Microalgal Technology: A Promising Tool for Wastewater Remediation

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Abstract

Many species of microalgae have excellent ability to remove nitrogen, phosphorus, heavy metals, pesticides, organic and inorganic compounds, and pathogens from wastewater. Microalgae species grow well in wastewater and may be used for treatment of municipal, industrial, agro-industrial, and livestock wastewaters. Furthermore, microalgae biomass is an excellent source of production of various valuable products. In this chapter, applications of microalgae for treatment of wastewater and production of valuable products are discussed.

Keywords

Phycoremediation · Microalgae · Wastewater · Bioenergy · Metabolites

2.1 Introduction

Pollution is the major threat to our environment which has resulted from increased mushrooming of industries and more urbanization. It affects our ecosystems, flora, fauna, and human health worldwide by contaminating soil, water, and air. Pollution arises due to increased concentrations of unwanted and harmful substances as the results of anthropogenic activities. Most of organic and inorganic constituents have

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been released in water bodies due to household, agriculture, and industries which have led to organic and inorganic pollution (Mouchet 1986; Lim et al. 2010). This pollution has greatly affected the availability and quality of water resources around the globe (Abdel-Raouf et al. 2012). Moreover, there are common incidences of discharging the wastewater into water bodies without proper treatment. There is no parallel connection between the planning and implementation of such discharges in municipal plans which are posing serious problems to public health. The wastewater contains nitrogen, phosphorus, heavy metals, pesticides, organic and inorganic toxins, and pathogens. The major reason for water pollution is the discharge of industrial waste and sewage without proper treatment. Several wastewater treatment plants discharge water that contains significant amounts of toxic metals and organic and inorganic compounds. Therefore, it is a major challenge to develop efficient wastewater treatment technologies.

Agriculture pollution is also a source of water contamination. Agrochemical residues containing high concentrations of insecticides/pesticides/fertilizers pose serious threat to aquatic ecosystems. Nitrate is identified as one of the most common sources of agriculture pollution that causes eutrophication (Abdel-Raouf et al. 2012).

Wastewater can be mainly categorized into household and industrial wastewater (Chiu et al. 2015). In the present world, one of the major challenges is the availability of clean and potable water for drinking and household. However, to meet this challenge, there is a need to develop different new methods for wastewater treatment (Bansal et al. 2018). One of the major resolutions can be phycoremediation which efficiently uses algae for treatment of wastewater (Bansal et al. 2018). Algae are eukaryotic organisms with great variety ranging from single cell to highly differentiated plants. Algae are efficient carbon fixer as it can utilize carbon to release oxygen into atmosphere (Rehnstam Holm and Godhe 2003). More than 50% of total photosynthetic activity can be attributed to algae, and it significantly affects the food chain (Day et al. 2017). Moreover, algae can be used to convert carbon dioxide to oxygen by utilization of carbon for its own growth. Thus, algal cells can be efficiently used for wastewater treatment because they can remove the organic compounds, metals, and nutrients left out in wastewater (Laurens et al. 2017). Heavy metals have been detected in industrial wastewater. Microalgae can efficiently remove/remediate heavy metal ions from the effluents. Kumar et al. (2015) have reviewed various biochemical mechanisms present in microalgal cells for removal of heavy metals. Algae can be used for production of value-added products along with safe cleaning of wastewater such as algal char to replace coal and production of effective biofuel, lipids, and active metabolites which can be used as colorants, preservatives, and medicines. Microalgal cells can be used to produce alternative bioenergy sources. It has been observed that microalgae due to high amount of polyunsaturated fatty acids (PUFA) have enormous potential to be used as biodiesel (VenkataMohan et al. 2015). They can be harnessed as valuable biodiesel sources due to their high cell densities and accumulation of large quantities of triacylglycerols. But some of the major problems using algal technology for wastewater treatment, including availability of space, sunlight, contamination, resilience time, etc., need to be managed before employing phycotechnology for wastewater treatment.

Some biotechnological strategies such as hyperconcentrated cultures, immobilized cell system, photobioreactors, and genetic engineering can be used to improve phycotechnology. Photobioreactors can be used to improve phycoremediation. A microalgae photobioreactor has been developed by Marbelia et al. (2014). In this bioreactor, they used lab effluent as input as growth media for *Chlorella vulgaris.* Photobioreactors can be used efficiently for growing high density of algae due to less washout problem, and dilutions can be maintained at optimal levels. Moreover, it has helped to achieve higher cell density and enabled high waste removal.

This chapter attempts to discuss the role of microalgae in phycoremediation of wastewater, current technologies used, and future technologies to improve the process further.

2.2 Adverse Effects of Wastewater on the Environment

The composition of wastewater reflects the lifestyles and technologies practiced in producing society (Gray 1989). It is a mixture of organic and inorganic materials and xenobiotic compounds. Major portions of sewage are carbohydrates, fats, proteins, volatile acids, etc. Major constituents of inorganic pollutants include various ions and heavy metals, viz., sodium, calcium, magnesium, chlorine, bicarbonate, and ammonium salts, which are among the causative agents of water pollution (Tebbutt 1983; Horan 1990; Lim et al. 2010). Various pollutant sources include untreated direct discharge of human wastes from household, municipal wastes, and agricultural leach outs including high concentrations of insecticides and pesticides. It includes the industrial drains containing higher concentrations of heavy metals (Horan 1990). Pollutants can be classified into two categories depending on their sources in biological and chemical wastes. Chemical wastes include various inorganic ions, heavy metals from industries, detergents from household, and agricultural leach outs containing insecticides and pesticides (Akpor 2011). Other than these different sources, pathogenic microorganisms such as bacteria, viruses, and protozoans are common problems which affect the quality of drinking water (Akpor 2011). Moreover, the largest contributor of pollution is discharge of effluents from wastewater treatment. There are a number of previous studies on the negative impact of these effluents, which may result into death of aquatic life, algal blooms, habitat destruction from sedimentation, debris, and toxicity from chemical contaminants and even can interfere with food chain (Canada Gazzette 2010).

The adverse effects of wastewater effluent on environment can be classified into two, that is, ecological and health impact (Akpor 2011). Wastewater includes a number of different inorganic pollutants such as nitrogen, phosphorus, and heavy metals (Larsdotter 2006). Major forms of nitrogen can be ammonium ions and nitrite and nitrate ions (Hurse and Connor 1999). Nitrogen in untreated wastewaters may be organic and inorganic (Sabalowsky 1999). Nitrate in water causes methemoglobinemia, which is the most significant health problem. Blood contains hemoglobin (iron-based compound), but in the presence of nitrite, it gets converted to methemoglobin that does not carry oxygen. Likewise, nitrogen is toxic to fish in its ammoniacal form and adds on to oxygen demand (Jenkins et al. 2003). However, it involves increased concentration of phosphorus in water which is an important constituent of living beings. But after assimilation high concentration of phosphorus in organisms poses major problem (Rybicki 1997). Increased concentration of phosphorus leads to eutrophication, a phenomenon which gives rise to algal bloom. This algal bloom has negative effect on wastewater treatment and can hinder the potability of drinking water. Thus, this type of major limitation can be removed by controlling the concentration of phosphate in wastewater discharge (Vanlarsdrecht 2005).

