up phosphorus, mainly orthophosphate as HPO_4^{2-} and $H_2PO_4^-$, and utilise it as an essential macro nutrient in the synthesis of various compounds such as nucleic acids, phospholipids, and proteins, etc. via phosphorylation. It is also vital for energy transfer during various metabolic activities within the cell since it forms a primary part of ATPs and ADPs (Conley et al. 2009). Excess amounts of orthophosphate taken up by microalgae during luxury uptake are stored as polyphosphate granules in the cell for future utilisation (Rasoul-Amini et al. 2014). While acid-soluble polyphosphate can be used for production of protein, nucleic acids, and various metabolic activities; acid insoluble polyphosphates are considered as a storage pool for future use when external phosphorus is exhausted or limiting (Powell et al. 2009). Indirect phosphorus removal also occurs due to precipitation of phosphate at high pH often observed with algal cultures (Nurdogan and Oswald 1995).

3 Phycoremediation of Heavy Metals

The metals in the aquatic environments come from both natural as well as anthropogenic sources; however, the release of significant amounts of toxic metals to the aquatic ecosystems due to extended industrial activities such as mining, agriculture, metal plating and paint industries, is of serious concern. Once, the metals are released to the aquatic environment, they remain in the water column for long durations and get deposited to the sediment systems. Therefore, the sediment systems acts as sinks for metals and significant amounts of metals are released to the aqueous medium from the sediment due to prevailing environmental factors. Thus the sediment system itself becomes a secondary source for the metals under certain environmental conditions. The major concern regarding these types of pollutants is that they get accumulated in the biological systems and aquatic food chain over a period of time and once beyond the thresholds, they have deleterious effects on the aquatic organisms and ultimately to the human beings through aquatic food chains (Gupta and Rastogi 2008; Babu and Gupta 2008).

Conventional methods such as precipitation, flotation, ion exchange, electrochemical and biological processes have long been practiced for metal removal from wastewater; however, most of these methods are often ineffective and/or are energy-intensive, thus too expensive for removal of low-level metal contaminants from water and wastewater. The conventional and physico-chemical treatments for the removal of heavy metals from the metal-contaminated water or sediment matrix is typical, therefore the need of the hour is the economically sound and environmentally sustainable technologies. Microalgal and cyanobacteria-based phycoremediation technologies have gained much attention recently as alternative bioremediation techniques over traditional methods for eco-friendly clean-up of metal-contaminated wastewater, industrial effluents and soil matrix (De Philippis et al. 2011; Sandau et al. 1996).

Of the physico-chemical or electrochemical treatments, phycoremediation through biosorption of toxic metals on live or dead algal biomass has emerged as an eco-friendly technology. Thus, phycoremediation is one of the most prominent technologies which can be used for metal removal/recovery from aqueous phase and eco-friendly disposal. Some of the microalgal and cyanobacterial species which are metal stress tolerant possess high resistivity towards metal toxicity, high surface binding and intracellular uptake due to inherent abilities. Such species, therefore, could serve as suitable and attractive alternatives for phycoremediation purposes (Terry and Stone 2002).

Various species of microalgae and cyanobacteria have been used for metal removal from water. It has been observed that significant proportions of toxic metals can be removed by algae and cyanobacteria. Earlier studies have demonstrated the potential application of cyanobacteria in *in situ* phycoremediation of heavy metals without external input of materials and energies (De Philippis et al. 2011; Doshi et al. 2007). The major proportion of the metals that are removed by algal species are mainly mediated through extracellular adsorption/diffusion or surface binding which is facilitated by the inherent properties of the algal cell walls. The extracellular surface binding of metal ions with the algal cell wall is rapid and better known as passive uptake followed by slow intracellular active uptake (Gupta et al. 2006). The algal cell wall exhibits chemical affinity to the metal ions due to the presence of various functional groups such as carboxyl, carbonyl, amino, amido, hydroxyl, sulfhydryl, sulphonate and phosphorus, etc. which confer negative charge to the cell surface and therefore facilitate surface binding with positively charged metal ions through adsorption, ion exchange, coordination, surface complexation, chelation and microprecipitation, etc. (Fourest and Volesky 1997). However, charge transfer between metal cations and carboxyl groups of algae plays a significant role (Sandau et al. 1996; Gupta and Rastogi 2008).

