Chapter 23
Biosorption of Heavy Metals and Dyes from Industrial Effluents by Microalgae

Wan-Loy Chu and Siew-Moi Phang

Abstract Discharge of industrial effluents containing heavy metals and dyes is of concern as the pollutants may adversely affect the environment by contaminating surface- and groundwater resources. Heavy metals and dyes are very persistent and may pose a threat to various organisms and human health. Physicochemical methods such as chemical precipitation and adsorption are commonly used to remove heavy metals, while flocculation, flotation, membrane filtration and activated carbon are used to treat dye wastewater. However, these conventional technologies are costly and may not be fully effective in removing heavy metals and dyes. Biosorption refers to the ability of living or dead biomass to sequestrate pollutants such as heavy metals and dyes through passive binding. The biosorption capacity of microalgae is due to their high surface-to-volume ratio and high binding affinity. The mechanisms involved in biosorption include ion exchange, complexation, precipitation and physical adsorption. Functional sites on the cell wall of microalgae involved in biosorption include carboxyl, imidazole, sulfhydryl, amino, phosphate and sulphate moieties. Despite the extensive research in this area, there has been limited success in commercializing the technologies using algal biosorbents. Detailed economic and market analyses are required to assess the feasibility of the technologies. Integration of wastewater treatment and biofuel production with heavy metal and dye removal using biosorption process would be an attractive approach. Apart from treating the wastewater, the microalgae can be harvested for biofuel production, and the residual biomass can be used for biosorption of heavy metals and dyes.

Keywords Biosorption · Heavy metals · Dyes · Microalgae · Bioremediation

W.-L. Chu (✉)
School of Postgraduate Studies, International Medical University, Kuala Lumpur, Malaysia
e-mail: wanloy_chu@imu.edu.my

S.-M. Phang
Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

Institute of Ocean & Earth Sciences (IOES), University of Malaya, Kuala Lumpur, Malaysia
e-mail: phang@um.edu.my

1 Introduction

Anthropogenic activities and industrialization have generated large quantities of aqueous effluents containing toxic metals as well as various organic pollutants including dyes. Industrial wastewaters containing heavy metals and dyes are of concern as they can cause detrimental effects on ecosystems and can be hazardous to human health (Bilal et al. 2018; Vikrant et al. 2018).

Effluents that contain high amounts of heavy metals include those from sources such as mining, plastic, fertilizer, textile, dyeing, paper and paint industries (Abbas et al. 2014). Contamination of drinking water resources due to discharge of industrial effluents laden with heavy metals is a great concern (Azimi et al. 2017). Furthermore, most of the heavy metal ions are toxic to living organisms, and thus, efficient removal of the toxicants before discharge is crucial (Dhankhar and Hooda 2011).

Effluents originating from textile, wool and tanning industries are a major source of dyes that pollute the aquatic ecosystems (Vikrant et al. 2018; Ghosh et al. 2016). There are over 100,000 types of commercially available dyes and pigments, with annual production of over 7×10^5 tons worldwide (Robinson et al. 2001). In the textile industry, up to 200,000 tons of these dyes are lost in the effluents due to inefficiency in the dyeing process (Ogugbue and Sawidis 2011). Contamination of water bodies by synthetic dyes is regarded as a global environmental problem that poses a threat to aquatic ecosystems as well as human health. Synthetic dyes can adversely affect growth and metabolic activities, especially the photosynthetic activity of microalgae, which play an important role as primary producers in the food chain. For instance, exposure to Congo red reduced the growth rate and adversely the photosynthetic efficiency of *Chlorella vulgaris* (Hernandez-Zamora et al. 2014).

Microalgae have been used in bioremediation of agroindustrial wastewaters, including the removal of pollutants such as heavy metals and dyes (Phang et al. 2015). *Chlorella vulgaris* grown in high rate algae ponds (HRAP) has been shown to have potential application for bioremediation of textile wastewater, especially in colour removal (Lim et al. 2010). Microalgae are known to have enormous potential for bioremediation of heavy metals due to their ability to concentrate metal ions (Suresh Kumar et al. 2015). One of the major processes involved in removing heavy metals and dyes by microalgae is through biosorption (Bilal et al. 2018). Algal biomass has high binding affinity for metals and dyes as the algal cell wall and exopolysaccharides contain diverse functional groups (Romera et al. 2006; Mohan et al. 2008; Maurya et al. 2014; Kumar et al. 2016).

The primary aim of this chapter is to review the current status of research on biosorption of metals and dyes using both living and non-living biomass of microalgae. This review will also highlight the potential applications of microalgae as biosorbents for bioremediation of industrial effluents containing metals and dyes.

2 Sources of Heavy Metal and Dye Pollutants

The major sources of wastewaters containing heavy metals are the electroplating, mining, tanning, metal finishing, electronic circuit, steel, aluminium and textile industries (O'Connell et al. 2008; Noreen et al. 2017). Other anthropogenic sources of heavy metals include landfill leachate, fertilizers and manure sewage sludge. Typical toxic metals found in industrial effluents include cadmium (Cd), copper (Cu), nickel (Ni), chromium (Cr), cobalt (Co), zinc (Zn) and lead (Pb). Effluents arising from electroplating industries are complex as they contain a variety of metal ions with counter anions, surfactants and organic/inorganic additives (Islamoglu et al. 2006). Such effluents contain high amounts of Cd (28,656 mg/L), with medium amounts of Zn, Cu and iron (Fe) and small amounts of Ni, Co and manganese (Mn). In addition, the total Cr in wastewater from chrome plating industries may range from 0.1 to 0.9 g/L in a diluted rinse discharge stream to 78–286 g/L in an electrolytic bath stream (Agrawal et al. 2006). In comparison, the raw effluent from copper smelting and refinery contain elevated levels of Cd (3810 μg/kg) and mercury (Hg; 760 μg/kg) (Chojnacka et al. 2004).

The concentrations of Cr from tanning operations may range from 0.5 to 4.0 g/L. High concentrations of arsenic (As; 54 mg/L), Fe (5.8 g/L) and Zn (1.6 g/L) along with SO_4^{2-} (17.7 g/L) have been reported in raw mining water from a former ore mining activity on the Kank Hill, Czech Republic (Doušová et al. 2005). In comparison, textile effluents contain high amounts of heavy metals such as Pb (0.13–0.25 mg/L) and Cd (0.10–0.20 mg/L) (Noreen et al. 2017). Animal husbandry, especially pig farming, is another source of heavy metal pollution, as pig manure may contain high levels of metals such as Cu (151.11 mg/kg) and Zn (538.29 mg/g) (Feng et al. 2018). The metals may cause a wide range of detrimental human health effects, including toxic effects on the nervous and reproductive systems (Pb), cardiovascular system (As) and respiratory and gastrointestinal systems (Cd) and carcinogenic effect (Cr) (Bilal et al. 2018).

There are many types of dyes, which can be broadly categorized into non-ionic (disperse dyes), anionic (direct, acid and reactive dyes) and cationic forms (basic dyes) (Vikrant et al. 2018). Azo dyes are the most common dye stuff used in the textile industry; the chromophoric azo groups (-N=N-) present in these dyes can undergo reductive cleavage resulting in the formation of highly toxic aromatic amines (Bruschweiler and Merlot 2017). The major sources of effluents containing dyes are from industries such as textile, paper, plastics and dyestuffs (Crini and Badot 2008; Vikrant et al. 2018). Such industries consume substantial amounts of water and use significant amounts of chemicals during manufacturing and dyes to colour their products. As a result, a considerable amount of coloured wastewater is generated. For instance, the apparent colour of textile wastewater may range from 169.67 to 1937.33 PtCo unit, with high levels of metals such as Pb, Cd, Zn and Mn (Lim et al. 2010). The effluents, if discharged untreated, are a major source of aquatic

pollution. The pollutants are not only toxic but also cause adverse impact on the aesthetic value of receiving water bodies. For instance, pulp and paper mills generate coloured effluents with high chemical oxygen demand (COD), biological oxygen demand (BOD) and suspended solid (mainly fibres) and contain toxic chemicals such as chlorinated phenolic compounds (Pokhrel and Viraraghavan 2004).

Efficient treatment is required before coloured wastewater is discharged as the presence of very small amounts of dyes in water is highly visible and undesirable. Wastewater containing dyes is not easy to treat as the chemicals consist of recalcitrant molecules, particularly azo dyes, that are resistant to aerobic digestion and stable to oxidizing agents (Fomina and Gadd 2014). Effluents containing metal complex dyes from textile and leather industries not only contaminate water bodies with synthetic dyes but also heavy metals such as Cr, Co, Cu and Ni (Ghosh et al. 2016).