Human and animal wastes are major sources for pathogenic organisms which are released in the wastewater, leading it to become a major reason for public health hazards. Most commonly occurring pathogenic microorganisms involve bacteria, viruses, and protozoa which contaminates the water resources (Kris 2007). These microorganisms are responsible for major waterborne diseases. Such severe waterborne diseases involve higher risk to human health. Some of the names include typhoid fever, shigellosis, salmonellosis, campylobacteriosis, and giardiasis. Hepatitis A is one of the virus-borne diseases due to drinking of contaminated water (WHO 2004), whereas other pathogens may cause critical diseases which have costly treatment such as stomach ulcers, etc. Viruses can be the most dangerous and harming pollutants present in water. Reasons for this may be high pathogenicity, difficulty in diagnosis, and high dose of antiviral compound requirement (Okoh et al. 2007). Bacteria are another form of pathogens by causing various types of health hazards associated with digestive systems and skin such as diarrhea, dysentery, and skin and tissue infections. Major disease-causing bacteria found in wastewater are different types of bacteria, such as *E. coli* O157:H7, *Listeria*, *Salmonella*, and *Leptospirosis*. *Giardia* and *Cryptosporidium* are among other protozoans causing serious diseases. More concentrations of nitrates can cause methemoglobinemia whose permissible limit has been set as 10 mg/mL by the US Environmental Protection Agency (EPA 2002). Nitrite can further interact with amine to form nitrosamines which are potent carcinogens. Thus, inorganic constituents such as nitrogen and phosphorus cause most favorable conditions for growth of such pathogenic organisms. The microbial toxins cause acute problems ranging from gastroenteritis to nervous system impairment. According to a health report from the World Health Organization, these pathogenic organisms can be a cause of liver cancer in humans.

2.3 Newer Approaches Over Conventional Wastewater

There are several approaches for cleaning wastewater, viz., conventional and advanced treatment (Fig. 2.1). Conventional approaches include various physical as well as chemical treatments. Chemical treatment is one of the most effective treatments for wastewater. The general purposes of the chemical treatment are to change the properties of water such as removal of suspended solids (turbidity) from the water, pH adjustment, and removal of dissolved material in the water, thus improving water quality. The prevalent methods in chemical treatment can be coagulation/flocculation, chlorination, chloramination, ozonation, and ultraviolet light (UV) (Gray 2002).

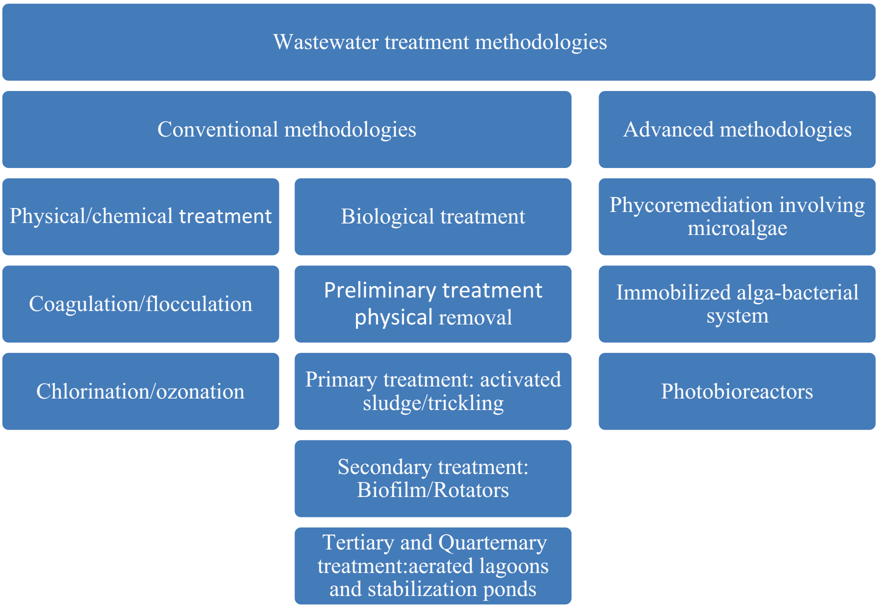

Fig. 2.1 Diagrammatic representation showing conventional and advanced methodologies for wastewater treatment

Flocculation aids in chemical and thermal destruction of pathogens and ultimate killing. Flocculation can contribute to the removal of various heavy metals and pathogenic organisms including *E. coli* (60–98%), viruses (60–90%), and *Giardia* (60–98%) (Tchobanoglous et al. 2003). The addition of alum (coagulating agent) increases the rate at which the suspended particles settle out flocculation. In bulk water treatment, the alum dose can be optimized (Gomez et al. 2006). Chlorination is the best known method of disinfection. It needs more contact time due to its high oxidation potential. Chlorine can react with NH^{4+}, NO^{2-}, H_2S, Fe^{2+}, and other organic compounds and leads to the formation of compound called trihalomethanes, always leading to toxicity problems. It is still the most commonly employed method in the treatment of wastewater with high organic compound concentration (Tchobanoglous et al. 2003).

Ozonation is used mainly in secondary wastewater treatment that has antimicrobial activity. Major drawbacks of ozonation include high cost involved and lack of maintenance. Moreover, there is always the possibility of microbial regrowth. The efficiency of UV and chlorine method has been found to be optimal for disinfection in wastewater. Treatment with UV light results in no toxicity but involves various limitations such as high cost, increased volume of sludge, and dewatering capability. The additional advantages of chemical processes are mineralization of nonbiodegradable components and reduction in size of reactor (Tchobanoglous et al. 2003).

Biological wastewater treatment can be classified as on-site and off-site treatment systems. Both treatments involve different conditions to be fulfilled before treatment. Moreover, both have different impacts on public health as well as environment (Akpor 2011). All biological methods use the metabolic activities of microorganisms to utilize the contaminants of wastewater, thereby reducing the BOD and COD values of wastewater effluent. Almost every biological method involves utilization of microorganisms for digesting inorganic constituents and improving the quality of wastewater. Then it is allowed to settle down as sludge which can be separated, and eventually BOD value can be lowered down. Moreover, the pathogenic organisms can be removed, and wastewater can be recycled efficiently (Abraham et al. 1997).

No single treatment is available for efficient treatment of wastewater. Primary, secondary, and tertiary treatment methods can be used for removal of contaminants and pathogens. Sometimes preliminary treatment can be used prior to primary treatment to increase the efficiency of the treatment methods. Moreover, most of the time, a combination of more than one method is required (Horan 1990). The stepwise biological treatment involves various steps. Preliminary treatment removes the coarse solid materials which will otherwise cause blockage and equipment damage. Very large particles and floating materials are removed by coarse removal which involves using bars (Tebbutt 1983). The next step is primary treatment which includes sedimentation processes. It enhances the settling of solids under gravity. Sedimentation tanks can contribute significantly by lowering the BOD value by 40% (Horan 1990). However, secondary treatment involves the reduction in organic matter. This involves the action of microorganisms such as heterotrophic bacteria and their digestive enzymes on utilization of organic matter for energy and growth. All processes can be divided into fixed film reactors and dispersed growth processes for the removal of the microbial population. Tertiary treatment is used to lower the concentration of organic ions. Biological and chemical methods can be used for tertiary treatment. Tertiary treatment is responsible for deciding factor of overall cost involved because it itself costs four times more than primary treatment (De la Noüe et al. 1992). Sometimes, the next step in quaternary treatment is also intended for removal of heavy metals, organic compounds, and soluble fractions.

2.4 Microalgal Species Involved in the Wastewater Treatments

Several species of microalgae have remarkable ability to remove nitrogen, phosphorus, heavy metals, pesticides, organic and inorganic toxins, and pathogens from wastewater. Example of these microalgae includes *Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas*, and *Arthrospira* (Abdel-Raouf et al. 2003; Rawat et al. 2013; Molazadeh et al. 2019).

Many microalgae species including *Chlorella, Scenedesmus, Euglena, Chlamydomonas, Oscillatoria,* and *Ankistrodesmus* have been demonstrated to grow efficiently in wastewater. Few microbial algae are known for heavy metal removal (Molazadeh et al. 2019). Examples are *Oscillatoria* spp. (for chromium removal), *Chlorella vulgaris (for* cadmium, copper, and zinc removal), *Chlamydomonas* spp.