This is also an important mechanism of algal and cyanobacterial cells to tolerate elevated metal concentrations (De Philippis et al. 2011). The rapid extracellular adsorption/diffusion of metals is followed by additional slow uptake of metal ions by irreversible surface binding through surface precipitation, covalent bonding, crystallisation on the cell surface or redox reactions (Wilde and Benemann 1993). In some cases, intracellular organelles and cell constituents such as protein and other materials also facilitate the diffusion and binding of metal ions into the algal cells. Past experience advocates that due to efficient membrane binding, the live algal biomass possess comparatively higher metal biosorption potential than the dead biomass. Moreover, the active metabolism also plays significant role in intracellular uptake (Terry and Stone 2002; Doshi et al. 2007). However, some of the studies have also demonstrated that even the dead algal biomass possesses similar or better adsorption capacity (Fourest and Volesky 1997). The passive metal uptake by algae is mainly facilitated by the physico-chemical properties of the surrounding water matrix whereas the intracellular uptake is mediated by cell metabolism (Raungsomboon et al. 2008). Schiewer and Volesky (1995) reported that metal ions bind first to the surface ligands with higher affinity, then to those with lower affinity. Studies of Ke et al. (1994) revealed that for binding of Ag+, pH-independent binding sites displayed a greater affinity than pH-dependent sites.

3.1 Factors Influencing Algal Sequestration of Metallic Ions

The chemical composition and functional groups and therefore ionic charges of algal cell wall vary from species to species; thus, the adsorption capacity also varies from species to species. The metal removal potential of algae is dependent on various physico-chemical factors, mainly pH, salinity and hardness of the water. The ionic charges of metal ions and chemical composition of the aqueous media also play significant roles (Wilde and Benemann 1993; Romero-González et al. 2001). In a study, Fourest and Volesky (1997) reported that the metal binding capacities of the seaweeds are directly proportional to their respective total carboxyl group content and the electromagnetivity of elements. Studies have demonstrated that pH of the medium is one of the important limiting factors in that biosorption of metal ions increases with increasing pH. Studies on surface charges showed that increasing pH increases the number of negative sites which facilitate binding of more metallic cations (Schiewer and Volesky 1995). Ionic strength also plays a significant role in metal adsorption/uptake. Metal removal is inversely proportional to the ionic strength, therefore, it increases with the decrease of ionic strength (Cho et al. 1994; Sandau et al. 1996).

3.2 Significance of Phycoremediation of Heavy Metals

The inherent properties of survival on radiant energy and rapid metabolism in the absence of organic carbon makes various algal species seem superior sequesters for metals than any other microorganisms such as bacteria and fungi. Moreover, various algal biomasses are low cost biosorption materials and possess comparatively high adsorption capacity and can be produced with the lowest energy consumption.

4 High-Rate Algal Ponds

The concept of high-rate algal ponds (HRAP) or raceway ponds (RWP) was first proposed by Prof. Oswald in late 1950s and 1960s as an improvement over existing oxidation ponds by providing paddlewheels for efficient mixing of algal biomass in the pond and by reducing the depth to 0.2–1 m (Oswald et al. 1957; Goldman 1979; Arbib et al. 2013). These improved designs provide much better conditions for algal growth and increased productivities in these ponds, which are usually designed as raceways, due to better light utilisation which in turn results in higher efficiencies of wastewater treatment with lower retention times of 4–10 days (García et al. 2000). A properly designed HRAP could efficiently remove nutrients as well as organic carbon from the wastewater. Nutrient removal is achieved directly due to algal growth, and also by indirect effects of such cultivation, namely ammonia stripping and phosphate precipitation due to increase in pH. Both direct and indirect removals are important in ponds. In addition, organic carbon removal is also achieved due to the presence of aerobic bacteria having symbiotic relationship with microalgae (García et al. 2000). Mixing is provided by paddle wheels which are designed to maintain horizontal flow velocities of 0.15–0.30 cm s^{-1} to overcome frictional losses while ensuring continuous flow (Chiaramonti et al. 2013).

Such ponds are the most commonly used systems for large-scale production of algal biomass (Chiaramonti et al. 2013). Raceway ponds could achieve productivities of up to 50 t ha^{-1} $year^{-1}$ (Rawat et al. 2011). However, their application for wastewater treatment as a primary objective rather than biomass production is considered to be more economically viable since large parts of capital and operational costs are recovered due to wastewater treatment itself. In addition, their water and energy footprints are lower (Park et al. 2011). HRAPs are also utilized in treating industrial or hazardous wastewaters such as effluents from tannery, mines, or zinc refinery (Rawat et al. 2011).