3 Technologies for Removal of Heavy Metals and Dyes

Efficient treatment of effluents containing metals is required before discharge of the wastewater to the environment. Conventional technologies used to remove heavy metals from aqueous solution include chemical precipitation, lime (calcium hydroxide) coagulation, solvent extraction, membrane filtration, reverse osmosis, ion exchange and adsorption (O'Connell et al. 2008; Kurniawan et al. 2006; Azimi et al. 2017). Chemical precipitation is most widely used for removal of heavy metals from electroplating wastewater; however, it is non-selective and produce large quantities of sludge (Kurniawan et al. 2006; O'Connell et al. 2008). Using this method, metals are precipitated by the addition of lime (calcium hydroxide), sulphide and caustic soda. Additional methods such as chemical extraction, bioleaching process, electrokinetic process and supercritical extraction need to be applied to remove the heavy metals from the sludge before disposal (Babel and del Mundo Dacera 2006).

Ion exchange is the second most widely used technique for metal removal from industrial effluents (O'Connell et al. 2008). In this technique, there is a reversible exchange of ions between the solid and liquid phase. A column of resin is used to remove ions from an electrolytic solution and releases other ions of similar charge in a chemically equivalent amount. Another widely used method is adsorption, which involves the physical adherence or binding of ions or molecules onto two-dimensional surface (Fomina and Gadd 2014). The common adsorbents used include activated carbon, carbon nanotubes and sawdust (Azimi et al. 2017). An advantage of the process is that the adsorbents can be regenerated by desorption. The disadvantages of the above-mentioned physicochemical techniques include the large amount of sludge generated, high chemical consumption and high operation costs (O'Connell et al. 2008).

Biosorption is an attractive method for removal of metal ions as it is comparable to the well-established ion-exchange resin-based treatment method (Volesky 2007). According to Gadd (2009), biosorption can be simply defined as *the removal of*

substances from solution by biological material. Biosorption involves physicochemical and metabolically independent process that is based on a variety of mechanisms including absorption, adsorption, ion exchange, surface complexation and precipitation (Fomina and Gadd 2014). The biological material used may include both living and dead microorganisms and their components. The biosorption process involves rapid and reversible binding of ions onto functional groups that are present on the surface of biomass (Michalak et al. 2013). Such process is independent of cellular metabolism, in contrast with bioaccumulation, which requires metabolic activity of living organisms (Davis et al. 2003).

The target sorbate removed from aqueous solution using biosoprtion include metals, dyes, fluoride, phthalates and pharmaceuticals. In the context of heavy metal removal, biosorption involves passive mechanisms of metal binding that are not driven by metabolism (Volesky 2007) compared to bioaccumulation, which involves active uptake of metals by living biomass (Chojnacka 2010). Metal removal by living biomass of microalgae may involve both adsorption and active uptake (Cheng et al. 2016; Kumar et al. 2016). For instance, adsorption kinetics of six metal ions (Al, Zn, Hg, Pb, Cu and Cd) on living cells of six species of microalgae showed that removal of the metals was through a combination of adsorption and accumulation (Schmitt et al. 2001).

Various physicochemical methods are used for the treatment of dyes, including oxidative remediation, adsorption, coagulation, membrane separation and ion exchange (Vikrant et al. 2018). Some of the shortcomings associated with such technologies include high operating/energy costs, generation of large amounts of sludge and production of damaging byproducts. Bioremediation is an attractive approach in the treatment of dye wastewater. For such purpose, the use of a consortium of microalgae, bacteria and fungi is more efficient than pure cultures for effective decolonization process (Forgacs et al. 2004). The individual strains may attack at different positions of the molecules or utilize the breakdown products from other strains. The use of low-cost and efficient solid materials as biosorbents for removing synthetic dyes from water and wastewater is regarded as a simple and economical method (Forgacs et al. 2004).

4 Use of Microalgae as Biosorbents for Removal of Heavy Metals and Dyes

According to the statistical review by Romera et al. (2006), algae have been less used as biosorbents compared to other kinds of biomass, especially fungi and bacteria. However, the interest in this field has increased significantly, as inferred from the publications produced in recent years. Amongst algae, the brown seaweeds, especially *Sargassum*, have been shown to be good biosorbents for heavy metals, which could be due to their abundant cell wall polysaccharides and extracellular polymers (Romera et al. 2006; Brinza et al. 2007; Wang and Chen 2009). The analysis by

Romera et al. (2006) showed that algal biomass has the highest sorption affinity for Pb, followed by Cd, Cu, Ni and Zn.

The use of algae as biosorbents has several advantages, including (1) diverse multifunctional groups on their surface, (2) relatively small and uniform distribution of binding sites on the surface, (3) less preparatory steps required, (4) less usage of harsh chemicals, (5) the algal biomass that can be easily produced and recycled and (6) good retention capacity (Bilal et al. 2018). It is advantageous to use microalgae instead of seaweeds as biosorbents as the former can be grown on a large scale to generate sufficient biomass. Furthermore, mass culture of microalgae can be integrated with other applications, especially for bioremediation of wastewater and production of biofuels (Chu 2017).

4.1   Biosorption of Heavy Metals

The potential of microalgae as a tool for remediation of heavy metals in industrial effluents has been reviewed by many authors (e.g. Mehta and Gaur 2005; Suresh Kumar et al. 2015). There are also critical reviews focusing on the use of microalgal biomass for removing heavy metals by biosorption (Vijayaraghavan and Balasubramanian 2015; Kumar et al. 2016; Bilal et al. 2018). The removal efficiencies of heavy metals by microalgae vary greatly with species, ranging from 0.17 to 1055 mg/g biomass, as compiled in an extensive review by Suresh Kumar et al. (2015). Various microalgae and cyanobacteria including green algae such as *Chlorella*, *Chlamydomonas* and *Desmodesmus* and diatoms such as *Phaeodactylum*, *Cyclotella* and *Aulosira* as well as cyanobacteria such as *Spirulina*, *Oscillatoria* and *Phormidium* have been assessed in terms of their capability to remove metals. A summary of various studies on the use of microalgae for biosorption of heavy metals is presented in Table 23.1.

Most studies related to metal sorption focussed on Cu(II), with *Chlorella vulgaris* being the most commonly used microalga, tested in various forms, ranging from non-living, living, free to immobilized cells (Suresh Kumar et al. 2015). Wilke et al. (2006) examined the biosorption abilities of 37 strains of algae in removing Cd(II), Pb(II), Ni(II) and Zn(II) from aqueous solution. The study showed that the cyanobacterium *Lyngbya taylorii* exhibited high uptake capacities for the metals, with the order of selective sorption as follows: Pb >> Ni > Cd > Zn. Recently, Dirbaz and Roosta (2018) assessed the biosorption capacities of four microalgae in removing Cd (II) and found that *Parachlorella* sp. showed the highest metal uptake (96.2 mg/g biomass). There have also been studies on biosorption of toxic elements, particularly radionuclides, by microalgae (Naya et al. 2003; Bilal et al. 2018). While there have been many reports on biosorption of heavy metals, most studies were based on defined media or synthetic metal solution rather than industrial effluents (Table 23.1).

Bakatula et al. (2014) reported the high sorption efficiency of Cu, Co, Cr, Fe, Hg, Ni, Zn and uranium (U) in single- and multi-ion solutions by the filamentous green

Table 23.1 Summary of the studies on the use of microalgae for biosorption of heavy metals

Microalgae	Heavy metals	Metal concentrations tested	Biomass used	Test system	Findings	References
Chlamydomonas reinhardtii	Hg(II), Cg(II) and Pb(II)	100 mg/L	Cells harvested from logarithmic phase cultures	Algal biomass (800 mg/L) transferred into metal ions in NaCl solution (25 mL), agitated magnetically	Biosorption capacity: Hg(II) – 72.2 mg/g; Cd(II) – 42.6 mg/g; Pb(II) – 96.3 mg/g; Optimum pH for maximum adsorption: Hg(II) and Cd (II) – pH 6.0; Pb(II) – pH 5.0; Followed Freundlich biosorption model	Tuzun et al. (2005)
Chlorella sp.	Cd (II)	10 mg/L	Algae immobilized in water hyacinth-derived pellets	Conical flasks	Maximal removal efficiency: 92.45% by the water hyacinth leaf biochar pellet immobilized with algal cells	Shen et al. (2018)
Chlorella minutissima	Cd, Cu, Mn and Zn	Cd: 0.2–0.6 mM; Cu: 0.2–1 mM; Mn: 2–6 mM; Zn: 2–6 mM	Dead (lyophilized) biomass	150 mL conical flask containing 50 mL metal solution	Maximum adsorption capacity: Cd – 35.36 mg/g; Cu – 3.28 mg/g	Yang et al. (2015)

(continued)

Table 23.1 (continued)