Table 2.1 Algae used in the remediation of the pollutants present in the wastewater

Pollutants	Algae used for bioremediation	References
Oil effluents	*Scenedesmus obliques* *Prototheca zopfii* *Ankistrodesmus* and *Scenedesmus quadricauda*	Rajasulochana et al. (2009) Walker et al. (1975) Abeliovich (1986); Pinto et al. (2003)
Textile waste effluents	*Chlorella vulgaris* *Chlorella pyrenoidosa*	El-kassas and Mohamed 2014; Jinqi and Houtian (1992)
Phenolics compounds	*Chlorella vulgaris, Spirulina*	Ismail et al. (2013)
Nitrate, organic, and inorganic phosphorous	*Oscillatoria, Synechococcus, Nostoc, Spirulina platensis* *Chlorella sorokiniana*	Dubey et al. (2011); Laliberte et al. (1997); Sawayama et al. (1992); Sawayama et al. (1998) and Ogbonna et al. (2000)
Copper- and iron-containing effluent	*Botryococcus braunii and Anabaena doliolum*	Rai and Mallich (1992)

(lead removal), and *Scenedesmus chlorelloides (*molybdenum removal). It is also noticed that tolerance to organic pollutants in wastewater varies from species to species (Molazadeh et al. 2019). *Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula*, and *Stigeoclonium* have been described as the most resistant genera to organic pollutants (Palmer (1974). Table 2.1 summarizes utilization of various pollutants of wastewater by microalgae.

Microalgae regulate eutrophication process by removing phosphorous and nitrogen components. Microalgae can be effective substitute for biological treatment which converts the organic as well as inorganic unwanted constituents to valuable biomass. Microalgal species have been widely used for treatment of municipal, industrial, agro-industrial, and livestock wastewaters. Algae harvested from the treatment pond may be a source of food and valuable products. Algae are able to accumulate toxic compounds including selenium, zinc, and arsenic in their cells and eliminate them from the aquatic environments. A variety of physical, chemical, and biological methods can be used at different stages of primary, secondary, or tertiary levels for wastewater treatment.

2.5 Factors Affecting the Wastewater Remediation

2.5.1 Carbon

Carbon is the most important constituent for microalgal growth and cell growth as it follows the autotrophic mode of nutrition. However, in other two modes, that is, heterotrophic and mixotrophic, it behaves as organic carbon. In *Chlorella protothecoides*, biomass as well as lipid content varied depending on the modes of nutrition. Heterotrophic mode resulted in 3.4 times more biomass and 4.2 times more lipid content than autotrophic mode (Yanna and Hyde 2002). Microalgae convert carbon dioxide into inorganic carbon source, and water acts as electron donor for production of glucose which gives rise to complex carbohydrates. Moreover, these microalgal cells are efficient for utilization of Na_2CO_3 and $NaHCO_3$. Other important constituents than carbon involve nitrogen and phosphorus which plays a significant role in growth and development of microalgae. There can be so many sources of nitrogen such as detergents, ammonium ions, nitrates, and nitrites present in wastewater which can be efficiently utilized for growth of microalgae.

2.5.2 pH

pH is an important abiotic factor that affects the microalgal growth. In the cultivation of the microalgae, the pH value increases due to the photosynthetic assimilations of the CO_2. pH is another factor responsible for availability of carbon (Azov 1982). Moreover, absorption of nitrogen increases pH of the medium. Mechanism involves reduction of nitrate to ammonia ions which produces hydroxyl ion (Xu et al. 2006). Increased pH induces precipitation of phosphate in the medium. However, this incidence can be lowered by process of respirations. pH is known to influence the growth rate of microalgae. The microalgae use the inorganic carbon and HNO_3 for the growth of the cell productivity. Depending on these parameters, pH value may vary from low to high in the alkaline region. pH from 7 to 9 is optimum for the algal growth. Carbon dioxide acts as buffer system in bicarbonate-carbonate for photosynthesis,

2.5.3 Salinity

Marine phytoplankton is tolerant to changes in salinity. The best algal growing conditions for most species are salinity levels that are lower than that of their native habitat. Lipids can perform both structural and storage functions as they can be used in synthesis of the cell membrane and storage products. The lipid contents and the composition of microalgae have been shown to change the responses to the environmental variables such as the light, temperature, and salinity. Microalgae have greater impact on lipid content due to salinity (Asulabh et al. 2012). However, increased salinity can negatively affect the photosynthetic activity which may be due to hindrance in electron transport chain (Zhang et al. 2012).

2.5.4 Temperature

It is also a very important factor for the growth of the microalgae. Usually microalgae grow profusely in elevated temperature, and growth stops beyond a critical temperature (Ras et al. 2013). Most common temperature range is from 16 °C to 27 °C. More heat along with humidity can result in less growth of microalga. After a critical temperature, growth rate decreases; however, effect of temperature may vary depending on the species.

2.5.5 Light

Microalgae are phototroph which means they obtain energy from the light. Some of the microalgae are capable of growing in dark conditions using simple organic compounds as the energy and the carbon sources. Light conditions directly affect the growth and the photosynthesis process of the microalgae. It was also reported that the conversion efficiency of the sunlight energy into chemical energy is 2% (Fontes et al. 1987). Direct sunlight can often be too intense and cause photoinhibition at the surface. At the same time, algal cells deeper down may suffer from photo-deprivation, as the radiation has been absorbed or reflected by cells closer to the surface. To deal with these challenges, cultivations must be designed with a large surface-to-volume ratio and adequate mixing of the algal mass to make sure all cells are illuminated for an appropriate amount of time (Christenson and Sims 2011). Algal cultures prevent from the light limitation to decrease the depth of the culture vessel. Moreover, depth also affects the productivity in light through inverse relationship.

2.5.6 Inhibitory Substance

Many substances act as inhibitory for cell growth as well as photosynthetic efficiency or process. Inhibitory substances include phenolics, heavy metals, herbicides, pesticides, substances in detergents, some microbes, household cleaning products, and personal care products. Ammonia is one of the inhibitors which reduces the microalgal growth in high temperature and pH (Aharon and Yosef 1976). Mechanism of toxicity by organic compounds is associated with inhibition of nutrient uptake, ultimately leading to permanent damage of cell membrane.

2.6 Problems Encountered During Wastewater Remediation

One of the important drawbacks of wastewater remediation by microalgae is that it requires spacious system for the growth of the algae and a good operation speed which is not fulfilled by present-day phycoremediation technologies. Downstream equipment used for the wastewater remediation is failing due to a build of large solid hairs and fibers during the primary treatments. The treated effluents are not giving the total nitrogen and phosphorous targets. Ammonia removal is a strictly aerobic process. If more ammonia is released into the wastewater remediation, then it results into more retention time and low food-to-microorganism ratio and affects pH buffering. Algae-treated wastewater is not meeting biochemical oxygen demand target due to organic overloading, low oxygen concentrations, and sludge accumulation and old sludge to the effluents. Loss of opportunity to maintain the fertility of the soil is achieved through wastewater rescue. This leads to the need to purchase the organic fossil fertilizers. The downstream processing parameters are very expensive for the harvesting and the recovery of the secondary metabolites. For short-term treatment processes, algal pond treatment can be better alternative for bioflocculation of the algae. Algal growth for the clearing of the wastewater requires the large amount of algae to grow in the water bodies and destabilize the ecosystem if the animals feed the large amount of the algae growing in the water bodies and leads to the death of the animals.