Despite these advantages, two critical limitations of raceway ponds are large land requirement and risk of culture contamination (Chiaramonti et al. 2013). Since these ponds are restricted in depth due to the need of efficient light penetration, the footprint is huge and may become a limiting factor for the applicability of this technology. In addition, the presence of various zooplanktons and protozoa that graze on microalgae may reduce the production and hence the treatment. Since these are open systems, variability in light and temperature affects the treatment efficiencies of these ponds. Other challenges associated with pond operation are evaporative losses and difficulty in maintaining carbon dioxide in the system required for algal growth (Singh and Sharma 2012). Another challenge in operating HRAPs is the entrainment of microalgae with the pond effluent due to poor settling properties, and which requires effective harvesting protocols.

5 Advanced Integrated Wastewater Pond Systems

The concept of natural wastewater treatment in ponds was explored in detail and an advanced pond system design was proposed by Oswald (1990) and his group at Berkeley to achieve comprehensive wastewater treatment with lower energy input. Such advanced integrated wastewater pond systems (AIWPS) are potentially applicable in developing nations where cost-effective treatment processes are in great demand to achieve sanitation goals effectively (Oswald 1990). Such advanced treatment ponds aim to maximise the applicability of conventional ponds while minimising their drawbacks. In comparison to conventional ponds, AIWPS require much less energy and resources including land. In addition, the issues of odour and sludge build-up of other treatment processes are also minimised (Oswald 1990).

Integrated ponds consist of at least four basic ponds which are designed to achieve different objectives. These objectives are similar to those in conventional wastewater treatment, namely, primary sedimentation, flotation, fermentation, aeration, secondary sedimentation, nutrient removal, storage and final disposal. These ponds include a facultative pond which consists of a deeper pit inside for anaerobic fermentation, a high rate algal pond, an algae settling pond, and finally a maturation pond.

5.1 Facultative Pond with Internal Fermentation Pit

The primary treatment of wastewater occurs in this facultative pond. A deep anoxic pit is provided within this pond to achieve fermentation of various solids, and eventually degrade them so as to minimise sludge build-up. Such pits are designed to avoid the intrusion of oxygen-rich water which might compromise the fermentation process. Raw sewage is allowed to enter at the bottom of this fermentation pit. The overflow velocities are kept low ($<1.5\ \text{m d}^{-1}$) which helps in settling of the solids. Since these velocities are less than settling velocities of helminth ova and parasite cysts, these are also trapped within this pit. As the solids settle and accumulate at the bottom of the pit, they create an anoxic layer which results in the fermentation of such solid biomass. The resulting biogas bubbles due to fermentation entrap some of the solids and carry them upwards in the pit. Such bubbles get bigger and finally detach from such solids which again settle to the bottom. This process results in a long sludge age within the pit, which eventually results in almost complete sludge digestion, and sludge removal is required over decades of operation. Carbon dioxide in the produced biogas reaches the upper layers within the pond where it helps in algal photosynthesis and eventual oxygen production. Photosynthetic oxygen results in oxygenic conditions in the upper water layers in the pond and aids in odour removal by aerobic degradation of causative compounds. The effluent from this pond is removed from the upper layers (at least 1 m below the water level to avoid passing of floating objects) with oxygen rich conditions. A properly designed pond of this nature could remove almost 100 % suspended solids and 60–70 % of the biochemical oxygen demand (BOD) from the influent wastewater (Oswald 1990).

5.2 High-Rate Algal Pond (HRAP)

The second pond in this system is a high-rate algal pond where algal growth is maximised with the application of paddlewheels for proper mixing and shallower depths for better light utilisation. High productivity of algae is achieved in these ponds with sufficient removal of nutrients at a shorter retention time. Oxygen production due to algal photosynthesis is utilised by aerobic bacteria to remove the remaining BOD in the wastewater. In addition, pH increases above 9–10 due to algal photosynthesis and helps in disinfection from most pathogenic bacteria. Part of this high pH and oxygen-rich effluent from HRAP is recycled to the top of first facultative pond to help with odour and pathogen removal.