Microalgae	Heavy metals	Metal concentrations tested	Biomass used	Test system	Findings	References
					Mn –21.19 mg/g	
					Zn –33.71 mg/g	
Chlorella vulgaris	Cd (II)	100 mg/L	Live and dead biomass	500 mL conical flasks (with 200 mL metal solution)	Maximum adsorption capacity	Cheng et al. (2016)
					Live biomass – 16.34 mg/g	
					Dead biomass – 16.65 mg/g	
Chlorella vulgaris	Fe(II)	30–300 ppm	Dried biomass (freely suspended cells and immobilized in Ca-alginate)	Flasks containing 100 mL defined medium spiked with metals or palm oil mill effluent (POME)	Biosorption capacity (defined medium)	Ahmad et al. (2018)
	Mn(II)				Suspended cells	
	Zn(II)				Fe(II) – 74.54 mg/g	
					Zn(II) – 69.19 mg/g	
					Mn(II) – 65.14 mg/g	
					Immobilized cells	
					Fe(II) – 128.83 mg/g	
					Zn(II) – 115.90 mg/g	
					Mn(II) – 105.29 mg/g	

Microalgae	Heavy metal/dye	Concentration	Biomass	Biosorption capacity	References
				Biosorption capacity (palm oil mill effluent, POME) Suspended cells Fe(II) – 15.23 mg/g Zn(II) – 11.03 mg/g Mn(II) – 9.43 mg/g Immobilized cells Fe(II) – 25.76 mg/g Zn(II) – 21.76 mg/g Mn(II) – 18.74 mg/g	
Desmodesmus multivariabilis; Scenedesmus acuminutus; Chloroidium saccharophilum; Stichococcus bacillaris	La	15–150 mg/L	Dead biomass	*Desmodesmus multivariabilis* – most efficient at adsorbing La Maximal sorption capacity $(q_{max}) = 100$ mg/g Affinity $= 4.55$ L/g	Birungi and Chirwa (2014)
Gelatinous colonies of cyanobacteria from paddy fields (*Cyanothece* spp., *Leptolyngbya* spp.	Cu(II), Cd (II) and Pb (II)	Cu(II) and Cd (II): 1, 2, 5, 10, 20, 30, 40 and 50 mg/L	Dried biomass	Maximum adsorption capacities (mg/g biomass) Cu (II): 27.78 Cd (II): 28.57	Tran et al. (2016)

Table 23.1 (continued)

Microalgae	Heavy metals	Metal concentrations tested	Biomass used	Test system	Findings	References
and *Phormidium* spp.)		Pb(II): 10, 20, 30, 40, 50, 80 and 100 mg/L			Pb (II): 76.92; Maximum desorption using 0.1 M HNO_3: >90%	
Oedogonium sp.	Cu, Co, Cr, Fe, Hg, Ni, Zn and U in single and multi-ion solutions	50–500 mg/L	Filamentous algal biomass collected from the dam receiving gold mine effluents	250 mL screw top polypropylene flasks	Single-ion solutions: Maximal adsorption capacity at pH 2–5 for Cr, Cu, Ni and Zn. Multi-ion solutions: adsorption capacity for the metals was constant over the pH 2–7 (except for Ni and U)	Bakatula et al. (2014)
Oedogonium sp. / *Nostoc* sp.	Pb(II)	0.1–0.8 g/L	Freshly collected from ponds, ditches, etc.; dried before use	Not described	Maximum Pb (II) biosorption capacity; *Oedogonium* sp. 145.0 mg/g; *Nostoc* sp. 93.5 mg/g	Gupta and Rastogi (2008b)
Parachlorella sp.	Cd(II)	18–180 mg/L	Biomass from cultured microalgae	Equilibrium study: 0.02 g biomass in 20 mL Cd(II) solution; Kinetic study: 1 g/L, 5 L shaking bioreactor at 1 rpm	Maximum uptake: 96.2 mg/g; Equilibrium: Langmuir model; Kinetics – pseudo-first order	Dirbaz and Roosta (2018)

					Biosorption capacity	
Phaeodactylum tricornutum	Cd (II)	Cd (II): 1, 5, 10, 25, 50, 75 and 100 mg/L	Live cells	Glass (Pyrex) bottles with 500 mL seawater	67.1 mg/g after 96 h with ~40% of this capacity in the intra-cellular fraction	Torres et al. (2014)
Scenedesmus obliquus	Cd (II)	Influent conc.: 2.5–7.5 mg/L Flow rate: 15 ml/min	Living cells immobilized in loofa sponge	Fixed-bed column	Adsorption capacity of 38.4 mg (breakthrough time at 15.5 h) was achieved at a flow rate of 5 ml/min with an influent concentration of 7.5 mg Cd/L	Chen et al. (2014)
Scenedesmus obliquus CNW-N	Cd(II)	25–200 mg/L	Algae grown in photobioreactor aerated with 2.5% CO_2; biomass harvested by centrifugation and concentrated by lyophilization	0.8 g dry weight/L suspended in 250 mL metal solution (flasks?)	Maximum biosorption capacity: 68.6 mg/g Optimum biosorption capacity: pH 6.0 Optimal temp: 30 °C Adsorption equilibrium Langmuir model Kinetics: pseudo-second order	Chen et al. (2012)
Scenedesmus quadricauda *Neochloris pseudoalveolaris*	Co(II), Cr(III), Pb (II), Cd(II), Ni (II) and Mn(II)	5–40 mg/L	Living cultures	50 mL polyethylene centrifuge tubes (10 mL metal ion solution)	Biosorption capacity Co(II) – 2.14–52.48 mg/g Cr(III) – 1.98–81.98 mg/g Pb(II) – 8.05–4.26 mg/g Cd(II) – 7.81–24.96 mg/g	Kizilkaya et al. (2012)

(continued)

Table 23.1 (continued)

Microalgae	Heavy metals	Metal concentrations tested	Biomass used	Test system	Findings	References
					Ni(II) – 2.17–55.71 mg/g	
					Mn(II) – 3.54–75.20 mg/g	
Spirogyra sp.	Pb(II)	100 and 200 mg/L	Collected from a pond, sun dried and then oven dried at 70 °C for 24 h	250 mL flask with 100 mL metal solution	Maximum adsorption capacity – 140 mg/g biomass at pH 5.0 in 100 min with 200 mg/L of initial concentration	Gupta and Rastogi (2008a)
					Equilibrium – Langmuir isotherm	
					Kinetics – pseudo-second order	
					Endothermic	
Spirulina platensis	Cr(III)	0–156.3 mg/L	Freshly harvested biomass	Conical flasks (50 mL metal solution)	Cr(III) uptake followed Langmuir isotherm model	Li et al. (2006)
					pH – the most important factor that influences metal uptake; more efficient at higher pH (i.e. pH 6)	

Spirulina platensis	Pb (II)	Dead biomass	20–200 mg/L	Conical flasks (100 mL metal solution)	>90% removal. Optimal conditions for maximal adsorption pH 3, 2 g adsorbent dose, 26 °C, 100 mg/L Pb initial conc., 60 min contact time	Al-Homaidan et al. (2016)
Spirulina sp.	Effluent from copper smelter and refinery, with elevated levels of Hg and Cd	Cells lyophilizate	Cd – 3810 ug/kg. Hg – 760 ug/kg	Cells lyopilizate (0.25 g) added to 1 L of effluent in aerated stirred tank reactor	Metal concentration factor: 80–4250. Bioaccumulation capacity: Cd – 463 ug/g biomass. Hg – 1340 ug/g biomass	Chojnacka et al. (2004)
Ulothrix zonata	Cu(II)	Algae collected from irrigated water channels; dried at 100 °C for 5–6 h	5–50 mg/L	250 mL flask. Algal suspension (10 g/L)	Rapid removal of Cu (II) in the first 20 min. Optimum pH for Cu (II) removal – 4.5	Nuhoglu et al. (2002)

alga *Oedogonium* sp., collected from a site receiving gold mine wastewater. The adsorption equilibrium fitted the Freundlich model. The test algal biomass could be repeatedly used for three cycles of adsorption/desorption. In another study, Birungi and Chirwa (2014) investigated the adsorption and desorption kinetics of lanthanum (La) on four freshwater microalgae. Amongst the species tested, *Desmodesmus multivariabilis* was found to be most efficient at adsorbing La (100 mg/g) and was the best desorbent, with metal recovery of higher than 99%.

The affinity of microalgae in removing metals by biosorption varies with the type of metals. For instance, the affinity of *Chlamydomonas reinhardtii* for selected metals can be ranked in the following order: Pb(II) > Hg(II) > Cd(II) (Tuzun et al. 2005). The same order of biosorption capacities was observed when the biosorbent was tested in multi-metal system consisting of the three metals. This could be attributed to competitive binding amongst the metal ions for binding sites on the algal surface. Rajfur et al. (2012) compared the biosorption capacities of the biomass of *Spirogyra* sp. in removing Cu(II) in static and dynamic system. In the static system, the algal biomass was placed in a perforated container immersed in a fixed volume of $CuSO_4$ solution, while in the dynamic system, there was continuous flow of the metal solution. The sorption parameters were influenced by the ratio of algal biomass to the volume of metal solution in the static system but not in the dynamic system.