2.7 Mechanism of Action of Microalgae During Wastewater Treatment

Microalgae are a type of microscopic photosynthetic organisms usually found in marine as well as freshwater environments. They possess a photosynthetic mechanism which is somewhat similar to land plants. Such photosynthetic capabilities of microalgae make them significant for treatments with microbial aids where higher concentration of nitrogen and phosphorus can be utilized in conversion of solar energy into biomass. General mechanism adopted by microalgae to treat wastewater includes assimilation, precipitation, biosorption, and bioaccumulation.

2.7.1 Assimilation

Wastewater contains phosphorous in organic as well as inorganic form. Most common forms of phosphorus in aqueous solutions are orthophosphates and polyphosphates which can be utilized by organism for production of biomass. In the next level, polyphosphates can be converted into orthophosphates. This process is usually quite slow. The removal of phosphorous from wastewater in the biological system comprises of the treatment of the influent wastewater which is incorporated into cell biomass and further involves cleaning with sludge wasting. Microalgal cells need phosphorus for metabolic processes such as ATP production, phospholipids, and nucleic acids. Algae can assimilate orthophosphates as inorganic ions with the aid of energy (Becker 1994). Microalgal cells can store the excess phosphorus in its storage (volutin) granules. These reserves can be used for prolonged growth of microalgal cells (Fogg 1975; Oliver and Ganf 2000). Therefore, it may be concluded that phosphorus is not associated with immediate effects on microalgal growth as compared to temperature and pH (Mostert and Grobbelaar 1987). Moreover, concentration of phosphorus may vary in wastewater ranging from 1 mg phosphorus per g dry mass. It has been reported that average concentration of phosphorus in algal cell is 13 mg phosphorus per g dry weight (Oswald 1988). Higher concentrations of phosphorus may not necessarily result in higher growth, whereas various different conditions can be optimized to increase the assimilation efficiency of microalgal cells. For instance, microalgal cells deficient in nutrients can result in better uptake and thus assimilation of phosphorus in less time span. It results in more efficient bioremediation. In turn, phosphorus assimilation depends on fixed carbon in algae. One of the various optimization strategies involves the starving conditions in bioreactor for enhancing the assimilation of other pollutants.

2.7.2 Precipitation

Carbon species are one of important constituents among others. Microalgae take up inorganic carbon in the form of carbon dioxide and bicarbonate ions during photosynthesis (Oswald 1988; Borowitzka 1988), which can be subsequently converted into carbon dioxide using carbonic anhydrase. When bicarbonate is used as carbon source, the pH in the medium increases. This pH increase, which can elevate the pH in algal cultures to values above 11, strongly affects the water chemistry. Phosphorus may as a result precipitate with available cations to form metal phosphates, where calcium phosphates are the most common. Besides being promoted by high pH values, precipitation reactions can be enhanced by higher concentrations of calcium and phosphorus along with high temperature (Song et al. 2002). Precipitation usually results in neutral pH and concentrations of phosphate and calcium to be 50 mg and 100 mg, respectively (Carlsson et al. 1997). In soft water which is usually with less concentration of 50 mg, raised levels of phosphate concentration can be used to induce precipitation. Carbonate enhances the production of amorphous calcium phosphate and promotes calcite formation from calcium at pH above 8.0. Various calcium phosphates can be present in the wastewater which may lie in molar ratios between 1 and 1.67. Some salts like amorphous calcium phosphate and octacalcium phosphate can act as precursor to hydroxyapatite (Arvin 1983). However, hydroxyapatite formation is inhibited by different ion concentrations such as magnesium, carbonate, and pyrophosphates ($P_2O_7^{4-}$) (Fergusson et al. 1973; Arvin 1983). The effect of magnesium is pronounced when the Mg/Ca ratio exceeds 0.45. At pH levels above 10.5, magnesium forms precipitates with hydroxide ions and loses its adverse effect on phosphorus solubility and in turn its utilization (Jenkins et al. 1971). Moreover, carbonate reduces the crystalline nature of calcium phosphate resulting in formation of amorphous calcium phosphate (Fergusson and McCarty 1971; Arvin 1983). It can be concluded that phosphorus precipitation is inversely correlated with carbonate concentration (Fergusson et al. 1973).

Chemical precipitation contributes significantly to phosphorus uptake by algal wastewater treatment and thus bioremediation (Doran and Boyle 1979; Moutin et al. 1992; Proulx and LessardP 1994; Mesple et al. 1996; Tam and Wong 2000). Particularly in areas with hard water, i.e., water with high concentrations of calcium and magnesium, this effect may be pronounced. One of the major effects is chemical stripping of phosphorus which can be specifically beneficial for algal growth with enhanced phosphorus removal as a result. It makes chemical sludge harvesting easier as compared to free-floating cells.

2.7.3 Biosorption

Metabolism-independent binding or adsorption of heavy metals to living or dead cells, extracellular polysaccharides, capsules, and slime layers is referred to as “biosorption.” Walls and envelopes of algae are very efficient in biosorption due to the charged groups present in them (Table 2.2). Algae can be immobilized in polyacrylamide gel and

Table 2.2 Parameters used for the algal cultivations

Operational parameters	Description	References
Inorganic carbon effect	CO_2 and HCO_3^-act as the inorganic source of carbon for the microalgae	De Morias and Costa (2007)
Salinity	High evaporation causes the salinity effect. High salinity can cause the cellular ionic stress and osmotic stress due to the selective ion permeability of the cell wall	Moheimani (2005); Salama et al. (2014)
Light	Strong illumination can inhibit the photosynthesis process	Kim et al. (2015)
pH effect	Higher photosynthetic activity can increase the pH. Neutral pH is favorable but pH as high as 10 and low as 4 are tolerate by some species	Moheimani (2005)

packed into columns or used in fluidized beds for the considerable binding of heavy metals such as zinc, cadmium, copper, lead, gold, and uranium from wastewater (Chojnacka et al. 2005).

Microalgae have enormous potential in cleansing water as they show a strong affinity toward polyvalent metal and dissolved metal ions in wastewater (De Bashan and Bashan 2010), for example, *Chlorella* and *Scenedesmus*.

Biosorption of metals by microorganisms proceeds through a two-stage pathway (Fig. 2.2):

1. An initial rapid, reversible, and passive adsorption onto the cell surface (where metal ions adsorb via electrostatic interactions to cell wall functional groups).
2. A less speedy, irreversible active process which involves the transport of metal cations across the cell membrane. The first stage occurs in both living and non-living cells, whereas the second one takes place only in living ones (Jjemba 2004; Sud et al. 2008).

Garnham et al. (1992) focused on removal of three metals (Zn, Co, and Mn) by *Chlorella salina* and showed that their uptake was essentially biphasic. The initial phase of biosorption is not dependent on physicochemical conditions such as light, temperature, and metabolic inhibitors. A slower phase of uptake followed that was instead dependent on metabolism and other abiotic factors. For those three metals, cellular compartment analysis indicated that large amounts were bound to intracellular components and to the cell wall itself. A higher concentration of each metal in the vacuole than in the cytosol was also observed, thus unfolding a possible mechanism of regulation of the free metal ion and detoxification. The capacity of biomaterials to adsorb metals depends on the composition of their cellular surface and is promoted by the presence of negatively charged functional groups, coupled with chemical composition of the outer solution undergoing treatment (Monteiro et al. 2011). This is especially true with regard to competing anionic groups and pH, which affect protolysis and consequently drive such changes. Microalgae are suitable chiefly as biosorbents owing to their natural abundance in seas and oceans