5.3 Algae Settling Pond

The use of paddle wheels in HRAP promotes the dominance of algal species with better settling characteristics in the HRAP, and this property is utilized for removing algae from effluent in algae settling ponds (Oswald 1990). The effluent from HRAP is sent to settling ponds, where algae settle to the bottom and clarified effluent is achieved. The water in this pond is required to be in quiescent condition to accelerate algal settling. Settled algae are rich in nutrients and can be utilized for further applications. At least two such settling ponds are operated in parallel to achieve periodic harvesting of settled algae without affecting the plant operation. The effluents from algal settling ponds are low in BOD and nutrients. However, enhanced disinfection might be necessary if effluent from such ponds is used for purposes where prolonged human contact is expected. In addition, additional algae harvesting processes might be necessary to achieve complete algae removal from the effluent.

5.4 Maturation Pond

Last of the ponds is a maturation pond where effluents from the settling pond are stored for additional 10–15 days to achieve enhanced levels of disinfection especially of faecal coliforms, while also acting as storage pond for irrigation applications. Additional BOD and nutrient removal is also achieved in this pond. In addition, these maturation ponds also act as a habitat for aquatic life. The effluents from such ponds are low in BOD, nutrients, and pathogens; and hence suitable for application in agriculture or other such objectives. In addition to domestic wastewater, such AIWPS technology has also been applied for treating industrial wastewaters such as tannery effluents (Tadesse et al. 2004). Such ponds have been also found effective in removing chromium (Tadesse et al. 2006), selenium (Green et al. 2003), etc. from wastewaters.

6 Symbiosis of Algae with Bacteria for Wastewater Treatment

Microalgal photosynthesis provides a unique opportunity for maintaining a mutually beneficial relationship with aerobic bacteria during wastewater treatment. The oxygen produced by microalgae fulfils the requirement of bacteria during degradation of organic carbon, and the carbon dioxide produced as a result of this degradation as well as bacterial respiration is an important substrate for photosynthesis. In addition, the excretion of organic carbon from microalgae also provides substrates for bacterial growth (Munoz and Guieysse 2006). However, such relationships are very complex in nature and include mutually beneficial or harmful effects.

Many researchers have observed such relationships to be species specific. Growth of microalga *Asterionella glacialis* was promoted with the addition of bacterial strain Pseudomonas sp. 022, while no effect on growth was shown with *Vibrio* sp. 05. Further study showed that *Pseudomonas* sp. 022 produced a glycoprotein that acted as a growth factor for *A. glacialis* (Riquelme et al. 1987). Similarly, bacterial strain *Spirillum* 7697 exhibited a 40-fold difference in taking up the extracellular products of algae *S. costatum* when compared to strain *Pseudomonas* HNY (Bell et al. 1974). Watanabe et al. (2008) studied the composition of metabolites from Chlorella sorokiniana and their relative uptake by its many bacterial symbionts. The growth of *Chlorella vulgaris* was found to increase when co-immobilized and co-cultured with *Azospirillum brasilense* (Gonzalez and Bashan 2000). In addition to mutual growth promotion, microalgae and bacteria also exhibit bactericidal and algicidal effects, respectively towards certain species. For example, the presence of bacterial strain *Flavobacterium* sp. 5N3 resulted in suppression of red tide plankton, *Gymnodinium mikimotoi* (Fukami et al. 1997).

These complex interactions between microalgae and bacteria also result in their changing behaviour within phycosphere in comparison to the surrounding milieu (Bell et al. 1974). For example, *Chlorella sorokiniana* IAM C-212 produces a polysaccharide gel, termed as sheath, under photoautotrophic conditions which is a suitable habitat for several symbiotic microorganisms as it ensures close proximity (Imase et al. 2008).

These symbiotic relations have been utilized in open ponds or closed photo-bioreactors for achieving enhanced removal of nutrients and organic carbon (Olguín 2012). In addition, various hazardous elements such as acetonitrile (Muñoz et al. 2005), salicylate (Muñoz et al. 2003b), phenanthrene (Muñoz et al. 2003a), etc. have also been successfully degraded with combined applications of algae and bacteria (Munoz and Guieysse 2006).

7 Utilisation of Wastewater-Grown Microalgae

Microalgal biomass (except those used for heavy metal remediation) can be utilized for biofuel production viz. biodiesel, bioethanol, biomethane, etc., as a feed in aquaculture and poultry, in fertilizers, pharmaceuticals, and nutraceuticals industry (Singh et al. 2014; Vanthoor-Koopmans et al. 2013). Recently, there has been renewed interest in microalgae as commercial sources of bioenergy and high-value products such as β-carotene, astaxanthin, docosahexaenoic acid, eicosahexaenoic acid, and phycobilin. However, high production cost is still a bottleneck for commercial scale production of lower value products (Singh et al. 2015). Integration of wastewater treatment with algae biomass production is one of the methods to reduce costs of microalgae mass cultivation (Ramanna et al. 2014).