Monteiro et al. (2009) compared the capacities of two strains of *Desmodesmus pleiomorphus*, one isolated from a polluted site and another from culture collection, in removing Cd(II). The Cd(II) removal capacities of live cells of the wild and culture collection strains were comparable, with maximal Cd(II) removal of 61.2 and 76.4 mg/g biomass, respectively. The metal ions were mainly removed by adsorption onto cell surface of the microalgae, with an initial fast uptake, reaching maximum removal after 1 day exposure. Doshi et al. (2006) assessed the efficiency of bloom algae, consisting predominantly of *Chlorella* sp., in removing Cd(II) and Ni(II) and methyl orange (azo dye) by biosorption. The study found that the algal biomass was more efficient in removing metal ions compared to the dye. In addition, Nuhoglu et al. (2002) reported the potential of using dried biomass of *Ulothrix zonata* collected from the wild as a low-cost biosorbent for removal of Cu(II). In another study, dried biomass of the filamentous alga, *Spirogyra*, was reported to be an efficient biosorbent for Pb(II) from aqueous solution (Gupta and Rastogi 2008a). Nayak et al. (2003) assessed the biosorption capacities of living cells of three genera of algae in removing heavy metals and toxic radionuclides. Amongst the algae, *Spirulina* showed the highest radionuclide accumulation at pH 8, while *Oedogonium* adsorbed highest amount of Hg at acidic pH.

Various pretreatments may be employed to enhance metal sorption capacity by modifying cell wall properties of the microalgae (Mehta and Gaur 2005). Physical treatments that can be used include heating/boiling, freezing/thawing, drying and lyophilization, while chemical treatments may include washing the biomass with detergents, cross-linking with organic solvents and acid and alkali treatments (Wang and Chen 2009). For instance, *Oedogonium hatei* biomass treated with 0.1 M HCl was found to be an efficient biosorbent for Ni(II) ions from aqueous solution (Gupta et al. 2010). Similarly, pretreatment with 0.1 mM HCl enhanced the sorption

capacity of biomass of *Chlorella vulgaris* in removing metals from single and binary metal solutions (Mehta et al. 2002b). It was suggested that acid pretreatment released the adsorbed cations, including metal ions from the algal biomass, freeing the sites for metal binding. Pretreatment with $CaCl_2$ is another method used for the activation of algal biomass for biosorption (Mehta and Gaur 2005). For instance, pretreatment with $CaCl_2$ enhanced Pb(II) sorption capacity of *Spirulina maxima* biomass by 84–92% (Gong et al. 2005).

Immobilized algal cells have also been used in removing metals and have been shown to be more efficient than free cells. Recently, Shen et al. (2018) reported the high efficiency of Cd (II) removal using *Chlorella* sp. immobilized in water hyacinth-derived pellets. A maximum bioaccumulation capacity of 13.81 mg/g of the complex of water hyacinth biochar immobilized with algal cells was attained. The study also found that intracellularly sorbed Cd(II) accounted for 34.8% of the total metal ions adsorbed. Recovery tests showed that both the algal cells and biochar pellets could be recycled and reused. In another study, a continuous fixed-bed biosorption process using *Scenedesmus obliquus* immobilized in loofa sponge was found to be feasible in removing Cd(II) (Chen et al. 2014). Repeated adsorption/desorption cycles showed that the fixed-bed system could be reused.

Saeed and Iqbal (2006) reported that biosorption of Cd(II) by *Synechococcus* sp. immobilized in loofa sponge (*Luffa* sp.) could be enhanced by 21% compared to free biomass. In another study, immobilized *Chlorella vulgaris* in Ca-alginate beads was found to have higher biosorption capacity in removing Fe(II), Mn(II) and Zn(II) compared to free-suspended cells (Ahmad et al. 2018). Immobilized cells of *Chlamydomonas reinhardtii* in Ca-alginate was found to be efficient in biosorption of Hg(II), Cd(II) and Pb(II) (Bayramoğlu et al. 2006). Similarly, Wan Maznah et al. (2012) found that *Chlorella* sp. immobilized in sodium alginate beads showed higher biosorption capacity in removing Cu(II) and Zn(II) than free biomass. In another study, Mohapatra and Gupta (2005) assessed the ability of immobilized cells of *Oscillatoria angustissima* to remove Co(II), Cu(II) and Zn(II) in single, binary and ternary metal systems. The affinity of single metal removal could be ranked as follows, Cu(II) > Co(II) > Zn (II), while in binary system, Cu(II) inhibited the sorption of both Zn(II) and Co(II).

Cheng et al. (2016) evaluated the biosorption capacity and kinetics of Cd(II) by both living and non-living biomass of *Chlorella vulgaris*. The study showed that both living and dead cells of *Chlorella vulgaris* had high adsorption capacity for Cd(II), removing 96.8% and 95.2% of the metal, respectively. The biosorption efficiency of Cd(II) in natural water by the alga was lower, with values of 61.6% and 81.5% for living and dead biomass, respectively. In another study, Yang et al. (2015) reported that removal of Cd, Cu, Mn and Zn by living cells and lyophilized biomass of *Chlorella minutissima* was mainly through intracellular accumulation and partly by extracellular adsorption. In another study, Wehrheim and Wettern (1994) showed that whole cells of *Chlorella fusca* accumulated more metal ions than isolated algal cell walls.

Torres et al. (2014) reported that *Phaeodactylum tricornutum* has the potential for bioremediation of Cd in saline habitats. The diatom is highly tolerant to cadmium

and could remove Cd(II) through biosorption and bioaccumulation. In another study, gelatinous colonies of mixed population of cyanobacteria from paddy fields were found to be efficient for the adsorption of Cu (II), Cd (II) and Pb(II) (Tran et al. 2016). The cyanobacterial biomass could be reutilized for three biosorption-desorption cycles with only slight decrease in their biosorption capacity. Gupta and Rastogi (2008b) assessed the biosorption capacities of dried biomass of two filamentous microalgae, *Oedogonium* sp. and *Nostoc* sp., in removing Pb(II). The study showed that *Oedogonium* sp. was more efficient than *Nostoc* sp. in removing Pb(II), and regeneration of both biosorbents with recovery up to 90% could be attained using 0.1 M HCl. Regeneration of the biosorbent is important to ensure the reusability of the biomass and recovery of the sorbate. For instance, Chen et al. (2012) showed that cadmium-loaded biomass of *Scenedesmus obliquus* could be regenerated, with 0.1 M HCl giving higher desorption efficiency than 0.1 M $CaCl_2$. However, treatment with $CaCl_2$ was preferred as the regenerated biosorbent retained good adsorption capability even after five consecutive adsorption/desorption cycles.

There are only few reports on the use of microalgae for biosorption of metals from real industrial effluents. In one study, freely suspended and immobilized *Chlorella vulgaris* was shown to be efficient in removing Fe(II), Mn(II) and Zn(II) from palm oil mill effluent (POME) by biosorption (Ahmad et al. 2018). Chojnacka et al. (2004) reported that *Spirulina* sp. could remove trace elements, especially Hg and Cd, from industrial effluent from copper smelter and refinery by biosorption and bioaccumulation. In another study, El-Sheekh et al. (2005) showed that *Nostoc muscorum* and *Anabaena subcylindrica* were able to grow in effluent from salt and soda factory and sewage wastewater, removing metals such as Cu, Co, Pb and Mn.

4.2   Biosorption of Dyes

A wide range of microalgae, including both unicellular and filamentous species, have been shown to have good biosorption capacity in removing various dyes, especially malachite green and methylene blue (Table 23.2). Most of the studies were done using non-viable algal biomass on synthetic dye solution. In one study, algal biomass of *Microspora* sp. after lipid extraction was found to be an efficient biosorbent for methylene blue, removing the dye up to 100% in 24 h when agitated at 150 rpm (Maurya et al. 2014). Defatted algal biomass from an oleaginous microalga, *Scenedesmus dimorphus*, was also evaluated for its efficiency in removing methylene blue by biosorption (Sarat Chandra et al. 2015). The maximum adsorption capacity of the defatted algal biomass was comparable to raw and acid-pretreated biomass. Waste residue from algal biodiesel industry has been shown to be useful as biosorbent for dye removal. For instance, Nautiyal et al. (2017) reported that biochar derived from *Spirulina platensis* after oil extraction for biodiesel was found to be an efficient biosorbent for methylene blue. In another study, Chen et al. (2018) showed that biochar derived from residual biomass of *Ulothrix zonata* after pigment extraction could be used as a low-cost biosorbent for malachite green, crystal violet and Congo red.