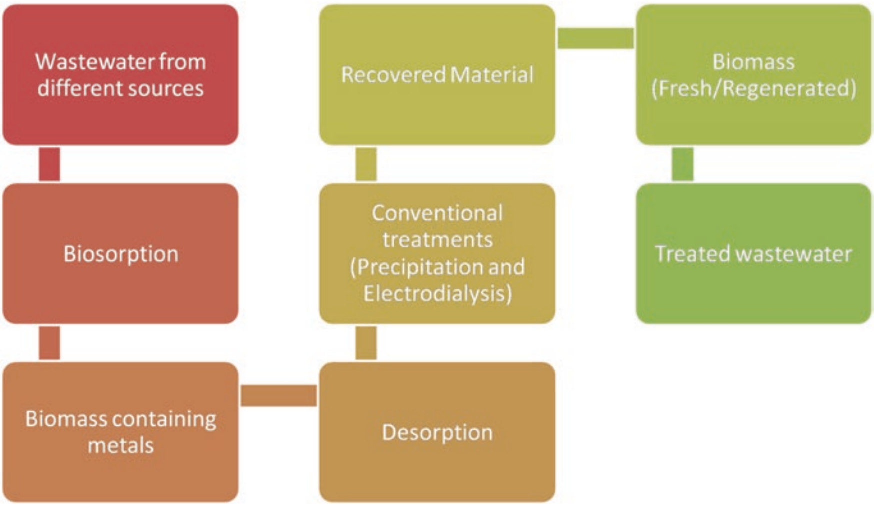

Fig. 2.2 Flowchart showing steps in biosorption

(which allows harvesting and culturing at relatively low cost) and their high sorption capacity when compared with other biological or physicochemical sorbents. Such a capacity spans the range of 25.4–389 mg/g under acidic/neutral pH values (from as low as 2 up to 7) while withstanding concentrations from 20 up to as high as 20,000 mg/L. These features are, on average, better than those claimed for other sorbents from biological or physicochemical origins. Therefore, microalgal biomass might be an economically feasible (besides technologically efficient) alternative to existing physicochemical methods of metal removal and recovery from wastewaters (Mehta and Gaur 2005; Romera et al. 2006) even though actual engineering/cost analyses have not been carried out in full. Note, however, that the yield of microalgal biomass on the original volume of (sea or fresh) water is relatively poor, unlike happens with, e.g., macroalgae, and harvesting is in addition difficult to achieve.

2.7.4 Bioaccumulation

Bioaccumulation is defined as intracellular accumulation of unwanted substances, which occurs in two stages: the first is similar to biosorption involving attachment of potentially toxic elements to the surface, and the second is active transport of metal ions into cells. Bioaccumulation is nonequilibrium process (Aksu and Dönmez 2000). The process is more complex than biosorption itself and requires metabolic activity of cells. It has been reported that metabolic processes support the bioaccumulation process and the following reaction takes place in the cell:

$$\mathrm{HCO_3} - \rightarrow \mathrm{CO_2} + \mathrm{OH}$$

The hydroxyl ions thus produced are present on cell surface which can scavenge the hydroxides of toxic and heavy metals by enhancing precipitation reactions. Therefore, these reactions aid in wastewater treatment processes.

Other processes such as biotransformations and biomineralization involve metabolic processes to remove the toxic ions and convert insoluble sulfides and phosphates into soluble ions so as to achieve effective removal (Lloyd 2002; Gavrilescu 2004). This property is used in the removal of ions of iron, manganese, and lead (Loukidou and Zouboulis 2005). This aspect of bioconversions includes batch systems for wastewater treatment (Aksu and Dönmez 2000). Bioaccumulation offers another important benefit that is separate; biomass harvesting step is not required, and both the treatment and harvesting can be performed simultaneously. Also, additional unit processes are reduced: harvesting, drying, processing, and storage (Aksu and Dönmez 2005). Bioremoval of pollutants present in wastewater has greater impact by operational conditions maintained during the process, as some of the pollutants have negative impact on growth of microalgal cells and thus the treatment process. Generally, the wastewater containing high load of pollutants cannot be treated by bioaccumulation which poses a major limitation to biological method of remediation. Moreover, energy source such as sucrose has to be supplied for providing energy to the growing cells for effective growth and removal (Aksu and Dönmez 2005). By employing effective methods of strain selection, those strains can be selected which can utilize the organic and inorganic pollutants and leads to bioaccumulation. Present-day practical application of bioaccumulation is that the majority of conventional municipal wastewater treatment plants based on living organisms have a significant contribution of bioaccumulation itself. However, much work is needed to confirm the significant contribution of bioaccumulation in municipal wastewater treatment plants (Aksu and Dönmez 2000).

In bioaccumulation, pollutants are transported across cell wall and membrane. Inside the cells are bound to intracellular structures (Kujan et al. 1995). It has been studied in previous work that bioaccumulation involves redox reactions to scavenge the unwanted constituents present in wastewater (Yilmazer and Saracoglu 2009). These metabolic processes are complex in nature, and different conditions such as pH, temperature, growth inhibitors, etc., affect the metabolic processes (Kujan et al. 1995). Metal ions cause toxicity by complexation with lipid content of cell membrane which causes damage in integrity (Yilmazer and Saracoglu 2009). It has been concluded that with increased concentration of pollutants, accumulation adversely affects the cell morphology and physiology (DeSiloniz et al. 2002). Major route for mechanism of action is through sulfhydryl groups of enzymes which can be easily attacked by metal ions, thus causing toxicity in the cell. Another route is by synthesis of low molecular weight proteins rich in thiol groups which can be synthesized in response to complexation of metal ions with these pollutants (Martin-Gonzalez et al. 2006). There are few reports on some adapted microorganisms which are better suited for bioaccumulation than non-adapted ones (Kocberber and Donmez 2007; DeSiloniz et al. 2002). Effective biotreatment results have been obtained by using the enriched cultures isolated from polluted environments (Kocberber and Donmez 2007). A study on bioaccumulation in which plasmid of *Escherichia coli* from pea with genes of metallothionein has been used showed improved accumulation of mercury (Deng and Wilson 2001).

2.8 Biotechnological Strategies for Improvement of Phycoremediation of Wastewater

All the problems faced during the wastewater treatment can be improvised by adopting various biotechnological strategies (Table 2.3) which can be explained in the following sections.

2.8.1 Immobilized Cell System

One of the major problems in the utilization of microalgae for the biological tertiary treatment of wastewater is their recovery from the treated effluent (Chevalier and De la Noüe 1985a; b). Among the ways of solving this problem which have been recently studied are immobilization techniques (De la Noüe and Proulx 1988). Immobilization of the cells provides better utilization as well as stability to the cells as compared to free cells. There are several reports on using immobilized cells in both batch and continuous culture systems (Hall and Rao 1989). Chevalier and De la Noüe (1985a, 1985b) discovered that *Scenedesmus* cells when immobilized using k-carrageenan were capable of bioaccumulating at same rates as that of free microalgal cells. There are numerous advantages related to using the immobilized living cells as compared to suspended cells. Immobilized microalgal cells on suitable

Table 2.3 Different biotechnological strategies used for improvement of phycoremediation

Biotechnological strategy	Microalgal sp. used	Approach used	References
Immobilized microalgal cells	*Phormidium laminosum*	Polymer foam was used as matrix for immobilization of microalgal cells	Sawayama et al. (1998)
Hyperconcentrated cultures	*Scenedesmus obliquus*	Algal biomass greater than 1.5 g/L. more biomass can sequester more carbon and thus result in energy-generating process along with wastewater treatment	Chevalier and De La Noüe (1985a, b)
Microalgal fixed biofilm	*Enterobacter cloacae* DT-1	Natural biofilm # 52 was used as feedstock for bioenergy production	Miranda et al. (2017)
Bacterial-algal ggconsortium	*Chlorella* sp. and bacterial sp.	Bacteria is known for efficient remediation of wastewater, and algal cells can be used for production of value-added compounds and energy production	Foladori et al. (2018)
Photobioreactors	*Dunaliella salina* and *Chlorella* sp.	Photosequencing batch reactor has been developed	

support are advantageous as cell retention time increases in the reactor (Travieso et al. 1992). Polymer coated form of *Phormidium laminosum* removes nitrate components up to 90% in a continuous-flow reactor (De la Noüe et al. 1990; Garbisu et al. 1991; Travieso et al. 1992; Sawayama et al. 1998). Sawayama et al. (1998) have reported that hollow fiber-immobilized cyanobacterial systems are easy to construct and immobilization does not take a long time. Markov et al. (1995) have observed that removal of inorganic nutrients from wastewater can be improved by immobilizing cyanobacteria on hollow fibers and hydrogen production was increased. In a similar study on direct generation of electricity using cyanobacterial species *Mastigocladus* and *Phormidium* immobilized on suitable matrix which has improved the process.