Several studies have been conducted on the utilisation of wastewater as growth medium for microalgal cultivation and the biomass produced can be utilized in several ways. Most of these studies are conducted on utilisation of wastewater as growth media for microalgal biomass production that can be used as biodiesel feedstock. Cho et al. (2013) studied the feasibility of wastewater for cultivation of *Chlorella* sp. in order to achieve high biomass production with low cost input. In their study the highest biomass (3.2 g L^{-1}) was obtained from the wastewater grown culture which was 1.72 higher than BG 11 medium. High lipid accumulation is a key factor for algal biodiesel production. Available nutrients in wastewater may not be sufficient for growth of microalgae. For production of commercial products such as biodiesel, bioethanol, pigments, fertilizers, wastewater may require supplementation with some nutrients.

Ramanna et al. (2014) studied the effect of different nitrogen sources on lipid accumulation of *Chlorella sorokiniana* while using domestic wastewater as growth medium. In their study they obtained maximum lipid accumulation of 61.7 % in the experiment with urea supplemented domestic wastewater. The fatty acid profile also confirms the suitability of their strain for biodiesel production. *Chlorella vulgaris* and *Botrycococcus teribillis* grown in domestic wastewater supplemented with glycerol as carbon source were reported as potential candidates for biofuel production. On the basis of biochemical compositions obtained, biomass was found suitable for production of biodiesel, bioethanol, biomethane and its utilisation as bio fertilizer (Cabanelas et al. 2013) (Table 1).

Microalgae can accumulate large amount of carbohydrates in the form of starch, glucose, cellulose, hemicelluloses, and various kinds of polysaccharides (Borowitzka 2013). These microalgal carbohydrates are conventionally used for biofuel production, especially for bioethanol (Vanthoor-Koopmans et al. 2013) and hydrogen (Beer et al. 2009). Recently these microalgal polysaccharides have been discovered as source of bioactive compounds. Specifically, algal polysaccharides contain sulphate esters called sulphated polysaccharides (e.g., fucoidan, carrageenans and agarans) (Spolaore et al. 2006; Yen et al. 2013). These sulphated polysaccharides have been shown to possess numerous medicinal activities, such as antioxidant, antitumor, anticoagulant, anti-inflammatory, and antiviral (Pulz and Gross 2004; Skjanes et al. 2013; Yen et al. 2013). A high-weight polysaccharide from *Chlorella pyrenoidosa* has very high immunostimulatory and antitumor effects with potential use in cancer therapy (Shi et al. 2007). Colourful appearance of the microalgae is because of the pigments which capture light and initiate photosynthesis (Spolaore et al. 2006). Carotenoids are pigments present in all classes of algae and serve as photo-protectors against the photo-oxidative damage resulting from excess energy captured by light-harvesting antenna (Cardozo et al. 2007). Astaxanthin is an oxidised form of carotenoid with high oxidation capacity (Qin et al. 2008). Astaxanthin has many applications in healthcare industry as it can be used for prevention and treatment of various conditions, such as chronic inflammatory diseases, eye diseases, skin diseases, cardiovascular diseases, cancers, neurodegenerative diseases, liver diseases, metabolic syndrome, diabetes, diabetic nephropathy and gastrointestinal diseases (Wayama et al. 2013). *Chlorella zofingiensis* was grown on waste molasses and examined for oil accumulation and astaxanthin production. A lipid productivity of 710 mg L^{-1} d^{-1} and astaxanthin 1.7 mg L^{-1} d^{-1} was obtained (Liu et al. 2012).

Arthospira is a well-known source for commercial products such as phycocyanin pigment and poly unsaturated fatty acids. Phycocyanin is a blue pigment with anti-oxidative property and known as immunity promoter in human and animals (Sarada et al. 1999). Arthospira was evaluated for its nutrient removal capacity and biomass productivity while growing on piggery wastewater. High biomass production (11.8 g L^{-1} d^{-1}) and high protein (48.9 %) content was obtained for Arthospira. The microalgal biomass was also evaluated for its suitability for fish feed and extraction of other valuable chemicals (Olguín 2003).