Table 23.2 Summary of studies on the use of microalgae for biosorption of dyes

Microalgae	Dye	Concentrations tested	Biomass used	Test system	Findings	References
Chlorella sp.	Malachite green	2.0–20.0 mg/dm^3	Waste biomass from algae-manufacturing industry	3 L stirred batch biosorption apparatus with four baffles	Biosorption capacity −9.45–33.7 mg/g Kinetic of removal followed pseudo-second-rate model	Tsai and Chen (2010)
Chlorella pyrenoidosa	Methylene blue in simulated textile wastewater	10–60 mg/L	Harvested from cultures grown in textile wastewater Wet and dried biomass	250 mL conical flask containing 100 mL textile wastewater added with methylene blue; 0.15 g biomass	Adsorption isotherms Langmuir and Freundlich Kinetics Pseudo-second order Adsorption capacity Dry biomass 7.2–29.2 mg/g Wet biomass 5.6–18.24 mg/g	Pathak et al. (2015)
Chlorella vulgaris	Remazol Black B (RB) Remazol Red RR (RR)	20–800 mg/L	Cultured in defined medium before harvesting, drying and homogenizing the biomass for the study	10 mL dried biomass suspension added to 90 mL dye solution; biosorbent load: 1 g/L	RB – adsorbed most effectively (419.5 mg/g) Biosorption capacity increased with increasing initial dye conc.	Aksu and Tezer (2005)

Table 23.2 (continued)

Microalgae	Dye	Concentrations tested	Biomass used	Test system	Findings	References
	Remazol Golden Yellow RNL (RGY)				Biosorption equilibrium models – Freundlich, Langmuir, Redlich-Peterson and Koble-Corrigan	
					RB – sorption increased with increasing temperature up to 35 °C	
					RR and RGY – sorption decreased with increasing temperature	
Chlorella vulgaris	Textile dyes (Supranol Red 3BW, Lanaset Red 2GA and Blue EBNA) and textile wastewater	0–60 mg/L	Cells immobilized in 1% κ-carrageenan and 2% alginate	Immobilized cells were grown in flasks containing Bold's Basal Medium added with dye or 40% textile wastewater supplemented with 1.47 mM NaNO$_3$	Highest % colour removal from Lanaset Red 2GA (44.0%)	Chu et al. (2008)
					% colour removal from Supranol Red 2GA and Lanset Red 2GA	
					Cells immobilized in 1% κ-carrageenan > cells in 2% alginate	
					% colour removal from textile wastewater	
					Immobilized > suspension cultures	

Chlorella vulgaris	Textile wastewater (TW) and Supranol Red 3BW	TW: 36 L, Supranol Red: 20 mg/L	Live cultures – 10% inoculum	High rate algae ponds (HRAP)	Colour removal up to 50%, with removal of NH_4–N (44.4–45.1%), PO_4–P	Lim et al. (2010)
					(33.1–33.3%) and COD (38.3–62.3%)	
					Sorption of dye – Langmuir and Freundlich models	
Microspora sp.	Methylene blue	20–2500 mg/L	Collected from coastal lagoons, used after oil extraction	500 mL flasks containing 200 mL solution	Dye removal – 5–7 g/L	Maurya et al. (2014)
				Fixed-bed class column (1.2 × 15 cm) packed with 0.3 g algal biomass	Dye removal –107.57 to 139.11 mg/g	
Pithophora sp.	Malachite green	20–100 mg/L	Collected from the wild (fountain)	30 mL dye in 50 mL beaker	Dye removal	Kumar et al. (2005)
			Thermally activated at 300 °C		Raw algae	
					64.4 mg/g	
					Thermally activated	
					117.6 mg/g	
Scenedesmus dimorphus	Methylene blue	1–5 mg/L	Harvested from high rate algae ponds	2 L flasks	Maximum biosorption capacity	Sarat Chandra et al. (2015)
			Raw, defatted and acid-pretreated biomass		Raw	
					6.0 mg/g	
					Defatted –	
					7.73 mg/g	
					Acid-pretreated	
					7.80 mg/g	

(continued)

Table 23.2 (continued)

Microalgae	Dye	Concentrations tested	Biomass used	Test system	Findings	References
Spirulina platensis	Methylene blue	30–200 mg/L	Residual biomass after oil extraction, sun and oven dried, followed by physical activation in a muffle furnace at 450 °C for 2 h (biochar)	250 mL flask	>80% dye removal in <5 min	Nautiyal et al. (2017)
				Biosorbent dosage: 1 g/100 mL	Uptake Biochar – 4.60 mg/g Raw biomass – 4.17 mg/g	
Spirogyra sp.	Azo dye	5, 10 and 15 mg/L	Collected from a eutrophic lake, dried under sunlight, biomass treated with 0.1 N HCl	100 mL dye solution in 250 mL glass bottles	Dye removal efficiency: 35.3–64.0%	Mohan et al. (2008)
				0.1 g biosorbent	Increased with increasing temperature (10–50 °C) Increased with decreasing pH (2.0–10) Isotherm: Langmuir model	
Spirogyra sp.	Reactive Yellow 22	Simulated synthetic dye effluent – 25, 50 and 100 mg/L	Collected from a pond, viable algae	100 mL simulated dye solution in bottle	Initial acclimatization of 12-h contact time required	Venkata Mohan et al. (2002)
				Biosorbent dosage: 0.5, 1.0 and 2.5 g/100 mL	Maximum dye uptake – 72 h of contact time Average removal capacity 0.4 g dye/g biomass (dry weight)	

Spirogyra sp.	Textile wastewater (TW) containing 0.22% Synazol Red dye	Raw TW	Collected from pond, dried at 80 °C for 20 h, pretreated by autoclaving (121 °C, 20 min)	100 mL TW in 250 mL flasks 0.2 g algal biomass	Maximal dye removal: 85% Three repeated runs using the same biosorbent showed the similar biosorption rate	Khalaf (2008)
Ulothrix zonata	Malachite green, crystal violet and Congo red	Malachite green 500 mg/L Crystal violet 200 mg/L Congo red 200 mg/L	Collected from the wild, biomass used was after pigment extraction, dried at 105 °C for 12 h, subjected to pyrolysis at 400 °C to produce biochar	10 mL dye solution in 30 mL glass reactor added with 0.005 g algal biochar	Maximal dye adsorption capacity Malachite green – 5306 mg/g; Equilibrium isotherm Freundlich model Kinetics:: Pseudo-second-order model	Chen et al. (2018)

Devi et al. (2014) reported that the biosorption capacity of dried biomass of *Spirulina platensis* in removing reactive blue 19 dye (96.9 mg/g) was higher than that of the seaweed *Gracilaria edulis* (82.3 mg/g). In addition, *Chlorella*-based biomass derived from algae-manufacturing waste was found to be an efficient low-cost biosorbent for the removal of malachite green (Tsai and Chen 2010). Pretreatment of the algal biomass has been shown to enhance biosorption of dyes in several studies. For instance, thermally activated *Pithophora* sp. at 300 °C could enhance the sorption capacity of the biomass in removing malachite green compared to raw algae (Kumar et al. 2005). Pretreatment by autoclaving is another method to enhance the colour removal capacity of algal biomass, as indicated in the studies on the removal of Synazol reactive dye by *Spirogyra* sp. (Khalaf 2008) and malachite green by *Cosmarium* sp. (Daneshvar et al. 2007).

Living microalgae and cyanobacteria have also been shown to be able to efficiently remove colour from dyes and to treat dye-containing effluent. For instance, three cyanobacteria, *Anabaena flos-aquae* UTCC64, *Phormidium autumnale* UTEX1580 and *Synechococcus* sp. PCC7942, were evaluated for their efficiency in degrading textile dyes (indigo, RBBR and sulphur black) and dye-containing effluent (Dellamatrice et al. 2017). The study showed that the cyanobacteria could be used for bioremediation of textile effluent, particularly in removing the colour and reducing the toxicity of the dyes. It is noteworthy that *Phormidium autumnale* UTEX1580 could completely degrade indigo dye after 19 days of incubation.