2.8.2 Hyperconcentrated Cultures

Hyperconcentrated cultures have also been employed for wastewater effluent remediation in which high algal biomass >1.5 g/L. Chitosan has been used for immobilizing algae using flocculent (Lavoie and De la Noüe 1983; Morales et al. 1985), whereas cell concentrations of up to 1.9 g/L dry weight have been obtained for *Oscillatoria* sp. grown on sewage sludge (Hashimoto and Furukawa 1989). Studies on hyperconcentrated cultures are very limited, and more work should be done to strengthen the work.

2.8.3 Genetic Engineering

Microalgae consist of characteristics which are helpful for using it for phytoremediation of wastewater, but still no one microalga is the most efficient one. Biotechnology can help in improving the bioremediation efficiency of microalgal cells by inserting the gene of interest in target cells (Guihéneuf et al. 2016). Mutagenesis has also been used for improving the microalgal cells. Selective mutagenesis can be performed using various physical and chemical mutagens. The genetically modified microalgal cells can be used to enhance the production of valuable products (Hlavova et al. 2015). Moreover, for the development of suitable genetically engineered algal strains capable of effective degradation of nitrogen and phosphorus, genome databases like NCBI and GenBank can be used for selection of suitable genes and data mining approach (NCBI directory 1995).

2.9 Microalgal-Bacterial Aggregate System for Wastewater Treatment

Different microorganisms can be complexed and used as aggregates for wastewater treatment. For example, microalgal-bacterial aggregates have been employed for wastewater treatment. Microalgal-bacterial consortium can be used due to synergistic effect in which microalga provides oxygen for the process and bacteria utilizes nitrogen due to nitrification-denitrification. Major problems associated with such aggregates are poor settlement of algal biomass and harvesting problem (Bansal et al. 2018). However, different conditions should be optimized and evaluated for effective wastewater treatment, and economic feasibility of the process can be determined (Quijano et al. 2017). In a similar study, Filadori and coworkers (2018) found enhanced nitrogen removal using energy efficient microalgal-bacterial consortium on real municipal wastewater. Photosequencing batch reactor (PSBR) has been developed for the removal of nitrogen. However, various kinetic characteristics should be evaluated, and mass balance analysis should be performed to improve the process further.

2.10 Development of Photobioreactors

Microalgal cells can be produced on a large scale for employing them in different applications such as bioremediation processes and bioconversion of biomass into valuable products and bioactive compounds (Gupta et al. 2015). A large number of efficient photobioreactors have been proposed that are very advantageous for mass cultivation of algae (Ugwu et al. 2008). Photobioreactor is a reactor with facility for light so as to grow photosynthetic microorganisms such as microalgae. Microalgal cultivation needs photobioreactor for different purposes. For generating high-value-added products, axenic cultivation of microalgae is needed. Until now, different types of PBRs have been invented and produced for algae cultivation during the past decades, and some of them have achieved large-scale commercial production (Singh and Sharma 2012; Fernández et al. 2013; Gupta et al. 2015). Both types of photobioreactors (open and closed ones) can be used for production of valuable products. Open bioreactors have been preferred due to limited control of physical and chemical conditions such as water, temperature, light, and pH. However, closed bioreactor can be used for large-scale production, but major limitation is less light and photosynthetic activity (Bansal et al. 2018).

Microalgal cultivation has been studied for over 70 years. Large-scale microalgal cultivation was firstly raised by the research of Carnegie Institute in 1952 (Burlew 1953). To deal with problems encountered in open system, closed vessels have been developed to achieve a better yield of microalgae biomass, which does not allow direct mass transfer between culture media and the atmosphere and is able to provide a controllable environment such as light, CO_2, temperature, and nutrients (Vasumathi et al. 2012; Wang et al. 2014). Closed photobioreactor can be used for production of various valuable products which can be used in biopharmaceuticals, cosmetics, human health, and biofuels which are produced from microalgae that became more and more important; therefore, the development of suitable and sustainable closed PBRs has a great potential. The current common closed PBRs generally include flat panel, vertical tube (bubble column and airlift), horizontal tube, stirred tank, and their modified configurations (Han et al. 2017). Most commonly used closed bioreactors for phycoremediation of wastewater are suspended system and fixed systems (Hoffman 2002).

Membrane bioreactors (MBR) are the most popular and an effective wastewater treatment technology used in the water treatment area. For the traditional PBR such as the flat bioreactor, microalgae can be easily washed out of the bioreactor. The membrane has a well-known function of excellent micro-size particle separation. Hence, applying membranes containing microalgae to treat wastewater allows decoupling the dilution rate (related to HRT) and biomass retention time (MRT). MBR was applied in the microalgae wastewater treatment processing, called microalgae MBR (MMBR) (Han et al. 2017).

2.11 Applications of Phycoremediation in Wastewater Treatment

Algae can be used in wastewater treatment for a range of purposes (Fig. 2.3) which are beneficial for the environment as well as for producing valuable products. Some of the applications of wastewater treatment by microalgae are given below:

2.11.1 Microalgae in Wastewater Treatment

2.11.1.1 Removal of Nutrients

Microalgae can be efficiently used to remediate the nutrients present in wastewater. Major nutrient is nitrogen which contributes to 10% of biomass and is the second most important nutrient to microalgal cells (Becker 1994). Nitrogen can be mainly present as ammonium ions and nitrate ions which can be easily accumulated by microalgal cells (Oliver and Ganf 2000). However, cyanobacteria can assimilate various amino acids such as arginine, glutamine, and asparagine and thus can fix nitrogen (Bhaya et al. 2000). Several species of microalgae can utilize excess of nitrogen. Phosphorus is the next significant macronutrient which can be utilized by algae in form of orthophosphate. Phosphates can be utilized by phosphatases and stored within the microalgal cells in polyphosphate granules which is known as assimilation. Thus, wastewater containing high amounts of phosphorous can be treated by using microalgal cultures (Fogg 1975; Oliver and Ganf 2000).

Moreover, microalgae can be efficiently used for tertiary and quaternary treatments as it can easily take up major nutrients for its growth.