Microalgae can synthesise numerous compounds that have nutraceutical value. Microalgae have become more ubiquitous sources of nutraceuticals due to the capability of producing necessary vitamins, essential elements and essential amino acids and Omega 6 (Arachidonic acid) and Omega 3 (Docosahexaenoic acid, eicosapentaenoic acid) fatty acids (Spolaore et al. 2006). *Chlorella* (lutein, vitamin B12), *Spirulina* (single cell protein), *Haematococcus* (antioxidant) and *Dunaliella* (β-carotene) are the most popular nutraceutical sources (Vanthoor-Koopmans et al. 2013).

Table 1 Utilisation of microalgal biomass grown in wastewater

Microalgal strain	Wastewater type	Biomass productivity	Lipid productivity or lipid content	Carbohydrate productivity	Other	Application	References
Scenedesmus sp.	Carpet mill	126.54 mg L^{-1} d^{-1}	16.2 mg L^{-1} d^{-1}				Chinnasamy et al. (2010)
Chlamydomonas reinhardtii	Municipal centrae	2000 mg L^{-1} d^{-1}	505 mg L^{-1} d^{-1}	–	–	Biodiesel	Singh et al. (2014)
Chlorella pyrenoidosa	Soyabeen process		400 mg L^{-1} d^{-1}	–	–	Biodiesel	Hongyang et al. (2011)
Dunaliella tertiolecta	Carpet mill untreated	28.3 mg L^{-1} d^{-1}	4.3 mg L^{-1} d^{-1}	–	–	Bioremediation, biodiesel	Chinnasamy et al. (2010)
Chlorella sorokiniana	Domestic wastewater with urea supplementation	200 mg L^{-1} d^{-1}	61.52 % lipid content	–	–	Biodiesel	Ramanna et al. (2014)
Chlorella vulgaris	Domestic waste water + glycerol (50 mM)	5.69 ton $year^{-1}$	894.2 kg $year^{-1}$	415.0 kg $year^{-1}$	–	Biodiesel, bioethanol, biomethane, fertilizer	Cabanelas et al. (2013)
Botrycococcus teribillis	Domestic waste water + glycerol (50 mM)	13.58 ton $year^{-1}$	1683.4 kg $year^{-1}$	1072.6 kg $year^{-1}$	–	Biodiesel, bioethanol, biomethane, fertilizer	Cabanelas et al. (2013)
Chlorella zofingiensis	Waste cane molasses	1550 mg L^{-1} d^{-1}	710 mg L^{-1} d^{-1}	–	1.7 mg L^{-1} d^{-1} astaxanthin	Bio-oil, pigments	Liu et al. (2012)
Arthospira	Piggery waste	11.8 g m^{-2} d^{-1}	–	–	48.9 % protein	Aquaculture, pigments	Olguín et al. (2003)

However, utilisation of wastewater grown biomass for human consumption, animal and fish feed and healthcare applications could face ethical and biosafety issues. Chemical and physical characterisation as well as microbiological assessment for pathogens of the products is important for safety considerations. The products must be examined to determine the potential for toxicity, the possibility for naturally occurring toxins (from the source organism), heavy metals, and hazardous levels of pathogenic microorganisms, as well as potential hazardous by-products formed from the degradation of certain macromolecules. Wastewater-grown microalgae may contain some heavy metals and pathogenic microorganisms which are critical in evaluating the toxicity of the products.

Microalgae components are valuable, with a wide range of applications. The carbohydrates present in microalgae are considered as an appropriate feedstock for various energy sources (bioethanol, biomethane, etc.) and source of various polysaccharides. The high lipid content in algal biomass makes it promising feedstock for biodiesel production, while the long-chain fatty acids, pigments and proteins have their nutraceutical and pharmaceutical applications (Table 1).

Integration of wastewater for generation of microalgal biomass reduces the production cost. Therefore, wastewater-grown microalgae deserve further investigations in particular for commercial viability, large scale cultivation, assessment of environmental and safety risk, ethical issues of converting the components of microalgae into biofuels and other valuable products.

8 Conclusion

With increased environmental awareness, the world has moved towards a zero waste strategy and valorisation of waste substrates. Phycoremediation of wastewater offers a significant avenue towards achieving this outcome. Despite the challenges associated with the technology, the lower cost and ease of operation make this technology attractive. The sheer versatility offered by algal wastewater treatment with regard to substrate and ability to derive value in terms of nutrient recycling and the potential for energy generation make phycoremediation essential for environmental protection.

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