In another study, *Chlorella vulgaris* grown in HRAP was found to remove up to 50% of the colour from textile wastewater and also reduced the load of other pollutants such as ammonia and phosphate (Lim et al. 2010). In addition, colour reduction up to 50% was achieved in the ponds containing textile dye alone (Supranol Red), which was attributed to sorption by the algal cells. The study concluded that the HRAP system growing *Chlorella vulgaris* could be an efficient system for the polishing of textile wastewater before discharge. Another study which highlighted the potential use of microalgae for bioremediation of textile wastewater and removal of dyes was by Pathak et al. (2015). In that study, *Chlorella pyrenoidosa* was found to be able to grow in textile wastewater, reducing phosphate, nitrate and BOD by 87%, 82% and 63%, respectively. Both wet (living) and dried (non-living) algal biomass from the cultures were further assessed for their ability to remove methylene blue in simulated textile wastewater. Dried biomass showed higher sorption efficiency due to its large surface area and high binding affinity for methylene blue compared to wet biomass. Recently, Dhaouefi et al. (2018) reported on the potential of an anoxic-aerobic photobioreactor for the treatment of synthetic textile wastewater involving microalgae-bacteria symbiosis. Efficient removal of carbon, nitrogen and phosphorus and heavy metals, as well as decolourization of the textile wastewater, was attained. However, the involvement of biosorption in the treatment system was not assessed in that study.

Apart from suspension cultures, immobilized microalgae have been shown to be efficient in removing colour from textile dyes and textile wastewater. For instance, *Chlorella vulgaris* immobilized in 2% alginate could remove 44% of the colour from the textile dye Lanaset Red 2GA at an initial concentration of 7.25 mg/L (Chu et al.

2008). The study also found that immobilized cells in alginate removed higher percentage of colour (48.9%) from the textile wastewater than the suspension cultures (34.9%).

5 Mechanisms and Equilibrium Modelling of Biosorption

Biosorption involve mechanisms such as adsorption, ion exchange and complexation/coordination (Gadd 2009). Ion exchange is the major mechanism of biosorption, which occurs through different functional groups present on the surface of the biomass (Verma et al. 2008). Through ion exchange, a readily exchangeable ion on the sorbent is replaced by another. Due to the weak attachment with the biomass, monovalent ions (H^+ and Na^+/K^+) are replaced with divalent ions of metals (Verma et al. 2008).

Variation in functional groups on the surface of the cell wall gives rise to the difference in biosorption mechanisms (Volesky 2007; Vijayaraghavan and Yun 2008; Wang and Chen 2009). Functional groups on cell wall of algae that are important for biosorption include carbonyl (ketone), carboxyl, sulfhydryl (thiol), sulfonate, thioether, amine, secondary amine, amide, imine, imidazole, phosphonate and phosphodiester (Volesky 2007). Eukaryotic algal cell wall consists of mainly cellulose, with potential metal-binding chemical groups including carboxylate, amine, imidazole, phosphate, sulfhydryl, sulphate and hydroxyl (Crist et al. 1981). The binding of metals with cell wall constituents of algae involves mainly ionic charge bonding while there is also covalent bonding with constituent proteins (Crist et al. 1981).

Recently, Ahmad et al. (2018) demonstrated through scanning electron microscopy (SEM)/energy-dispersive X-ray (EDX) analysis that sulphate, carboxyl and hydroxyl groups were involved in biosorption of metals by *Chlorella vulgaris*. In comparison, Fourier transform infrared (FTIR) spectra showed that binding of Cd (II) by *Chlamydomonas reinhardtii* involved mainly complexation to carboxylic functional groups (Adhiya et al. 2002). In another study, FTIR analysis revealed that the presence of amino, carboxyl, hydroxyl and carbonyl groups were responsible for the sorption of Pb(II) by the biomass of *Spirogyra* sp. (Gupta and Rastogi 2008a). Similarly, the main chemical interactions involved in the biosorption of Cd(II) by *Scenedesmus obliquus* were ion exchange between the metal ions and the hydrogen atoms of carboxyl, hydroxyl and amide groups of the algal biomass (Chen et al. 2012). Pores present on the surface of the algal biomass are also important in facilitating the sorption of metal ions. In another study, Li et al. (2006) investigated the process and mechanism of Cr(III) uptake using biomass of *Spirulina platensis*. The study showed that initially, Cr(III) was adsorbed to the unoccupied, negative sites on the surface of algal cell wall by electrostatic attraction. This was followed by chemical complexation and ion exchange and then binding to algal cell components such as proteins, polysaccharides and lipids.

In relation to biosorption, metal ions can be divided into A, B and borderline subcategories depending on their affinities to bind to cellular ligands, which are classified into Categories I, II and III (Volesky 2007; Wang and Chen 2009). Metal ions of Class A (e.g. Al, La and Ca) bind to Category I ligands through an oxygen atom. In comparison, metal ions Class B (e.g. Cu, Co, Zn and Cd) connect to ligands of Category II (e.g. RNH_2, -CO and $=N^-$) and Category III (e.g. S^{2-}, CO and CN^-). Borderline cations (e.g. Fe^{2+} and Mn^{2+}) can bind to various atoms of ligands from Categories I, II and III.

The cell wall biosorptive component of cyanobacteria consists of mainly peptidoglycan, with some species also produce extracellular mucilaginous polysaccharides (Fomina and Gadd 2014). Negatively charged exopolysaccharides produced by cyanobacteria have been shown to be an important chelating agent in removing positively charged metal ions from aqueous solution (De Philippis et al. 2011). For instance, Okajima et al. (2009) developed the megamolecular polysaccharide sacran, containing carboxylate and sulphate groups from the extracellular matrix of *Aphanothece sacrum*, for biosorption of metals such as indium and lead.

The mechanisms involved in dye biosorption include surface adsorption, chemisorption, diffusion and adsorption-complexation (Crini and Badot 2008). Amongst these, chemisorption involving the exchange of electrons is the main mechanism involved in the adsorption of anionic dyes in acidic conditions. Various kinds of interactions, such as chemical bonding, ion exchange, hydrogen bonds, hydrophobic interactions, van der Waals force, physical adsorption, aggregation mechanisms and dye-dye interactions, may also be involved. According to Crist et al. (1981), dye removal by adsorption on an adsorbent material may involve the following four steps: (1) bulk diffusion (migration of dye from the bulk of the solution to the adsorbent surface), (2) film diffusion (diffusion of dye through the boundary layer to the adsorbent surface), (3) pore diffusion or intraparticle diffusion (transport of the dye from the surface to within the pores of the particle) and (4) chemical reaction (adsorption of dye at an active site on the surface of the sorbent via ion exchange), complexation and/or chelation.

Maurya et al. (2014) demonstrated that the biosorption of dye by de-oiled algal biomass involved chemisorptions via surface active charges in the initial phase followed by physical sorption by occupying pores of the biomass. Similarly, Tsai and Chen (2010) concluded that the biosorption of malachite green by *Chlorella* biomass was due to the electrostatic interactions between the negatively charged surface area and positively charge dye molecule. In addition, the removal of azo dye by biomass of *Spirogyra* sp. was suggested to be due to the combined effect of chemical and ion-exchange sorption phenomena (Mohan et al. 2008).

While most studies tested on pure dye solution, Venkata Mohan et al. (2002) assessed the ability of *Spirogyra* to remove Reactive Yellow 22 azo dye from simulated dye effluent. The authors suggested that the mechanisms involved not only biosorption but also bioconversion and bioagulation. After being adsorbed onto the cell surface, the dye molecules diffuse into the algal cells and undergo subsequent bioconversion. The dye molecules in the aqueous phase coagulate with the biopolymers released as metabolic intermediates during metabolic conversion of the

dye. In another study, Chen et al. (2018) showed that dye adsorption by algae can be enhanced by subjecting the biomass to pyrolysis (800 °C), which could be due to the increased porosity and surface area values. At high temperature, the algal biomass is well carbonized because volatile matter such as cellulose and hemicellulose is removed.

A biosorption isotherm, the plot of uptake (Q) versus the equilibrium solute concentration in the solution (C_f), is often used to evaluate the sorption performance (Vijayaraghavan and Yun 2008). Two most commonly used modelling to explain, represent and predict the experimental behaviour of biosorption are the Langmuir and the Freundlich isotherms. The Langmuir isotherm is represented as follows: $Q = (Q_{max}b_LC_f)/(1+b_LC_f)$, where Q_{max} = maximum achievable uptake by a system, b_L = affinity between the sorbate and sorbent and C_f = equilibrium solute concentration. The Langmuir constant (Q_{max}) is often used to compare the performance of biosorbents. According to this model, there are a finite number of uniform adsorption sites and absence of lateral interactions between adsorbed species. In comparison, the Freundlich isotherm is represented as $Q = K_F C_f^{1/nF}$, where K_F corresponds to the binding capacity, while nF characterizes the affinity between the sorbent and sorbate. The isotherm is used to characterize the sorption to heterogenous surfaces or surfaces supporting sites with various affinities (Gadd 2009). The biosorption isotherms may vary with the type of algal biomass used. For instance, the biosorption equilibrium of metal biosorption by *Synechococcus* sp. fitted the Langmuir adsorption isotherm (Saeed and Iqbal 2006). In comparison, Aksu (2001) showed that biosorption of Cd (II) by *Chlorella vulgaris* fitted well both the Langmuir and Freundlich models.