2.11.1.2 Reduction of Biological and Chemical Oxygen Demand (BOD/COD)

Algae are more efficient carbon fixer as well as scavenge excess nutrients at effective cost. It can relieve the biological oxygen demand of wastewater by oxygen produced by photosynthetic process (Laliberte et al. 1994). Microalgae are considered to be better for remediation process because it results in less toxic waste as well as are nonpathogenic. Algae can remediate the wastewater by the use of enzymes for conversion and degradation of pollutants (Oswald 1988). More recalcitrant metals and xenobiotic compounds can be remediated by algal metabolism. Many

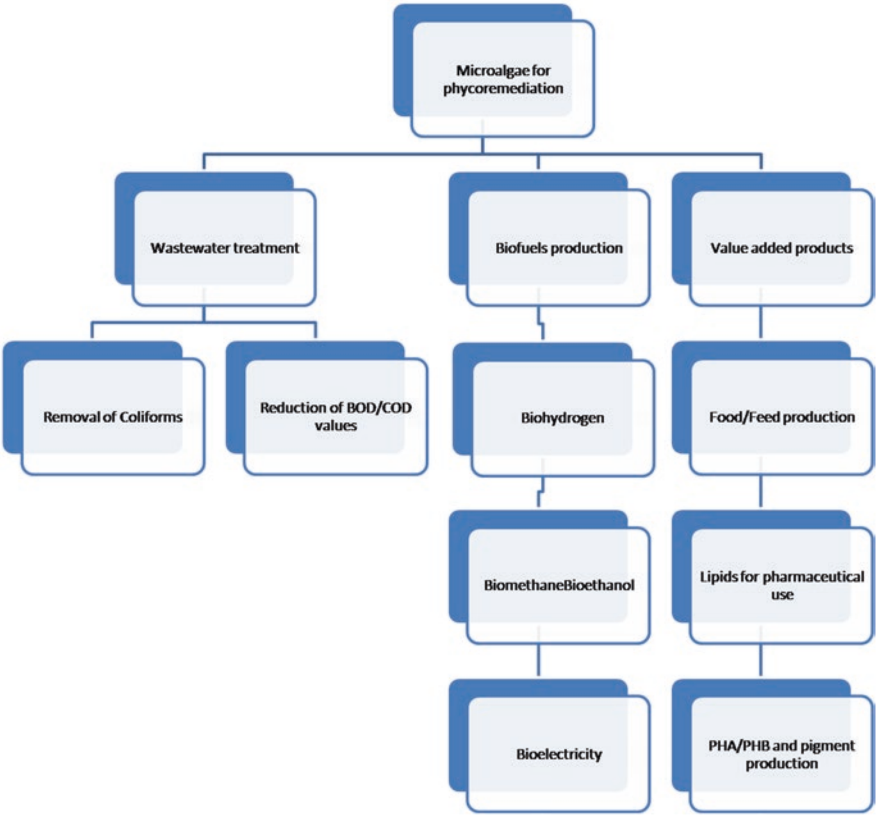

Fig. 2.3 Applications of microalgae for wastewater treatment and production of valuable compounds

researchers have studied microalgae as possible solution for environmental problems (Yoshida et al. 2006). The growth of algal cells in natural waters makes it fit for human consumption. Important algae species which can be employed for wastewater treatment are *Chlorella, Scenedesmus, Synechocystis, Gloeocapsa, Chroococcus, Anabaena, Lyngbya, Oscillatoria,* and *Spirulina* (Palmer 1974).

However, due to excess BOD values of wastewater, the dissolved oxygen can be depleted which results in anaerobiosis and thus has adverse effect on aquatic life. Hence, its removal is necessary. Colak and Kaya (1988) have found that possibilities of biological wastewater treatment by algae eliminated BOD and COD by 68.4% and 67.2%, respectively.

2.11.1.3 Removal of Coliform Bacteria

Microalgae can result in scanty growth of fecal coliforms (FC) due to less availability of dissolved oxygen in wastewater. This affects the growth of coliforms. Most of the coliform bacteria are responsible for pathogenic outbreaks which make water unfit for drinking purposes. The efficiency of microalgae for bioremediation is decided on the basis of coliforms it removes (Sebastian and Nair 1984).

In a study carried out by Curtis et al. (1992), different necessary conditions have been checked out for growth of coliforms in the wastewater. Sunlight can damage fecal coliform by depleting the oxygen concentration. This depletion occurs in the presence of higher pH range which is usually greater than pH 8.5 (Colley Davies et al. 2000). Moreover, pH along with oxygen gives rise to photooxidation process responsible for killing most of fecal coliforms (Maynard et al. 1999). Major significant factors have been found to be increased pH and oxygenation (Van der Steen et al. 2000). Ansa et al. (2011) have observed that simulation of algal cells in lake conditions can effectively reduce the coliforms.

2.11.1.4 Heavy Metal Removal

Microalgal cells possess molecular mechanism which can differentiate between normal and heavy metals (Vela et al. 2006). Moreover, they can be used for easy recovery of heavy metals using different desorption chemicals (Figueira et al. 1999; Rajamani et al. 2007). Algal cells have higher affinity for metals which makes them more suitable for metal removal from wastewater (De Bashan and Bashan 2010). In particular, the mechanism by which microorganism removes metals from solution includes:

1. Extracellular accumulation/precipitation.
2. Cell-surface sorption or complexation by live or dead biomass.
3. Intracellular accumulation that requires microbial activity (Cossich et al. 2002).

Mechanism for biosorption involves the entrapment of heavy metals in the cellular structure and subsequent absorption on binding sites of cells. This method is also termed as “biosorption” or “passive uptake” (Malik 2004). Heavy metals can interfere with the metabolic processes of cell and thus cause bioaccumulation or active uptake.

2.12 Formation of Valuable Products

Extensive use of fossil fuels due to rapid industrialization has resulted in increase in global warming, thereby changing climatic conditions. There is aroused interest in utilizing microalgal cells for efficient alternative energy sources known as biofuels. Microalgae are thus extensively used for the production of biomass which in turn can be used for biofuel production. Microalgae have several advantages for biofuel production as these cells have short generation time, rapid growth, high lipid content, and minimal land requirements. Moreover, wastewater stream can be utilized as nutrient feed for these microalgal cells for conversion of biomass to bioenergy.

2.12.1 Biomass Production

Microalgal cells can be easily grown using the wastewater stream as they contain various constituents that serve as nutrients for the cells. Biomass thus produced can be used in a number of beneficial ways. As biomass can be used for accumulation of heavy metals that are toxic and after extraction of lipid left out, biomass can be used for animal feed or for increasing fertility of soil (Pittman et al. 2011). The biomass can be utilized for production of biofuels and can be converted into many valuable products such as biofertilizers, biofilms, and biopolymers, and recent report is on using them for production of electricity (Gouveia et al. 2016).

2.12.2 Bioethanol Production

Generally, two methods are employed for the production of bioethanol from microalgal biomass, namely, fermentation (biochemical process) and gasification (Singh and Gu 2010). Microalgae are rich in carbohydrates and proteins which can be used as carbon sources for fermentation, so microalgae can replace the requirement of food crops. This provides the scope for utilization of microalgae in the third-generation biofuel production, as using the food crops will cause the scarcity of the same. Moreover, there is a temporary prohibition on the use of food crops for the production of bioethanol due to food security and availability of agricultural land which can be easily resolved using microalgal cells. Therefore, microalgae are generating a lot of interest as biomass feedstock for bioethanol production (Harun et al. 2010). Fermentation of the microalgal biomass is catalyzed by microbes such as bacteria, yeast, and fungi, and the main by-products are CO_2 and water. The spent biomass after fermentation is used for anaerobic digestion process for methane production so in essence all the organic matter is accounted for (Singh and Gu 2010; Harun et al. 2010). Bioethanol production using microalgal cells is still in initial stage, and more studies are required to evaluate the utilization of microalgae for conversion of biomass to bioethanol (Harun et al. 2010).

2.12.3 Biomethane Production

The interest in biomethane production emanates from the fact that biomethane fermentation technology produces valuable products such as biogas (Singh and Gu 2010; Harun et al. 2010). Biogas is a mixture of methane (55–75%) and CO_2 (25–45%) and is produced via anaerobic digestion of microalgal biomass by anaerobic microorganisms (Singh and Gu 2010; Harun et al. 2010). Biomethane can be used as fuel gas and can also be used to generate electricity, while the spent biomass is used to make biofertilizers (Singh and Gu 2010). Biogas production in turn depends on several factors such as temperature, pH, organic load, and retention time in reactors (Harun et al. 2010). Though microalgae hold enormous potential for biogas production, more studies are needed to strengthen the possibilities of using them on commercial scale for biogas production (Singh and Gu 2010).