6   Factors Affecting Biosorption of Metals and Dyes by Microalgae

Factors that influence biosorption process include physical and chemical properties of metal ions (e.g. molecular weight, ionic radius and oxidation state), properties of biosorbent (e.g. structure of the biomass surface) and the process parameters (e.g. pH, temperature and concentrations of biosorbent and sorbate) (Davis et al. 2003). Amongst these factors, pH is a key factor that influences the dissociation of sites, solution chemistry of metal ions, hydrolysis, complexation by organic and/or inorganic ligands, redox reactions and precipitation as well as the speciation and the biosorption affinity of metal ions.

Maximum adsorption capacity of metals by algal biomass occurs mainly at acidic pH. For instance, non-viable biomass of *Nostoc muscorum* removed highest amount of [Cr(VI)] at pH 3.0 (Gupta and Rastogi 2008c). Similarly, the maximum biosorption of Cd(II) by both free and immobilized cells *Synechococcus* sp. was at pH 4.0 (Saeed and Iqbal 2006). In comparison, maximum removal of Hg(II), Cd (II) and Pb(II) by immobilized cells of *Chlamydomonas reinhardtii* occurred at pH 5.0–6.0 (Bayramoğlu et al. 2006). The biosorption of Cd(II) by *Chlorella vulgaris*

increased with pH up to 4.0 and then decreased with further increase in pH (Aksu 2001). The low biosorption at extremely low pH (2–3) was postulated to be due to the association of hydronium ions (H_3O^+) with cell wall ligands, which restrict the binding of metal ions because of the repulsive force. In general, increasing pH enhances sorption of cationic dyes or basic dyes but reduces that of anionic metals or acidic dyes (Vijayaraghavan and Yun 2008). For instance, sorption of azo dye (an acidic dye) by non-viable biomass of *Spirogyra* sp. was found to be higher at lower pH (Mohan et al. 2008).

Increasing temperature generally enhances biosorption by increasing surface activity and kinetic energy of sorbate, but this may also damage the physical structure of the biosorbent (Park et al. 2010). For instance, Aksu (2001) showed that biosorption capacity of *Chlorella vulgaris* in removing Cd(II) decreased from 85.3 to 51.2 mg/g with an increase in temperature from 20 to 50 °C. It was postulated that as Cd(II) biosorption is normally an exothermic process, the capacity is higher at low temperature. In contrast, temperature variations from 5 to 40 °C did not affect the biosorption capacities of immobilized cells of *Chlamydomonas reinhardtii* in removing Hg(II), Cd(II) and Pb(II) ions from aqueous solution (Bayramoğlu et al. 2006). In addition, increased agitation speed may enhance biosorptive removal rate of the pollutant by minimizing mass transfer resistance (Park et al. 2010). However, when the mixing speed is too high, it may reduce the biosorption capacity. For instance, uptake of Cd(II) by *Parachlorella* sp. decreased when mixing speed was increased to 250 rpm due to damage of the algal cells. Apart from pH and temperature, culture age may affect biosorption capacity of microalgae in removing metal ions. For instance, Mehta et al. (2002a) found that older cultures of *Chlorella vulgaris* showed higher Cu(II) adsorption capacity than exponentially growing cultures, suggesting that there may be new/additional sites in older cells.

The increase of initial pollutant concentration may increase the quantity of biosorbed pollutant per unit weight of biosorbent (Fomina and Gadd 2014). For instance, the total amount of Cd(II) removed by *Desmodesmus pleiomorphus* increased with increasing initial metal concentration (Monteiro et al. 2009). Similarly, the amounts of Cu(II) adsorbed by *Ulothrix zonata* increased with increasing concentration of the sorbate (Nuhoglu et al. 2002). In addition, the presence of other pollutants may affect biosorption efficiency, as they may compete for the binding sites. The biosorptive removal of the target pollutant may be reduced due to increasing concentration of competing pollutants. The competitive effect of multi-metal ions on biosorption capacity may be influenced by the concentration of algal biomass tested. For instance, competitive effects on biosorption of Cu(II) and Cd (II) were only observed in *Scenedesmus abundance* at the lowest algal concentration tested (15.1 mg/L).

The concentration of algal biomass used may affect the removal of heavy metals by biosorption. For instance, the capacity of *Scenedesmus abundance* in removing Cd (II) and Cu(II) increased with decreasing concentration of algal biomass (Terry and Stone 2002). Similarly, the amount of Cu(II) adsorbed to *Ulothrix zonata* increased from 38 to 160 mg/g with the decrease of algal biomass from 1.0 to 0.1 g/L (Nuhoglu et al. 2002). In another study, the adsorption capacity of *Scenedesmus obliquus* for Cd

(II) decreased with increasing adsorbent dosage, but the removal efficiency was nearly 100% at dosage higher than 0.6 g (Chen et al. 2012). Similarly, the removal of Pb (II) by *Spirogyra* sp. increased from 31.2% to 80% with the increase of adsorbent dose from 0.05 to 10 g/L (Gupta and Rastogi 2008a).

Maurya et al. (2014) assessed the influence of multiple factors on biosorption efficiency of de-oiled algal biomass in removing methylene blue based on an artificial neural network model. The results showed that the relative importance and ranking of the input variables for dye removal efficiency are as follows: temperature > agitation speed > contact time > pH > initial dye concentration > adsorbent dose. In another study, efficiency in the removal of Acid Red 274 dye by *Spirogyra rhizopus* was influenced by initial pH, temperature and initial dye and algal biomass concentrations (Özer et al. 2006). The optimum conditions for dye removal by the alga were at initial pH 3.0, temperature 30 °C and algal concentration 0.5 g/L.

The removal of azo dye (Reactive Yellow 22) by *Spirogyra* was also found to be dependent on the concentrations of dye and algal biomass (Venkata Mohan et al. 2002). An incremental increase of 10% dye removal was observed for every 0.5 g increase of biomass, which could be attributed to the more surface area for sorption with the increased biomass. In addition, the authors observed that there was increase and then decrease in pH during the dye removal process, which could be attributed to the interaction between the cell surface and dye molecule. In comparison, highest sorption of Synazol (anionic dye) from textile wastewater by *Spirogyra* sp. occurred at pH 3.0, with very little removal between pH 6.0 and 8.0 (Khalaf 2008). Similarly, highest sorption capacity of dried biomass of *Chlorella vulgaris* in removing three vinyl sulfone-type reactive dyes (Remazol dyes) was at pH 2.0 (Aksu and Tezer 2005). The sorption capacity was also affected by temperature, with maximum capacity at 25 or 35 °C, depending on the type of dyes.

7 Living Versus Non-living Algal Biomass for Biosorption

The use of non-living algal biomass is preferred to living cells for removal of metals and dyes by biosorption. It is advantageous to use non-living biomass as there is the absence of toxicity limitations, absence of requirements for growth media and nutrients, easy recovery of the sorbates, easy regeneration and reuse of biomass, possibility of easy immobilization of dead cells and easier mathematical modelling of uptake of sorbates (Dhankhar and Hooda 2011). However, there may be a need to use living cells for overall removal of heavy metals and other pollutants. For instance, the use of metal-resistant microalgae is desirable to ensure better removal of metals and other pollutants, involving a variety of processes including bioprecipitation, biosorption and continuous uptake of metals after physical adsorption (Malik 2004). Such processes may lead to simultaneous removal of toxic metals, organic pollutants and other inorganic impurities. Metabolic processes are important in bioremediation systems such as sewage treatment, biofilm reactors for pollutants and anaerobic digestion, where biosorption is a component of the overall process (Gadd 2009).

There have been reports on the use of both living and non-living algal biomass for biosorption of heavy metals. For instance, Kızılkaya et al. (2012) and Alam et al. (2015) showed that living biomass of *Scenedesmus quadricauda* and *Neochloris pseudoalveolaris* was effective in removing Co(II), Cr(III), Pb(II), Cd(II), Ni(II) and Mn(II) from aqueous system by biosorption. Adhiya et al. (2002) reported that lyophilized and living cells of *Chlamydomonas reinhardtii* showed similar ATR-FIR spectra, suggesting that lyophilization did not change the chemical composition of the cell surface, including cell wall. However, thermally inactivated cells of *Desmodesmis pleiomorphus* showed lower Cd(II) adsorption than living cells at the highest metal concentration tested (5 mg/L) (Monteiro et al. 2009). In another study, living cells of *Scenedesmus abundans* were found to be more efficient in removing Cu(II) and Cd(II) than non-living algae (Terry and Stone 2002). The use of microalgae with self-flocculating ability as biosorbent is advantageous as it reduces the harvesting costs. For instance, Alam et al. (2015) reported the potential use of a self-flocculating strain of *C. vulgaris* as an efficient biosorbent for the removal of Cd (II), with a maximum sorption capacity (144.93 mg/g), which was much higher than the non-flocculating strain (84.03 mg/g).

Both living and non-living algal biomass have also been assessed for their ability to remove dyes (Daneshvar et al. 2007). For instance, decolourization of malachite green dye by living cells of *Cosmarium* sp. was mainly by biodegradation (Daneshvar et al. 2007). However, autoclaved dead biomass could also remove the dye by 63%, comparable to living cells (74%). It was suggested that autoclaving ruptured the cells, increasing the surface area for sorption and exposing more binding sites on the cell wall.

8   Scaling Up and Commercialization of Biosorption Technologies Based on Microalgae

Biosorption technologies based on microalgae for removal of metals and dyes have not been fully developed for large-scale applications in industries (Ghosh et al. 2016). Most studies on biosorption were conducted using synthetic solution under controlled laboratory based on single species of algae. Actual industrial effluent is much more complex, consisting of metals and dyes together with other organic compounds and salts. The test algae may not be efficient in removing the dyes or metals on a larger scale using actual effluent. Lim et al. (2010) evaluated the potential application of *Chlorella vulgaris* for bioremediation of textile wastewater using HRAP. Apart from 41.8% to 50.0% of colour removal, there was also significant reduction in other pollutants such as ammoniacal nitrogen, phosphate and carbon oxygen demand (COD) from the wastewater. The use of HRAP to produce high-density cultures is well established as an efficient system for treating agroindustrial effluents (Phang et al. 2001, 2015; Mustafa et al. 2011).

Batch culture studies are important to gather sufficient data before scaling up of the biosorption system (Kumar et al. 2016). Continuous flow studies, such as those conducted using packed bed column, appears to be more efficient and economically feasible than batch operation for metal sorption. For instance, Saeed and Iqbal (2006) reported that 63.7% removal of Cd(II) could be attained using immobilized *Synechococcus* sp. packed in a fixed-bed column bioreactor with continuous liquid flow system. Fixed-bed column containing algal biomass can be used for biosorption of heavy metals and/or dyes for final polishing of industrial effluents before discharge. The effluent is passed through a column with biosorbent, which can be regenerated when the maximal sorption capacity is reached (Zabochnicka-Świątek Magdalena 2014). The columns can also be arranged in parallel for sorption and desorption processes to occur without interruption in a continuous flow system (Gadd 2009). Fixed-bed column systems have been used mainly with seaweed rather than microalgal biomass. For instance, Ibrahim and Mutawie (2013) developed a fixed-bed column using biomass from red seaweeds for the removal of Cu(II), Zn(II), Mn(II) and Ni (II) from industrial effluent from chemical fertilizer factory. Batch and continuous stirred up tank reactors are also used in large-scale biosorption systems (Ghosh et al. 2016). In continuous stirred tank reactors, the inflow and outflow of medium and substrate are kept equal, while agitation and aeration are applied to mix the medium content.

Despite the extensive research, commercialization of biosorption technologies based on microalgae is still limited (Fomina and Gadd 2014). In the 1990s, a biosorbent, AlgaSORB™, based on *Chlorella* immobilized in silica or polyacrylamide gels was developed and commercialized for wastewater treatment (Garnham et al. 1997). The biosorbent can efficiently remove metal ions from dilute solution of 1 to 100 mg/L, reducing the concentration to below 1 mg/L. The biosorbent resembles an ion-exchange resin, which can undergo more than 100 biosorption/desorption cycles. Another biosorbent that has been commercialized is Bio-fix, which consists of a variety of biomasses, including *Sphagnum* peat moss, algae, yeast, bacteria and/or aquatic flora immobilized in high-density polysulphone. The granular Bio-fix has been tested for the treatment of acid mine waste, particularly for removal of Zn(II) (Garnham et al. 1997).

9 Future Directions of Research

Integrating biosorption technologies with other applications of microalgae, especially for biofuel production and CO_2 fixation, would be a way forward in attempts to develop cost-effective and environment-friendly bioremediation system for removing heavy metals and dyes from industrial effluents. Along this line, Yang et al. (2015) showed that *Chlorella minutissima* was effective in removing Cd, Cu, Mn and Zn ions from artificial wastewater. The elevated levels of Cd and Cu also induced lipid accumulation, generating useful algal biomass for biofuel production. The potential use of residual algal biomass after lipid extraction as biosorbents

should be further worth explored. For instance, waste biomass of green algae after oil extraction has been shown to be useful as a low-cost biosorbent for Pb(II), Cu(II) and Co (II) (Bulgariu and Bulgariu 2012). In addition, de-oiled biomass of *Microspora* sp. has been shown to be useful as biosorbent for dye, utilizing the waste stream from algal biofuel production (Maurya et al. 2014). Microalgae which are able to fix CO_2 efficiently are advantageous for use as biosorbents. For instance, Chen et al. (2012) showed that an efficient CO_2-fixing microalga, *Scenedesmus obliquus* CNW-N, could be used as a useful biosorbent to remove Cd(II) from aqueous solution, with a maximum capacity of 68.6 mg/g. The microalga could be first grown with continuous feeding of 2.5% CO_2 to generate high-density biomass as biosorbent for Cd(II) removal.

Most of the reported biosorption studies on microalgae were based on unialgal cultures or biomass derived from single species. Although such studies are useful in providing insights into the mechanistic aspects of biosorption, there is a need to assess the efficiency of the microalgae in removing heavy metals or dyes from real industrial effluents. Efficient treatment of industrial effluents involves not just a single algal species but requires the symbiotic involvement of microalgae with other microorganisms such as bacteria (Dhaouefi et al. 2018). Thus, there is a need for more biosorption studies based on consortium of microalgae and other microorganisms grown in industrial effluents. The potential of co-culturing microalgae with bacteria or yeasts to generate high amounts of biomass as biosorbents should be further explored. Such concept has been proposed as a strategic approach to enhance biomanufacturing processes based on algae (Padmaperuma et al. 2017). Further, the potential application of biofilms as biosorbents is another area that needs further investigations. The biofilm community, consisting of microalgae, cyanobacteria and other bacteria, is known to produce extracellular polymeric substances (EPS), which could enhance the biosorption capacity for heavy metals (Comte et al. 2008).

Detailed economic and market analyses are required to assess the feasibility of using algae-based biosorption technology for the removal of heavy metals and dyes (Srinivasan and Viraraghavan 2010). In addition, detail life cycle analysis (LCA) is needed to assess the environmental impacts if the biosorption component is incorporated into an algal biofuel production system integrated with wastewater treatment (Mu et al. 2014). In developing further the industrial use of algae-based biosorption technology, there is a need to adopt a multidisciplinary approach in which chemists, biologists and engineers work together. Apart from engineering principles involved in designing the biosorption system, there is a need of better understanding of biological processes involved and the chemistry involved in the binding of sorbates with cell materials. Furthermore, with the advent of "omic" tools, genomic and metabolomic approaches can be applied to enhance the biosorption capacity of microalgae in removing metals and dyes. For instance, genetically engineered bacteria with modified metal-binding peptides on their cell surface have shown improved affinity and selectivity for biosorption of target metals (Mosa et al. 2016). However, such approach has not been explored yet in attempts to enhance the biosorption capacity of microalgae.

While the use of dead algal biomass as biosorbents is advantageous, the potential of hybrid technology combining both living and non-living cells should be further explored (Wang and Chen 2009). Combining biosorption with other biotechnological processes such as bioaccumulation, bioreduction and bioprecipitation is important for effective treatment of real effluents. The use of living algal cells is desirable in bioremediation of dye-containing wastewater, as efficient removal of dyes involves not only biosorption but also biodegradation (Vikrant et al. 2018). For instance, the decolourization of dyes by *Chlorella vulgaris* and *Nostoc linckia* involves azo dye reductase enzyme, which was induced when the microalgae were treated with G-Red and methyl red (El-Sheekh et al. 2009).

10 Concluding Remarks

There have been extensive studies on the use of microalgae as biosorbents for heavy metals and dyes reported in the literature, as highlighted in this chapter. However, most of the studies were based on metal or dye solution rather than real industrial effluents. Such experimental studies have provided much insights into the mechanistic aspects of biosorption of metals and dyes by microalgae, particularly on adsorption equilibrium and kinetic modelling. Despite that, commercialization of biosorption technologies based on microalgae is still limited. For efficient bioremediation of industrial effluents, especially in removing heavy metals and dyes, combination of biosorption technologies with other processes such as bioconcentration and bioconversion, using both living and non-living algal biomass would be a strategic approach. More studies focusing on the integration of biosorption technologies with other applications, particularly using the microalgae for biofuel production and CO_2 biofixation, would be the way forward.

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