2.12.4 Biochar Production

Biomass obtained from microalgal cells can be used as important alternative of energy due to its high efficiency to fix carbon dioxide and easy availability that can be very useful in present scenario of energy crisis around the globe. Algal biomass can be converted into biochar that involves slow pyrolysis to prepare a carbon-rich product used for increasing alkalinity of acidic soils. Moreover, high concentrations of nitrogen and phosphorus give additional advantage to increase fertility of soil for agriculture (Chaiwong et al. 2013). In some previous studies, this biochar due to the presence of functional groups and inorganic elements helps it to be used as absorbent for wastewater remediation (Yu et al. 2017). Functional groups present on microalgal biochar are responsible for better biosorption for various organic molecules. Biochar has potential to be used for increasing fertility and alkalinity of soil for agricultural processes.

2.12.5 Microalgae in Bioelectrochemical Systems

Due to huge energy demands, there is a constant need to find alternative energy resources which are clean, renewable, and cost-effective as well as environmentally friendly. Microbial fuel cells (MFCs) are such energy-generating systems that fulfill all the above characteristics. Microbial fuel cells are based on important property of microalgal cells to fix atmospheric carbon dioxide and produce oxygen by photosynthesis that can enhance the cathodic reaction (Saba et al. 2017). These microalgal cells can also act as efficient electron acceptors and can behave as electron acceptors at cathodic end and electron donors at anodic end. They can be used for removal of various organic and inorganic constituents from wastewater (Gude et al. 2013; Wu et al. 2014; Commault et al. 2014). Baicha et al. (2016) reviewed microalgal cells as MFCs for bioproduction of electricity and concluded that carbon dioxide can play a significant role in biomass cultivation. Along with MFCs, microalgal cells can be also employed as microbial desalination cells (MDCs) and bioelectrochemical systems (BES). In another study, Saba et al. (2017) have reviewed the effect of several parameters on energy production from MFCs.

The major limitation in MFC is the low current flow; however, considerable amount of energy is generated (Otadi et al. 2011). Photosynthetic activity of microalgal cells can be used to generate electricity like bioelectrochemical system. Thus, solar energy can be converted to electricity using microalgal cells. These play a significant role in power generation and can consume the light at night generated in daylight (Soni et al. 2016).

2.12.6 Microalgal Biofilms

Biofilms are produced when microalgal cells are covered by surface molecules such as exopolysaccharides. Algal biofilms are films which are generated by colonization of microalgal cells on illuminated surfaces in the presence of humid conditions and nutrients (Leadbeater and Callow 1992; Jarvie et al. 2002). Algal biofilms can adapt the change in environment and survive all the adverse conditions as single cell or in clumps (Menicucci 2010). Microalgal cells can be used in a wide number of applications ranging from agriculture, alternative energy sources, and personal care products and nutraceuticals (Pulz and Gross 2004; Mata et al. 2010). Algal biofilm is used for removing water impurities; thus, algae are significant due to nutrient removal from wastewater due to their enhanced nitrogen (N) and phosphorous (P) metabolism ability. Algal biofilms can be employed in wastewater treatment as well. It avoids expensive harvesting techniques used in suspension cultivation like centrifugation and flocculation (Gross et al. 2016).

2.13 Other Applications of Microalgae

2.13.1 Production of Secondary Metabolites

Microalgae share some of the common properties like plants, and thus they can be used for production of some important secondary metabolites, viz., carotenoids, sterols, proteins, lectins, oils, unsaturated fatty acids, antioxidants, fibers, and amino acids. Their potential can be explored for commercial production (Cardozo et al. 2007a, b; Rosenberg et al. 2008; Ioannou and Roussis 2009; Ibañez et al. 2012).

2.13.2 Sulfated Polysaccharides

Marine algae can be used as source for sulfated polysaccharides (SPs) with so many structural variants (Wijesekara et al. 2011; Zhang et al. 2012). But most common sulfated polysaccharides are fucoidan and laminarins derived from brown algae, carrageenan from red algae, and ulvan obtained from green algae (Li and Kim 2011). Some of previous studies have been carried out with objective of using these sulfated polysaccharides in food, feed, pharmaceutical, and beauty industry (Li and Kim 2011). Some of the studies have confirmed the role of sulfated polysaccharides as antiviral compounds against enveloped viruses (Baba et al. 1998; Zhu et al. 2003). In a similar study, anti-HIV activity has been reported in microalgal and cyanobacterial extracts. Moreover, the antiviral activity depends on the molecular weight as well as grade of sulfation in the compounds (Witvrouw and De Clercq 1997). Antiviral compounds were extracted with anti-HIV activities from *Fucus vesiculosus*. It showed water solubility and high anti-HIV activity (Béress et al. 1993). Likewise, different algae Phaeophyta, Rhodophyta, and Chlorophyta have been explored for antiviral activity (Zhu et al. 2003).

2.13.3 Proteins and Amino Acids

Rhodophyta (red algae) have enormous amount of proteins as compared to other types of microalgae (Mendis and Kim 2011). For instance, phycobiliproteins (PBPs) are generally present in red algae and cyanobacteria (Sekar and Chandramohan 2008). PBPs possess high solubility, stability, and fluorescent properties (Su et al. 2010). These can perform several important functions such as light harvesting reactions in cyanobacteria and formation of cryptomonads and cyanelles (Glazer 1994). There are extensive reports of previous studies on PBPs, and they are known to contain medicinal properties such as antitumor, anti-inflammatory, serum lipid-reducing properties, and antiviral properties. Moreover, these can be utilized for adsorption of pollutants from the body (Romay et al. 2003; Sekar and Chandramohan 2008).

2.13.4 PHA and PHB Production

Novel compounds such as sustainable polymers like PHAs (polyhydroxyalkanoates) and PHBs (polyhydroxybutyrates) can also be produced from algal biomass that is significantly used for production of bioplastics. These polymers can be used successfully in nanotechnology as efficient nanomaterials and scaffolds in tissue engineering (Verma et al. 2019; Bansal et al. 2018).

2.14 Conclusion and Future Prospects

It can be concluded that phycoremediation is one of the safe methods that can be used for treating wastewater. It not only produces the clean water but provides various valuable products as well as better alternative energy sources such as biofuels. Though there are numerous studies on using microalgae in production of these valuable products, still many challenges have been encountered such as land and space requirements, algal contamination with bacterial cells, eutrophication, etc. These problems can be resolved by using photobioreactor which is very efficient novel biotechnological approach. As one of the major problems is availability of clean water for human use, microalgal technology can help humanity in a great way.

Microalgae can be effectively employed for removal of metal ions and can be used as recombinant systems for protein expression for higher plants and animals (Hempel et al. 2011). In some of previous works, *Chlamydomonas reinhardtii* and the diatom *Phaeodactylum tricornutum* have been utilized as model expression systems. They can also be used for preparation of nanoparticles using metal oxides. Microalgae can reduce the pollutant load in environment and avoid the problems that can affect human health care (Fawcett et al. 2017). Moreover, these cells can be efficiently used for alternative energy sources and production of biofuels. Unlike petroleum-based fuels like diesel and petrol, biofuels are rapidly biodegradable. Microalgae can also serve as clean electricity producers for bioenergy production

(Clarens et al. 2011). Along with so many wonderful properties, these cells can be used for production of some of novel compounds such as PHA and PHB which has revolutionized the tissue engineering approaches in human health care.

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